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CHAPTER 61

Liver

STRUCTURE AND FUNCTION

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Liver Structure

The hepatic lobule is the anatomic unit of the liver. In the anatomic model, liver lobules are organized into irregular polygons demarcated by connective tissue and composed of plates of hepatocytes radiating outward from the central vein to the portal triads (Figure 61-1). The hepatic acinus is the functional unit of the liver. In the functional model hepatocytes are instead oriented around the afferent vascular system (portal veins and hepatic arteries) just as they anastomose into sinusoids (Figure 61-1), and the central vein is at the periphery of the acinus instead of centrally located as in the anatomic model. The acinus is divided into three contiguous zones (1, 2, and 3) that correspond to distance from the arterial blood supply. Those hepatocytes in closest proximity to the arterioles (zone 1) receive the greatest oxygen content, but are also first in line to be affected by toxins transported from the gut to the portal vein. Zone 3 hepatocytes reside at the periphery of the acinus near the central vein, and zone 2 hepatocytes are interspersed between zone 1 and zone 3 hepatocytes. The anatomic model is perhaps easier to understand, but the functional model serves as a better foundation for understanding liver pathology.[1] In either model portal venous and arterial blood flow centripetally, that is, toward the central vein, whereas bile flows centrifugally, that is, away from the central vein. Hepatocytes extract nutrients and oxygen from portal and arterial perfusion, respectively, and produce bile acids and other bile constituents that are transported from hepatocytes into bile canaliculi, ductules, and ducts.

Biliary Tract Structure

The basic elements of the biliary tract are the hepatic canaliculi, bile ductules, intralobular ducts, interlobular ducts, hepatic ducts, cystic duct, gallbladder, common bile duct, and the pancreaticobiliary sphincter (of Oddi).[2] There are many variations on this central theme, the most important of which are (a) the pancreaticobiliary sphincter is a common physiologic and anatomical channel at the duodenal papilla in the cat[3] and (b) there are many anatomic variations in the feline gallbladder, from single gallbladder to bilateral gallbladders, body duplication, fundic duplication, complete duplication, septate, and Y-shaped gallbladder.[4]

Cells of the Liver

Hepatocytes

Hepatocytes account for 60% to 80% of the liver cell mass (see Table 61-1) and contribute to a wide range of metabolic activity, including carbohydrate, protein, lipid, nucleic acid, porphyrin, metal, vitamin, glutathione, hormone, and xenobiotic metabolism; coagulation factor synthesis; biliary secretion; and immune surveillance.[1,5] Hepatocytes have an eosinophilic cytoplasm reflecting numerous mitochondria, and basophilic stippling caused by large amounts of rough endoplasmic reticulum and free ribosomes. Hepatocyte nuclei are round with dispersed chromatin and prominent nucleoli. Anisokaryosis is common and often reflects various degrees of polyploidy, a normal feature of more than 50% of hepatocytes. The average life span of the hepatocyte is 5 to 6 months reflecting their ability to regenerate. Hepatocytes are organized into plates separated by vascular channels (sinusoids), an arrangement supported by a reticulin (collagen type III) network. The sinusoids have a discontinuous, fenestrated endothelial cell lining. The endothelial cells have no basement membrane and are separated from the hepatocytes by the space of Disse, which drains lymph into the portal lymphatics. Hepatocytes are supported by a number of other cell types, which account for 40% of the liver cell mass.

Cholangiocytes

Representing 3% to 10% of liver cell mass, cholangiocytes are also known as biliary epithelial cells.[6] They secrete water, bicarbonate, and cations into the bile in the physiologic state, but they may also participate in the immune response as antigen-presenting cells in disease states. The biliary tract is a convergent system of canals that begins in the canaliculi, followed by the bile ducts, and ending with the common bile duct. Bile secretion depends on the function of membrane transport systems in hepatocytes and cholangiocytes and on the structural and functional integrity of the biliary tract. The hepatocytes, constituting the most abundant liver cell population, generate the so-called primary bile in their canaliculi. Biliary canaliculi are blind tubular structures, with a very high surface-to-volume ratio that by means of osmotic gradients favors the formation of bile flow. Cholangiocytes, which constitute 3% to 10% of the liver cells, modify the canalicular bile by secretory and reabsorptive processes as bile passes through the bile ducts, and they are responsible for approximately 30% of bile volume. In contrast to hepatocytes, where secretion is constant and poorly controlled, cholangiocyte secretion is broadly regulated.[5,6]

Table 61-1 Cells of the Liver and Their Functions

Cell Type	Other Names	Functions	Cell Markers
Hepatocytes	Liver cells	Intermediary metabolism	Albumin, cytokeratin 8 and 18
Cholangiocytes	Biliary epithelial cells	Line the bile ducts, secretion	Cytokeratin 7 and 19
Kupffer cells	Browicz-Kupffer cells, stellate macrophages	Phagocytosis of pathogens and particles	ED-1 and ED-2
Stellate cells	Ito cells, vitamin A–storing cells, lipocytes	Storage of vitamin A; production of myofibroblasts in injury	GFAP, desmin; α-smooth muscle actin
Natural killer (NK) cells	Pit cells, large granular lymphocytes, γδ T cells	Immune surveillance–infection, cancer	CD3
Vascular endothelial cells	Endothelial cells	Line blood vessels	CD34 and CD31
Lymphatic endothelial cells	Endothelial cells	Line lymphatic vessels	Podoplanin
Smooth muscle cells	Myocytes	Regulation of microcirculation	Myocardin, α-smooth muscle actin
Portal tract fibroblast	Fibroblasts	Integrity of portal triads, supporting function	Vimentin
Stem cells	Progenitor cells, oval cells	Bi-potential progenitor cell for hepatocytes and biliary epithelial cells	α-Fetoprotein

GFAP, Glial fibrillary acidic protein.
Adapted from Wallace K, Burt AD, Wright M: Liver fibrosis. *Biochem J* 411:1, 2008.

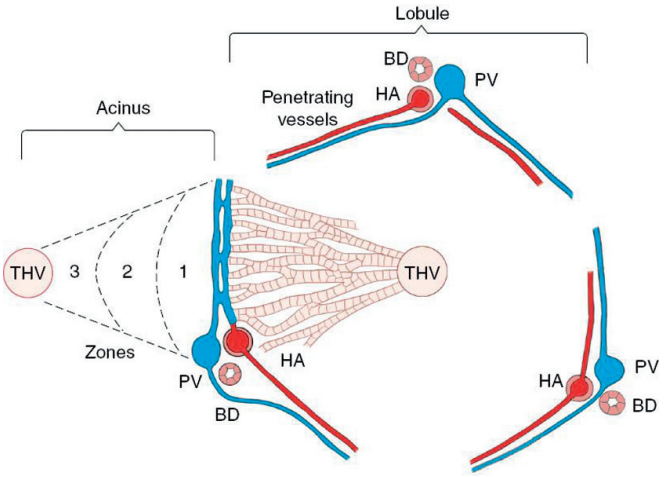

Figure 61-1 The anatomic unit of the liver is the hepatic lobule. The functional unit of the liver is the hepatic acinus. BD, bile duct; HA, hepatic artery; PV, portal vein; THV, terminal hepatic venule. (From Crawford JM: The gastrointestinal tract. In: Cotran RS, Kumar V, Robbins SL, editors: *Robbin's Pathologic Basis of Disease*, Philadelphia, 1994, Saunders.)

Kupffer Cells

Also known as *Browicz-Kupffer cells* or *stellate macrophages*, these cells represent 2% to 5% of the liver cell mass, and are specialized macrophages localized to the sinusoids as part of the mononuclear phagocyte system. Kupffer cells begin their development in the bone marrow with the genesis of promonocytes and monoblasts into monocytes, and then on to peripheral blood monocytes, completing differentiation into Kupffer cells within the liver. In health, Kupffer cells are involved in the metabolism of erythrocyte hemoglobin. During perfusion of the liver, senescent red blood cells are phagocytized by the Kupffer cells, and the hemoglobin molecule is further metabolized into its component parts. Globin chains and amino acids are reutilized; the iron-containing portion of heme is removed, transported, and stored; and heme is further oxidized into bilirubin, conjugated with glucuronic acid within hepatocytes, and secreted into the bile. Kupffer cells also express a complement receptor of the immunoglobulin family, without which the liver cannot clear complement system–coated pathogens.

In disease states, Kupffer cells contribute to the pathology of ethanol and other toxic principles through production of inflammatory mediators, activation of Toll-like receptors, and elaboration of tumor necrosis factor (TNF-α).[7] Kupffer cell activation is responsible for early ethanol-induced liver injury, common in chronic alcoholics. Ethanol activates the Toll-like receptor 4 and CD14, receptors on the Kupffer cell that internalize the endotoxin lipopolysaccharide. Internalization activates the transcription of TNF-α and production of superoxide (a prooxidant). TNF-α then enters the stellate cell in the liver, leading to collagen synthesis and fibrosis. Fibrosis eventually causes cirrhosis or loss of function of the liver (see the role of the stellate cell, which is discussed in "Stellate Cells" section that follows).

Stellate Cells

Hepatic stellate cells (HSCs) (also referred to as vitamin A–storing cells, lipocytes, interstitial cells, fat-storing cells, and Ito cells) exist in the space between parenchymal cells and liver sinusoidal endothelial cells of the hepatic lobule and store 50% to 80% of vitamin A in the whole body as retinyl palmitate in lipid droplets in the cytoplasm.[7-10] In physiologic conditions, these cells play pivotal roles in the regulation of vitamin A homeostasis. In pathologic conditions, such as hepatic fibrosis or liver cirrhosis, HSCs lose vitamin A and synthesize a large amount of ECM components, including collagen, proteoglycan, glycosaminoglycan, and adhesive glycoproteins. The morphology of these cells also changes from that of the star-shaped stellate cell to that of the fibroblast or myofibroblast. HSCs are now considered to be targets of therapy of hepatic fibrosis or liver cirrhosis.[11] Activation of HSCs, a key event in liver fibrosis, is caused by diminished adipogenic transcription.[12] Wnt signaling inhibits antiadipogenic activation of HSCs and liver fibrogenesis; wnt antagonism inhibits HSC activation and liver fibrosis.[9]

Pit Cells

Also known as natural killer (NK) cells or large granular lymphocytes, pit cells represent 1% of liver cell mass, and serve as part of the immune surveillance mechanism in the hepatic sinusoids. Pit

cells belong to the group of sinusoidal cells, together with Kupffer, endothelial, and fat-storing (stellate) cells. Pit cells use the FasL Fas ligand (FasL) and perforin/granzyme pathway to kill target cells. FasL on effector cells binds the Fas that is present on the target cell membrane, which results in oligomerization of Fas and activation of caspase 8. Perforin and granzymes, of which granzyme B is the most potent, reside in granules of the cytotoxic lymphocytes and are released by exocytosis. Intracellular delivery of granzyme B results in the initiation of the caspase cascade by proteolytic activation of caspase 3, either directly or through a mitochondrial-dependent pathway. Caspases play a central role in the execution of apoptosis.

Endothelial Cells

Lymphocyte recruitment from the circulation into tissue is dependent on the ability of the lymphocyte to recognize and bind molecules on the endothelial cell surface that promote transendothelial migration. A multistep model of leukocyte adhesion to vascular endothelium has been described and is broadly applicable, although the details of the signals involved differ between tissues.[13,14] In a generally accepted model, tethering or rolling receptors expressed on endothelial cells capture free-flowing leukocytes. These receptors may be either selectins or members of the immunoglobulin superfamily. Once captured, the leukocyte can receive activating messages presented by endothelial cells in the form of chemokines that activate specific G-protein–coupled receptors on the leukocyte surface. Occupancy of these receptors triggers a cascade of intracellular signals that results in the presentation of high-affinity integrin receptors on the leukocyte surface that bind to immunoglobulin family of counterreceptors on the endothelium to promote leukocyte arrest on the vessel wall. In the presence of the appropriate migratory signals the leukocyte will migrate across the endothelium into tissue, where it follows a hierarchy of chemotactic signals toward the focus of inflammation.

Smooth Muscle Cells

Representing 2% to 5% of the liver cell mass, smooth muscle cells are located primarily in the hepatic artery and portal vein and their tributaries, and serve primarily to regulate the hepatic microcirculation.

Hepatic fibrosis is a common outcome of hepatic injury in the dog. Activated fibroblasts that develop myofibroblastic characteristics play an essential role in hepatic fibrogenesis, and are comprised of three subpopulations: (a) portal or septal myofibroblasts, (b) interface myofibroblasts, and (c) the perisinusoidally located HSCs.

Stem Cells

It is difficult to arrive at a universally applicable definition of a stem cell because some of the defined properties of a stem cell can be exhibited by the stem cells in some tissues or organisms but not in others. In spite of that, a generally acceptable consensus defines a stem cell as an undifferentiated cell that has capacity to self-renew, for production of progeny in at least two lineages, for long-term tissue repopulation after transplantation, and for serial transplantability. In addition, stem cells exist in a mitotically quiescent form and clonally regenerate all of the different cell types that constitute the tissue in which they exist. They can undergo asymmetrical cell division, with production of one differentiated (progenitor) daughter and another daughter that is still a stem cell. The offspring of stem cells are referred to as progenitor cells, also named as transit amplifying cells and therefore cannot be serially transplanted, and are classified as early and late. The early progenitor or stem/progenitor cells have multilineage potential and similar characteristics to stem cells. The late progenitor cells have differentiated further and produce progeny in only a single lineage. Although they divide rapidly, they are capable of only a short-term tissue reconstitution and they do not self-renew.[15]

Early studies in hepatocyte turnover and liver regeneration showed that the parenchymal cell, the hepatocyte, was the primary and only cell involved in tissue renewal. However, new studies of liver regeneration, hepatocarcinogenesis, liver transplantation, and various cell lines show that a variety of cell types participate in maintaining hepatocyte number and mass. Recent studies indicate the presence of both intrahepatic and extrahepatic stem/progenitor cell populations that serve to maintain the normal organ and to regenerate damaged parenchyma in response to a variety of insults. The intrahepatic compartment most likely derives primarily from the biliary tract, particularly the most proximal branches, that is, the canals of Hering and smallest ductules. The extrahepatic compartment is at least in part derived from diverse populations of cells from the bone marrow. Embryonic stem cells are considered as a part of the extrahepatic compartment.[16] The precise role(s) of each of these individual cells remains to be determined, but it is clear that in the aggregate they confer the vast regenerative capacity of the liver .

Liver Function

Metabolism

The liver is involved in many aspects of intermediary metabolism.[1]

Carbohydrates

The liver is at the center of carbohydrate metabolism through its role in maintaining normoglycemia. Glucose is the primary energy source for most mammalian cells, and its metabolism is tightly regulated to guarantee that a sufficient supply is available to glucose-dependent organs, particularly the brain. Glucose can be made available from two sources: absorption of dietary glucose from the intestine, and release of glucose from organs such as the liver and kidney. Early in fasting, the majority of endogenous glucose is generated by glycogenolysis where glycogen in the liver is converted to glucose-6-phosphate under the regulation of debranching enzyme, hepatic glycogen phosphorylase, and phosphorylase kinase. With more prolonged fasting, endogenous glucose is generated by gluconeogenesis from certain substrates such as amino acids, lactate, and glycerol. Both processes generate glucose-6-phosphate, which must then be dephosphorylated in order to transport glucose out of the cell.

- Early fasting: glycogen → glycogenolysis → glucose → normoglycemia
- Prolonged fasting: amino acids → gluconeogenesis → glucose → normoglycemia

The enzyme responsible for the dephosphorylation of glucose-6-phosphate is glucose-6-phosphatase-α. Alterations in quantity, location, or activity of glucose-6-phosphatase, such as those seen in type 1 glycogen storage diseases, effectively result in a lack of all endogenous glucose production and severe hypoglycemia develops during periods of fasting.[17]

Proteins

The liver is an important site of protein metabolism. Amino acids and proteins absorbed from the intestine or produced in the body are delivered to the liver. The liver deaminates amino acids and

converts them to carbohydrates and lipids.[18-21] Deamination produces α-keto acids, which can be metabolized for energy or used for synthesis of monosaccharides and fatty acids.[20] The liver synthesizes amino acids from intermediates of carbohydrate and lipid metabolism by amination and transamination.[21] Examples of amino acid transaminations include:

- Alanine + α-ketoglutarate ↔ pyruvate + glutamate
- Aspartate + α-ketoglutarate ↔ oxaloacetate + glutamate

The liver synthesizes many proteins, including albumin and fibrinogen, most α globulins, and some of the β globulins. Prothrombin and clotting factors V, VII, VIII, IX, and X are produced in the liver, as well as ceruloplasmin, ferritin, and many serum enzymes.

Lipids

Lipid metabolism and transport is organized into three basic transport systems: (a) exogenous transport, which is associated with the metabolism of exogenous (dietary) lipids, (b) endogenous transport, which is associated with the metabolism of endogenously produced lipids, and (c) reverse transport, which is associated with the transport of lipids from the periphery (e.g., skeletal muscle, adipose, connective tissue) to the liver.

Exogenous Transport. Triglyceride is the major dietary lipid, along with cholesterol, phospholipids, and fat-soluble vitamins.[22] The digestion of dietary lipids begins in the proximal GI tract with the action of lingual and gastric lipases, and is completed in the small intestine with the actions of pancreatic lipase, cholesterol ester hydrolase, and phospholipase A_2. Lipid digestion and absorption is more complicated than carbohydrate and protein digestion and absorption because of lipid solubility characteristics, and involves emulsification of lipids by bile salts, hydrolysis by pancreatic lipase and colipase, solubilization of fatty acids and monoglycerides into mixed micelles, absorption, reesterification, chylomicron formation, and transport into the intestinal lymphatics or portal capillaries. Chylomicrons containing short- and long-chain triglycerides, and the newly incorporated B-100 apoprotein, are preferentially absorbed into the intestinal lymphatics where they are transported into the cisterna chyli, thoracic duct, and systemic circulation where they acquire apolipoproteins C and E from circulating high-density lipoproteins (HDLs). Apolipoprotein (apo) C-II activates lipoprotein lipase in the capillary beds of adipose and skeletal muscle, where they are stored as is or hydrolyzed into free fatty acids, β-monoglyceride, and glycerol. The cholesterol-rich remaining particles (now referred to as chylomicron remnants), return apo C-II molecule to HDL and are recognized by specific hepatic apo E and apo B-100 receptors that rapidly remove them from the circulation by endocytosis. The cholesterol found in chylomicron remnants can be used in very-low-density lipoprotein (VLDL), lipoprotein synthesis, bile acid formation, or cholesteryl storage.

Endogenous Transport. While chylomicrons are the apoprotein responsible for transport of dietary lipids, VLDLs, intermediate-density lipoproteins, low-density lipoproteins (LDLs), and HDLs are instead involved in the metabolism of endogenously produced lipids. Triglycerides and cholesterol produced by the liver combine with phospholipids, apo B-100, and apo B-48 to form VLDLs. When secreted from the liver, VLDLs acquire the apo C and apo E from HDL. VLDL apo C-II activates lipoprotein lipase located in the capillary beds, where once again triglyceride hydrolysis takes place with the production of free fatty acids and glycerol. The VLDL molecules remaining after hydrolysis of VLDL triglycerides are either removed from the circulation by the liver or undergo further transformation by lipoprotein lipase and/or hepatic lipase to form intermediate-density lipoproteins and LDLs. LDLs, which are relatively depleted of triglyceride and enriched in cholesteryl esters and phospholipid, circulate in the blood and bind to specific LDL receptors that are widely distributed throughout tissues in order to deliver cholesterol. HDLs produced by the liver play an important role as donors and acceptors of apo C, apo E, and various lipids from other lipoproteins in the circulation.

Reverse Transport. HDLs play an important role in the reverse transport of cholesterol from the periphey to the liver. Lecithin cholesterol acyl transferase esterifies HDL cholesterol and cholesteryl esters move to the core of the HDL molecule to allow more free cholesterol to be absorbed into the particle. Continued absorption of free cholesterol and subsequent esterification by lecithin cholesterol acyl transferase leads to the formation of the larger, cholesteryl ester-rich HDL2s. HDL2 molecules continuously acquire cholesteryl esters, resulting in the formation of the HDL1 molecules. On HDL1, cholesteryl esters are transferred from tissues to the liver for disposal or reuse, and not to LDL or VLDL molecules (as in humans), which transfer cholesterol to peripheral tissues. This function of HDL1s may account for the lower incidence of atherosclerotic disorders in dogs compared with humans.[23,24]

Nucleic Acids

Pyrimidine biosynthesis is one of the classic roles of the liver in nucleic acid metabolism. More recently, microRNAs have been impugned in the normal development and regeneration of the liver, as well as in hepatic pathology. microRNAs are small noncoding RNAs that regulate both messenger RNA and protein expression of target genes, which results in alterations in messenger RNA stability or translation inhibition. microRNAs influence at least one-third of all human transcripts and are known regulators of various important cellular growth and differentiation factors. microRNAs recently emerged as key regulatory molecules in chronic liver disease.[25]

Porphyrins

Porphyrins are intermediates of the heme biosynthetic pathway. Porphyrins are found in hemoglobin, myoglobin, cytochromes, catalase, and peroxidase enzyme. The liver and biliary tract serve as an excretory route for the porphyrins.

Metals

The liver stores iron, which can be toxic in excessive amounts (hemochromatosis). The amount of iron in the body is largely determined by regulation of its absorption in the upper small intestine. Iron is stored intracellularly as ferritin in a number of tissues, with the liver having a large storage capacity. When the capacity of the liver is exceeded, iron accumulates as hemosiderin.

The liver incorporates copper into specific copper proteins such as cytochrome c oxidase, mitochondrial monoamine oxidase, and ceruloplasmin. Mobilization of copper from hepatocytes takes place by at least two mechanisms: ceruloplasmin and bile secretion. Cholestatic liver disease is associated with secondary copper retention, which may then induce hepatocyte injury.[26,27]

Vitamins

The liver is importantly involved in vitamin metabolism. The liver produces bile for absorption of fat-soluble vitamins (A, D, E, K), and the liver is an important site for vitamin storage.

Vitamin A is stored in both stellate cells and hepatocytes. Approximately 95% of total body vitamin A is stored in the liver, representing a 1- to 2-year supply. The liver continues to release vitamin A to maintain normal blood concentrations despite reductions in its content. Liver and plasma vitamin A concentrations are reduced by malnutrition, liver disease, and intestinal malabsorption, but signs of deficiency do not appear until abnormalities become severe.

Fat-soluble vitamins A, D, E, and K require normal bile secretion for absorption. Vitamin K is particularly essential for synthesis of the prothrombin-complex clotting factors.

Water-soluble vitamins, with the exception of vitamin B_{12} (cobalamin), are readily absorbed from the small intestine. These vitamins are used primarily as coenzymes in metabolic processes. Vitamin phosphorylation, occurring primarily in hepatocytes, is required to produce some coenzymes. Thiamine is phosphorylated to thiamine pyrophosphate, for example, primarily in the liver and kidney. Nicotinic acid is a precursor in pyridine nucleotide synthesis, and an initial step in its conversion is nicotinamide synthesis in the liver. Pyridoxine is phosphorylated to its active form in the liver, as is the transformation of pantothenic acid to coenzyme A. Folic acid is converted to its active form in the liver. Large amounts of all water-soluble vitamins except vitamin C are stored in the liver.

Glutathione

Glutathione is synthesized in most if not all mammalian cells. The liver is particularly active and has relatively high levels of glutathione. Glutathione performs a variety of physiologic and metabolic functions, including thiol transfer reactions that protect cell membranes and proteins; thiol-disulfide reactions involved in protein synthesis, protein degradation, and catalysis; reduction of capacity; detoxification of hydrogen peroxide, organic peroxides, free radicals, and foreign compounds; and metabolism of various endogenous compounds.

Bile Secretion

Biliary secretions provide (a) a source of bile acids for fat digestion and absorption, (b) an excretory route for metabolites and xenobiotics, and (c) additional HCO_3^- for buffering of H^+ ion in the duodenum. Bile acids are the major components of bile accounting for about one-half to two-thirds of the total solutes. Bile also contains water, electrolytes, cholesterol, phospholipids, hormones, protein, and bilirubin (Figure 61-2).

Bile components are synthesized, stored, and secreted from the hepatocytes into the biliary ductal system.[28] In the absence of neural or hormonal input (as in the fasting state), the gallbladder is relaxed, the terminal biliary ductal sphincter (sphincter of Oddi) is contracted, and bile is largely stored in the gallbladder. While stored in the gallbladder, water and large portions of the electrolytes are reabsorbed by the gallbladder mucosa, concentrating the remaining constituents. During feeding, neural (acetylcholine) and hormonal (cholecystokinin) mechanisms activate gallbladder contraction, biliary ductal sphincter relaxation, and emptying of bile into the duodenum. Secretin and bile salts stimulate bile salt–independent and bile salt–dependent bile flow, respectively.[2,5,28]

Bile acids are synthesized from the cholesterol nucleus to which are attached a five- or eight-carbon side chain with a terminal carboxylic acid, and hydroxyl groups positioned at the C3, C7, or C12 carbon atom positions (Figure 61-3, *A* and *B*). The major primary bile salts are cholic acid and chenodeoxycholic acid in about equal molar quantities. When these primary bile acids are secreted into the lumen of intestine, a portion of each is dehydroxylated by intestinal bacteria to produce the secondary bile acids, deoxycholic acid, and lithocholic acid.[2,5,28] Prior to secretion, bile acids are conjugated with taurine and/or glycine to form tauro- and glycoconjugated bile salts (see Figure 61-3, *C*). Conjugation lowers the pK_a to well below the physiologic range of biliary and intestinal pH, and conjugated bile acids become ionized anions (referred to as bile salts) rather than undissociated bile acids. In the ionized form, they are less likely to be absorbed by the small intestine and so maintain a higher intraluminal concentration appropriate for emulsification, digestion, and absorption of lipids. Dogs and cats conjugate primarily with taurine. Dogs can convert to glycine conjugation if taurine is deficient, but cats cannot. Cats are obligate taurine conjugators, and have an essential dietary taurine requirement.[2,5,28]

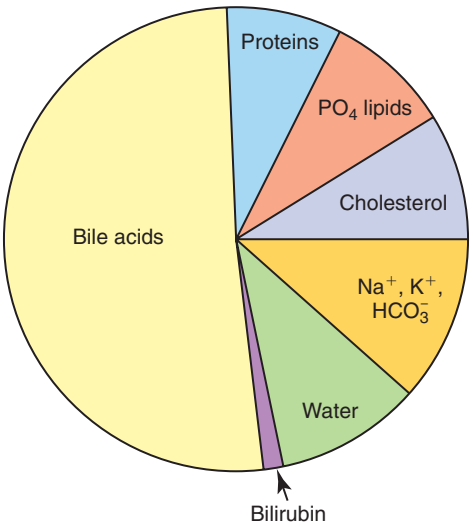

Figure 61-2 The chemical components of bile: bile acids, proteins, phospholipids, cholesterol, water, Na^+, K^+, HCO_3^-, and bilirubin.

Bile salts are amphipathic molecules with polar and nonpolar domains imparting two important functions. Bile salts have an initial detergent effect on fat particles in food permitting the breakup of fat globules into smaller sizes. This is the initial emulsification phase of bile salts that facilitates intraluminal lipid hydrolytic digestion. Bile salts further assist in the absorption of fatty acids, monoglycerides, cholesterol, and other lipids through the formation mixed micelles. These micelles serve to transfer digested lipids across the unstirred layer of the mucosa.

Following emulsification and micellarization of fat, most of the secreted bile salts are transported along the GI tract to the ileum where they are absorbed into ileal enterocytes and portal blood flow via Na^+–bile salt cotransporters.[2,28]

Coagulation Factors

The liver plays an important role in maintaining hemostasis. The liver produces procoagulant, anticoagulant, and fibrinolytic proteins, and also removes normal and abnormal clotting factors from the circulation.[29]

Hepatocytes synthesize most of the clotting factors including clotting factor I (fibrinogen), II (prothrombin), V, VII, IX, X, XI, and XIII. The site of biosynthesis of factor VIII remains controversial, but it is probable that the liver plays an important role in this factor, too. The liver is also responsible for the activation of the vitamin K–dependent factors II, VII, IX, and X and protein C. In addition to the production and activation of coagulation factors, the liver is also essential for the clearance of activated coagulation

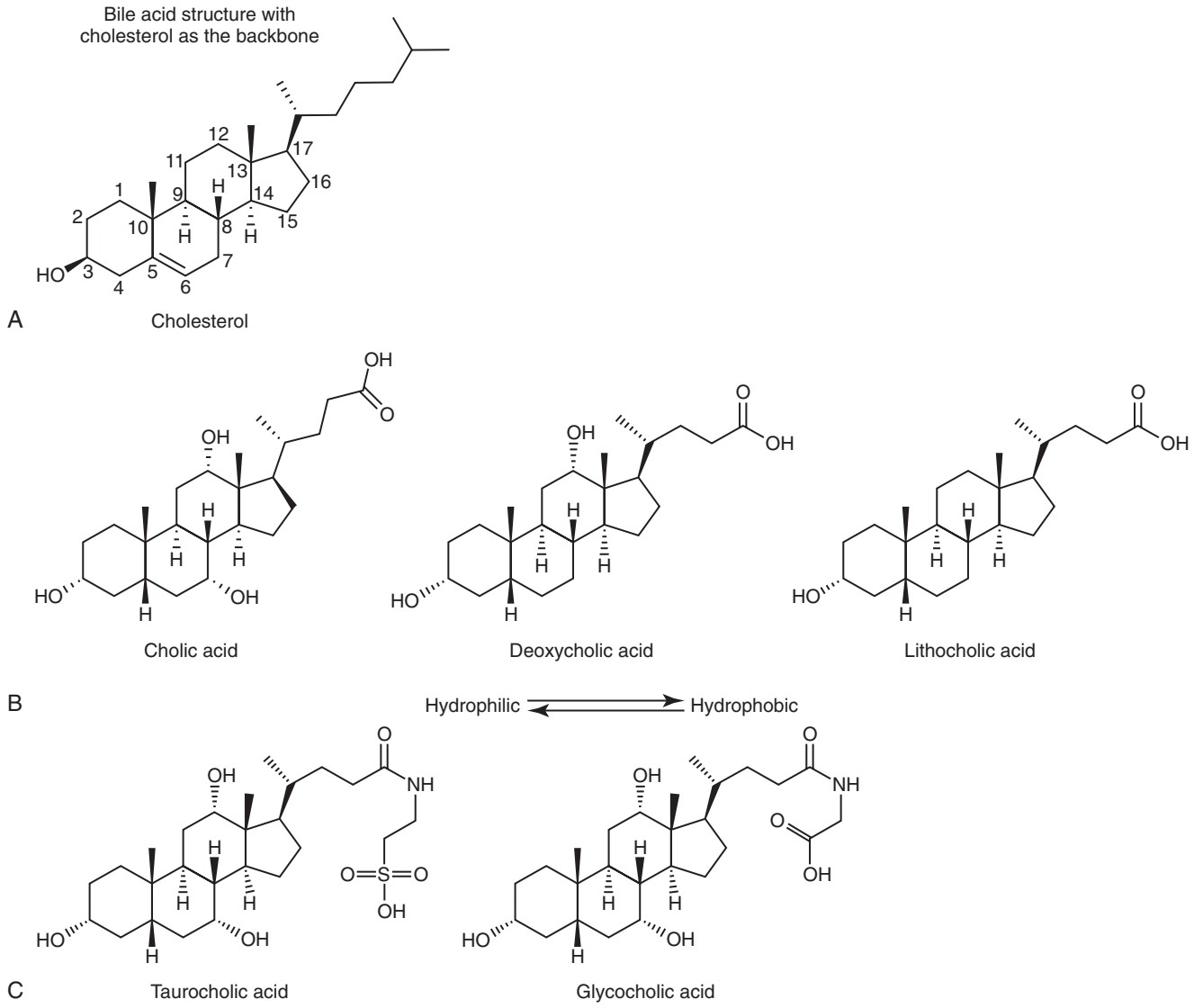

Figure 61-3 **A,** Cholesterol serves as the chemical backbone in bile acid synthesis. **B,** Hydrophilicity and hydrophobicity of bile acids. **C,** Glycine and taurine conjugation of bile acids.

products and the production of clotting factor inhibitors, such as antithrombin and α_1-antitrypsin, as well as fibrinolytic proteins like plasminogen.

In liver disease, factor and inhibitor synthesis and clearance of activated factors in both the coagulation factors and fibrinolytic system may be impaired. The extent of coagulation abnormalities depends upon the degree of disturbed liver function.[29] Patients with hepatic failure may present with the entire spectrum of factor deficiencies and may even develop disseminated intravascular coagulation.

In a study of 42 dogs with histologically confirmed liver disease, one or more coagulation abnormalities were found in 57% of dogs with liver disease.[29] Activated partial thromboplastin time was significantly prolonged in dogs with chronic hepatitis with or without cirrhosis. Mean platelet numbers, antithrombin, and factor IX activity were significantly lower in dogs with chronic hepatitis with cirrhosis, compared to dogs with other hepatopathies. D-Dimers were not significantly increased in any group. Only three dogs, all with different histologic diagnoses, satisfied the criteria for disseminated intravascular coagulation. Hemostatic abnormalities were primarily seen in dogs with chronic hepatitis plus cirrhosis, which may be a result of reduced synthesis rather than increased consumption of coagulation factors.

Detoxification

Xenobiotic Agents

Numerous foreign compounds, including drugs, are so hydrophobic that they would remain in the body indefinitely were it not for hepatic biotransformation. Cytochrome P450 (P450 or CYP) comprises a superfamily of enzymes that catalyze oxidation of a variety of xenobiotic chemicals such as drugs, toxic chemicals, and carcinogens, as well as endobiotic chemicals including steroids, fatty acids, prostaglandins, and vitamins. The cytochrome P450 enzymes in families one to three mediate 70% to 80% of all phase I–dependent metabolism of clinically used drugs and participate in the metabolism of a huge number of xenobiotic chemicals. There are 57 known active *P450* genes and 58 pseudogenes in the human genome. With 54 active genes, dogs are phylogenetically closest to the human. Although there are many similarities between dogs and humans, there also are many important differences.[30-33] Dogs present an

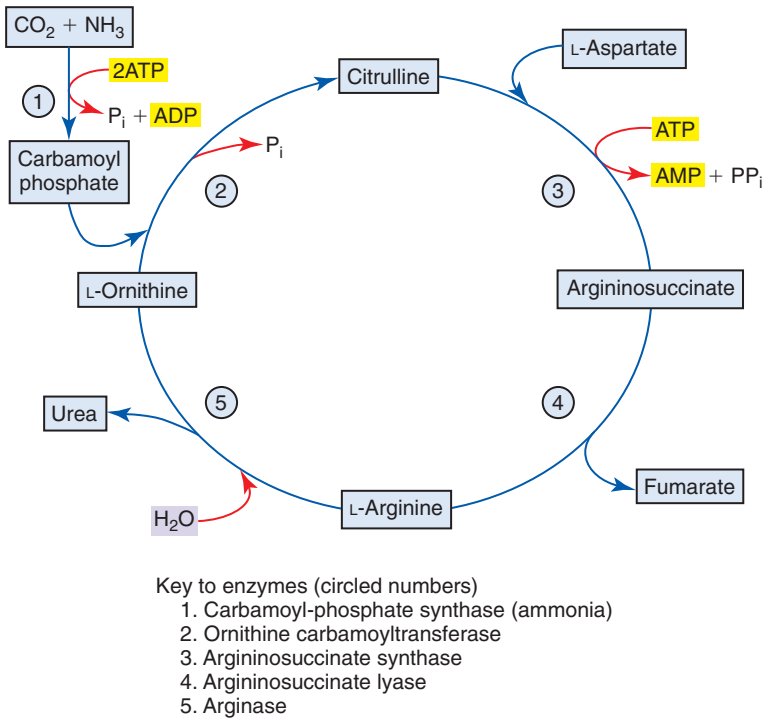

Figure 61-4 Urea cycle. Transformation of toxic ammonia into nontoxic urea.

interesting challenge in the assessment of P450-mediated drug–drug interactions because most of the enzymes have not been completely characterized, diet and aging induce significant changes in gene expression, and dogs are often treated off-label with a number of human drugs with little idea of risk for drug–drug interaction.

Drug metabolism takes two general forms: phase I metabolism (modification reactions) and phase II metabolism (conjugation reactions). Phase I metabolism typically subjects a drug to oxidation or hydrolysis. It involves the cytochrome P450 (CYP) enzymes, which facilitate reactions that include *N*-, *O*-, and *S*-dealkylation; aromatic, aliphatic, or *N*-hydroxylation; *N*-oxidation; sulfoxidation; deamination; and dehalogenation. Phase II metabolism conjugates the drug to hydrophilic substances, such as glucuronic acid, sulfate, glycine, or glutathione. Phase I metabolism usually precedes phase II metabolism, but this is not always the case.[34]

The liver is an important site of drug toxicity and oxidative stress because of its proximity and relationship to the GI tract. Seventy-five percent to 80% of hepatic blood flow comes directly from the GI tract and spleen via the main portal vein. Portal blood flow transports nutrients, bacteria and bacterial antigens, drugs, and xenobiotic agents absorbed from the gut to the liver in a more concentrated form. Drug-metabolizing enzymes detoxify many xenobiotics but might activate the toxicity of others. Hepatic parenchymal and nonparenchymal cells may all contribute to the pathogenesis of hepatic toxicity.

The toxicity of drugs can be considered in five contexts: on-target toxicity, hypersensitivity and immunologic reactions, off-target pharmacology, bioactivation to reactive intermediates, and idiosyncratic drug reactions.[35,36]

Ammonia

Ammonia is an important by-product of amino acid metabolism. Organisms that cannot easily and quickly remove ammonia usually have to convert it to some other substance, like urea or uric acid, which are much less toxic. Insufficiency of the urea cycle occurs in some genetic disorders (inborn errors of metabolism), or more typically, in liver failure. The result of liver failure is accumulation of nitrogenous waste, mainly ammonia, which leads to hepatic encephalopathy.

The GI tract, particularly the colon, is the most important source, through the action of bacterial urease on endogenous urea or dietary amines. Ammonia produced by colonic bacteria enters the portal circulation and is transported to the liver for urea cycle transformation (Figure 61-4). Ammonia is transformed into urea in the urea cycle in the overall equation:

$$2\,NH_3 + CO_2 + 3\ \text{adenosine triphosphate} + H_2O \rightarrow \text{urea} + 2\,ADP + 4\,Pi + AMP + 2\,H$$

where NH3 = ammonia; adenosine triphosphate = adenosine triphosphate; ADP = adenosine diphosphate; P_i = inorganic phosphate; and AMP = adenosine monophosphate.

Endogenous Hormones

Mineralocorticoids (aldosterone), glucocorticoids (cortisol, corticosterone), and sex steroids (androgens, estrogens, progesterone) are metabolized by the liver. Changes in the concentrations of total and free cortisol and of the binding capacity of corticosteroid-binding globulin have been reported in canine liver disease. As a consequence of hypercortisolemia, dogs with liver disease and hepatoencephalopathy have clinical and biochemical characteristics of PDH, including polyuria, high basal cortisol levels, and α-melanotropin.[37,38] Chronic hypercortisolism is associated with impaired osmoregulation of the release of vasopressin and inadequate urinary concentration.[39]

Immune Surveillance

The multiple physiologic functions of the liver require an immune response that is locally regulated. Pathogenic microorganisms must be efficiently eliminated, while the large number of antigens derived

from the GI tract must be tolerated. The liver favors the induction of tolerance rather than the induction of immunity. Although hepatocytes constitute the major cell population of the liver, direct interaction of hepatocytes with leukocytes in the blood is unlikely. Sinusoidal endothelial cells, which line the hepatic sinusoids and separate hepatocytes from leukocytes in the sinusoidal lumen, and Kupffer cells, the resident macrophage population of the liver, can directly interact with passenger leukocytes. In the liver, clearance of antigen from the blood occurs mainly by sinusoidal endothelial cells through very efficient receptor-mediated endocytosis. Liver sinusoidal endothelial cells constitutively express all molecules necessary for antigen presentation (CD54, CD80, CD86, major histocompatibility complex [MHC] classes I and II, and CD40) and can function as antigen-presenting cells for $CD4^+$ and $CD8^+$ T cells.[40,41] Thus, these cells probably contribute to hepatic immune surveillance by activation of effector T cells. Antigen-specific T-cell activation is influenced by the local microenvironment. This microenvironment is characterized by the physiologic presence of bacterial constituents such as endotoxin and by the local release of immunosuppressive mediators such as interleukin-10, prostaglandin E_2, and transforming growth factor-β.[42]

Regeneration

Liver regeneration after partial hepatectomy is a very complex and well-orchestrated phenomenon. It appears to be carried out by the participation of all mature liver cell types.[43,44] The process is associated with signaling cascades involving growth factors, cytokines, matrix remodeling, and several feedbacks of stimulation and inhibition of growth related signals.[45,46] The liver manages to restore any lost mass and adjust its size to that of the organism, while at the same time providing full support for body homeostasis during the entire regenerative process. In situations when hepatocytes or biliary cells are blocked from regeneration, these cell types can function as facultative stem cells for each other.

Gene expression in the regenerating liver is a multistep process with at least two critical steps: the transition of quiescent hepatocytes into the cell cycle ("priming"), and the progression beyond the restriction point in the G_1 phase of the cell cycle. Hepatocytes must first be primed before they can fully respond to growth factors. As many as 70 different genes participate in the early response to hepatectomy, but tumor necrosis factor (TNF), interleukin (IL)-6, and interleukin-22 (IL-22) appear to be the major cytokines involved in the priming of hepatocytes.[47] The proliferative effect of TNF on hepatocytes is further influenced by reactive oxygen species, nitric oxide, and glutathione content, and multiple transcription factors (e.g., nuclear factor kappa B, STAT3, AP-1, and C/EBPβ) play major roles in the initiation of early liver regeneration.[47] Progression through the cell cycle beyond the initiation phase requires growth factors, primarily hepatocyte growth factor and transforming growth factor-α (TGF-α). The subsequent expression of cell-cycle genes establishes the stage at which replication becomes growth factor-independent and autonomous. At this point, the hepatocyte is irreversibly committed to replicate and the cell cycle replication machinery takes over.

The proliferation of hepatocytes advances from periportal to pericentral ares of the lobules, as a wave of mitoses. Hepatocytes surrounding the central veins are the last ones to undergo cell replication. Proliferation of biliary epithelial cells occurs a little later than hepatocytes. Proliferation of endothelial cells starts at 2 to 3 days and ends around 4 to 5 days after partial hepatectomy. The kinetics of proliferation of stellate cells is incompletely understood. The regenerative capacity of the residual hepatocytes may restore liver mass and function after as much as 65% to 70% hepatectomy and it takes place over 7 to 14 days in most animal species.[48] A small wave of apoptosis in hepatocytes occurs at the end of regeneration.

HISTORY AND PHYSICAL EXAMINATION

Hein P. Meyer and Jan Rothuizen

Clinical Importance

The liver is the second largest organ in the body and performs an estimated 1500 essential biochemical functions.[1] These diverse functions include drug metabolism; removal of exogenous and endogenous toxins (e.g., ammonia, food antigens); synthesis of vital substances such as albumin and blood clotting factors; protein, fat, and carbohydrate metabolism; vitamin storage and activation; glycogen, triglyceride, and mineral (e.g., copper, iron) storage; activation, conversion, secretion, deactivation, and excretion of various hormones; bile salt synthesis; conjugation and excretion of bilirubin in bile; among others. Symptoms (defined here as abnormalities noted by the owner), clinical signs (defined here as abnormalities found during the physical examination), and diagnostic results reflect impairments in these functions. Hepatitis represented approximately 1% of the clinical population of the companion animal teaching hospital of Utrecht University. Box 61-1 summarizes the most common liver diseases with their possible etiologies in dogs and cats.

History of Dogs and Cats with Liver Disease

A properly taken history is pivotal to defining the most clinically relevant problems that need to be resolved. A structured interview process and understanding the basics of communication are important success factors to retrieve this crucial information. Fortunately, the knowledge about communication in the medical profession and the focus on the veterinary curriculum, has increased considerably during the last few years.[2,3]

Some basic principles should be kept in mind to understand the symptoms in dogs and cats with diseases affecting the hepatic parenchyma, portal vasculature, and the biliary system. First, for most of its functions, the liver has a tremendous (approximately 80%) reserve capacity and a remarkable potential to regenerate.[4] Symptoms occur only when progressive disease exhausts hepatic reserves. Diseases often remain subclinical for lengthy periods of time; symptoms may be relatively mild and nonspecific because the liver reserve prevents overt abnormalities. Symptoms such as lethargy, vomiting, or mild polyuria and polydipsia (PU/PD) may alert the clinician that a liver disorder could be developing. Serious symptoms may indicate loss of hepatic reserves. The onset of symptoms may be acute, but they may be the end result of a disease that has been present for many weeks or months. Because no specific physical abnormalities occur with most liver diseases, it is important to remember that liver disease may be present when symptoms of illness are unexplained or nonspecific. Sensitive and specific laboratory tests may easily detect such liver diseases.[5]

Second, most liver diseases cause similar signs and symptoms (Table 61-2). One of these is acholic feces, which occurs nearly exclusively in dogs with common bile duct obstruction.[6] Owners

Box 61-1 Causes of Acute Liver Disease in Dogs and Cats

Infectious Agents

Viral

- Infectious canine hepatitis (canine adenovirus I)
- Canine and feline herpesvirus (neonates)
- Coronavirus (feline infectious peritonitis virus)
- Feline *Calicivirus* (virulent form)

Bacterial

- Extrahepatic infections, septicemia, and endotoxemia
- Cholangitis
- *Clostridium piliforme* (Tyzzer disease)
- *Helicobacter canis* (dog)
- *Leptospira* spp.
- Liver abscess

Fungal

- *Histoplasma capsulatum*
- *Coccidioides immitis*
- Others

Protozoal

- *Toxoplasma gondii*
- *Neospora caninum*
- *Babesia* spp.
- *Cytauxzoon felis*

Rickettsial

- *Ehrlichia* spp.
- *Rickettsia* rickettsiae

Parasitic

- Liver flukes[a]
- Heartworms and caval syndrome

Drugs and Anesthetics

Anticonvulsants and Sedatives

- Diazepam (cats)
- Phenobarbital[a] (dogs)
- Primidone[a] (dogs)
- Phenytoin (dogs)

Antiinflammatory and Analgesic Drugs

- Glucocorticoids (dogs)
- Acetaminophen (dogs and cats)
- Carprofen and other nonsteroidal antiinflammatory drugs (dogs)

Antimicrobials and Parasiticides

- Diethylcarbamazine (dogs)
- Doxycycline (dogs)
- Griseofulvin (cats)
- Itraconazole (dogs and cats)
- Ketoconazole (dogs and cats)
- Mebendazole (dogs)
- Oxibendazole (dogs)
- Sulfonamides (dogs)
- Terbinafine (dogs)
- Tetracycline (dogs and cats)
- Thiacetarsamide (dogs)

Anesthetics

- Halothane (dogs)
- Methoxyflurane (dogs)

Miscellaneous

- Amiodarone[a] (dogs)
- Azathioprine (dogs)
- Danazol (dogs)
- Glipizide (cats)
- Lomustine[a] (dogs)
- Methimazole (cats)
- Methotrexate (dogs)
- Mithramycin (dogs)
- Mitotane (dogs)
- Phenazopyridine (dogs)
- Stanozolol (cats)

Herbal and Dietary Supplements

- α-Lipoic acid
- Black cohosh
- Comfrey[b] (pyrrolizidine alkaloids)
- Chaparral leaf[b]
- Chinese herbal medicines[b] (Jin Bu Huan, Ma huang)
- Kava[b]
- Pennyroyal oil
- St. John's wort

Biologic Toxins

- Aflatoxin
- *Amanita* mushrooms
- Blue-green algae
- Cycads (Sago palms)
- Hornet stings
- *Indigofera linnaei* (legume)

Food Additives

- Xylitol (sugar substitute)(dogs)

Chemicals

- Carbon tetrachloride
- Dimethylnitrosamine
- Dinitrophenol
- Pine oil
- Heavy metals (e.g., copper, lead, iron, arsenic)
- Organochloride pesticides
- Phenols
- Many others

Metabolic Disorders

- Acute pancreatitis
- Hemolytic anemia and disseminated intravascular coagulation
- Hepatic copper accumulation[a]
- Inflammatory bowel disease
- Feline hepatic lipidosis

Neoplastic Disorders

- Carcinoma (biliary, pancreatic)
- Lymphoma
- Malignant histiocytosis

Hypoxic/Ischemic Disorders

- Shock
- Liver lobe torsion
- Thromboembolic disease
- Congestive heart failure

Miscellaneous

- Trauma
- Heat stroke

[a]More likely to present with chronic rather than acute liver disease.
[b]Documented in humans, may occur in dogs and cats.

Table 61-2 Common Clinical Signs in Dogs with Liver Disease

Liver Disease with Relative Frequency (%)	PERCENTAGE OF DOGS AFFECTED BY DISEASE WITH SIGNS												
	Apathy, Depression	Inappetence	Reduced Endurance	Vomiting	Diarrhea	Weight Loss	Hepatic Encephalopathy	Polyuria/ Polydipsia	Dysuria	Anesthesia Intolerance	Acholic Feces	Distended Abdomen	Retarded Growth
Acute hepatitis (3)	44	49	–	61	21	12	–	11	–	–	–	–	–
Chronic hepatitis (10)	18	29	14	43	33	39	–	49	–	–	–	–	–
Cirrhosis (7)	25	53	39	61	37	58	9	56	–	–	–	55	–
Lobular dissecting hepatitis (2)	58	21	29	32	20	45	22	39	–	–	–	65	–
Reactive hepatitis (25)	10	34	–	48	77	39	–	9	–	–	–	–	–
Destructive cholangiolitis (1)	76	82	–	68	21	66	–	49	–	–	9	8	–
Portosystemic shunt (16)	99	68	62	31	12	81	91	52	3	9	–	–	39
Portal vein thrombosis (1)	25	10	–	38	44	15	5	32	–	–	–	33	–
Portal vein hypoplasia (4)	49	13	22	16	21	14	38	45	–	–	–	60	8
Liver cell carcinoma (4)	15	26	18	74	14	32	–	19	–	–	–	25	–
Metastatic tumor (10)	24	54	17	67	27	60	–	38	–	–	–	14	–
Malignant lymphoma (14)	18	75	32	70	21	85	–	55	–	–	–	5	–
Cholecystitis/ choleliths (1)	–	65	–	93	19	30	–	–	–	–	–	–	–
Extrahepatic cholestasis (2)	10	72	–	81	37	54	–	46	–	–	16	–	–

Figures are from the Utrecht University Clinic population. The relative frequencies are based on 2500 referred cases.

may note the light-gray appearance of stool, which in combination with icterus is virtually diagnostic for extrahepatic cholestasis. Different combinations of clinical signs and symptoms may occur with any liver disease. Statistically, one disease may be associated with a typical pattern of signs and symptoms in dogs and cats. However, overlapping patterns are so great that it is useless to try to identify the exact disease based on clinical signs and symptoms alone (Table 61-2). Clinical signs and symptoms associated with liver diseases of cats are similar to those in dogs, except for PU/PD, which is not clinically overt in most cats. Certain liver diseases cause neurobehavioral signs associated with hepatic encephalopathy,[7] and these signs may wax and wane in their frequency and severity. Any medication given may appear to be effective because of the natural fluctuation of signs. Therefore signs of hepatic origin may be easily missed. Seizures alone are never caused by hepatic encephalopathy; if they do occur, they occur in combination with other signs seen with this syndrome.[5] Furthermore, very few, if any, medications to treat liver diseases have been tested in double-blind, placebo-controlled studies, making decisions about the best therapeutic regimens difficult.[8]

It is usually not possible to differentiate between hepatic diseases and diseases of other organs based on symptoms and clinical signs. Signs associated with hepatic diseases are nonspecific; similar signs may occur in diseases of many other organ systems, most notably the GI, neurologic, renal, and hematologic systems (see Table 61-2).[9] Of the GI tract–related symptoms, nausea expressed as vomiting in acute cases or reduced, irregular appetite with occasional vomiting and weight loss over time, is very common in liver and biliary diseases of dogs and cats. For biliary diseases these are always the most prominent symptoms. Diarrhea, however, is not a major symptom of liver disease, and in cases in which diarrhea is the leading symptom the liver is only rarely the causative organ (except for rare cases with complete common bile duct obstruction). A rare sign (not included in Table 61-2) is an ulcerative form of dermatosis, so-called superficial necrolytic dermatitis, hepatodermal, or hepatocutaneous syndrome. This syndrome occurs rarely in dogs with liver cirrhosis and nodular hyperplasia and whose pathogenesis is poorly understood.[10] This symptom and the more common symptoms of lethargy, inappetence, vomiting, diarrhea, weight loss, PU/PD, and neurobehavioral symptoms are frequently associated with diseases of other organs. Therefore the history often discloses symptoms that may suggest hepatic disease, but may also be caused by other disorders.

Two main reasons account for the nonspecificity of liver-related symptoms. First, the liver is the central organ for many metabolic and detoxifying pathways; consequently, failing liver function may cause dysfunction of other organs. One example is hepatic encephalopathy; metabolic dysfunctions of the liver cause neurotransmitter dysfunctions of the brain, resulting in neurobehavioral signs.[11] Second, toxic factors resulting from diseases of other organ systems (especially from the GI tract) often secondarily affect the liver. Examples include hepatic lipidosis in diabetes mellitus, steroid-induced hepatopathy in Cushing syndrome, reactive hepatitis in GI diseases, and centrolobular liver necrosis in acute, severe anemia.[5] Therefore signs and symptoms of liver disease may be hidden within signs of other organ dysfunction, and vice versa. Because clinical and physical examination findings may be compatible with hepatic disease, and because laboratory tests to detect hepatic disease are also abnormal with primary and secondary hepatopathies, it is often necessary to make a histologic diagnosis of the liver disorder to resolve this dilemma.[12,13]

Lack of specific physical examination findings may prevent recognition of a primary liver disease. Most dogs with illnesses causing the clinical signs listed in Table 61-2 are candidates for having a primary hepatopathy. In all such cases, further diagnostic studies should be performed to confirm or exclude liver disease (Figure 61-5).

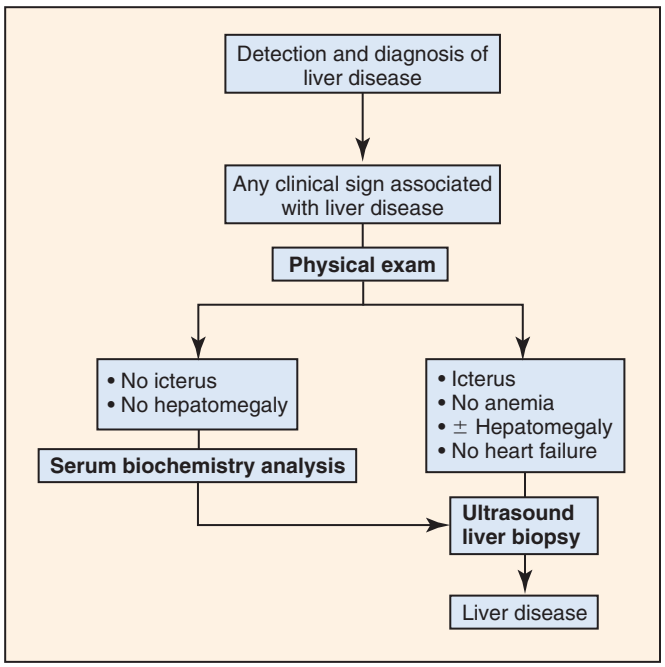

Figure 61-5 Algorithm for the detection and diagnosis of liver disease.

Predispositions

Breed, sex, age, and drugs may predispose dogs and cats to hepatopathies. The presence of numerous risk factors should be a stimulus for an extended diagnostic workup; other diseases should be investigated in the absence of such suspicions. Caused by hypersensitivity to sulfonamides, destructive cholangiolitis is the most common drug-induced liver disease.[14] A recent history of therapy with sulfonamides or other potentially hepatotoxic drugs, combined with icterus makes this condition likely and should prompt immediate discontinuation of the medication. Breed associations may occur when a disease is (in part) determined by genetic factors. Breeders may, by chance, increase the incidence of hepatic diseases by familial selection. Because dog breeds may represent more or less closed populations in a country, breed predispositions may vary among countries. Therefore this section only mentions generally applicable predispositions; locally, other breed associations may be more pertinent.

Chronic hepatitis and cirrhosis, both of which are, as a rule, different stages of one disease, occur more frequently in certain breeds.[15] Hepatitis may develop at any age, but typically not before 2 years of age. Only lobular dissecting hepatitis tends to occur at a young age (i.e., often before 1 year of age).[16] Breeds associated with hepatitis are Doberman Pinschers, Bedlington Terriers, West Highland White Terriers, American and English Cocker Spaniels, Labrador Retrievers, and many other breeds. Recent copper excretion studies have shown that hepatitis is caused by copper retention and not vice versa in Doberman Pinschers.[17] The cause of copper retention remains unclear; many of the tested candidate genes (including Murr 1, the affected gene in Bedlington Terriers) were excluded as monogenetic causes for copper-associated subclinical hepatitis in

Doberman Pinschers.[17] The hepatitis in Doberman Pinschers is sex linked, confined to females, and aggressive.[18] It is responsive to medication with penicillamine,[19] but may terminate in micronodular cirrhosis. This form of cirrhosis, predominantly seen in copper toxicosis, differs from other forms of chronic hepatitis, in which patients typically develop macronodular cirrhosis with large hyperplastic nodules. Hepatitis is overrepresented in female Doberman Pinschers by a factor of 10; a study in Finland showed that approximately 10% of Doberman Pinschers may be affected.[20] Inherited copper toxicosis is also a well described entity in Bedlington Terriers worldwide.[21] Both sexes may be affected. Clinical signs usually develop after 4 years of age as a result of the gradual accumulation of copper. It is caused by a defect in the Murr 1 gene, leading to a severely decreased excretion of copper by hepatocytes. Other affected breeds are West Highland White Terriers (particularly in the United States), Skye Terriers, Dalmatians, Anatolian Shepherd dogs, and Labrador Retrievers.[22-26] Siamese cats may also be predisposed to copper-associated hepatopathies.[9] Although essential for life, copper is usually ingested to excess and must be eliminated by the liver to prevent toxicity. The central role of the liver in copper homeostasis makes it vulnerable if elimination processes fail.[27] Furthermore, increased copper levels add to the oxidative stress, which is an important component in chronic inflammatory and cholestatic diseases in dogs.[25] Spaniels seem to have the form of chronic hepatitis unrelated to copper toxicosis and develop macronodular cirrhosis when left untreated. No sex predisposition exists, but there seems to be a worldwide overrepresentation of hepatitis in this breed.

Congenital portosystemic shunts (CPSS) are seen in both sexes in various breeds. Intrahepatic shunts predominate in large breeds, whereas extrahepatic shunts predominate in small and toy breeds. Although CPSS are most likely inherited in some fashion in all affected breeds, this has only been proven in Irish Wolfhounds[28] and Cairn Terriers.[29] Worldwide predispositions occur in Irish Wolfhounds, Australian cattle dogs, Labrador Retrievers, Dachshunds, Yorkshire Terriers, Cairn Terriers, Maltese Terriers, and Miniature Schnauzers.[7,9] In the United States, an increased prevalence of shunts has also been reported to occur in German Shepherd dogs, Doberman Pinschers, and Golden Retrievers. CPSS occur most often in mixed-breed cats; however, Persian and Himalayan cats are frequently overrepresented. Clinical signs are usually seen in young dogs and cats (<1 year old) with congenital shunts.[5,9]

Pathogenesis of Common Symptoms of Primary Liver Diseases

Vomiting

Vomiting is one of the most common symptoms noted in dogs and cats with liver disease. Vomiting may be caused by direct stimulation of the vomiting center via the chemoreceptor trigger zone in the fourth ventricle by (endo)toxins that are not cleared by the liver.[30] This typically occurs when toxins from the GI system bypass the liver and access other body systems. Vomiting is common in all conditions that share portosystemic shunting and liver dysfunction (e.g., congenital shunts and acquired shunts because of hepatitis, fibrosis, cirrhosis, and portal vein hypoplasia or thrombosis). Hepatic diseases that cause an abnormal liver shape may reposition the upper GI tract and induce nausea and vomiting by vagal stimulation. Causes include hepatic tumors, especially liver cell (or hepatocellular) carcinomas, and unilateral collapse and contralateral hypertrophy, which may occur with thrombosis of a main branch of the portal vein. The gallbladder and larger bile ducts have a rich sympathetic innervation; therefore dilation (e.g., extrahepatic cholestasis), cholecystitis, or cholelithiasis should be suspected in vomiting dogs and cats.[5]

Vomiting is also common in upper GI disease. In many GI diseases, translocation of bacteria and endotoxins may cause secondary, nonspecific, reactive hepatitis.[30] This occurs frequently in dogs, but rarely in cats. Reactive hepatitis is characterized by intrahepatic canalicular cholestasis, liver cell necrosis, and an exudative inflammatory reaction. Clinical signs, symptoms, and diagnostic results associated with primary liver disease and reactive hepatitis are similar; therefore, a further diagnostic workup is important to reveal the primary cause.

Diarrhea

Small bowel-type diarrhea occurs frequently with hepatic diseases (see Table 61-2). Two primary mechanisms may account for clinical signs. First, cholestatic diseases (intrahepatic or extrahepatic caused by common bile duct obstruction) disrupt the normal enterohepatic cycle of bile acids; therefore less bile reaches the duodenum.[30,31] Decreased resorption of dietary fat may cause hyperosmotic intestinal contents and diarrhea. However, studies in rats show that cholestasis must be severe before steatorrhea as a result of disruption of the enterohepatic bile acid cycle occurs. Another mechanism for diarrhea in liver disease is increased resistance to portal blood flow, resulting in portal hypertension and congestion of splanchnic organs. Intestinal vasculature congestion reduces intestinal water resorption and increases intestinal volume content. This is the predominant mechanism underlying diarrhea in diseases such as chronic hepatitis, lobular dissecting hepatitis, portal vein thrombosis, and portal vein hypoplasia.[5] Alternatively, when the primary cause of diarrhea is intestinal disease, the liver may be affected secondarily. In those cases, the hepatic macrophage system should remove the increased absorption of (endo)toxins or bacteria by the affected intestinal wall. Increased exposure, however, can lead to secondary, nonspecific, reactive hepatitis. Endotoxins also effectively inhibit bile formation and flow, leading to cholestasis. It is therefore common to find increased plasma liver enzyme activities and bile acid levels in cases of reactive hepatitis; clinical icterus may even be apparent. The cause of diarrhea can be determined only by further diagnostic methods, including histologic evaluation of liver biopsy specimens. Reactive hepatitis resolves rapidly when the primary disease is treated successfully. In the authors' experience it is very rare to find diarrhea as single or the leading symptom in cases of liver disease. If present, it is usually one of the less prominent symptoms in the spectrum of other more prominent symptoms such as apathy, PU/PD, or vomiting. One may therefore elect to follow liver laboratory values after treatment of the intestinal disease and perform a liver biopsy if liver parameters fail to improve within a few weeks.

Hepatic Encephalopathy and Related Anesthesia Intolerance

Hepatic encephalopathy is a complex of neurobehavioral signs resulting from portosystemic shunting of blood in combination with a reduction of functional liver mass.[11] It may occur in animals with CPSS or in those with APSCAPSC because of portal hypertension. Diseases associated with the latter form are chronic hepatitis, cirrhosis, portal vein hypoplasia, lobular dissecting hepatitis, and portal vein thrombosis.[32] Cats, because of their dependence on some essential amino acids (e.g., arginine), may develop hepatic encephalopathy without portosystemic shunting, especially when they have a severe form of hepatic lipidosis.[33]

Hepatic encephalopathy is caused by derangement of neurotransmitter systems caused by defective metabolic processes in the liver.[34] Inadequate metabolism of ammonia and aromatic amino acids by the liver may reduce the excitatory glutamatergic and monoaminergic neurotransmitter system tones, respectively.[35] In addition, there is an increased tone of the inhibitory γ-aminobutyric acid (GABA) system.[36] These neurotransmitter derangements make anesthesia risky in some animals with liver disease. The liver inactivates many anesthetics and the unforeseen delay of recovery from anesthesia may suggest an underlying liver disease as a cause for nonspecific clinical signs. This occurs especially in dog and cats with portosystemic shunting, either congenital or acquired. In addition to reduced hepatic clearance, anesthetics exert their action via various neurotransmitter systems in the brain, which may already be functioning abnormally as a consequence of hepatic encephalopathy.[34] This is especially true of drugs that act via the GABA-benzodiazepine pathway. That pathway is already overstimulated and may provoke an exaggerated and prolonged anesthetic effect.

Polyuria and Polydipsia

PU/PD is one of the most frequent signs (50% of cases) in dogs with liver disease, but is less common in cats. PU/PD is most common in diseases associated with congenital or acquired portosystemic shunting and, therefore, with hepatic encephalopathy. In affected dogs, abnormal neurotransmitter disturbances lead to increased secretion of adrenocorticotropic hormone (ACTH) from the anterior and intermediate pituitary lobes.[37] Chronically elevated ACTH stimulates increased levels of free cortisol. Increased levels of free cortisol, in turn, affect the posterior lobe of the pituitary creating an increased threshold for the release of arginine vasopressin.[38,39] Thus, a higher plasma osmolality is required to stimulate antidiuresis through arginine vasopressin, and before reaching that threshold, affected dogs become thirsty and start drinking.[38]

PU/PD is not only present in cases with hepatic encephalopathy, but also frequently in all other liver diseases. This may be caused by certain bile acids, which are often increased in plasma of animals with liver diseases. Bile acids may inhibit the activity of 11β-hydroxyl steroid dehydrogenase.[40] This enzyme protects the aldosterone receptor from occupation by cortisol, by converting cortisol into cortisone, which cannot bind to the receptor. Present in plasma in tenfold excess compared with aldosterone, cortisol can occupy and stimulate the aldosterone receptor thereby inducing pseudohyperaldosteronism and PU.[41]

Reduced hepatic formation of urea is another possible but undocumented mechanism that may play a role in the pathogenesis of PU/PD. In urea deficiency states, the kidney does not have sufficient urea available to build up an osmotic gradient in the medulla. Apart from this mechanism, PD also occurs in liver diseases not associated with hepatic encephalopathy (e.g., extrahepatic cholestasis and liver tumors). The mechanism is unclear; however, nausea with an impulse to drink and compensation of water loss by vomiting and diarrhea may play a role.[5]

Dysuria

Dysuria may occur as a result of insufficient liver function when nonmetabolized uric acid is excreted by the kidneys and precipitates to form uroliths. Such calculi are seen in dogs but rarely in cats.[42] There are two main categories of liver dysfunction that cause ammonium urate urolithiasis. Most frequently it is caused by congenital portosystemic shunting, whereby the liver is underdeveloped and fails to metabolize uric acid into allantoin. In urine, uric acid flocculates easily in the presence of high ammonia concentrations to form ammonium urate. Affected dogs usually have clinical signs related to shunting and liver dysfunction, such as hepatic encephalopathy, PU/PD, or vomiting. In the other category, the enzyme uricase, which forms allantoin, is inactive because of an inborn error affecting only this function. Ammonium urate urolithiasis occurs commonly in Dalmatians but may occur in other breeds.[43] Affected dogs only have signs related to urolithiasis (e.g., pollakiuria, stranguria, dysuria).

Acholic Feces

An owner may note acholic feces, which can provide a direct clue to the underlying diagnosis. Steatorrheic feces that do not contain normal bile pigment are seen only when bile flow into the intestinal tract is completely disrupted, usually as a consequence of extrahepatic obstruction of the common bile duct.[6] Destructive cholangiolitis is the only intrahepatic process severe enough to seriously disrupt bile flow. The latter disease is caused by a hypersensitivity reaction to sulfonamide-containing drugs. The smaller bile ductules become necrotic and liver lobuli may be disconnected from the biliary tract. Affected dogs have a history of recent medication with sulfonamides. Acholic feces contain excess fat because resorption is impaired. The lack of normal black-brown fecal pigments occurs because their precursor, bilirubin, does not reach the duodenum. Therefore, the feces from affected animals are gray-white and soft. Animals with this condition often are icteric. The presence of icterus reduces the likelihood of exocrine pancreatic insufficiency as a diagnosis.

Abdominal Distention

Abdominal distention may occur in dogs and cats with liver disease for several reasons. First, ascites is a frequent finding associated with liver disease in dogs as a result of portal hypertension, but is less common in cats. Abdominal distention may also result from organ enlargement, which in the case of liver disease may include the liver and, in the spleen in the presence of portal hypertension. In contrast to dogs, cats often have hepatomegaly with liver disease.

Other Symptoms

Nonspecific symptoms, such as apathy, reduced appetite or anorexia, and weight loss, may occur in dogs and cats with liver disease. Retarded growth is common in young animals. These problems reflect the central role of the liver in many metabolic and detoxifying functions. In addition, nausea, inappetence, vomiting, and diarrhea can result in a catabolic state, which, in turn, may aggravate hepatic encephalopathy. Signs of early hepatic encephalopathy include depression and other nonspecific problems. Anemia, another common finding in liver disease (see below), can cause general malaise. Dogs with liver cell carcinoma often are hypoglycemic,[44] which may be the primary problem underlying apathy and weakness. Production of insulin-like growth factors by the tumor may be responsible.

Physical Examination and Signs of Liver Diseases

As with historical findings, physical examination findings rarely provide enough information to pinpoint the liver as definitive cause of the presenting problems. Possible findings include icterus, hepatomegaly, splenomegaly, ascites, and pale mucous membranes. Petechiae of the skin or mucous membranes occur infrequently. Of these possible findings, only icterus and hepatomegaly are more or less specific for liver diseases; other abnormalities on the physical examination occur more frequently with diseases of other organ systems.

Ascites and hepato- and splenomegaly may have been noted by the owner as abdominal enlargement.[5] Biochemistry analyses are an integral part of the diagnostic process for liver diseases. Most of these analyses are not a decisive factor in the diagnosis of liver disease, but serve to rule out liver disease from the differential diagnosis.[8]

Icterus

Icterus is the most frequently encountered specific abnormality noted on the physical examination in dogs and cats with liver disease. However, only approximately 20% of dogs with hepatobiliary diseases and 30% to 40% of cats are icteric. Icterus results from bilirubin accumulation in the blood and extravascular space as a result of increased production, reduced clearance, impaired conjugation by the liver, and/or impaired bile flow. In most cases, a combination of these factors is involved. Cholestasis is predominant; therefore conjugated bilirubin is the fraction present in greatest quantity. Hemolysis alone does not result in icterus with normal liver function. When hemolysis is severe, however, it may result in such a degree of portal hypoxia that the centrolobular zones of the liver lobules become necrotic. In those cases, icterus results from the combination of increased production and reduced liver function and cholestasis.[45] If hemolysis is the primary cause of icterus, it must be severe, and the mucous membranes will be extremely pale. Primary liver diseases that may cause icterus are commonly accompanied by hemolysis. Whereas the erythrocyte lifetime is reduced to 6 to 10 days in dogs with severe primary hemolytic disease; it is 20 to 60 days (normally 100 days) in hepatobiliary disease. Increased production of bilirubin and liver dysfunction with cholestasis result in a combined conjugated and unconjugated hyperbilirubinemia in dogs and cats with primary hepatic or hemolytic disease.[46] Icterus caused by hemolytic disease is characterized by pale mucous membranes, whereas the mucous membranes in animals with primary liver disease are normal or only slightly pale. The combined evaluation of icterus and the color of the mucous membranes immediately reveal the nature of the underlying process.

Pale Mucous Membranes

As previously discussed, most hepatobiliary diseases are accompanied by increased degradation of red blood cells. The mechanisms behind hemolysis in liver disease are not completely clear. Hypersplenism and reduced portal blood flow due to portal hypertension may drastically prolong the transit time of erythrocytes through the spleen, with a greater chance that they will be trapped when they are slightly abnormal. Increased fragility of red cell membranes may be a result of the high bile acid levels in most liver diseases, whereas a reduced clearance of enteral endotoxins and bacteria by the liver may also induce immune-mediated hemolysis. Apart from hemolysis, nonregenerative anemia also may occur as part of the syndrome of anemia of chronic disease as an expression of catabolism and slight deficiencies of iron and B vitamins. Although common in liver diseases,[12] anemia, in contrast to icterus, is nonspecific.

Hepatomegaly

Like icterus, hepatic enlargement is a distinct sign of an abnormal liver. In dogs, most liver diseases do not cause hepatomegaly. Exceptions include tumors of the liver, liver congestion, and secondary liver involvement in metabolic diseases. Examples of the latter conditions are glycogen accumulation in the liver in Cushing disease, fatty liver with diabetes mellitus, and rare cases of amyloidosis of the liver.

The more chronic liver diseases of dogs tend to reduce liver size, and acute diseases cause little change in size. Liver enlargement as a result of congestive heart disease can, in most cases, be recognized easily by physical examination of the circulatory system. Measurement of central venous pressure is diagnostic. The exception is liver congestion caused by a thrombus in the caudal vena cava proximal to the liver, which is assessed by other methods. When the liver is overtly enlarged because of congestion, ascites is usually present. Ascitic fluid has the typical slightly hemorrhagic appearance of congestive fluid. Dogs with enlarged livers and no signs of congestive disease often have liver cancer, which may be primary, metastatic, or a form of malignant lymphoma. With most tumors, the liver is diffusely enlarged, but primary hepatocellular carcinomas or adenomas may cause enlargement of the affected lobe only. Bile duct carcinomas disseminate easily over the biliary system and usually cause pronounced icterus and hepatomegaly.

Most cats with hepatic disease develop pronounced enlargement of the liver. In cats, liver enlargement occurs with cholangitis, hepatic lipidosis, amyloidosis, hepatic tumors (primarily malignant lymphoma), and congestive disease. When the liver is involved in feline infectious peritonitis, it may not be enlarged. Cats with CPSS have small livers.

Splenomegaly and Ascites

Splenomegaly and ascites in association with liver disease are nonspecific findings. They occur especially with hepatic diseases causing portal hypertension. Both findings are frequent in dogs but rare in cats. There is a positive undulation test with distinct ascites; slight ascites can be found with ultrasonography rather than physical examination. The liver may be enlarged with central causes of venous congestion. Canine liver diseases associated with portal hypertension include chronic hepatitis and cirrhosis, portal vein hypoplasia, and lobular dissecting hepatitis. Portal hypertension is sometimes seen with cirrhosis because of advanced cholangiohepatitis in cats. In these diseases, hepatic encephalopathy is also common. Portal vein thrombosis is a prehepatic cause of portal hypertension that usually causes ascites. Although as a rule the liver is small in these cases, unilateral obstruction of a main branch of the portal vein may cause hypertrophy of the rest of the liver, which may be palpable.

Conclusion

Diseases of the hepatic parenchyma, hepatic vasculature, and biliary tract are relatively common in dogs and cats. Because the symptoms and signs accompanying liver disease are quite nonspecific, and the liver may be secondarily involved in diseases of other organs, liver disease can easily go undetected. Therefore, after taking a thorough history and performing a physical examination, it is critical to perform additional biochemical tests with the highest possible diagnostic accuracy whenever liver disease is included in the differential diagnosis. If liver disease cannot be ruled out based on these examinations, additional testing is necessary to define the type of liver disease, most notably ultrasonography of the cranial abdominal quadrant and examination of a liver biopsy specimen. The algorithm in Figure 61-5 summarizes this approach.

Diagnosis usually depends on histopathologic examination of liver tissue, especially for parenchymal liver diseases, many biliary tract diseases, and tumors of the liver or biliary tract. Although biopsy methods are beyond the scope of this chapter, excellent sources exist.[13] One note of caution: blood coagulation testing is vital before collecting a liver biopsy specimen for histopathology. Most animals, for example, have one or more abnormal coagulation tests.[47-52] Factors involved may include vitamin K deficiency, reduced

production of clotting factors, some degree of disseminated intravascular coagulation (DIC), and severe protein deficiency.[13,52] It should be noted that taking a fine-needle aspiration biopsy should be considered safe, even if abnormalities in coagulation are present. However, its diagnostic value is limited as the liver architecture is lost.

Diagnosis of circulatory hepatic diseases depends on information obtained from laboratory results, ultrasonography (most reliable diagnostic test to date), and histopathology.[13] A recent paper found that fasting ammonia concentration was superior to fasting bile acids for diagnosing portosystemic shunting in dogs.[53] Ammonia is easily measured in practice with dry chemical methods, some of which provide reliable results.[54] Another recent publication showed ultrasonography to be a reliable diagnostic method to noninvasively characterize the underlying hepatic disease in dogs with hyperammonemia.[55]

DIAGNOSTIC EVALUATION

Jonathan A. Lidbury and Jörg M. Steiner

Diagnostic evaluation of the hepatobiliary system has several aims: (a) to determine if hepatobiliary disease is present, (b) to assess liver function, (c) to determine if liver disease is primary or secondary, (d) to definitively diagnose hepatobiliary disease, and (e) to monitor response to treatment. Despite the apparent clarity of these aims, hepatobiliary disease can present a diagnostic challenge for a number of reasons. First, as clinical signs can be nonspecific, hepatobiliary disease should be a consideration when evaluating any patient with signs of systemic disease. Dogs and cats with hepatobiliary disease may not have any clinical signs. Furthermore, because of the liver's central role in metabolism and detoxification of endogenous toxins and xenobiotic agents, a number of extrahepatic diseases can secondarily affect the liver. It is important to distinguish these *secondary hepatopathies* from diseases that originate in the liver (*primary hepatopathies*). Additionally, serum markers of hepatocellular damage, cholestasis, and hepatic function can be abnormal in the absence of hepatobiliary disease. Finally, the liver's large reserve capacity means that detectable loss of liver function often occurs late in the course of disease. Thus, when assessing a patient with suspected hepatobiliary disease, it is important to consider the clinical presentation, results of laboratory testing, diagnostic imaging findings, and the results of cytologic and/or histopathologic evaluation together.

Laboratory Testing of the Liver

Hepatic Enzymology

Hepatic enzymes can be divided into markers of hepatocellular damage and markers of cholestasis. Serum alanine aminotransferase (ALT) and aspartate transaminase (AST) activities are the two most commonly measured markers of hepatocellular leakage, while serum alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT) activities are the two most commonly measured markers of cholestasis.

Although increased serum hepatic enzyme activities are considered to be sensitive, they are not specific for primary liver disease because they are produced by extrahepatic tissues. The relative importance of these extrahepatic *isoenzymes* varies, but their extrahepatic release can lead to increased serum activities. Also, the production of some hepatic enzymes can be induced by certain hormones and drugs, leading to an increase in their serum activities in patients without primary hepatic disease. Additionally, serum hepatic enzyme activities can be increased as a consequence of secondary hepatopathies.

The magnitude of hepatic enzyme activity increases may aid in the assessment of the severity or the extent of hepatic injury but should not be considered to be prognostic. The liver has a large capacity for regeneration, so even in cases of severe hepatic injury, with dramatically raised hepatic enzyme activities, a full recovery is possible. This is especially true when the injury is acute. Conversely, in cases of chronic end-stage liver disease, such as cirrhosis, serum hepatic enzyme activities may not be markedly increased, or may even be within the reference interval as a result of the replacement of hepatocytes with fibrous tissue. Consequently, serial evaluation of serum hepatic enzyme activities is more useful for assessing prognosis than measurement at a single point in time. Consistent decreases of a previously increased activity are considered a favorable sign in acute liver injury, whereas a decreasing hepatic enzyme activity in a patient with chronic liver disease that is clinically deteriorating suggests loss of hepatocytes because of fibrosis. It is important to note that serum hepatic enzyme activities do not provide an assessment of liver function.

Markers of Hepatocellular Damage

ALT is an enzyme found primarily in the cytosol of hepatocytes. ALT is released into the serum when hepatocyte membrane permeability is increased, or if there is hepatocellular necrosis. ALT is considered to be the most liver-specific enzyme. ALT is also produced by cardiac muscle, skeletal muscle, and the kidneys.[1] Apart from the hepatic form, only the muscle isoenzyme is clinically significant. Although uncommon, severe muscle injury can result in an increased serum ALT activity. Hepatic microsomal induction in response to some drugs can also produce small increases in ALT activity.

Some controversy exists regarding the serum half-life for ALT in dogs. The mean serum half-life of ALT was reported as being 149 minutes in one study[1] and 59 hours in another.[2] The serum half-life of ALT is generally believed to be shorter in the cat than in the dog. A mean serum half-life of 207 minutes was reported in an experiment involving three healthy cats.[3] The shorter half-life in cats means that increases in serum ALT activity are considered more clinically important in this species. As ALT is metabolized in the liver its serum half-life may be longer in patients with liver disease.[4]

Increased cell membrane permeability in the absence of hepatocyte destruction can cause a rapid increase in serum ALT activity. Because of this, ALT activity is a considered to be a highly sensitive marker of hepatocyte injury. This also means that an increased ALT activity does not imply severe or irreversible hepatocellular injury. The highest increases in ALT activity are seen during acute hepatic inflammation or necrosis, but because of the capacity for the liver to regenerate these do not indicate irreversible damage. Consequently, a single measurement of ALT activity does not provide an accurate prognosis. However, the degree of the ALT activity increase is believed to have some correlation with the number of hepatocytes that have been injured. Cholestasis can also result in an increased serum ALT activity because of hepatocellular damage caused by the accumulation of bile acids. Certain drugs can lead to increases in serum ALT activity. These are usually minor, for example, phenobarbital used at therapeutic doses frequently leads to small increases in serum ALT activity, in the absence of hepatic insufficiency. These increases are thought to occur as a result of subclinical hepatic injury rather than induction of hepatic microsomal enzymes.[5] Toxic doses

of phenobarbital can cause dramatic increases of serum ALT activity and hepatic insufficiency. Prednisone and other glucocorticoids can cause an induction of ALT (and steroid hepatopathy) and consequently small increases in serum ALT activity. Serum ALT activity can also be increased with any secondary hepatopathy. However, a persistently increased serum ALT activity, even with an apparently normal liver function, is an indication for further diagnostic testing.

Serial evaluation of serum ALT activity can be helpful to prognosticate but must be done while considering the patient's clinical signs and other laboratory values. In general, a declining serum ALT activity after acute liver injury is considered a good sign.

AST is another aminotransferase enzyme that is used as a marker of hepatocellular leakage. AST is found in skeletal muscle, the brain, liver, kidney, cardiac muscle, and to a lesser extent within other tissues.[6] The extrahepatic isoenzymes of AST are relatively more important than they are for ALT. Muscle disease can cause an increase in serum AST activity. Because of this, AST is considered less liver specific than ALT. However, by looking at serum AST activity in conjunction with the activities of other hepatic enzymes and muscle enzymes, it is usually possible to differentiate increases caused by muscle damage from increases caused by hepatic damage.

Again, there is controversy regarding the serum half-life of AST. In dogs one study[1] reported the half-life to be a mean of 263 minutes; another study reported a mean of 22 hours.[2] One study reported the mean half-life to be 78 minutes for cats.[7] Unlike ALT, a considerable proportion of AST (approximately 30%) is found within hepatocyte mitochondria rather than the cytosol.[8] The cytosolic fraction of AST is released into the serum from hepatocytes when cell membrane permeability is increased, or in case of hepatocellular necrosis. In contrast, the mitochondrial fraction is only released during hepatocellular necrosis. Release of AST from hepatocytes into the serum parallels the release of ALT. Therefore, like serum ALT activity, serum AST activity is considered a sensitive marker for hepatocyte injury. It has been suggested that increased AST activity may be more sensitive than increased ALT activity for the detection of hepatocellular injury in cats.[9] Corticosteroids and phenobarbital may cause mild increases in serum AST activity. Because of the considerable overlap in the information provided by the measurement of serum ALT and AST activities, measurement of serum AST activity may be redundant.

Markers of Cholestasis

ALP is an enzyme bound to the membranes of the hepatocytes that comprise the bile canaliculi and the sinusoidal membranes. It is considered a sensitive marker for cholestasis, especially in the dog, but is not liver specific. Cholestasis, canalicular cell necrosis, and increased hepatic synthesis may lead to the release of this enzyme into the circulation. Synthesis of this enzyme can be induced by certain drugs, most notably corticosteroids. The possibility that an increase in serum ALP activity could be caused by extrahepatic disease, or could be induced by glucocorticoids in the dog, can make the interpretation of this finding challenging.

In the dog a wide variety of tissues exhibit ALP activity, including intestinal mucosa, kidney (cortex), bone marrow, pancreas, testicle, brain, lung, kidney (medulla), lymph node, liver, skin, spleen, skeletal muscle, and cardiac muscle.[6] There is disagreement in the literature regarding the relative contributions of ALP activity from each of these tissues in cats.[10-12] There are two genes encoding ALP in the dog. Different forms of ALP arising from the same gene are called *isoforms*. Differences among these isoforms arise because of differing posttranslational processing. Liver ALP (L-ALP), bone ALP (B-ALP), and kidney ALP (K-ALP) are transcribed from the tissue nonspecific ALP gene. The other gene encodes intestinal ALP (I-ALP) and probably glucocorticoid-induced ALP (G-ALP).[13] In dogs the serum half-lives of placental ALP, K-ALP, and I-ALP are less than 6 minutes.[14] In cats the serum half-life of I-ALP is less than 2 minutes. The half-lives of placental ALP and K-ALP are also assumed to be short in the cat as they have similar structures to I-ALP. Because of this, only L-ALP, B-ALP, and, in the dog but not the cat, G-ALP, are believed to contribute significantly to serum ALP activity. The serum half-life of L-ALP is approximately 70 hours in the dog[14,15] and 6 hours in the cat.[16]

L-ALP is bound to the membranes of the hepatocytes by glycosylphosphatidylinositol linkages. Cleavage of these links by glycosylphosphatidylinositol-phospholipase allows the enzyme to be released into the bloodstream.[17] As bile acids have detergent-like properties; accumulation of bile acids during cholestasis facilitates this process. Cholestasis can also result in the induction of synthesis of L-ALP (and G-ALP in the dog). Consequently, serum ALP activity is often severely increased in patients with cholestatic disorders. In the dog, ALP is considered to be a sensitive marker for cholestasis with a sensitivity of 85%.[18] The short half-life of L-ALP in cats means that increases in ALP during cholestasis are not as high as in the dog. Consequently, ALP is a less-sensitive marker of cholestasis in the cat than in the dog, with a reported sensitivity of only 48%.[19] However, the shorter half-life in cats and the absence of G-ALP means that any increase in ALP activity should be considered clinically important in this species. An increased serum ALP activity does not differentiate between intrahepatic or extrahepatic cholestasis. A wide variety of liver diseases can cause intrahepatic cholestasis. This is generally caused by hepatocyte swelling, causing obstruction of the small bile canaliculi. The increase of ALP following a hepatic insult is delayed compared to rises in markers of hepatocellular leakage. The reason for this is that it takes time for the enzyme to be synthesized and released into the systemic circulation. ALP often remains increased for some time after the resolution of liver injury.

B-ALP is released into the bloodstream as a result of the activity of osteoblasts. Therefore any condition that results in increased bone formation can lead to increased serum ALP activity. In animals that are skeletally immature, mild increases in serum ALP activity are commonly observed. Animals with increased osteoblast activity, such as those with hyperparathyroidism, neoplasia involving bones, and osteomyelitis, may have mild to moderate increases in ALP activity. These causes of increased B-ALP activity are unlikely to be confused with primary liver disease because the increases are smaller than would be expected with cholestasis, and because bone diseases are often clinically apparent. Finally, an increased serum activity of B-ALP was reported in a family of asymptomatic Huskies.[20]

In dogs, but not in cats, *G-ALP* and tissue-nonspecific ALP may be induced by corticosteroids. G-ALP is believed to be an isoform of I-ALP with a prolonged serum half-life that is produced by the liver.[13] Posttranslational glycosylation of the G-ALP is believed to be responsible the prolonged half-life. Induction of G-ALP may cause an increase in total serum ALP activity after administration of exogenous corticosteroids. Synthesis of this isoenzyme can also be induced by the administration of anticonvulsant drugs, such as phenobarbital. Similarly, hypercortisolemia frequently causes an increased serum ALP activity because of induction of G-ALP. However, in dogs with excess serum concentrations of endogenous or exogenous corticosteroids, hepatocyte swelling caused by glycogen accumulation (vacuolar hepatopathy) may lead to intrahepatic cholestasis, another potential contributor to increased serum ALP

activity. Before diagnosing primary liver disease in a dog with an increased serum ALP activity, induction of G-ALP by endogenous or exogenous steroids should be ruled out. Recently a group of Scottish Terriers were found to have increased serum ALP activity with no identifiable underlying cause.[21]

It is technically possible to selectively measure the activity of G-ALP using techniques such as levamisole inhibition. Measurement of G-ALP activity was initially investigated as a way to differentiate increases in ALP caused by corticosteroids from those caused by cholestasis. Unfortunately, measuring G-ALP is not clinically useful as G-ALP activity may be increased in a variety of conditions, including hepatic disease, diabetes mellitus, hypothyroidism, and pancreatitis.

GGT is a glycoprotein enzyme that is bound to the membranes of those hepatocytes that form the bile canaliculi and bile ducts and also periportal hepatocytes. In comparison to ALP its distribution includes more distal areas of the biliary tract, but measurement of serum GGT activity is not useful to distinguish between intrahepatic and extrahepatic cholestasis. GGT is also produced by a number of extrahepatic tissues. Most of the GGT activity in serum is thought to be a result of the hepatic isoenzyme. Colostrum also contains GGT, which is responsible for the mild increases in serum GGT activity that are seen in suckling animals.[22]

Changes in serum GGT activity generally parallel those in serum ALP activity, in that activity is often increased in patients with cholestasis. Because GGT is also induced by glucocorticoids, its activity may be increased in patients with hyperadrenocorticism or those exposed to exogenous steroids. In dogs, an increased serum GGT activity is considered to be more specific, but less sensitive than ALP activity for the presence of liver disease.[18] In cats, measurement of serum GGT activity is more sensitive but less specific for the detection of liver disease than ALP. Cats with hepatic lipidosis may be an exception to this as they often have a normal serum GGT activity but an increased serum ALP activity.[19]

Markers of Protein Metabolism

The liver plays a central role in protein metabolism. It is responsible for the synthesis of plasma proteins, deamination of amino acids, conversion of ammonia to urea, amino acid synthesis, and interconversion of amino acids.[23] Consequently, in patients with hepatic disease these functions may be compromised.

Plasma Proteins

Albumin is an important plasma protein that is produced exclusively by the liver. The rate of albumin synthesis must equal the rate of albumin loss to maintain serum albumin concentrations. Mild decreases in serum albumin concentration can occur from a variety of conditions. However, the differential diagnoses for severe hypoalbuminemia (<2 g/dL) are limited to hepatic insufficiency, severe exudative skin disease, protein-losing enteropathy, and protein-losing nephropathy. It is possible to determine the cause of severe hypoalbuminemia from a combination of clinical findings, measurement of the serum globulin concentration, urinalysis (including protein creatinine ratio), tests of GI protein loss, and tests of liver function. As albumin contributes significantly to colloid oncotic pressure,[24] severe hypoalbuminemia can lead to ascites, pleural effusion, and/or subcutaneous edema. The liver has a large reserve capacity for the synthesis of albumin and albumin has a serum half-life of approximately 7 days in dogs.[25] Consequently, hypoalbuminemia is a relatively insensitive marker for hepatic insufficiency and is only likely to be seen in patients with advanced chronic liver disease or *portosystemic shunts* (PSSs).

Globulins are produced in the liver, but not exclusively so. The liver produces α-globulins and β-globulins, whereas lymphoid cells produce immunoglobulins (γ-globulins). Hepatic insufficiency rarely leads to a decrease in serum globulin concentration. Conversely, inflammatory liver disease may be associated with hyperglobulinemia because the nonimmunoglobulin fraction produced by the liver includes several *acute-phase proteins* (C-reactive protein, haptoglobin, and serum amyloid A). The hepatic synthesis of these proteins is increased during systemic inflammation[26-29] possibly leading to a rise in the total serum globulin concentration. Additionally, immunoglobulin production may be increased in infectious, neoplastic, or autoimmune diseases.

Coagulation factors (except factor VIII), anticoagulation factors (antithrombin and protein C), and the fibrinolytic protein plasminogen, are all synthesized by the liver. The liver is also the site of activation of the vitamin K-dependent clotting factors: II, VII, IX, X, and protein C. Furthermore, as bile acids are needed to emulsify fat and aid in the absorption of vitamin K from the intestine, vitamin K malabsorption may develop secondary to cholestasis. Consequently, hepatobiliary disease can affect hemostasis in more than one way.

In canine and feline liver disease, coagulation parameter abnormalities have been reported in specific clotting factor activities, prothrombin time, aPTT,[30-32] proteins induced in the absence of vitamin K,[33,34] fibrin degradation products, fibrinogen, and protein-C activity.[35] These abnormalities of hemostasis are not specific for liver disease but may support its presence. Patients with liver disease may develop DIC, which can be difficult to distinguish from coagulopathy because of reduced hepatic synthesis of clotting factors alone. Although spontaneous bleeding seldom occurs in patients with liver disease, the assessment of the coagulation status of these patients is important, especially when an invasive procedure such as a liver biopsy is being considered.

A recent study investigated the diagnostic value of serum protein C as a marker for hepatobiliary disease and portosystemic shunting in dogs. Serum protein-C measurement was reported to aid in the differentiation of portal vein hypoplasia without portal hypertension (formerly called microvascular dysplasia) from portosystemic shunt (PSS). Dogs with portal vein hypoplasia without portal hypertension had a significantly higher serum protein-C concentration than those with portosystemic shunting.[35]

Protein Catabolism

Urea is produced from ammonia in the liver, released into the systemic circulation, and subsequently excreted by the kidneys. Serum urea nitrogen concentration may be close to or below the lower limit of the reference interval in patients with hepatic insufficiency, PSS,[36] or urea cycle enzyme deficiencies. However, serum urea nitrogen concentration may also be decreased because of medullary solute washout caused by diuresis, malnutrition, or a protein-restricted diet, and is a normal finding in neonates. In a patient with liver disease, a high fasting serum urea nitrogen concentration relative to the serum creatinine concentration suggests GI hemorrhage.

Ammonia (NH_3) is produced in small intestinal enterocytes from the catabolism of glutamine and in the colon as a consequence of bacterial deamination. Ammonia is a highly diffusible gas and passes readily through the bowel wall into the bloodstream. In the blood, at a pH of 7.4, most of the ammonia exists in the form of ammonium ions (NH_4^+). The ammonium is transported in the blood from the intestines through the hepatic portal circulation to the liver. The extraction of ammonia from the portal circulation is highly efficient. Endogenous ammonia is produced from the breakdown of

nitrogenous substances in the body, especially glutamine. In the liver the ammonium is converted to urea by the enzymes of the urea cycle, or is used during the conversion of glutamate to glutamine.[37] Urea enters the circulation and is excreted by the kidneys. Ammonium that is not removed by the liver enters the systemic circulation.

The liver has a large reserve capacity for the conversion of ammonia into urea. Because of this, plasma ammonia measurement is a relatively insensitive marker for hepatic insufficiency. However, measurement of blood ammonia concentration is a sensitive test for congenital PSSs and APSC shunts (also known as acquired PSSs). This is because when portosystemic shunting occurs, the ammonia absorbed from the intestines bypasses the liver and reaches the systemic circulation directly. The sensitivity of plasma ammonia measurement for the detection of PSS is reported to be between 81% and 100% in dogs[38-41] and 83% in cats.[41] The measurement of postprandial venous ammonia is more sensitive than the measurement of fasting ammonia (sensitivities of 91% and 81%, respectively) for the detection of congenital PSS.[42] However, the sensitivity for detecting dogs with hepatocellular disease is only 36%. Generally, hyperammonemia is considered specific for hepatic insufficiency or PSS. However, although they are uncommon, urea-cycle enzyme deficiencies may also cause an increased blood ammonia concentration. These enzyme deficiencies can be hereditary as a result of the absence of a particular enzyme[43] or secondary to cobalamin or arginine deficiency.[44,45] Arginine deficiency is especially relevant in cats with hepatic lipidosis. Ammonia is one of the substances that cause *hepatic encephalopathy* (HE). Therefore blood ammonia measurement is a useful marker for HE. However, other substances can also cause HE and the plasma ammonia concentration of a patient with HE may be within the reference interval.

Ammonium ions are labile in plasma, so sample handling is critical when measuring plasma ammonia concentration. Samples should be collected, placed immediately on ice, and the plasma separated from the red blood cells as soon as possible. The plasma must be kept cooled and should be analyzed within 30 minutes of collection. These handling requirements have meant that ammonia measurement has been mainly confined to practices with immediate access to a commercial laboratory. Measurement of plasma ammonia is available on an in-house dry chemistry analyzer (VetTest, Idexx Laboratories, Westbrook, ME) although this method was only considered to reliably agree with a reference method for serum ammonia concentrations greater than 150 μM.[46] A recent study found that a point of care blood ammonia analyzer (PocketChem BA, Menarini Diagnostics, Florence, Italy) may be suitable for the measurement of blood ammonia concentrations in dogs and cats.[47]

Ammonia tolerance tests (ATTs) have been investigated in an attempt to increase the sensitivity of ammonia measurement for detecting hepatic insufficiency and PSS. However, the oral administration of ammonium salts can cause vomiting and potentially worsen HE signs. Ammonium chloride or sulfate can also be given rectally, which is less likely to produce adverse. This method is sensitive for the detection of PSS in dogs.[48]

Markers of Lipid Metabolism

The liver plays a central role in lipid metabolism and is responsible for oxidation of fatty acids, synthesis of cholesterol, synthesis of lipoproteins, and synthesis of fatty acids from proteins and carbohydrates.[23]

Serum cholesterol concentrations may be increased, normal, or decreased in patients with liver disease. Increased or decreased fasting serum cholesterol concentrations are not sensitive or specific for hepatobiliary disease in dogs or cats. In patients with severe hepatic insufficiency or PSS[49] serum cholesterol, concentration may be decreased as a consequence of impaired hepatic synthesis. Hypocholesterolemia might also occur as a result of inadequate dietary intake, maldigestion, malabsorption, or hypoadrenocorticism. The serum cholesterol concentration of patients with hepatobiliary disease may be within the reference interval. Patients with cholestatic disease can become hypercholesterolemic.[50] Fasting hypercholesterolemia also may be observed in patients with various endocrinopathies, obesity, protein-losing nephropathy, pancreatitis, or primary hyperlipidemias.

Serum triglyceride concentration may be increased or normal in patients with liver disease. However, an increased fasting serum triglyceride concentration is not a sensitive or specific marker for hepatobiliary disease in dogs or cats. A mild increase in serum triglyceride concentration may develop in patients with cholestasis. There is some evidence that hypertriglyceridemia is associated with gallbladder mucocele formation.[51] Hypertriglyceridemia is associated with increased serum hepatic enzyme activities in Miniature Schnauzers.[52] Increased fasting serum triglyceride concentrations are also observed in patients with endocrinopathies, obesity, pancreatitis, and primary hyperlipidemias.

Markers of Carbohydrate Metabolism

The liver plays a central role in carbohydrate metabolism and is responsible glycogen storage, conversion of galactose and fructose into glucose, gluconeogenesis, and the synthesis of many compounds from carbohydrates.[23]

Blood glucose measurement is not a sensitive or specific marker for liver disease. The liver has a large reserve capacity for glucose production. Consequently, hepatic insufficiency must be severe for hypoglycemia to occur. Hypoglycemia occurs in a proportion of patients with congenital PSS.[53] Hepatic neoplasia can also lead to hypoglycemia. This is thought to be caused by release of insulin-like substances.[54] A variety of extrahepatic conditions can also lead to hypoglycemia.

Other Tests of Liver Function

Bilirubin is a yellow pigment produced from the breakdown of *heme*-containing compounds. Measurement of serum bilirubin concentration can be used to assess liver function. Hyperbilirubinemia can be the result of hepatobiliary or extrahepatic disease. Icterus is the yellowish pigmentation caused by the retention of bilirubin in the soft tissues. Laboratory assessment is the most sensitive way to detect increased serum bilirubin concentrations. Hyperbilirubinemia is classified as *prehepatic*, *hepatic*, or *posthepatic* in origin. Bilirubin may be artifactually increased by in vitro hemolysis or by the administration of synthetic hemoglobin polymers. When assessing a hyperbilirubinemic patient it is critical to localize the underlying cause.

Bilirubin is the major product of the degradation of heme-containing compounds by cells of the *mononuclear phagocyte system*. Bilirubin is released from the mononuclear phagocyte system and is transported in the plasma. The bilirubin is reversibly bound to albumin as it is water insoluble. The unconjugated bilirubin is absorbed through the hepatocyte cell membranes and is bound to glucuronic acid (conjugation). Conjugated bilirubin is water soluble and is actively excreted from the hepatocytes into the bile canaliculi, eventually being excreted into the intestines. Once in the intestine some of the bilirubin is converted into urobilinogen by bacteria. Some of the urobilinogen is reabsorbed from the intestines, but most of this is immediately reexcreted by the liver. When

exposed to air the urobilinogen remaining in the intestines is altered and oxidized into the brown pigment sterocobilin.[23]

Prehepatic hyperbilirubinemia is caused by increased production of bilirubin as a result of hemolysis. The liver has a large reserve capacity for bilirubin excretion so, for hemolysis to cause hyperbilirubinemia, hepatic bilirubin clearance must be decreased.[55] This occurs if the hemolytic anemia results in hepatocyte dysfunction because of hypoxia. If hepatic hypoxia occurs serum hepatic enzymes activities are often increased. Prehepatic hyperbilirubinemia is mainly distinguished from other causes of hyperbilirubinemia by the presence of severe anemia. Other supportive evidence includes the presence of a regenerative erythroid response, characteristic changes in red blood cell morphology, and possibly the detection of red blood cell bound antibodies.[56]

Hepatic hyperbilirubinemia is caused by a decreased rate of hepatocyte bilirubin uptake, conjugation, or excretion (as a result of *intrahepatic cholestasis*). Usually, hepatocyte dysfunction and intrahepatic cholestasis occur concurrently. Hepatic enzyme activities (both hepatocellular leakage markers and cholestatic markers) are often increased, although they can also be increased with both prehepatic and posthepatic hyperbilirubinemia. Because of the hepatic reserve capacity, hepatic disease must be severe in order to cause hyperbilirubinemia. A range of primary and secondary hepatopathies can cause hepatic hyperbilirubinemia. Hepatic hyperbilirubinemia can usually be distinguished from prehepatic hyperbilirubinemia by assessment of the patient's hematocrit, and from posthepatic hyperbilirubinemia by abdominal ultrasound. Other markers of hepatic insufficiency, when present, provide additional support for the presence of hepatic hyperbilirubinemia.

Posthepatic hyperbilirubinemia is a result of extrahepatic bile duct obstruction. This is often caused by pancreatic inflammation or, much less commonly, neoplasia. The main diagnostic tool for documenting extrahepatic bile duct obstruction is abdominal ultrasound. Typically, extrahepatic bile duct obstruction leads to dramatic increases in serum cholestatic enzyme activities (compared to hepatocellular leakage enzyme activities) and hypercholesterolemia. When the bile duct is completely obstructed, acholic (pale-colored) feces may be noted. Rupture of the biliary tract frequently leads to hyperbilirubinemia as bilirubin accumulates in the abdomen.

It is possible to measure the concentration of serum conjugated bilirubin. However, this test is not considered to be clinically useful for distinguishing between prehepatic, hepatic, or posthepatic hyperbilirubinemia, and is thus rarely performed.

Bilirubin can covalently (nonreversibly) bind to albumin. This biliprotein cannot be cleared by the liver and thus persists in the plasma. Biliprotein has a serum half-life comparable to albumin. This is of clinical importance because it means hyperbilirubinemia (and icterus) may persist for several weeks after the resolution of its cause.[57]

Bile acids (or bile salts when they are deionized) are formed from cholesterol in the liver and are the major constituent of bile. *Serum bile acids* (SBAs) measurement is a useful test of liver function in dogs and cats. SBAs are either measured as a fasting sample (after withholding food for 12 hours) or by collecting paired fasting and 2-hour postprandial samples.[58] Both of these tests are simple to perform and safe. Enzymatic measurement of the concentration of total bile acids in serum has become widely available and has replaced other techniques such as radioimmunoassays.[59,60] Once collected, the samples of serum can be stored at room temperature, making it possible to send them to an outside laboratory for evaluation. Lipemia and hemolysis of the blood samples should be avoided as both can interfere with the assay. Increased SBA concentrations

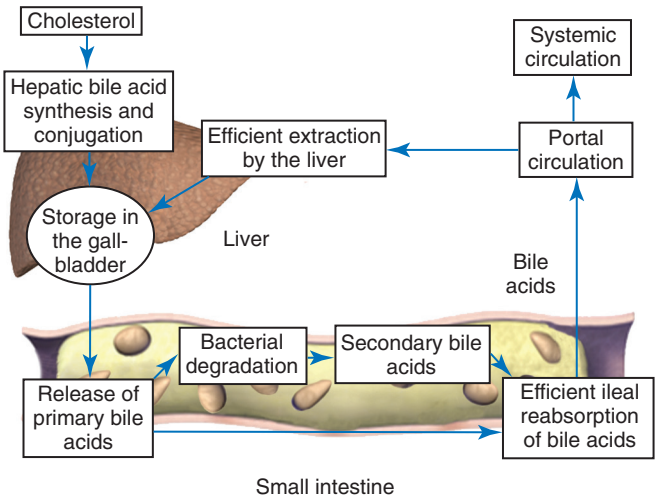

Figure 61-6 The enterohepatic circulation of bile acids.

(fasting or postprandial) suggest hepatic dysfunction, PSS, or cholestasis, but they are not specific for any particular liver disease.

Bile acids are exclusively synthesized in the liver from cholesterol. Nearly all of the bile acids that are produced by the hepatocytes are conjugated to an amino acid. In both dogs and cats conjugation is primarily to taurine, but dogs may also convert to a conjugation with glycine.[61] In contrast, even taurine depleted cats conjugate their bile acids almost exclusively to taurine.[62] The conjugated bile acids produced by the liver are called *primary bile acids*. These are secreted in the bile, and then stored in the gallbladder. *Cholecystokinin* is released from endocrine cells in the small intestine. This hormone stimulates gallbladder contraction and the flow of bile into the duodenum. When the gallbladder contracts the bile acids are released into the intestines. Spontaneous gallbladder contraction also occurs during the interdigestive phase.[63] Bile acids act as ionic detergents, aiding the emulsification of dietary lipids and their subsequent intestinal absorption in micelles.[64]

Bile acids are recycled in a process known as *enterohepatic circulation* (Figure 61-6). Primary bile acids are lipid insoluble and thus are only absorbed from the intestines when they bind to specific high affinity ileal mucosal receptors.[64] This ileal reabsorption is very efficient. The reabsorbed bile acids enter the portal circulation and upon reaching the liver they are efficiently extracted from the plasma and subsequently reexcreted. The total bile acids pool can be recirculated several times in a day. Consequently, the rate of hepatic bile acid synthesis and the fasting serum bile acids concentration in dogs and cats with normal hepatic function is low. Because of the increased release of stored bile acids during the postprandial period, small increases in total SBA concentration occur in animals with normal hepatic function.

Primary conjugated bile acids can undergo bacterial deconjugation in the intestinal lumen. The resulting unconjugated bile acids are called *secondary bile acids*. These are readily absorbed from the colon by passive diffusion. First-pass extraction and reexcretion of secondary bile acids is less efficient than that for primary bile acids. Consequently, secondary (unconjugated) bile acids are often present in postprandial serum samples.[65]

Hepatobiliary disease can cause increased SBA concentrations by interfering with hepatocellular function, by causing decreased bile flow (cholestasis), or by altering the hepatoportal blood flow. The main clinical use of SBA measurement is to assess hepatic function in patients suspected to have hepatic disease, with serum

bilirubin concentrations that are within the reference interval. Measurement of postprandial SBA concentration does not seem to have an advantage over fasting SBA concentrations or *vice versa*. Sensitivity can be increased by collecting paired preprandial and two-hour postprandial samples.[66] Numerous studies show that SBA measurement is a useful test for diagnosing hepatobiliary disease, including PSS in dogs and cats.[41,66-70] A recent study found the sensitivity of fasting SBA measurement for diagnosing PSS (using a cutoff value of 20 µmol/L) to be 93% for dogs and 100% for cats. The reported specificities were 67% for dogs and 71% for cats.[41] However, the sensitivity of SBA measurement for detecting hepatic insufficiency is lower than that for detecting PSS.

Measurements of SBA concentrations have several limitations. First, this test does not allow differentiation between various types of hepatobiliary disease. Also, measurement of serum bile acids in a patient with proven cholestasis is of no clinical benefit. Additionally, there is limited utility in measuring SBAs concentrations in patients with hyperbilirubinemia, although potentially SBA measurement could be useful in distinguishing prehepatic from hepatic or posthepatic causes of hyperbilirubinemia. With prehepatic causes of hyperbilirubinemia, the bile acid concentrations should be within the reference interval. However, in most cases prehepatic hyperbilirubinemia is easily distinguished by the presence of severe anemia. It should also be noted that the magnitude of increases of SBA concentration are not correlated with prognosis or disease severity.

It is important to note that fasting SBA concentrations may be higher than the upper limit of the reference interval or higher than the postprandial value because of spontaneous contraction of the gallbladder in the fasting state, or because of delayed gastric emptying. This could result in an increased fasting SBA concentration in the absence of hepatobiliary disease. Increased fasting and postprandial serum bile acids concentrations can be the result of increased bacterial deconjugation of primary bile acids into secondary bile acids.[71] False-negative results may occur if enterohepatic circulation of bile acids does not occur from a lack of gallbladder contraction. This could be a problem if a patient is anorectic, does not eat enough food, consumes a diet with insufficient protein or fat, vomits the test meal, or has delayed gastric emptying.

Ceruletide is an injectable cholecystokinin analogue that has been used to stimulate gallbladder contraction when using SBA measurement to diagnose hepatobiliary disease.[72,73] This test circumvents many of the factors that influence postprandial SBA concentrations. *Urine bile acids* measurement has been described in dogs and cats. The diagnostic performance was similar to that of SBA measurement in both species. This test does not offer any advantages over SBA measurement.[74-77]

Excretion of exogenous tracers, such as the anionic cholephilic dyes, bromsulphalein, and indocyanine green have been used historically to assess hepatic function in veterinary patients. However, these tests are considered unreliable and have been replaced by the measurement of SBA concentrations.

The metabolism of exogenous substances can be used to assess liver function. A variety of substances have been investigated as markers for hepatic metabolism in human medicine. Assessment of the metabolism of *aminopyrine* has been investigated in dogs and to a lesser extent in cats. The ^{13}C-labeled aminopyrine demethylation blood test involves intravenous administration of ^{13}C-labeled aminopyrine to the subject. The aminopyrine is metabolized by the liver, resulting in the production of $^{13}CO_2$. This is measured in the blood by fractional mass spectroscopy.[78,79] Further investigation of the utility of this test for assessment of liver function is needed. A recent study did not support the use of a ^{13}C-labeled galactose breath test for assessment of liver function in dogs.[80]

The metabolism of endogenous substances has been investigated to find possible markers of hepatic cellular metabolism. Dogs with hepatic disease (hepatitis and neoplasia) had significantly higher serum L-phenylalanine concentrations than did healthy dogs and those with nonhepatic diseases.[81] Further investigations are needed to determine the utility of these tests for assessing liver function in veterinary patients.

Urinalysis

Urine specific gravity can be decreased in patients with hepatic insufficiency or PSS. This can be caused by an inability to fully concentrate urine, resulting in PU, or from primary PD.

Bilirubin is commonly measured semiquantitatively in canine and feline urine using urine dipsticks. Bilirubinuria (<2+ on a dipstick) can be a normal finding in dogs (especially males).[82] Bilirubinuria in dogs without hemolytic or hepatobiliary disease can occur as a consequence of the loss of unconjugated bilirubin that is bound to albumin in proteinuric patients and renal filtration of small amounts of conjugated bilirubin that has leaked from the liver. Additionally, the renal tubular cells of male dogs have the enzymes needed to produce and conjugate bilirubin. As cats have a higher renal threshold for bilirubin than dogs, bilirubinuria should always be considered abnormal in cats. Bilirubinuria in cats and excessive bilirubinuria in dogs implies hemolytic or hepatobiliary disease. Because dogs have a relatively low renal threshold for bilirubin, bilirubinuria is often detected before bilirubinemia or jaundice.

Ammonium biurate crystals are detected in the urine sediment by light microscopy. Uric acid is a product of purine catabolism and is converted to allantoic acid by hepatic urate oxidase. In cases with severe hepatic insufficiency or PSS, the serum uric acid concentration may be higher than the renal threshold. This combined with hyperammonemia may lead to ammonium biurate precipitation in the urine. Urate urolithiasis seems to be more common in patients with PSS than those with other types of hepatic dysfunction. Between 40% and 70% of dogs with PSS were found to have urate crystalluria.[83] However, it should be noted that urate crystalluria is not specific for hepatobiliary disease.

Hematology

The erythrocyte series may be affected by hepatobiliary disease, resulting in erythrocyte dysmorphias and anemia. These abnormalities are suggestive of, but are not specific for, hepatobiliary disease.

Patients with hepatobiliary disease can be anemic as a result of blood loss, in which case signs of a regenerative response are normally present within 3 days of hemorrhage. Acute, severe hemorrhage may occur in patients with hepatobiliary disease following invasive procedures such as liver biopsy or as a consequence of hemorrhage from a hepatic neoplasm or hepatic rupture. Less-severe anemia may occur as a result of GI bleeding.[84] Chronic GI blood loss may eventually lead to iron-deficiency anemia. This is characterized by microcytic hypochromic erythrocytes and a variable regenerative response. Additionally, hepatobiliary disease may lead to anemia of chronic disease, which is typically nonregenerative with normocytic normochromic erythrocytes.

Red blood cell morphologic changes are sometimes observed in dogs with hepatobiliary disease. Poikilocytosis, characterized by the presence of acanthocytes and target cells, may be seen in patients with chronic hepatic disease. This is thought to be a result of altered phospholipid metabolism. Patients with PSS can have microcytic red blood cells. This is more common in dogs than in

Table 61-3 Typical Patterns of Clinicopathologic Changes Associated with Liver Disease in the Dog

Laboratory Test	Acute Hepatitis/ Hepatic Necrosis	Chronic Hepatitis	Cirrhosis	CPSS	Biliary Tract Obstruction	Nonobstructive Biliary Tract Disease	Hepatic Neoplasia
ALT	↑↑-↑↑↑	↑-↑↑↑	N-↑↑	N-↑	N-↑↑	N-↑↑	N-↑↑
ALP	↑-↑↑	↑-↑↑	N-↑↑	N-↑	↑↑↑	↑-↑↑↑	N-↑↑
Total bilirubin	N-↑↑↑	N-↑↑	N-↑↑↑	N	↑↑-↑↑↑	N	N-↑
Preprandial SBA	N-↑↑	N-↑↑	↑-↑↑↑	N-↑↑	↑↑-↑↑↑	N	N-↑
Postprandial SBA	N-↑↑	N-↑↑	↑-↑↑↑	↑↑-↑↑↑	↑↑-↑↑↑	N	N-↑
Ammonia	N-↑↑	N-↑↑	N-↑↑	↑-↑↑↑	N	N	N-↑

↑, Mild increase; ↑↑, moderate increase; ↑↑↑, severe increase; ALP, serum alkaline phosphatase activity; ALT, serum alanine aminotransferase activity; CPSS, congenital portosystemic shunt; N, within the reference interval; SBA, serum bile acid concentration.

cats.[83] Microcytosis also occasionally occurs in patients with hepatocellular disease. Altered iron metabolism is thought to lead to a delay in red blood cell precursors gaining a sufficient amount of hemoglobin to be released into the circulation. This delay leads to the precursors undergoing an extra cell division in the bone marrow, resulting in microcytosis.[85] Microangiopathy can occur as a result of hepatic neoplasia or DIC, and may lead to the formation of schistocytes.

The leukocyte series may be affected by hepatobiliary disease in a variety of ways. The resultant abnormalities are inconsistent and are not specific for hepatobiliary disease. Leukocytosis, leukopenia, and sometimes an inflammatory leukogram may be present when infectious and, less commonly, inflammatory or neoplastic processes affect the hepatobiliary system. A leukocytosis was found to be present in 44% of dogs with chronic hepatitis.[86]

The thrombocyte series is occasionally affected by hepatobiliary disease, but changes are both inconsistent and nonspecific. Mild to moderate thrombocytopenia may occur in patients with severe liver disease.[86] This may be the result of a decreased production of thrombopoietin by the liver. Disseminated intravascular coagulopathy associated with liver disease also may lead to thrombocytopenia. Additionally, infectious diseases affecting the liver, such as leptospirosis may result in thrombocytopenia.[87]

Other Diagnostic Tests for Hepatobiliary Disease

Genetic testing for copper hepatotoxicosis has been developed in Bedlington Terriers. Affected Bedlington Terriers have an autosomal recessive defect of their COMMD1 gene. Dogs with a homozygous affected genotype develop copper hepatopathy as a result of impaired biliary excretion of copper. Initially, a microsatellite marker that is in linkage disequilibrium with the mutation was discovered and used to identify affected dogs and select dogs homozygous unaffected dogs for breeding.[88] Subsequently, a mutation of the COMMD1 gene (a deletion of exon 2) was identified as the cause of the condition in the majority of Bedlington Terriers.[89] A genetic test for this disease has become commercially available (VetGen, Ann Arbor, MI). This test is run alongside the linked marker as a small proportion of Bedlington Terriers do not have the deletion but are nevertheless affected by the disease. These dogs are likely to have a rare second mutation of their COMMD1 gene which the linkage markers may track.

Hyaluronic acid is a major constituent of the ECM and hyaluronic acid (HA) concentration in blood has been used as a marker for hepatic fibrosis in humans. A recent study investigated the use of HA concentration as a marker for hepatic disease in dogs.[90] This study found that blood HA concentrations were significantly higher in dogs with liver disease than in dogs with extrahepatic disease, and were higher in dogs with cirrhotic liver disease than in dogs with noncirrhotic liver disease. Blood HA concentration may prove be a useful marker for hepatic fibrosis in dogs but further studies are necessary to evaluate its clinical utility.

Patterns of Clinicopathologic Change Associated with Liver Disease

Histopathologic analysis of liver biopsies or identification of a shunting blood vessel is often required to definitively diagnose hepatic disease. However, the pattern of laboratory test abnormalities, particularly when interpreted in conjunction with the patient's clinical presentation, and the results of diagnostic imaging, can increase or decrease a clinician's index of suspicion for specific liver diseases (Table 61-3). It is important to note that there is considerable overlap between the patterns for different diseases. To avoid misinterpretation and misdiagnosis when evaluating a patient for liver disease, it is essential to consider the limitations of the laboratory tests discussed above.

Diagnostic Imaging of the Liver

Diagnostic imaging is an important part of the investigation of hepatobiliary disease in dogs and cats. Diagnostic imaging may help to determine whether or not hepatobiliary disease is present, identify the cause of a secondary hepatopathy, aid in the diagnosis of specific hepatobiliary diseases, and provide prognostic information. However, with the exception of diagnosis of a PSS, imaging seldom yields a definitive diagnosis. Radiography and abdominal ultrasound are the most frequently used imaging modalities for assessment of the hepatobiliary system in dogs and cats, but alternative imaging techniques are now being used more frequently.

Abdominal Radiographs

Abdominal radiographs allow assessment of hepatic size, shape, opacity, and location in most patients.[91] Radiography may also allow identification of extrahepatic abnormalities that affect the liver. However, radiographs provide limited information about the hepatic parenchyma. It is important to note that patients with hepatobiliary disease often have normal abdominal radiographs.

Radiography allows subjective assessment of liver size. Cranial displacement of the gastric axis may be observed on lateral abdominal radiographs when microhepatia is present. However, subtle microhepatia is unlikely to be appreciated radiographically. PSSs and hepatic cirrhosis are the most common conditions causing microhepatia. Mild bilateral renomegaly may also be

appreciated radiographically for patients with PSS. Urate uroliths can be radiolucent so they might not be visible on plain abdominal radiographs.

Hepatomegaly can be generalized or focal. Generalized hepatomegaly can be caused by a number of conditions including neoplasia, vacuolar hepatopathies, congestion, or amyloidosis. Focal hepatomegaly can be caused by neoplasia, abscesses, granulomas, or a liver lobe torsion. Radiographic signs associated with hepatomegaly are rounded hepatic borders, caudal displacement of the gastric axis, and extension of the hepatic silhouette beyond the costal arch. Radiography does not allow appreciation of mild hepatomegaly. Additionally, it can be normal for the hepatic silhouette to extend beyond the costal arch in brachycephalic breeds, chondrodystrophic breeds, neonatal animals, or geriatric animals.[91]

The liver is normally appreciated as an area of homogenous soft-tissue opacity on radiographs. Radiolucent areas within the liver indicate accumulation of gas within the hepatic parenchyma, biliary tract, or portal vasculature. Gas in the parenchyma of the liver can be associated with an hepatic abscess.[92] Gas in or around the gallbladder has been reported in dogs with emphysematous cholecystitis.[93] Although uncommon in dogs and cats, if choleliths or choledocholiths contain enough calcium, they may be appreciated as mineral opacities within the hepatic silhouette.[94] Mineralization of the gallbladder wall can be associated with a biliary adenocarcinoma in the dog.[95] Parenchymal mineralization can be associated with granulomas, abscesses,[96] hematomas, neoplasia, or hepatic necrosis.[91]

Angiography allows the visualization of the hepatoportal vasculature, including abnormal vessels. This often provides a definitive diagnosis of PSS and is indicated in patients that are suspected of having a PSS, where the shunt cannot be adequately evaluated by abdominal ultrasound. Anatomical characterization of congenital PSS is important when planning attenuation, and angiographic procedures often allow this. There are several techniques for mesenteric portography. Operative mesenteric portography is commonly performed immediately prior to surgical attenuation of a congenital PSS and involves catheterization of a mesenteric vein and injection of a contrast agent. Operative contrast portography allows evaluation of the portal vasculature before and after shunt attenuation. This technique has the disadvantage of being relatively invasive. Cranial mesenteric portography can be accomplished less invasively by using ultrasound guidance to percutaneously catheterize the splenic vein.[97] However, this can be technically demanding and may not be possible in smaller patients. Transvenous retrograde portography has been described and involves the catheterization of the jugular vein.[98] This technique allows selective catheterization of the shunting vessel and measurement of portal pressures (Figure 61-7). Transvenous retrograde portography has been applied during percutaneous transjugular coil embolization of intrahepatic shunts.[99] Percutaneous splenoportography involves percutaneous injection of contrast media into the spleen. This technique is simple to perform, but there is a risk of complications such as splenic infarction or hemorrhage.

Abdominal Ultrasound

Abdominal ultrasonography is the most commonly used imaging modality for evaluating small animal patients with suspected hepatobiliary disease. Ultrasonography allows assessment of the hepatic parenchyma and biliary tract. Evidence of extrahepatic disease causing a secondary hepatopathy may also be detected. Additionally, ultrasound guidance is often used when collecting samples for cytologic and histologic evaluation of the liver.

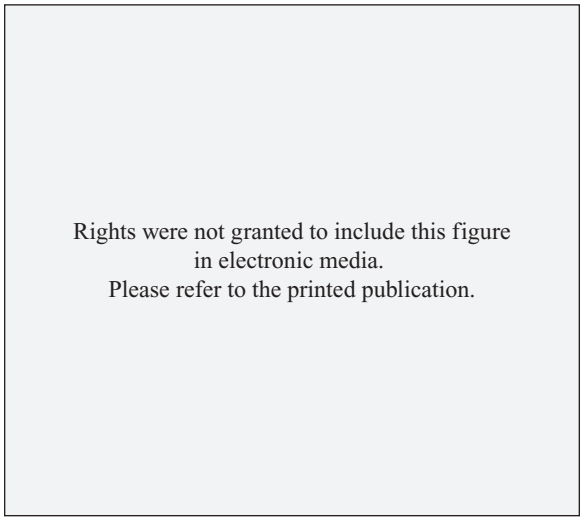

Figure 61-7 Lateral transvenous retrograde portogram of a dog with a single extrahepatic shunt. A balloon-tipped catheter positioned immediately cranial to the diaphragm was used for contrast injection. There is retrograde filling of some hepatic veins (*arrowheads*), there is also a large PSS that filled in a retrograde fashion (*arrows*). (From Miller MW, Fossum TW, Bahr AM: Transvenous retrograde portography for identification and characterization of portosystemic shunts in dogs. *J Am Vet Med Assoc* 221:1586, 2002.)

The size of the liver can be subjectively assessed by abdominal ultrasonography. The findings of a small liver and cranial displacement of the stomach suggest microhepatia. Hepatomegaly is another subjective finding and can be generalized or focal. The finding of rounded liver lobe margins suggests hepatomegaly.

Hepatic parenchymal changes can be classified as being diffuse, multifocal, or focal. A wide variety of disease processes can cause diffuse changes to the hepatic parenchyma. These changes can be isoechoic, hypoechoic, hyperechoic, or of mixed echogenicity. In some cases the architecture of the liver will not be altered, but in other cases changes will occur. Examples of diseases in which the echogenicity of the hepatic parenchyma is diffusely changed but no changes in architecture occur include cholangitis, neoplasia, hepatic lipidosis, other vacuolar hepatopathies, toxic hepatopathy, and early micronodular hyperplasia with various degrees of fibrosis.[100] Hyperechogenicity of the liver compared to the falciform fat, poor visualization of the intrahepatic blood vessels, and increased attenuation of the ultrasound beam have been used as criteria for the sonographic diagnosis of feline hepatic lipidosis.[101] Diseases where the hepatic architecture is altered are easier to detect sonographically. These include neoplasia, micronodular hyperplasia, and chronic hepatitis with fibrosis. Cystic structures, abscesses, hematomas, and granulomas are examples of focal parenchymal liver disease. These lesions are usually easily detected sonographically. Sonography seldom allows a definitive diagnosis of hepatic parenchymal disease to be made. In one study the overall accuracy of ultrasound for discrimination among different categories of diffuse liver disease was 36.5% for dogs and 54.6% for cats. Hepatic lipidosis in cats could be diagnosed slightly more accurately than other diffuse hepatic diseases.[102] Cytologic or histologic evaluation of a hepatic tissue sample is usually needed to make a definitive diagnosis.

Hepatic neoplasia, whether primary or metastatic, can be diffuse, multifocal, or focal in its distribution. Round cell tumors are the most likely tumor type to diffusely infiltrate the liver. These tumors can cause hypoechoic, hyperechoic, or mixed-echoic changes, or

may not affect the echogenicity of the liver at all. Neoplasia can also lead to the appearance of nodules within the hepatic parenchyma. Malignant liver nodules have a variable appearance and size and can be difficult to distinguish from nonmalignant conditions such as cysts, hematomas, benign hyperplastic nodules, granulomas, or abscesses. The finding of one or more target lesions in the liver or spleen had a positive predictive value of 74% for detecting malignancy, and thus should not be considered a specific finding.[103] Cytologic or histologic evaluation of a tissue sample is needed to differentiate between malignant and benign liver nodules. Tumors, such as hepatoma or hepatocellular carcinoma, can also focally infiltrate the liver.

Contrast-enhanced harmonic ultrasound allows assessment of tissue perfusion patterns. Gas-filled microbubbles are administered intravenously to the patient. The microbubbles are relatively echogenic. When they reach the tissue of interest, they produce a more potent harmonic signal than the surrounding tissue. This technique allows enhanced differentiation between tissues with varying perfusion patterns. In one study the sensitivity of contrast enhanced ultrasound for differentiation between benign and malignant liver nodules in dogs was reported to be 100% and the specificity was reported to be 94.1%.[104]

Sonography is also a valuable tool for the evaluation of the biliary system. Biliary disease can be classified as being obstructive or nonobstructive.

The term *cholangitis* refers to a group of nonobstructive biliary diseases, which are more common in cats than in dogs. Typical ultrasound findings in cats include a hypoechoic hepatic parenchyma and prominent portal vasculature.[105] Additional findings can include evidence of pancreatic inflammation, thickening of the gallbladder wall, and dilation of the intrahepatic and extrahepatic biliary system. It is important to note these changes are not always present. Cytologic or histologic confirmation and bacterial culture are needed to confirm this diagnosis. Generalized gallbladder wall thickening can occur as a result of cholecystitis, cholangitis, or hepatitis. However, the gallbladder wall can also appear to be thickened when peritoneal effusion or hypoproteinemia are present. Gall bladder wall masses can be identified sonographically as a focal thickening of the gallbladder wall. Sonography has also been used to assess gallbladder motility in dogs.[106] It should be noted that gravity dependent gallbladder sludge can be found in dogs without hepatobiliary disease, so this finding should be considered incidental.[107]

Abdominal ultrasound is the most commonly used imaging modality for the detection of biliary obstruction in dogs and cats. Findings consistent with biliary obstruction include common bile duct distention, intra- and extrahepatic bile duct distention, and/or gallbladder dilation. A retrospective study showed that common bile duct dilation greater than 4 mm was 97% sensitive for the detection of biliary obstruction in cats.[108] Sonography can also aid in identifying the cause of biliary obstruction. Biliary obstruction can be classified as being luminal or extraluminal. Extraluminal causes include nonneoplastic pancreatic disease, abdominal adhesions, and, rarely, pancreatic neoplasia. Luminal causes include gallbladder mucocele, biliary neoplasia, inflammation, and cholecystolithiasis. Biliary tract obstruction can progress to biliary rupture and bile peritonitis. Sonographic signs of biliary rupture include loss of gallbladder wall continuity, free peritoneal fluid, and signs of localized peritonitis. Gallbladder mucoceles occur in the dog, but have not been described in cats. Mucoceles have a variable sonographic appearance; typical findings include a stellate or finely striated bile pattern with a hypoechoic rim, which is not gravity dependent, and

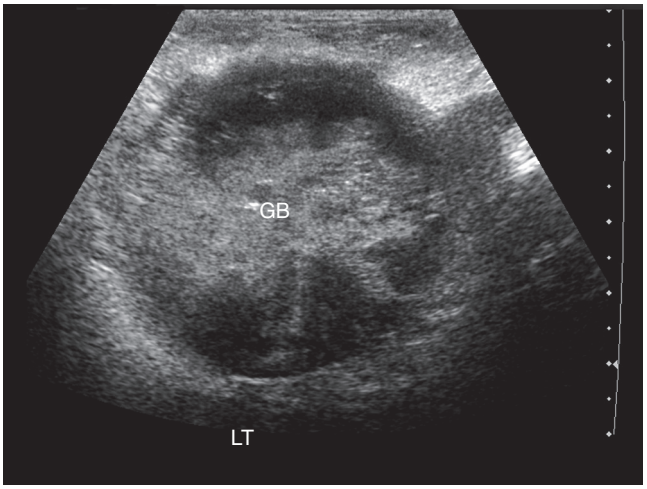

Figure 61-8 Abdominal ultrasound image of a dog with a gallbladder mucocele. There is organized hyperechoic bile within the distended gallbladder (*GB*). The periphery of the bile is stellate in appearance and the gallbladder wall is thickened.

distention of the gallbladder (Figure 61-8). Gallbladder mucoceles can also lead to biliary obstruction, which might also be appreciated sonographically. The sensitivity of ultrasound for the detection of a gallbladder wall rupture in dogs with gallbladder mucoceles is reported to be 85.7%.[109]

Abdominal ultrasound can be used to assess the liver for vascular disease. Congenital PSS is classified as being intrahepatic or extrahepatic. Although angiographic techniques are considered to be the gold standard for the detection and characterization of PSS, abdominal ultrasound is being used increasingly for this purpose. Sonographic assessment of the portal vasculature is time-consuming and highly operator dependent. Because of this there should be a high index of suspicion for PSS before performing these studies. Secondary findings consistent with PSS include mild bilateral renomegaly, urolithiasis (because of urate crystalluria), and microhepatia. Ascites and hepatic parenchymal changes are not consistent with congenital PSS. Extrahepatic shunts typically occur in small-breed dogs and arise from the splenic vein or the right gastric vein while intrahepatic shunts typically occur in larger breeds of dogs and arise from the right or left portal branch. An intrahepatic PSS is usually easier to detect sonographically than an extrahepatic PSS in dogs. Cats typically have single extrahepatic shunts with a wider degree of anatomical variation than in the dog. Ultrasonography has been reported to have a sensitivity of 92% and a specificity of 98% for detecting PSS in dogs.[110] Portal hypertension can develop as a result of chronic hepatitis with fibrosis, hepatic arterioportal fistulas, portal vein thrombosis, primary portal vein hypoplasia, extraluminal compression of the portal vein, or after ligation of a congenital PSS. The finding of hepatofugal or reduced velocity hepatopetal blood flow using Doppler ultrasound is consistent with portal hypertension.[111] However, not all patients with portal hypertension will have these changes. Ascites frequently, but not always, develops secondary to portal hypertension and this can be readily detected on abdominal ultrasound examination. Acquired portosystemic collaterals (also known as acquired PSS) may develop when sustained prehepatic or hepatic portal hypertension is present.[112] Sonography may allow detection of portal hypertension and APSCAPSC although APSC vessels are more difficult to identify than congenital PSS. Posthepatic portal hypertension does not result in the development of

APSC vessels, but can lead to a distention of hepatic veins and ascites.

Nuclear Scintigraphy

Nuclear scintigraphy involves administering a radioactive tracer substance (radiopharmaceutical) to the patient, which localizes to a specific organ or tissue. The radioactive decay of this substance is detected by a gamma camera and used to form images. Scintigraphy has been used to detect PSS and to assess gallbladder emptying in small animals. However, specialized equipment and a license for the use of radioisotopes are required. Consequently, availability of this imaging modality is currently limited to academic institutions and specialty referral hospitals.

Technetium-99m pertechnetate is the most commonly used radiopharmaceutical for assessing the portal circulation of small animal patients. Two techniques have been described: per-rectal portal scintigraphy and transsplenic portal scintigraphy. By analyzing the radiation emitted from regions of interest drawn over the patient's liver and heart, PSS can be detected and a shunt fraction can be calculated. This allows for the minimally invasive diagnosis of PSS, differentiation of PSS from portal vein hypoplasia without portal hypotension (previously known as microvascular dysplasia), and comparison of the degree of shunting before and after shunt attenuation. Transsplenic portal scintigraphy is preferred over per-rectal portal scintigraphy as it is simpler to perform, uses lower doses of the radiopharmaceutical, and is more sensitive (Figure 61-9). Transsplenic portal scintigraphy is 100% sensitive and specific for the diagnosis of congenital PSS, and significantly more likely than per-rectal portal scintigraphy to detect shunt number and termination in dogs.[113]

Nuclear scintigraphy has been used to quantify liver function and to assess biliary tract patency in dogs. In a retrospective study hepatobiliary scintigraphy was found to be 83% sensitive and 94% specific for the detection of extrahepatic biliary obstruction in dogs and cats.[114]

Computed Tomography and Magnetic Resonance Imaging

Computed tomography (CT) (Figure 61-10) and magnetic resonance imaging (MRI) have been used to detect hepatic parenchymal neoplasia in humans. Compared to abdominal ultrasound these techniques have an improved accuracy for the diagnosis of hepatic neoplasia in humans. However, there is limited data in the veterinary literature evaluating their diagnostic performance. In one study the diagnostic accuracy of CT for detecting hepatic masses was not found to be significantly different from that of abdominal ultrasound in dogs.[115] In another study MRI was found to have a sensitivity of 100% and a specificity of 86% for the differentiation between benign and malignant liver lesions in dogs.[116]

CT angiography is being used increasingly in dogs for the diagnosis of congenital PSS and other hepatic vascular diseases. It offers the advantage of being less invasive than operative angiography, allows for improved assessment of the portal vasculature, and allows the creation of a three-dimensional reconstruction. The vasculature detail afforded by CT angiography is particularly useful when planning attenuation of a congenital PSS. The diagnostic utility of CT angiography for detecting and characterizing PSS in dogs was shown to compare favorably to that of other techniques, including surgical exploration.[117] Transsplenic CT portography has been described in dogs without PSS. This technique offers more intense enhancement of the splenic and portal veins than CT angiography.[118] MRI angiography diagnoses PSS in dogs with a sensitivity of 80% and a specificity of 100%.[119] The disadvantages of CT and MRI include their limited availability, cost, and the need for anesthesia.

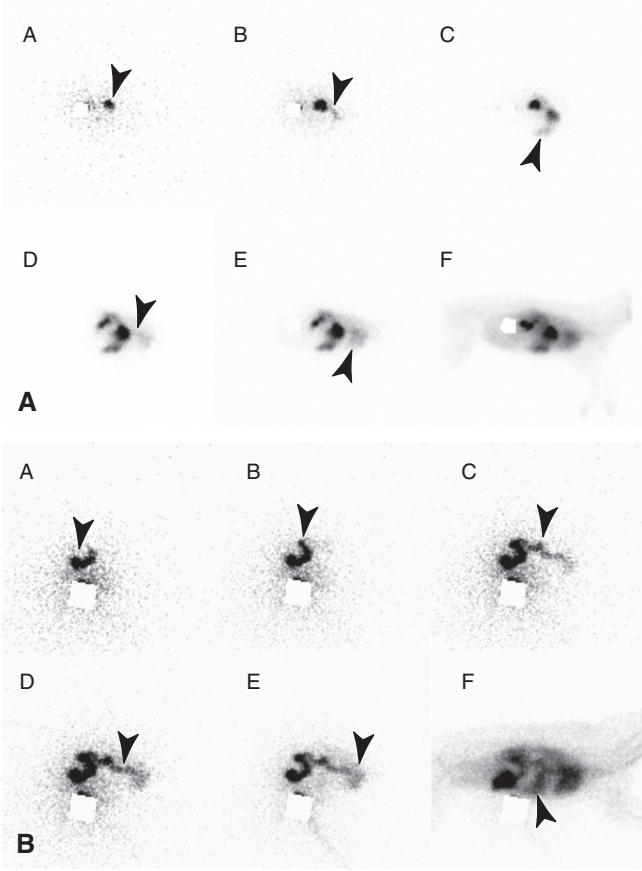

Figure 61-9 **A,** Transsplenic portal scintigraphy of a dog with normal portal vasculature. Images were acquired following ultrasound-guided intrasplenic injection of 2 mCi ^{99m}technetium pertechnetate. The radionuclide exits the spleen via blood flow in the splenic vein then enters the portal circulation. Blood first reaches the liver before entering the caudal vena cava and heart. Frames a to e document progression of radionuclide from the spleen to the heart: (a) splenic injection site; (b) portal vein; (c) liver; (d) caudal vena cava; (e) heart. Frame f is a summed image providing anatomic landmarks for reference. **B,** Transsplenic portal scintigraphy of a dog with a portocaval shunt. Images were acquired following ultrasound-guided intrasplenic injection of 2 mCi ^{99m}technetium pertechnetate. The radionuclide exits the spleen via blood flow in the splenic vein then enters the portal circulation. Blood bypasses the liver and enters the caudal vena cava and heart. Frames a to e document progression of the radionuclide from the spleen to the heart: (a) splenic injection site; (b) portal vein; (c) shunting vessel; (d) caudal vena cava; (e) heart. Frame f is a summed image providing anatomic landmarks for reference. (Courtesy of Dr. Benjamin D. Young, Texas A&M University, College Station, TX.)

Cytologic Evaluation of the Liver

Although cytologic evaluation of the liver provides a definitive diagnosis, often histologic examination is also required. There are a variety of techniques to collect cytologic samples of the hepatobiliary system. Abdominal effusion, when present, can be collected percutaneously. Fine-needle aspirates (FNA) of the liver can be collected percutaneously under ultrasound guidance. Cholecystocentesis can also be performed percutaneously with ultrasound

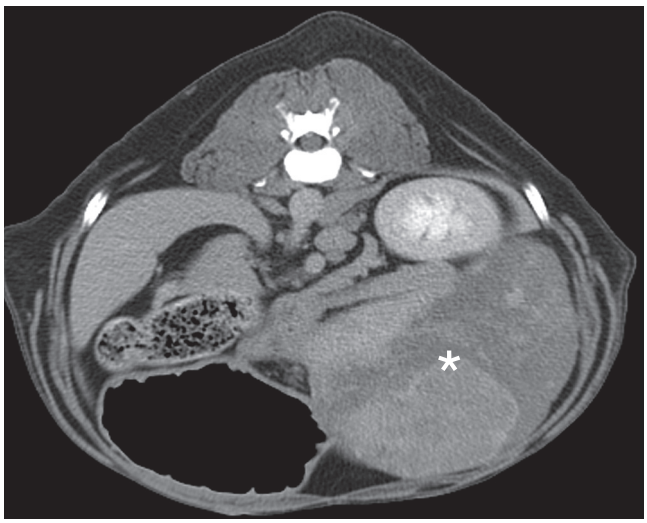

Figure 61-10 Abdominal computed tomography image of a dog with a massive hepatocellular carcinoma. There is a large irregularly shaped mass associated with the ventrolateral right side of the liver (*).

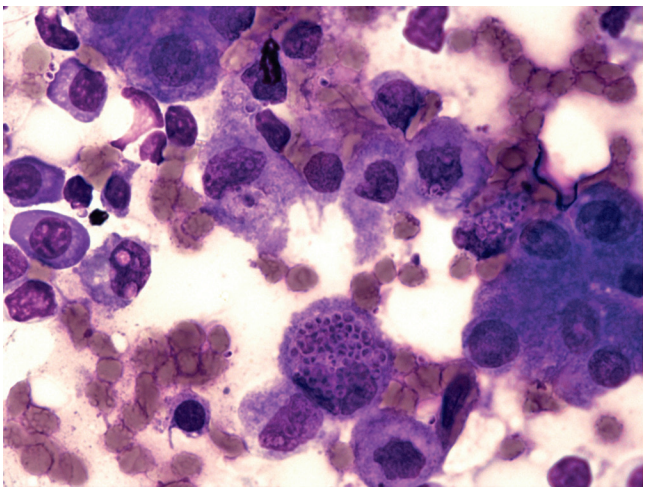

Figure 61-11 **Fine-needle aspirate from the liver of a cat with disseminated histoplasmosis.** Erythrocytes, hepatocytes, lymphocytes, plasma cells, and macrophages containing large numbers of *Histoplasma capsulatum* organisms can be seen (*). Diff-Quik 100× objective. (Courtesy of Dr. Kathrin F. Burke, Texas A&M University, College Station, TX.)

guidance. These techniques are minimally invasive and the risk of complications is relatively low but caution should be exercised in patients with bleeding disorders.

Liver disease can cause abdominal effusion by several mechanisms. In cases with hepatic insufficiency, severe hypoalbuminemia (<1.5 g/dL) can occur. This can lead to the formation of a pure transudate as the result of a reduced plasma colloid oncotic pressure. Increased capillary hydrostatic pressure because of portal hypertension may lead to formation of a pure or modified transudate. Hepatic neoplasia can also lead to a formation of a modified transudate. Biliary tract rupture can lead to bile peritonitis and abdominal effusion. An exudate with a bilirubin concentration greater than twice that of the plasma is suggestive of bile peritonitis.[120]

Cytologic evaluation of hepatic FNA can aid in making a diagnosis of liver disease. Suppurative, mixed inflammatory, lymphocytic and, more rarely, eosinophilic patterns of inflammation can be appreciated cytologically. Each pattern of inflammation suggests a group of possible diagnoses. The finding of dark green or black bile casts suggests cholestasis. Infectious diseases such as histoplasmosis can be definitively diagnosed based on the cytologic finding of the infectious agent (Figure 61-11). Hepatocellular vacuolation can be classified as being caused by lipid or not. Lipid vacuolation of hepatocytes is characterized by colorless cytoplasmic vacuoles. Severe lipid vacuolation is suggestive of hepatic lipidosis in cats (Figure 61-12). However, feline hepatic lipidosis often occurs secondary to another disease process. A group of cats with cytologic findings suggestive of hepatic lipidosis were reported to have underlying infiltrative liver disease.[121] Nonlipid vacuolation is characterized by generalized hepatocyte swelling and lacy vacuolation (Figure 61-13). Vacuolar hepatopathy occurs secondary to a wide variety of extrahepatic disease processes in dogs.[122] Metastatic tumors and round cell tumors, such as lymphoma (Figure 61-14), affecting the liver can often be diagnosed cytologically. Additionally, cytologic evaluation can aid in distinguishing liver nodules because of extramedullary hematopoiesis from those caused by neoplasia. However, it is not possible to distinguish hepatic nodular hyperplasia from hepatic adenoma or well-differentiated carcinoma cytologically. Some cases of hepatocellular carcinoma can be diagnosed cytologically if criteria for malignancy are present.

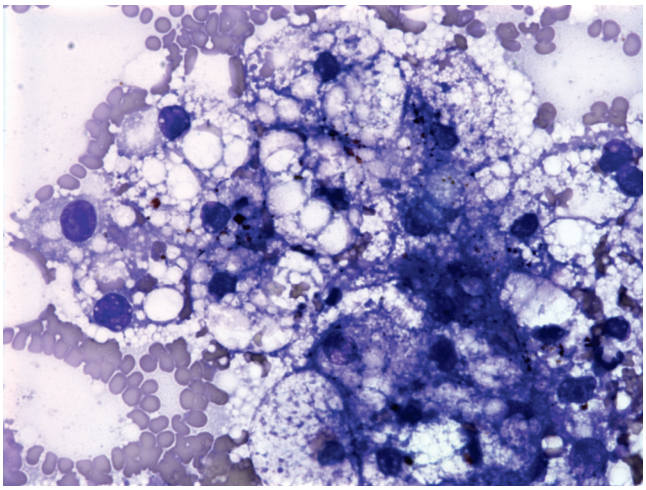

Figure 61-12 **Fine-needle aspirate from the liver of a cat with hepatic lipidosis.** The cytoplasm of the hepatocytes is severely distended by many variably sized clear vacuoles (both microvesicular and macrovesicular type) consistent with lipid that has been cleared during the staining procedure. Diff-Quik 100× objective. (Courtesy of Dr. Mark C. Johnson, Texas A&M University, College Station, TX.)

The findings above may aid in making a diagnosis of liver disease but are not a substitute for histopathologic analysis, as cytologic specimens do not allow assessment of the hepatic architecture. Furthermore, only a tiny proportion of the liver is sampled when cytologic samples are examined. These limitations are reflected by the results of a retrospective study that found the overall agreement between the histopathologic and cytologic diagnosis of liver disease to be 30.3% for dogs and 51.2% for cats.[123]

Cytologic evaluation of bile can also be useful for the diagnosis of biliary disorders, particularly in cats. World Small Animal Veterinary Association (WSAVA) Standards for the Clinical and Histological Diagnosis of Canine and Feline Liver Disease suggest that the cytologic evaluation of bile forms part of the minimum diagnostic requirement for cats with extrahepatic cholestasis and for dogs

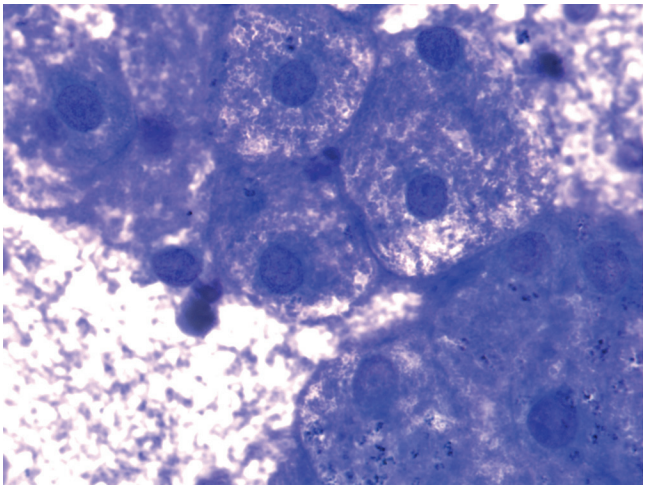

Figure 61-13 Fine-needle aspirate from the liver of a dog with glycogen deposition hepatopathy. Hepatocytes are distended and the cytoplasm is less dense than normal (cytoplasmic rarefaction) consistent with increased glycogen storage. Diff-Quik 100× objective. (Courtesy of Dr. Mark C. Johnson, Texas A&M University, College Station, TX.)

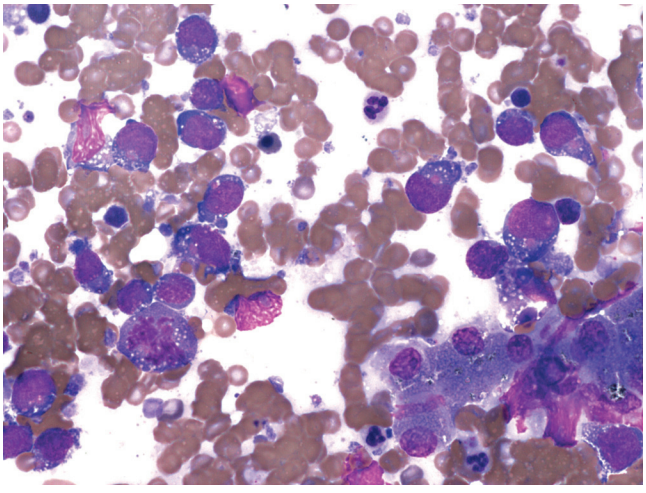

Figure 61-14 Fine-needle aspirate of the liver from a dog with T-Cell lymphoma (confirmed by immunohistochemistry. The predominant nucleated cell population consists of large atypical lymphoid cells. Hepatocytes (*lower right*) and occasional neutrophils are also present. Diff-Quik 60× objective. (Courtesy of Dr. Kathrin F. Burke, Texas A&M University, College Station, TX.)

and cats suspected to have cholangitis.[124] The finding of neutrophils and bacteria on bile cytology supports a diagnosis of feline neutrophilic cholangitis. Cytology is essential for the diagnosis of this disease, as cats with neutrophilic cholangitis may not have typical hepatic histopathologic changes and it can be difficult to distinguish these cats from those with lymphocytic cholangitis. Bile should also be submitted for aerobic and anaerobic bacteriologic culture.

Histopathologic Evaluation of the Liver

Histopathologic evaluation is required to make a definitive diagnosis of most liver diseases. Histopathologic evaluation of the liver allows a morphologic and sometimes an etiologic diagnosis to be made (see Chapter 29). In addition to routine staining with hematoxylin and eosin, a variety of other staining techniques can be employed to demonstrate hepatic pathology. To optimize the value of histopathologic evaluation of the liver, particular attention should be paid to specimen collection, specimen handling, and communication between the clinician and the pathologist.

Although liver biopsy is considered to be relatively safe, the patient should be assessed for bleeding disorders before this procedure. This assessment should include a platelet count, coagulation times, and a buccal mucosal bleeding time. Liver biopsies can be collected in a number of ways. Each method has advantages and disadvantages, and there is controversy in the veterinary literature as to which technique is optimal. Laparotomy allows collection of relatively large wedge biopsies, with direct visualization. This technique does not require specialized equipment or training, and excessive bleeding can be readily identified. However, laparotomy requires general anesthesia and is the most invasive biopsy technique. Percutaneous needle biopsy techniques have been described. These techniques may be possible under heavy sedation and are the least-invasive method for collecting liver biopsies. Ultrasound guidance is often used, allowing biopsy of focal lesions. It is also possible to biopsy tissue that is deeper within the hepatic parenchyma than is possible with other techniques. However, the specimens that are collected are relatively small and may be inadequate for accurate assessment in some patients. A prospective study showed that there was agreement between the histomorphologic diagnoses made upon examination of needle biopsies and those made on wedge biopsies collected during laparotomy or necropsy for only 48% of dogs and cats.[125] Excessive hemorrhage after biopsy may not be identified immediately. Laparoscopy allows collection of biopsies using forceps with laparoscopic guidance. This technique requires general anesthesia, but is less invasive than laparotomy. The biopsies collected are larger than needle biopsies and excessive bleeding can be visualized. However, laparoscopy requires specialized equipment and training. The use of biopsy forceps may result in crushing artifact and the tissue collected may be too superficial to identify lesions that lie deeper within the hepatic parenchyma.[126] Regardless of the technique used, a tiny proportion of the organ is sampled and, because liver disease can affect the hepatic parenchyma in a heterogeneous manner, sampling error is possible. To reduce the effect of sampling error, several biopsies from different areas of the liver should be collected and focal lesions should be specifically biopsied.

The clinician should provide the pathologist with all the pertinent information from the patient's history, physical examination findings, the results of laboratory testing, and the findings from diagnostic imaging. In turn the histomorphologic diagnosis that the pathologist makes should be interpreted by the clinician along with the other clinical data. When the histopathologic diagnosis does not fit the clinical picture, the pathologist should be consulted and when necessary a second opinion should be requested. Variation in the assessment of hepatic pathology between pathologists was highlighted by a study that found agreement between examiners for only 44% of needle biopsies and 65% of wedge biopsies examined.[125] Hopefully, the adoption of WSAVA Standards for the Clinical and Histological Diagnosis of Canine and Feline Liver Diseases since the aforementioned study will reduce this interobserver variation.

Quantification of hepatic metal concentrations requires submission of tissue for flame atomic absorption spectroscopy. Although zinc has a role as an antioxidant, hepatic copper and iron retention can lead to oxidative liver injury. Copper is the most frequently

measured of these metals and quantification is essential for the diagnosis of hepatic copper retention. These measurements are usually performed on freeze-dried pieces of liver. Specimens for metal measurement should not be stored in saline and should be kept in metal-free containers. Recently, it was shown that measurements of the concentration of copper and iron, but not zinc, can be ascertained from deparaffinized-archived liver tissue.[127]

BIOPSY TECHNIQUES

Keith Richter

Hepatobiliary diseases can be challenging to diagnose. Although diagnostic tests that employ biochemical, molecular biologic, serologic, functional, as well as imaging techniques are capable of establishing the etiology of some chronic or acute liver diseases, in most instances the gold standard for definitive diagnosis and the assessment of stage and severity of liver diseases is the histologic evaluation of a liver sample. Recent advances in imaging technology, the use of multiple imaging modalities, and newer biopsy methods have resulted in improvement in the ability to safely procure hepatic tissue for evaluation. There are several means of obtaining hepatic samples including fine-needle aspiration, ultrasound-guided biopsy, laparoscopy, and laparotomy. All techniques have both advantages and disadvantages, which should be carefully considered before choosing the appropriate sampling method.

Indications

Many biochemical tests are available to evaluate the anabolic and/or catabolic function of the liver and the hepatic circulation. These include measurement of concentrations of bile acids, ammonia, bilirubin, and the ability to excrete organic dyes. Other tests of hepatic function include measurement of serum albumin, glucose, urea nitrogen, and clotting factor analysis. Hepatic function can be markedly abnormal despite maintenance of the hepatocellular membrane and therefore normal serum activities of hepatic enzymes. Examples include PSSs, terminal cirrhosis, and metastatic hepatic neoplasia. Likewise, the liver can continue normal anabolic or catabolic function despite severe hepatocyte leakage of intracellular enzymes because of its marked reserve capacity. This can occur, for example, in certain cases of hepatocellular necrosis, blunt abdominal trauma, or primary hepatic neoplasia. Thus, the limitations of serum hepatic enzyme activities must be taken into consideration. Hepatocellular leakage enzyme activities include ALT and AST. Enzyme activities that increase with biliary tract obstruction include serum ALP and GGT.

No laboratory test identifies a specific problem, helps determine specific therapeutic management, or predicts an outcome. This is because different diseases produce similar alterations in hepatic function or in laboratory tests. Once biochemical tests identify the presence of hepatic disease, the diagnosis must be pursued further. In some instances, diagnostic imaging can reveal specific abnormalities (e.g., PSSs and extrahepatic bile duct obstruction). When results of imaging do not give a specific etiology, the next step is often to pursue a morphologic diagnosis obtained by analysis of a biopsy specimen. Often it is a judgment call as to when to pursue hepatic biopsy. In cases with severe clinical signs and/or severe biochemical abnormalities, biopsy is usually warranted early in the course of the evaluation. In patients that are asymptomatic and have abnormal biochemical testing, repeat evaluation is sometimes warranted. A general guideline is to obtain a liver biopsy in asymptomatic patients if there are moderate to severe elevations in serum hepatic enzyme activities that persist for at least 3 months, or if there are mild to moderate elevations in serum hepatic enzyme activities that persist for at least 6 months. If clinical signs of hepatic disease develop, then biopsy should not be unreasonably delayed. If there are concurrent elevations in serum bile acids, biopsy should also not be delayed.

Other indications for hepatic biopsy are ultrasound imaging abnormalities. If there are focal hepatic masses or diffuse echotextural changes, a biopsy may be warranted, depending on results of laboratory testing. In one study, abdominal ultrasound findings alone were not reliable for obtaining a diagnosis of infiltrative hepatic disease with diffuse changes in echogenicity (either hypoechoic or hyperechoic, uniform or mottled).[1] In another study, sonographic detection of a hepatic mass greater than or equal to 3 cm, ascites, abnormal hepatic lymph node(s), and abnormal spleen were predictive of liver neoplasia based on cytology.[2] Conversely, sonographic detection of hepatic nodules less than 3 cm was predictive of vacuolar hepatopathy on cytology. Thus several sonographic findings, alone or combined, may be predictive of liver ultrasound-guided fine-needle aspiration cytology results. In light of the fact that ultrasound-guided fine-needle aspiration cytology of the liver has limitations , the results of ultrasound and cytology should be adjuncts to other findings.

Another indication for hepatic biopsy is the need to assess response to therapy. In cases of chronic hepatitis in dogs, it is often difficult to determine if there is ongoing inflammation and resolution/progression of fibrosis during long-term therapy. This is particularly true when the patient is receiving glucocorticoid therapy as these medications cause variable increases in serum ALP and transaminase activities independent of the underlying disease. Repeat or serial hepatic biopsy analysis is often helpful to guide therapeutic decisions in these cases.

Prebiopsy Considerations

Among the most serious complications of liver biopsies are hemorrhage, infections, and injury to the adjacent viscera. Consequently the clinician must take into account the clinical question, the appropriate invasive biopsy method, and methods of managing postbiopsy complications. Postbiopsy hemorrhage is often the first concern, although it is unclear as to what the best predictor of hemorrhage is in patients about to undergo hepatic sampling. In one study of 200 human patients in which bleeding was evaluated laparoscopically, there was no correlation between any in vitro coagulation test and "liver bleeding time."[3] Other studies in man have used laparoscopy and ultrasonography to assess hepatic bleeding time following needle biopsy, and most have shown similar poor correlation between coagulopathies and hepatic bleeding times. Similar studies have not been reported in veterinary medicine. There also have been studies in human and veterinary medicine evaluating risk factors for bleeding complications (as opposed to "hepatic bleeding times").[4,5] Bigge et al. correlated coagulation profile findings and bleeding complications after ultrasound-guided biopsies in 310 dogs and 124 cats.[5] There was no apparent correlation between coagulation parameters and major complications following liver biopsy. Studies show that clotting times assessing proteins induced by vitamin K antagonism are more sensitive in detecting coagulopathies in patients with hepatic disease.[6,7] The

proteins-induced-by-vitamin-K-antagonism test is more than twice as sensitive in dogs and more than three times as sensitive in cats in detecting coagulopathies compared with prothrombin time (PT) and aPTT.[6,7] However, in a pilot study performed by me, hepatic bleeding times assessed via laparoscopy did not correlate with proteins induced by vitamin K antagonism times. Thus it appears that indices of coagulation in the peripheral blood are generally unreliable guides of the risk of bleeding after liver biopsy, and hence, are of limited value in determining contraindications to this procedure. This lack of correlation may be explained by the high concentration of clotting factors in the hepatic parenchyma and by mechanical compression of the needle tract by the elastic tissue within the liver. In most cases of significant hemorrhage, technical errors such as damaging a large vessel are the cause rather than persistent oozing from a needle biopsy site. Controlled studies in veterinary patients will be necessary to make final conclusions regarding postbiopsy hemorrhage in the patient with a coagulopathy.

In one study of normal dogs, biopsies taken from the left lateral hepatic lobe using a biopsy punch, biopsy needle, ligature method, laparoscopic biopsy forceps, and ultrasonically activated scalpel resulted in minimal hemorrhage (<2 mL).[8] However, this investigation did not assess the risk of hemorrhage in dogs or cats with hepatic disease. These risks will be discussed later under each sampling method. With the exception of fine-needle aspirations, each patient should have a prebiopsy packed cell volume and 3 and 6 hours postbiopsy packed cell volume for close monitoring of potential hemorrhage.

Fine-Needle Aspiration

Fine-needle aspiration involves obtaining a small amount of hepatic tissue for cytologic analysis, and is typically performed in conjunction with, and guided by, ultrasound. Ultrasound imaging helps determine if there is a diffuse abnormality (e.g., increased or decreased echogenicity, diffuse mottling) or if there are focal abnormalities (e.g., discrete nodules, cysts, masses, or focal areas of heterogenous mottling). An appropriate site to be sampled is chosen. Often multiple sites are chosen to represent different lobes, and in the case of focal lesions, to sample more than one area of abnormal tissue and sample seemingly normal tissue. The sites are also chosen based on accessibility. For example, a solitary nodule in the dorsocranial aspect of the liver in a large deep-chested dog would be impossible to reach with a 1.5-inch needle. A lesion adjacent to the gallbladder or caudal vena cava would involve considerable risk. The clinician would need to decide whether the relative risk of sampling such lesions is the appropriate decision, or whether other methods of sampling would be more appropriate such as laparoscopy or laparotomy. Figure 61-15 depicts a typical setup for fine-needle aspiration. Usually a 22-gauge, 1.5-inch needle is used. For most patients, the procedure is performed without sedation or local anesthetic. If it is determined that the animal is moving too much during the initial ultrasound examination, a sedative may be necessary (or an anesthetic in extreme cases). The needle is inserted without a syringe using ultrasound guidance. The needle is rapidly agitated in and out (sometimes referred to as mimicking the action of a sewing machine) and simultaneously twisted multiple times for a few seconds to obtain a sample. This method relies on capillary action rather than suction to get tissue into the needle, resulting in less hemodilution. After removing the needle from the liver, a syringe is attached and cells are expelled onto a glass slide for cytologic examination. Often three to five separate attempts are made to increase the sample size and diversity.

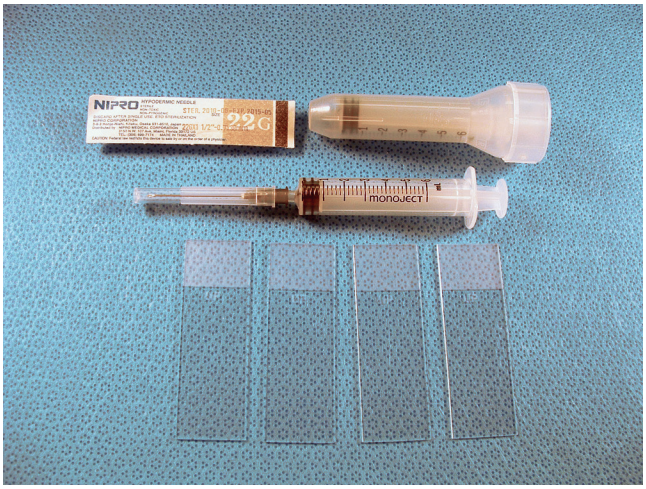

Figure 61-15 Fine-needle aspirate setup including 6-mL syringes, 1.5-gauge 22-inch needles, and glass slides.

Advantages and Disadvantages of Fine-Needle Aspiration

Fine-needle aspiration has several advantages. Little to no sedation is usually required. Because the size of the needle is so small, there is little risk of hemorrhage. Therefore multiple sites can easily be sampled. The procedure is rapid and can usually be performed on an outpatient basis. There is also less cost to the client.

The primary disadvantage of fine-needle aspiration is its questionable accuracy. The sample size often limits the number of available cells to obtain an accurate diagnosis, and hemodilution makes it difficult to assess whether inflammatory cells were present in the liver or peripheral blood. There are several important elements used to interpret pathologic information including lobular architecture, presence and location of inflammation within a lobule, presence and severity of fibrosis, metal accumulation, vascular abnormalities, and lobule heterogeneity. These criteria cannot be accurately determined using a cytologic sample obtained using FNA. Several studies have compared fine-needle aspiration cytology with biopsy with histopathology.[9-12] In one study with a total of 34 cases, there was good correlation in 35% of cases, partial correlation in 35% of cases, and no correlation in 30% of cases.[9] Poor correlation was found with a variety of histologic changes, including vacuolar change, lipidosis, cholestasis, inflammation, and neoplasia. In a similar study with 97 cases, complete agreement between fine-needle aspiration and histopathology was seen in only 30% of cases in dogs: 25% agreement with inflammation, 14% agreement with neoplasia (mainly carcinoma), and 64% agreement with vacuolar hepatopathy.[10] In cats, there was overall agreement in 51% of cases: 27% agreement with inflammation, 33% agreement with neoplasia (lymphoma), and 64% agreement with vacuolar hepatopathy. Although vacuolar hepatopathy was the most sensitive diagnosis, it was also the most common misdiagnosis using cytology. In another study, the best correlation between hepatic cytology and biopsy was seen with lipidosis, lymphoma, and carcinoma, whereas the worst performance was seen with inflammatory and fibrotic disorders.[11] Another study found high sensitivity and specificity with fine-needle aspiration in detecting inflammatory hepatic disease in dogs.[12] However, further information was not provided such as the severity of the inflammation or other histopathologic features. Additionally, for noninflammatory hepatic disease, cytology was inaccurate in 76% of cases.

Summary of Fine-Needle Aspiration

Although fine-needle aspiration is easy to perform, involves little risk, and little to no sedation, the information is of little value if it is inaccurate as often as it is accurate. There is institutional bias regarding its accuracy, which may relate to the experience and expertise of the cytologists. Given its clear limitations, fine-needle aspiration is best used as an adjunctive diagnostic modality in conjunction with other techniques or clinical findings, and does not replace histopathology. The clinician must be aware of its inherent inaccuracy before undertaking fine-needle aspiration and relying on the cytologic findings.

Ultrasound-Guided Biopsy

Ultrasound-guided hepatic biopsy uses a cutting-type needle as a sampling tool. Automated needles are preferred and should be either completely automated or semiautomated. These are spring-loaded needles similar in style to the manual Tru-Cut needle. Completely automated needles thrust the inner obturator (containing the biopsy tray or specimen notch) followed by the outer cutting sheath into the liver in a fraction of a second. These needles can be operated with one hand while the other hand operates an ultrasound transducer to allow precise placement of the biopsy instrument. There is minimal displacement of the liver, a shorter intraparenchymal phase, and a more reliable yield of tissue. This allows a smaller diameter needle to be used and a lighter degree of sedation in some cases. Using the rapid cutting action, the hepatic tissue tends to be less fragmented. Semiautomated needles require manual placement of the internal obturator into the liver, followed by an automatic thrusting of the outer cutting sheath by a spring-loaded mechanism. These needles have the additional advantage of control over the final needle position, as the tip of the needle can be precisely localized before the outer cutting sheath is deployed. I generally use a 16-gauge needle for ultrasound-guided hepatic biopsy. Figure 61-16 depicts a typical setup for ultrasound-guided biopsy.

In most dogs, the liver can be biopsied using local anesthesia and minimal sedation. Most cats require general anesthesia to safely obtain tissue. It must be emphasized that the degree of sedation must be tailored to each individual patient. A careful ultrasound examination is performed prior to biopsy. This allows planning of the procedure based on echo pattern, lesion size, proximity to other organs, proximity to blood vessels, determination of cystic or solid tissue, and optimal approach of the needle path. Care must be taken prior to taking samples to ensure that vessels and other organs are not within the path of the needle. For diffuse lesions, the transducer is typically placed caudal and to the left of the xiphoid, and aimed at the left medial or lateral lobes. In patients with a small liver, it may be difficult to adequately visualize the needle without gastric gas interference. Placing these animals in a 45-degree right lateral oblique position can reduce this interference. If the animal is under general anesthesia, an assistant can compress a rebreathing bag to hold the animal in deep inspiration, which serves to move the diaphragm and liver caudally to improve visualization. The area is surgically prepared. The ultrasound transducer is covered with sterile wrap and sterile lubricant is used to enhance skin contact. A small stab incision is made in the skin at the desired needle insertion site. While one hand maneuvers the transducer, the other hand advances the needle into the liver under direct ultrasound visualization. The image should be optimized to maximize the chance of recognizing the needle within the liver. To allow distinction of the needle from other echogenic structures, the needle can be gently moved in and out with minimal movements (attempting to move the liver within the abdominal cavity rather than the needle within the liver). Occasionally the needle cannot be seen, and indirect evidence of organ penetration must be used such as movement of the liver or visualization of movement at the liver border. The needle is then directed so the trajectory will avoid other structures when it is fired. The needle is then fired, and immediately removed. For most cases, four to five samples are obtained, and are submitted for aerobic/anaerobic culture, histopathologic evaluation, and metal (copper, zinc, and iron) quantification. In one study, liver tissues with high metal concentrations had significantly lower copper and iron in needle-core versus wedge biopsy specimens.[13] Consequently the value of needle-core biopsy specimens for measurement of metal concentrations is questionable. Careful examination for post-biopsy hemorrhage is then performed. External digital pressure may be used to help control hemorrhage in smaller patients. Usually an abdominal compression wrap is ineffective for controlling hemorrhage.

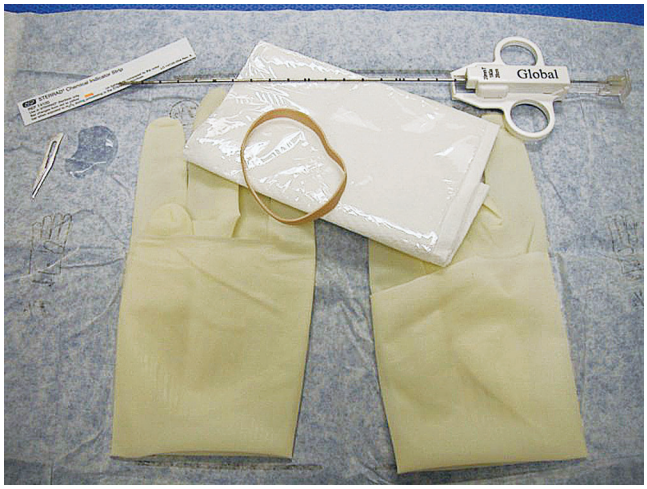

Figure 61-16 Ultrasound-guided biopsy setup including sterile gloves, sterile wrap and lubricant, number 11 surgical blade, and 16-gauge needle biopsy instrument.

Advantages and Disadvantages of Ultrasound-Guided Biopsy

Ultrasound-guided biopsy has many of the advantages of fine-needle aspiration, including the need for minimal sedation in some patients, the ability to sample multiple sites, and low to moderate cost to the client. Additionally, tissue is obtained for histopathology.

One disadvantage of ultrasound-guided biopsy is the risk of bleeding (especially when multiple sites are sampled and larger-gauge needles are used). In one study, 96 percutaneous transabdominal hepatic needle biopsy samples were obtained with no adverse consequences noted[14]; however, this study was performed in normal dogs, and still carries high risk. Additional disadvantages of ultrasound-guided biopsy include the needing sedation or anesthesia in some patients, difficulty of imaging small livers, difficulty of obtaining liver tissue in patients with fibrosis, and, most importantly, the obtaining of samples that have a questionable representation of the underlying hepatic pathology. The diagnostic accuracy of needle biopsy has been questioned by many clinicians, observing that results of needle biopsy analysis often do not adequately reflect the clinical and laboratory features of the patient. This questionable accuracy is in most part a result of potential for sampling error. This method still results in a relatively small sample size, possible

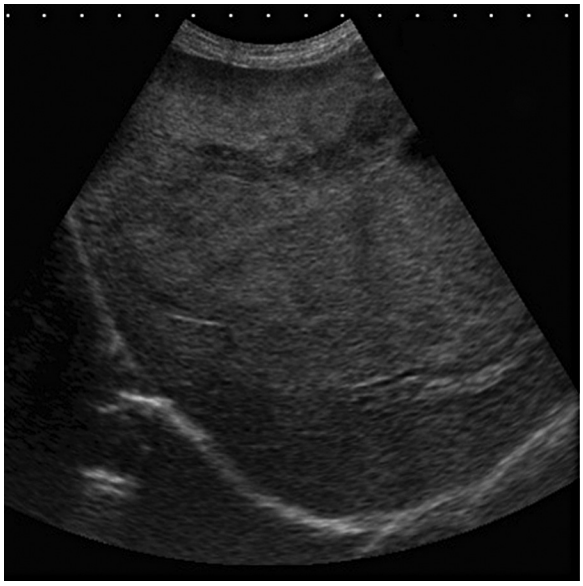

Figure 61-17 Ultrasound image of a focal hepatic mass (hepatocellular carcinoma) in a Pomeranian dog. This mass is amenable to ultrasound-guided biopsy.

fragmentation of fibrous tissue, and may not enable sampling of abnormalities located in other lobes (the left medial or lateral lobes are generally sampled because of their ease of imaging). In one study, percutaneous hepatic sampling using core biopsies resulted in 92% diagnostic quality samples, however these were not compared with large wedge biopsy to assess the accuracy of this method.[15] In another study, the diagnostic accuracy of the Tru-Cut–type needle biopsy was compared with the gold standard of surgical wedge biopsy of the liver in 124 patients.[16] The overall discordance between the two methods was 53% in dogs and 50% in cats, with a greater than 60% discrepancy occurring with chronic hepatitis or cirrhosis, cholangitis/cholangiohepatitis, portosystemic vascular anomalies, microvascular dysplasia, fibrosis, and miscellaneous disorders. These disorders are the most commonly seen among dogs and cats with hepatobiliary disease. The greatest accuracy was with neoplasia (80% concordance). Figure 61-17 is an example of a mass amenable to an ultrasound-guided needle biopsy. Use of a 14-gauge versus 18-gauge needle may reduce this discordance as it raises the number of portal triads sampled from an approximate mean of four to seven, though larger needles carry the risk of increased hemorrhage.

Summary of Ultrasound-Guided Biopsy

In summary, ultrasound-guided hepatic biopsy is relatively easy to perform, but involves more risk to the patient (primarily bleeding). Like fine-needle aspiration, ultrasound-guided biopsy has questionable accuracy. The accuracy may be increased by using a larger-gauge needle, but this carries a greater risk of postbiopsy hemorrhage. If the patient is suspected of having inflammatory disease, vascular abnormalities, or significant fibrosis, or is at risk for hemorrhage, laparoscopy or laparotomy should be considered.

Laparoscopic Biopsy

Chapter 28 provides a detailed description of laparoscopic liver biopsy. Briefly, laparoscopy is performed under general anesthesia with the patient in dorsal recumbency tilted 45 degrees to the left. A Veress needle is placed at the level of the umbilicus into the peritoneal space through a stab incision using a number 11 scalpel blade. After ensuring no obstruction and negative pressure using the infusion and aspiration of saline, the abdomen is insufflated with carbon dioxide gas and maintained at a pressure of approximately 12 mm Hg. A scope port (cannula) is then placed 4 cm right lateral to the Veress needle. The Veress needle is then removed and replaced with an instrument port. Hepatic sampling is achieved using a "spoon" or oval cup biopsy forceps. Multiple samples are obtained under direct visualization, and samples are submitted for aerobic/anaerobic culture, histopathologic evaluation, and metal (copper, zinc, and iron) quantification. Following procurement of all the biopsy specimens, the sites are inspected for hemorrhage. The abdomen is then decompressed, and lidocaine and bupivacaine are infused into the peritoneal cavity through either port. Both the instrument and scope ports are removed, and the port site incisions are closed using either a cruciate or simple interrupted pattern in the body wall, subcutaneous tissue, and skin.

Advantages and Disadvantages of Laparoscopy

This technique enables gross evaluation of the entire liver, extrahepatic biliary system, and surrounding structures while obtaining multiple large specimens of liver. The ability to obtain multiple samples decreases the risk for sampling artifact in cases of regional diversity within the liver. Additionally, by directly visualizing the hepatic parenchyma, the clinician can correlate the histopathologic findings and clinical data with the gross appearance of the liver to render the most accurate diagnosis. This method also enables the visualization of smaller masses and irregularities that may not be evident with ultrasonographic imaging. These masses can also be individually sampled. Laparoscopy also gives the clinician an excellent view of the liver regardless of the hepatic size or conformation of the patient, making it an easy method to sample the liver in patients that are difficult to image with ultrasound.

There is generally minimal bleeding during this procedure, even in patients with in vitro coagulopathies. Using a "spoon" or oval-cup biopsy forceps typically results in a marked decrease in the amount of hemorrhage when compared with needle biopsies. Any hemorrhage can be directly visualized for adequate clot formation. If hemorrhage persists, direct pressure using a blunt probe for 5 minutes can be used. If the site continues to bleed, electrocautery can be applied to the biopsy site or a topical hemostatic agent (Gelfoam) can be placed directly on the biopsy site using laparoscopic forceps.

Disadvantages of laparoscopy include the need for expensive equipment, the need for extensive training, the need for general anesthesia in most cases, and higher cost to the client.

Summary of Laparoscopy

Laparoscopy gives the clinician the advantages of a laparotomy (large sample size, ability to best direct sampling, and ability to take multiple samples, thus resulting in the highest diagnostic accuracy), though with a relatively minimally invasive procedure. The complication rate (especially hemorrhage) is far less than with ultrasound-guided biopsy in my practice. For these reasons, it is my method of choice for obtaining hepatic biopsy specimens in most cases.

Surgical Biopsy

Wedge biopsy via laparotomy is another potential method for obtaining hepatic biopsies. If a random liver biopsy is needed and a section of liver is protruding, a guillotine suture can be used. A preformed encircling ligature of 4-0 monofilament absorbable suture material is placed around the protruding section of liver.

The ligature is then tightened until it has crushed the hepatic parenchyma. After completing several throws in the knot, the sample is excised 1 to 2 mm distal to the ligature using Metzenbaum scissors or a scalpel blade. If a specific area of liver is needed, a sample can be obtained using the transfixation method or a biopsy punch. The transfixation method entails placing a ligature through the liver lobe approximately 8 to 10 mm from its edge. The ligature is tightened to crush through the hepatic parenchyma along one border of the desired biopsy specimen. An additional throw is made at a right angle to the first ligature, and this throw is tightened to crush the parenchyma of the second border of the specimen. The sample is removed 1 to 2 mm distal to the crushed area using a scalpel blade or Metzenbaum scissors. If the desired area does not lie near the edge of a liver lobe, a 6-mm biopsy punch can be used. The biopsy punch should be inserted into the hepatic parenchyma ensuring not to penetrate the opposite surface. If the biopsy site is close to the hilus, extra caution must be used so that no more than half of the thickness of the liver is penetrated. The biopsy sample is removed from the liver using scissors. Hemorrhage can be controlled by filling the defect with a topical hemostatic agent (Gel Foam) and applying digital pressure for 3 to 5 minutes, or by suturing the hepatic capsule with fine, absorbable monofilament suture in a cruciate pattern. Akin to laparoscopy, this method has similar advantages and disadvantages as listed previously. Although it is more invasive, it allows easier biopsy of other abdominal organs (such as intestine and mesenteric lymph node) and the ability to perform therapeutic maneuvers (such as hepatic mass removal or biliary diversion).

PARENCHYMAL DISORDERS

Susan E. Johnson

Inflammation and Necrosis

Acute Hepatitis and Acute Hepatic Necrosis

Etiology

Hepatocyte death (necrosis and apoptosis) in dogs and cats occurs secondary to a broad variety of insults, including infectious agents, drugs and toxins, hypoxia, immunologic events, and metabolic disorders. Hepatic necrosis and acute inflammation often occur together and the relationship between these two processes is complex. Acute inflammation may be the primary event, or necrosis of hepatocytes can be followed by a substantial inflammatory response, the "hallmark" of necrotic cell death.[1] The term *acute hepatitis* traditionally has been used when infectious agents cause hepatocellular necrosis, even though in the early stages, hepatic inflammation can be minimal or absent.[2] Controversy exists among veterinary pathologists regarding the preferred terminology (*acute hepatitis* versus *acute hepatic necrosis*), when necrosis predominates and is caused by noninfectious insults such as toxins or ischemia.[2] For the purposes of this discussion, lesions of acute hepatitis and acute hepatic necrosis are discussed together, recognizing that the primary contributions of each lesion may be variable, depending on the cause, host response, and passage of time. Acute hepatitis, a form of primary hepatitis, should be differentiated from "nonspecific reactive hepatitis," a response of the liver to a variety of extrahepatic disorders that is characterized by focal inflammation without necrosis.[2] Nonspecific reactive hepatitis is discussed in a later section of this chapter.

Acute hepatitis and necrosis are common morphologic hepatic lesions in dogs and cats presenting with acute liver disease caused by infectious, toxic, metabolic, and ischemic disorders (Box 61-1). However, acute liver disease can also be associated with other pathologic processes such as severe hepatic lipidosis (cats), granulomatous hepatitis (fungal infections), intrahepatic cholestasis (bacterial cholangitis, leptospirosis), and malignant infiltration (lymphoma, malignant histiocytosis). Canine adenovirus I, canine and feline herpesvirus in the neonate, *Clostridium piliforme*, and *Toxoplasma gondii* are specific examples of infectious agents that cause acute hepatic necrosis (with variable inflammation), often as part of a multisystemic disorder.[2] Although leptospirosis is a well-recognized infectious cause of acute liver disease in dogs, hepatic necrosis is an uncommon histologic feature and hepatic lesions are typically characterized by cholestasis, liver cell dissociation, and nonspecific reactive hepatitis.[3]

Despite the large number of potential causes of acute hepatitis and necrosis, a specific etiology is often not determined.[4,5] In a recent case series of 101 dogs with primary hepatitis (acute and chronic hepatitis) that were presented to a referral clinic, 21 dogs were diagnosed with morphologic features of acute hepatitis.[4] A cause could not be determined in the majority of these cases, although increased hepatic copper was detected in five dogs with acute hepatitis, suggesting that copper accumulation could be a significant contributing factor.[4]

Pathophysiology

Despite numerous potential causes of hepatocyte death, two general mechanisms are recognized: apoptosis and necrosis.[2] These two mechanisms have traditionally been considered to be distinct events. However, it now appears that apoptosis and necrosis are alternate outcomes of the same initiating causes and signaling pathways.[1] Apoptosis is adenosine triphosphate–dependent (caspase-dependent) programmed cell death that causes shrinkage of the cell (apoptotic bodies or acidophil bodies) with orderly resorption of cellular contents, minimal leakage of cellular components, and minimal secondary inflammation.[1,2] Necrosis occurs when depletion of adenosine triphosphate results in cellular swelling, loss of integrity of the cell membrane and cell lysis, with release of cell contents and secondary inflammation.[1,2]

Diffuse hepatic necrosis is the most consistent histological lesion detected in dogs and cats with acute liver failure.[5] Acute liver failure (ALF) is a rare clinical syndrome (usually fatal) that occurs when a sudden severe insult to the liver compromises at least 70% of functional hepatic mass. Liver cell death exceeds hepatic regenerative capacity, resulting in clinical signs of liver failure.[5] The clinical and laboratory features of ALF are not specific for the inciting cause but reflect disruption of one or more major hepatic functions.

Once hepatocellular injury has occurred (and assuming the patient survives), the morphologic hepatic response to injury may include parenchymal regeneration, fibrosis, and ductular proliferation.[2] Nearly complete hepatic regeneration is possible if hepatocyte injury is limited and the reticulin network remains intact.[2] With severe parenchymal destruction or extensive loss of hepatocytes, periportal ductular proliferation, hepatic fibrosis, postnecrotic scarring, and regenerative hepatic nodules are more likely.[2] Dogs with acute hepatitis may also progress to chronic hepatitis.[4]

Clinical Examination

The clinical presentation of dogs and cats with acute hepatitis and necrosis varies with the underlying cause and the extent and severity of the hepatic lesions. The spectrum of hepatic involvement may

include (a) subclinical (biochemical abnormalities only), (b) clinical signs of acute liver disease, or (c) the clinical syndrome of ALF. When liver injury is mild (focal necrosis and inflammation), clinical signs may be absent, mild, or related to an underlying cause in another organ system. In this setting, hepatic involvement may not be recognized until biochemical evaluation reveals increased liver enzyme activity or mild hyperbilirubinemia. It has been suggested that many dogs with acute hepatitis are not recognized clinically, because signs are mild and self-limiting, and dogs recover spontaneously regardless of treatment.[6] Clinical signs of acute hepatitis include acute onset of lethargy, anorexia, vomiting, diarrhea, PU, and PD, in a previously healthy animal. These are nonspecific findings of acute liver disease, which overlap those of other systemic disorders. The finding of icterus on the physical examination is a more specific indicator of hepatobiliary disease, especially in the absence of anemia.

Dogs and cats with acute diffuse hepatic necrosis often present with ALF. [5] In addition to the signs of acute liver disease described above, animals in ALF show signs of HE (depression, behavioral changes, dementia, ataxia, pacing, circling, blindness, hypersalivation, seizures, and coma) and clinical evidence of a bleeding tendency (melena, hematemesis, or cutaneous and mucosal hemorrhages), which suggest severe hepatic dysfunction.[5] Signs of ALF are rapidly progressive (over hours to days) and this clinical syndrome is often fatal, with reported mortality varying from 25% to 100%.[5]

With acute hepatic disease, the history typically reveals acute onset of signs in a previously healthy animal. However, liver failure that is recently recognized may not necessarily be recent in onset. With occult chronic liver disease, clinical signs may be vague and go unrecognized by the owner until a final phase of hepatic decompensation. The owner should be questioned about any subtle signs of chronic illness that would suggest the underlying liver disease may be chronic rather than acute, and that the current illness may be an exacerbation or decompensation of chronic liver disease. Dogs and cats with ALF are generally in good nutritional status compared with those with chronic hepatic disease. Findings of cachexia, emaciation, ascites, or edema suggest a more protracted illness and are characteristic of chronic rather than acute liver disease. It is important to make a distinction between acute and chronic liver disease as the intensive supportive care indicated in ALF might not be warranted in chronic end-stage liver disease. The long-term prognosis is better for acute hepatitis than chronic hepatitis.[4]

Diagnosis

An initial database consisting of complete blood cell count, serum chemistry, and urinalysis should be obtained in dogs and cats with acute liver disease. Liver enzyme elevations are a common finding in dogs and cats with acute hepatitis and necrosis. With mild hepatic injury or focal hepatic necrosis, increased ALT activity may be the only finding on an otherwise unremarkable biochemical profile. ALT and AST activities are moderately to markedly increased, because of enzyme leakage from damaged hepatocytes.[7] Although ALT activity increases with many hepatic diseases, the largest magnitude of increase is seen with acute hepatic necrosis and roughly correlates with the number of involved cells.[7] ALT activity may be increased as much as 100 times the upper range of normal, with increases in AST activity that parallel but are generally lower (30 times the upper limit of normal) than the ALT. It should be noted that some recognized hepatotoxins (aflatoxin and microcystin in blue-green algae) are not associated with severe or protracted increases in ALT activity because of toxin-suppressed transaminase synthesis.[7] Increased activity of the cholestatic liver enzymes, alkaline phosphatase, and GGT, also commonly occur with acute hepatitis and necrosis, but the magnitude of the increase is much less than for the ALT and AST.

Abnormalities in biochemical tests such as hyperbilirubinemia, increased SBAs, hypoglycemia, and hyperammonemia indicate compromised hepatic function. Hyperbilirubinemia and bilirubinuria support more significant hepatic injury once prehepatic (hemolytic) causes have been discounted. Primary biliary tract disorders including posthepatic mechanisms of hyperbilirubinemia should also be considered in the differential diagnosis. Other considerations for hypoglycemia in conjunction with acute liver disease include xylitol toxicity (excess insulin release) and sepsis. Hypoalbuminemia usually suggests chronic rather than acute liver disease, because of the long serum half-life of albumin. If azotemia is detected, dehydration, GI blood loss, and concurrent renal damage (e.g., leptospirosis, nonsteroidal antiinflammatory drugs [NSAIDs]) should be considered. Interpretation of azotemia is facilitated by concurrent urinalysis. Renal injury is supported by findings of cellular or granular casts, glucosuria, isosthenuria, and proteinuria. The complete blood cell count may reveal an inflammatory response suggesting underlying infectious or inflammatory disorders, and it is also useful for ruling out hemolytic anemia as cause of jaundice. Documentation of a coagulopathy is required for the clinical diagnosis of ALF. Laboratory findings indicative of a coagulopathy include prolonged PT and activated partial thromboplastin time (aPTT), decreased fibrinogen, increased fibrin degradation products, and thrombocytopenia.

Abdominal radiographs are often unremarkable in dogs and cats with acute hepatitis and necrosis. The liver may appear normal or increased in size. On abdominal ultrasound, the liver may appear normal or hypoechoic. Thoracic and abdominal imaging may be helpful to evaluate for other causes of acute hepatic disease, and biliary tract disorders.

Because dogs and cats with acute hepatitis and necrosis present with nonspecific signs of acute liver disease, the clinician should maintain a broad perspective regarding the many potential diseases and processes that can acutely affect the liver. Prior to obtaining a liver biopsy, ancillary testing (cytology or biopsy of more accessible lesions, infectious disease titers or molecular tests, diagnostic imaging) should be performed to evaluate for systemic disorders with secondary hepatic effects or multisystemic infections, thus providing a diagnosis of other causes of acute liver disease in a less-invasive manner.

When acute hepatitis or hepatic necrosis is suspected (or confirmed by liver biopsy), a thorough history is essential to identify exposure to potential hepatotoxins and infectious agents. The owner should be questioned regarding recent medications, including prescription and over-the-counter drugs, and alternative medicines such as herbal and dietary supplements. The potential for exposure to chemicals or hepatotoxins (*Amanita* mushrooms, blue-green algae, Sago palms, aflatoxins, or xylitol) should be assessed (for more details, see "Drug and Toxin-Induced Liver Injury" section). Other pertinent historical questions include current vaccination history (canine adenovirus, leptospirosis), travel history (fungal infections or tick-borne diseases), and exposure to other animals (infectious causes).

Liver biopsy is required to document the presence of acute hepatitis and necrosis; evaluate for specific causes; and differentiate acute from chronic disease. In patients with mild (or absent) clinical signs and liver enzyme elevations that correspond to recent medication administration, a liver biopsy may be postponed, the medication discontinued, and clinical signs and liver enzymes monitored for

improvement over a 2- to 3-week period. For patients with ALF and coagulopathy, the clinician must carefully weigh the benefits of histologic characterization versus the risk of excessive bleeding from the procedure.

Acute hepatitis is characterized histologically by a mononuclear or mixed inflammatory pattern, accompanied by hepatocellular apoptosis or necrosis.[2] Necrosis should be further characterized by the pathologist as to the morphologic pattern of injury (focal, multifocal, confluent, bridging, massive, or piecemeal) because the pattern of necrosis may provide insight into the pathogenesis of the lesion.[2] For example, because centrilobular hepatocytes have an abundance of cytochrome P450 enzymes, these hepatocytes are preferentially affected in drug-induced hepatotoxicity, when cytochrome P450 metabolism of the parent drug results in toxic metabolites.[8] Quantitative copper analysis and histochemical staining for copper are recommended, as copper accumulation may be an underappreciated cause of acute hepatitis in dogs.[4] Infectious causes of acute hepatitis may be diagnosed on liver biopsy, or by additional tests performed on liver tissue (culture, immunohistochemistry, polymerase chain reaction [PCR], virus isolation; Table 61-4).

Table 61-4 Infectious Diseases and the Liver

Classification/ Organism	Species	Hepatic Lesions	Tissue Tropism	Diagnosis
Viral				
Canine adenovirus I (infectious canine hepatitis)	D	Centrilobular necrosis; neutrophilic and mononuclear cell infiltrates; intranuclear inclusions in hepatocytes and Kupffer cells; gallbladder edema; chronic hepatitis?	Liver, vascular endothelial cells	Virus isolation, PCR, immunohistochemistry, histopathology
Herpesvirus (neonates)	D	Multifocal hemorrhagic necrosis; intranuclear inclusions	Kidneys, liver, lung, spleen, lymph node	Virus isolation, fluorescent antibody techniques; PCR, EM, histopathology
Canine acidophil cell hepatitis	D	Acute or chronic hepatitis; cirrhosis; acidophil cells characterized by angular shape, acidophilic cytoplasm and hyperchromatic nucleus	Liver	Histopathology
Coronavirus (feline infectious peritonitis)	C	Pyogranulomatous and granulomatous hepatitis; multifocal hepatic necrosis	Macrophages; vascular endothelium; peritoneum, liver, lymph nodes, kidneys, CNS, eyes	Immunohistochemistry on effusions or lesions with infected macrophages
Calicivirus (virulent form)	C	Massive or centrilobular hepatic necrosis; individualization of hepatocytes	Macrophages; vascular endothelium	Virus isolation, PCR
Bacterial				
Leptospira interrogans	D	Acute cholestasis, liver cell dissociation, and nonspecific reactive hepatitis; chronic hepatitis	Kidneys, liver, vascular endothelium	Serology, PCR on urine, histopathology
Clostridium piliforme (Tyzzer disease)	D, C	Multifocal periportal hepatic necrosis; intracellular filamentous organisms demonstrated on Giemsa or Warthin-Starry stain	Liver, ileum, colon	Histopathology
Sepsis and endotoxemia	D, C	Intrahepatic cholestasis, mild periportal lymphocytic infiltrate, scattered foci of macrophages or neutrophils, and occasional necrotic hepatocyte		Blood and extrahepatic tissue cultures; response to treatment of systemic infection
Mycobacterium spp.	D, C	Granulomatous hepatitis; acid-fast organisms demonstrated on special stains	Lungs, lymph nodes, GI tract; skin (varies with species)	Histopathology; cytology, culture; direct fluorescent antibody; PCR
Bartonella henselae and *clarridgeiae*	D	Granulomatous hepatitis and peliosis hepatis (*Bartonella henselae*); hepatic disease (*Bartonella clarridgeiae*)	Liver, lymph nodes, myocardium, joints	Serology, culture, PCR on liver tissue
Helicobacter spp. (enterohepatic)	D, C	Multifocal necrotizing hepatitis (dog); cholangitis (cats)	Liver and biliary tract	EM, PCR
Abscess (aerobic and anaerobic organisms)	D, C	Unifocal or multifocal hepatic abscesses	Primary liver or multisystemic infection	Aerobic and anaerobic cultures; histopathology

Continued

Table 61-4 Infectious Diseases and the Liver—cont'd

Classification/ Organism	Species	Hepatic Lesions	Tissue Tropism	Diagnosis
Fungal				
Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis, others	D, C	Granulomatous or pyogranulomatous hepatitis; fungal organisms seen on Grocott or Gridley silver stains	Varies with organism	Serology, cytology, histopathology, urine antigen testing (*Blastomyces*)
Protozoan				
Toxoplasma gondii	D, C	Hepatic necrosis; cholangitis (cats)	Lungs, CNS, liver, pancreas, heart, eyes	Serology, histopathology, PCR
Neospora caninum	D	Hepatic necrosis, neutrophils, hemorrhage	CNS, muscle, nerves, liver	Serology, histopathology, PCR
Cytauxzoon felis	C	Schizont-laden macrophages in the lumen of small vessels in liver; granulomatous hepatitis	Liver, spleen, bone marrow	Organism seen on blood smears; histopathology
Leishmania infantum	D	Multifocal, mild to moderate, granulomatous to pyogranulomatous inflammation; chronic hepatitis: lymphocytic plasmacytic portal inflammation with mild fibrosis	Skin, hemolymphatics; spleen, liver, kidneys	Organisms seen on cytology or histopathology; serology, culture, PCR
Babesia canis, Babesia gibsoni	D	Hepatitis, focal necrosis, bile stasis	Red blood cells	Cytology, serology, PCR
Hepatozoon canis	D	Hepatitis; mononuclear and neutrophil infiltration; meronts	Lymph nodes, marrow, liver, spleen, lungs	Serology, histopathology
Sarcocystis canis	D	Necrosuppurative and eosinophilic hepatitis; vasculitis; schizonts	CNS, liver, skin	Histopathology, EM
Rickettsial				
Ehrlichia canis	D	Portal hepatitis; lymphocytes, plasma cells, macrophages; morulae in mononuclear cells	Monocytes; macrophages	Serology, PCR, immunohistochemistry
Rickettsia rickettsiae	D	Focal hepatic necrosis	Endothelial cells; skin, CNS, heart, kidney	Serology, direct fluorescent antibody staining of tissues
Parasitic				
Visceral larval migrans (*Toxocara* migration)	D	Subcapsular and parenchymal granulomas with fragments of parasitic larvae; portal areas with eosinophils; lymphocytes	GI tract	Histopathology, fecal examination
Heterobilharzia americana (schistosomiasis)	D	Granulomas and schistosome ova; portal fibrosis	Liver, GI tract, lymph nodes	Histopathology, fecal examination, fecal PCR, serology
Dirofilaria immitis (Caval syndrome)	D, C	Passive congestion, cavernomatous change of hepatic veins, centrilobular necrosis and fibrosis, microfilaria in sinusoids with occasional small nodular aggregates; microthrombi	Heart, lungs, liver	Serology, microfilaria identification in blood; thoracic radiographs, echocardiography
Liver flukes: *Platynosomum concinnum, Amphimerus pseudofelineus; Opisthorchis, Metorchis*; others	D, C	Chronic cholangitis (eosinophils; lymphocytes, plasma cells, neutrophils); dilated bile ducts; periductal and portal fibrosis	Biliary tract; pancreas	Identification of ova on fecal exam or cytology of bile; direct visualization at surgery or necropsy
Alveolar echinococcosis (*Echinococcus multilocularis*)	D	Cystic hepatic masses containing amorphous debris; granulomatous inflammation	Intestine	Serology, fecal examination, histopathology
Algae				
Prototheca zopfii	D	Granulomatous hepatitis; organisms stained by periodic acid-Schiff or methenamine silver stain	Eyes, colon, CNS, kidneys, liver	Cytology or histopathology of affected tissues; culture

C, cat; CNS, central nervous system; D, dog; EM, electron microscopy; MAT, microscopic agglutination test; PCR, polymerase chain reaction.

Unfortunately, in most cases, routine liver biopsy is unlikely to reveal a specific cause of acute hepatitis.[2,4] Findings of inflammation and necrosis/apoptosis accompanied by nodular regeneration and fibrosis suggests chronic rather than acute hepatitis. The long-term prognosis is better for acute hepatitis than for chronic hepatitis.[4]

Treatment

If a probable cause of acute hepatitis and hepatic necrosis can be determined, then specific treatment is directed at the primary etiology (e.g., discontinuing potentially hepatotoxic medications, treating for leptospirosis with doxycycline, or chelating hepatic copper with penicillamine). In most cases specific therapy is unavailable and treatment is directed at more general supportive and symptomatic treatment of liver disease. Glucocorticoid therapy is not typically indicated in the treatment of acute hepatitis.[4] Empirical treatment with antioxidants such as *S*-adenosylmethionine (SAMe; 20 mg/ kg PO q24h), milk thistle (Siliphos; 3 to 6 mg/kg PO q24h), or vitamin E (10 to 15 IU/kg q24h) may be warranted, as oxidative stress is believed to play a role in drug (carprofen, potentiated sulfonamides, diazepam, methimazole, lomustine, others), and toxin (aflatoxin, organic solvents, and heavy metal toxicity) induced hepatic injury.[9] SAMe and milk thistle have additional cytoprotective properties that could be beneficial in necroinflammatory hepatopathies and hepatotoxicity. Antioxidants and cytoprotective agents are discussed in more detail in Chapters 40 and 46, respectively. Liver biochemistries should be monitored to assess patient response to therapy. Repeat liver biopsy performed 6 to 8 weeks after the initial diagnosis has been recommended, to confirm that acute hepatitis has improved or resolved, or to document a progression toward chronic hepatitis.[4,6] It has been suggested that most dogs with mild idiopathic acute hepatitis (not in ALF) recover after several days, regardless of treatment.[6]

For patients with ALF, aggressive supportive treatment is required. Goals of therapy are to treat the underlying cause when possible, allow adequate time for hepatic regeneration and repair, and prevent or control complications of liver failure, such as hypoglycemia, coagulopathy and anemia, HE, GI ulcers, and septicemia. Intravenous *N*-acetylcysteine (NAC), a glutathione source/antioxidant, is the antidote of choice for treatment of acetaminophen toxicity. NAC also appears to have additional potential benefits (improved systemic hemodynamics and tissue oxygen delivery), and should be considered for use in any dog or cat with ALF.[9] The optimal dose regimen when NAC is used for this purpose has not been determined. Treatment of complications of liver failure are discussed in "Complications of Liver Disease" section.

Prognosis

The prognosis for recovery in dogs with acute hepatitis is good, as most dogs recover uneventfully.[4] However, there is a potential for dogs with acute hepatitis to develop chronic disease.[4] If animals present with signs of advanced liver failure (e.g., HE, coagulopathy, hypoglycemia), the prognosis is guarded. If the animal survives, hepatic lesions such as periportal ductular proliferation, hepatic fibrosis, postnecrotic scarring, and regenerative hepatic nodules are likely.[2] If a hepatic drug reaction is suspected, reexposure of the patient to the suspect drug should be avoided.

Hepatic Abscesses

Etiology

Hepatic abscesses from bacterial infection of the liver occur uncommonly in dogs and cats.[10-13] Abscesses may form as solitary or multiple macroscopic masses or microabscesses. In newborn animals, Gram-positive and Gram-negative bacteria cause hepatic abscesses, presumably related to postpartum umbilical infections.[14] In adult animals, Gram-negative enteric bacteria (especially *Escherichia coli*) and anaerobes (especially *Clostridia* spp.) are most commonly identified; multiagent infections are frequent.[10,12] Other organisms such as *Yersinia* spp., *Actinomyces* spp., *Nocardia asteroides* can also cause hepatic abscesses as part of a systemic infection.[14]

Pathophysiology

The pathogenesis of hepatic abscesses in dogs and cats is unclear. Hepatic abscesses are usually associated with extrahepatic infections or regional hepatic parenchymal damage. Small numbers of bacteria, including *Clostridium* spp., can be cultured from liver tissue of healthy dogs. Hypoxia of hepatic tissue caused by hepatic neoplasia, liver lobe torsion, or trauma may predispose to abscess formation, because small numbers of existing anaerobes (e.g., *Clostridium* spp.) can proliferate under these conditions.

Other potential sources of bacteria include hematogenous spread (via the umbilical vein, hepatic artery, or translocation of intestinal bacteria into the portal blood), ascension via bile ducts, penetrating abdominal and caudal thoracic wounds, and direct extension from local suppurative diseases. Concurrent diseases or potential predisposing factors in dogs include systemic infections (pneumonia, pyelonephritis, prostatitis, pyometra, endocarditis), gallbladder rupture, pancreatitis, diabetes mellitus, liver lobe torsion, coexisting hepatic disease such as hepatic neoplasia (infected necrosis), long-term phenobarbital administration, long-term corticosteroid administration, and previous surgical biopsy.[10,11] Concurrent diseases in cats include cholecystitis, pyothorax, and hepatic neoplasia.[12] Solitary abscesses are more common in dogs, whereas cats are more likely to be septic and have multiple hepatic abscesses.[11,12] No association with feline leukemia virus or feline immunodeficiency virus infection has been made.[12] Solitary liver abscesses are more likely to involve the right liver lobe in cats and the left liver lobe in dogs.[11,12]

Clinical Examination

When adult dogs and cats are diagnosed with hepatic abscesses, they are usually older than 8 years of age.[10-12] Clinical signs are nonspecific and can be attributed to sepsis, inflammation, and hepatic dysfunction. The most common signs are anorexia, lethargy, vomiting, and diarrhea.[10,11] Clinical signs of hepatic involvement may be overshadowed by signs of the associated disease process (e.g., neoplasia, pyelonephritis, pancreatitis). Dogs with hepatic abscess may have a history of failure to respond to antibiotics or improvement that relapsed when antibiotics are discontinued.[10]

Physical examination findings are often vague and include depression, dehydration, fever, abdominal pain, hepatomegaly, abdominal mass, and abdominal effusion.[10-12] Hypothermia is a more common finding than fever in cats with hepatic abscesses.[12] Because the clinical findings are vague and nonspecific, hepatic abscesses often go undetected until an abdominal ultrasound is performed or they rupture and are discovered during laparotomy. Rupture of a hepatic abscess leads rapidly to peritonitis, septic shock, and death.

Diagnosis

Clinicopathologic abnormalities are consistent with an inflammatory hepatic disease. Potential findings on the complete blood count include neutrophilia with a left shift (or neutropenia and degenerative left shift if rupture occurs), mild anemia, and thrombocytopenia.[10,12] Increased ALT and ALP activity are common findings although the ALT may be in the normal range.[10] Liver enzyme elevations are a less-consistent finding in cats with hepatic abscesses

(increased ALT and ALP activity occurred in less than 50% of cats).[12] Other potential biochemical findings include hyperglobulinemia, mild hyperbilirubinemia, and hypoglycemia (sepsis). Laboratory abnormalities may also reflect the associated disease processes (e.g., hyperglycemia with diabetes mellitus, increased pancreatic lipase immunoreactivity with acute pancreatitis). If an abscess ruptures, cytology of the abdominal infusion reveals septic suppurative inflammation.

Abdominal radiographs may be normal or reveal hepatomegaly, hepatic mass lesion, or decreased abdominal detail or effusion associated with secondary peritonitis. With proliferation of gas-producing organisms, radiolucent areas may be seen in the liver. Ultrasonographic examination permits earlier detection of hepatic abscesses.[11] Ultrasonographically, a liver abscess appears as a hypoechoic or anechoic structure with irregular, hyperechoic margins.[11,13] The ultrasonographic pattern is similar to that seen with hepatic hematomas, cysts, neoplasia, and biliary cystadenoma. Gas may be seen within the abscess.[11] If abscess rupture has occurred, concurrent abdominal effusion may be detected. Additional ultrasonographic findings may reflect associated disorders such as pancreatitis, cholecystitis, or pyelonephritis. Ultrasound-guided fine-needle aspiration of a suspected liver abscess can be safely performed to obtain samples for cytology and culture to confirm the diagnosis.[11] If ultrasonography is not available, the diagnosis of hepatic abscesses is usually established during exploratory laparotomy (or at necropsy).

An attempt should be made to isolate and identify the organism(s) associated with abscessation so that appropriate antibiotic therapy can be instituted based on sensitivity testing. Aerobic and anaerobic cultures can be performed on abscess contents (by fine-needle aspiration), abdominal exudate, blood or hepatic tissues.

Treatment

Treatment of hepatic abscesses consists of surgical resection or drainage of focal lesions, administration of appropriate antibiotics, correction of associated fluid, electrolyte, and acid–base imbalances, and identification and treatment of any underlying disease process. Treatment of large unifocal hepatic abscesses has typically involved surgical resection of affected tissue, which may necessitate partial or full lobectomy.[10,12] If perforation and peritonitis are present, surgical abdominal drainage and lavage are indicated. Ultrasound-guided percutaneous drainage of a solitary abscess may resolve the abscess or allow stabilization until surgical resection can be performed.[11] The successful management of focal hepatic abscesses (up to 8 cm in diameter) by ultrasound-guided percutaneous drainage and alcoholization has been described in five dogs and one cat.[13]

Broad-spectrum combination antibiotic therapy (directed toward both aerobic and anaerobic bacteria) should be initiated as soon as cultures have been obtained. Results of a Gram stain on the exudate may provide preliminary information as to type of organism and guide the empirical choice of potentially effective antibiotics. Recommendations for broad-spectrum antimicrobial coverage of hepatobiliary infections include either a fluoroquinolone combined with amoxicillin/clavulanate or a fluoroquinolone combined with penicillin and metronidazole, until culture results are available. The dose of metronidazole should be adjusted in animals with hepatic dysfunction (7.5 mg/kg PO q8-12h). Antibiotic therapy should be continued for at least 6 to 8 weeks. Response to treatment can be monitored with serial ultrasound examinations and repeated blood work.

Prognosis

Historically, hepatic abscesses have carried a grave prognosis, with an overall reported mortality rate of approximately 50% in dogs[10] and 79% in cats.[12] The survival rate appears to be better when solitary abscesses are detected.[12,13]

Granulomatous Hepatitis

Granulomatous hepatitis is characterized histologically by focal or multifocal aggregates of activated macrophages with an epithelioid appearance, usually accompanied by lymphocytes and plasma cells.[14] This inflammatory response is distinct from that encountered in canine chronic hepatitis. Systemic infectious diseases are an important cause of granulomatous hepatitis and this lesion has been described with fungal infections (histoplasmosis, coccidioidomycosis, many others), bacterial infections (*Mycobacteria, Bartonella, Nocardia, Actinomyces, Rhodococcus*), protozoal diseases (cytauxzoonosis, leishmaniasis); parasitic diseases (visceral larval migrans, schistosomiasis, alveolar echinococcus, *Hepatozoon americana*), and disseminated protothecosis (see Table 61-4).[15,16] In cats, feline infectious peritonitis (coronavirus) is an important cause of multisystemic granulomatous or pyogranulomatous inflammation.

Other causes of granulomatous inflammation include a local response to foreign material (crystalline material, sutures, plant material) or a drug reaction. In humans, granulomatous liver lesions have been associated with administration of diltiazem, sulfonamides, quinidine, allopurinol, interferon-α, and phenytoin.[17,18] However, drug therapy as a cause of granulomatous hepatitis in dogs and cats has not been specifically reported. Granulomatous lesions in the liver has been described in a small number of dogs with lymphangiectasia, lymphosarcoma, and histiocytosis.[19] Many cases of granulomatous hepatitis are idiopathic.[16] Hepatic lipogranulomas ("fatty cysts"), which are often found in dogs with congenital portosystemic shunt, are aggregates of pigment-laden foamy macrophages and should not be confused with granulomatous hepatitis.

Clinical findings with granulomatous hepatitis are highly variable, depending on the underlying cause. When granulomatous hepatitis is identified on liver biopsy, special stains for fungal and mycobacterial organisms should be performed. Other diagnostics to either identify an organism (cytology, culture, fecal exam, PCR) or detect antibodies against the organism (serology) vary widely with the underlying agent (see Table 61-4). If a cause cannot be found after a thorough diagnostic evaluation, consideration should be given to presumptive treatment for undiscovered infectious agents such as atypical mycobacteria, *Bartonella* spp., or systemic fungal infection. Corticosteroids or other immunosuppressant agents should only be used when diagnostic testing and empirical treatment have been unsuccessful, as steroid-induced immunosuppression may exacerbate an underlying infection.[19]

Eosinophilic Hepatitis

Eosinophilic hepatitis occurs rarely in dogs and cats.[2] Potential causes include visceral larval migrans (*Toxocara)*, schistosomiasis, liver fluke infections, *Sarcocystis canis*, and possibly, fungal infections (see Table 61-4).[2] With parasitic causes, eosinophils are often located at or near the site of the parasitic lesion in the liver. Dogs and cats with systemic allergic, parasitic (heartworms), or hypereosinophilic syndromes, may also have scattered eosinophils in the liver, a variant of nonspecific reactive hepatitis.[2] Hepatic drug-induced liver injury should also be considered. Phenytoin and minocycline are associated with eosinophilic infiltrates in humans with drug-induced liver injury.[18,20] Potentiated sulfonamides have been suggested to cause drug-induced eosinophilic hepatitis in dogs,[2] although a more typical pattern is acute hepatocellular necrosis or a cholestatic hepatopathy.[21] When eosinophilic infiltrates are identified, efforts should be directed at diagnosing parasitic causes (fecal,

heartworm test; see Table 61-4), systemic eosinophilia, and hypersensitivity reactions. If no specific cause can be determined, empirical treatment with fenbendazole should be considered, followed by corticosteroid therapy as described for idiopathic chronic hepatitis.

Nonspecific Reactive Hepatitis

The term *nonspecific reactive hepatitis* is used to describe the slight to moderate widespread inflammatory infiltrates of the liver that occur secondary to a spectrum of extrahepatic disease processes.[2] Lesions of nonspecific reactive hepatitis are associated with febrile and inflammatory disorders, especially those involving the GI tract and pancreas, or they may represent residual evidence of a previous intrahepatic inflammatory disorder.[2] Inflammation occurs in portal or parenchymal areas and necrosis is absent. Neutrophils predominate with acute extrahepatic disorders, whereas mononuclear inflammation occurs with chronic extrahepatic disorders or residual hepatic inflammation. The liver may be secondarily affected by systemic disorders because of changes in liver blood flow, portal blood delivery of bacteria, drugs, hormones, cytokines, or other substances from the GI tract, or activation of intrahepatic Kupffer cells (monocyte-macrophage system) involved in the hepatic immune response. It may be challenging to differentiate nonspecific reactive hepatitis from resolving acute hepatitis or mild chronic hepatitis, without supportive clinical information.

Clinical signs in dogs and cats with nonspecific reactive hepatitis are usually referable to the extrahepatic disorder. Liver enzyme elevations (ALT—two times the upper limit of normal; ALP—three- to fourfold increases) are common, thus mimicking primary hepatic disease. However, tests that reflect liver function, including serum bile acids, are usually normal. It is important to consider extrahepatic disorders that can secondary affect the liver, prior to focusing on primary hepatic disease. Treatment is directed at the underlying extrahepatic disorder.

Canine Chronic Hepatitis

Chronic hepatitis, a heterogeneous group of inflammatory-necrotizing diseases of the liver, occurs commonly in dogs, but is rare in cats. Cholangitis, which is inflammatory liver disease that targets the biliary tract, rather than hepatocytes, is more common in cats but also occurs in dogs.The term *chronic hepatitis*, rather than *chronic active hepatitis* or *chronic persistent hepatitis*, is recommended.[2,15] If the etiology is known, it should be included as an adjective, such as "drug-induced chronic hepatitis," or "copper-associated chronic hepatitis"; otherwise, it is considered "idiopathic chronic hepatitis."

Chronic hepatitis in dogs is defined based on histopathologic features of hepatocellular necrosis or apoptosis associated with inflammation and evidence of regeneration and fibrosis.[2] Lymphoplasmacytic inflammation is characteristic, but a neutrophilic component may be present.[2] The histopathologic features of chronic hepatitis are similar, regardless of the underlying cause. Chronic hepatitis has the potential to progress to cirrhosis.[15,16] Recommendations have been made to include a clinical component to the definition of chronic hepatitis, such as documenting an increase in ALT activity along with histologic evidence of hepatic inflammation for a minimum of 4 months. However, many dogs with chronic liver disease are not clinically apparent until the advanced stages, so duration can be difficult to evaluate. The early stages may not be recognized unless biochemistries are monitored for hepatic injury.

A familial predisposition to develop chronic hepatitis has been suggested by demographic studies, pathologic surveys, and clinical case series (see Chapter 62). Breeds of dogs at increased risk for chronic hepatitis include the Bedlington Terrier,[22,23] West Highland White Terrier,[24,25] Doberman Pinscher,[26-28] American and English Cocker Spaniel,[29-33] Skye Terrier,[34] Dalmatian,[35] Labrador Retriever,[36-38] and English Springer Spaniel.[39] Unfortunately, with the exception of hereditary copper-associated liver disease in Bedlington Terriers, information is lacking for most of the breed-related disorders. Female dogs appear to be at increased risk in some studies,[4,40] while others report that male and female dogs are equally affected.[41,42] Within particular breeds, sex differences have been noted (female Doberman Pinschers, Labrador Retrievers, and English Springer Spaniels; male Cocker Spaniels).[26,29,36,38,39] Dogs diagnosed with chronic hepatitis are generally 4 to 7 years of age, but adult dogs of any age (or breed) can be affected.[4,29,41]

Etiology and Pathogenesis

Ideally, canine chronic hepatitis should be classified on an etiologic basis. However, with the exception of copper-associated liver disease in Bedlington Terriers, the cause, pathogenesis, natural history, optimal treatment, and prognosis of these disorders are unknown (Table 61-5). Idiopathic chronic hepatitis is the most common clinical diagnosis.[4,32,40,41]

Infectious Causes. Viral infections are a common cause of chronic hepatitis in humans, but are not currently recognized as an important etiology in dogs. In humans, viruses have the potential

Table 61-5 Causes of Canine Chronic Hepatitis

Category	Cause	Breed Predisposition
Infectious	CAV-1	
	Acidophil cell hepatitis	
	Leptospira spp.	
	Leishmania infantum	
Metabolic	Copper associated	Bedlington Terrier
		West Highland White Terrier
		Skye Terrier
		Doberman Pinscher
		Dalmatian
		Labrador Retriever
		Other breeds?
	α_1-Antitrypsin deficiency?	English (and American?) Cocker Spaniel
Toxic	Phenobarbital, primidone, phenytoin	
	Oxibendazole-diethylcarbamazine	Doberman Pinscher
	Lomustine	
	Carprofen?	
	Aflatoxicosis	
	Transient protoporphyria	German Shepherd
Autoimmune		Doberman Pinscher?
Idiopathic		Any breed
		Cocker Spaniel
		West Highland White Terrier
		Labrador Retriever
		English Springer Spaniel
		German Shepherd

to cause hepatitis either because of a persistent hepatic infection or as a transient infection that triggers an immune response because of a cross-reaction between the virus and liver antigens.[43] In an attempt to identify infectious causes of canine hepatitis, PCR screening of liver tissue was performed in 98 dogs with various stages of hepatitis to look for canine adenovirus type 1, *Hepadnaviridae*, hepatitis A virus, hepatitis C virus, hepatitis E virus, *Helicobacter* spp., *Leptospira* spp., and *Borrelia* spp.[44] Based on negative results, the authors concluded that canine hepatitis is not typically caused by these infectious agents.[44] However, dogs that are experimentally infected with canine adenovirus type I, but are partially immune, can develop chronic hepatitis that progresses to cirrhosis.[45] The virus could not be detected beyond the first week postinfection, although the disease progressed over a period of months.[45] Canine adenovirus antigen has been demonstrated by immunohistochemical techniques in formalin-fixed liver sections from five of 53 dogs with various hepatic inflammatory lesions, suggesting that canine adenovirus 1 (CAV-1) may play a role in spontaneous chronic hepatitis.[46] In contrast, PCR and immunohistochemistry failed to detect canine adenovirus in liver tissue of 45 dogs with chronic liver disease.[47] Whether CAV-1 is a significant cause of chronic hepatitis under natural conditions is unknown.

Another proposed viral cause of chronic hepatitis and cirrhosis is the "canine acidophil cell hepatitis virus," reported from Great Britain in the 1980s.[48,49] This transmissible agent, most likely a virus, is distinct from CAV-1. It was transmitted experimentally by subcutaneous injection of serum or liver extracts from affected dogs, resulting in experimentally induced acute and chronic hepatitis. No further studies have been published to clarify the nature of this infectious agent or the associated hepatitis.

Canine leptospirosis is typically associated with acute cholestatic hepatic disease and acute renal failure. However, persistent infection can cause chronic hepatitis in the absence of azotemia.[50,51] *Leptospira* serovar *grippotyphosa* was incriminated as a cause of chronic hepatitis in a kennel of American Foxhounds, based on serologic evidence and demonstration of spirochetes in the liver.[50] *Leptospira* serogroup *australis* (serovars *australis*, *bratislava*, and *muenchen*) infection was suspected to cause chronic hepatitis in 16 young Beagle dogs in a breeding colony routinely vaccinated against leptospirosis serogroups *canicola* and *icterohaemorrhagica*.[51]

Canine leishmaniasis has been associated with histologic evidence of chronic hepatitis,[52] but clinical features suggestive of hepatic involvement (hepatomegaly, ascites, or icterus) were absent. Histologic findings revealed granulomatous hepatitis in most dogs, but some dogs had marked portal infiltration with lymphocytes and plasma cells, and mild portal fibrosis. *Leishmania* amastigotes were routinely identified in macrophages in liver or other affected tissues. *Bartonella clarridgeiae* DNA was amplified from a liver biopsy of a Doberman Pinscher with copper-associated chronic hepatitis, although the significance of this finding is unclear.[53]

Copper Accumulation. Copper is an essential trace element in diets and is required for a number of physiologically important enzymes. Cells have highly specialized and complex systems for maintaining intracellular copper concentrations. At toxic concentrations, free intracellular copper initiates oxidative damage causing hepatocellular necrosis and inflammation.[54,55] Normal copper metabolism has been reviewed in detail elsewhere.[54,56]

Copper accumulation in the liver can be associated with significant hepatic injury resulting in acute hepatitis, chronic hepatitis, and cirrhosis (Figure 61-18).[4,54,57] It is one of the few well-documented causes of canine chronic hepatitis. In one study, copper-associated

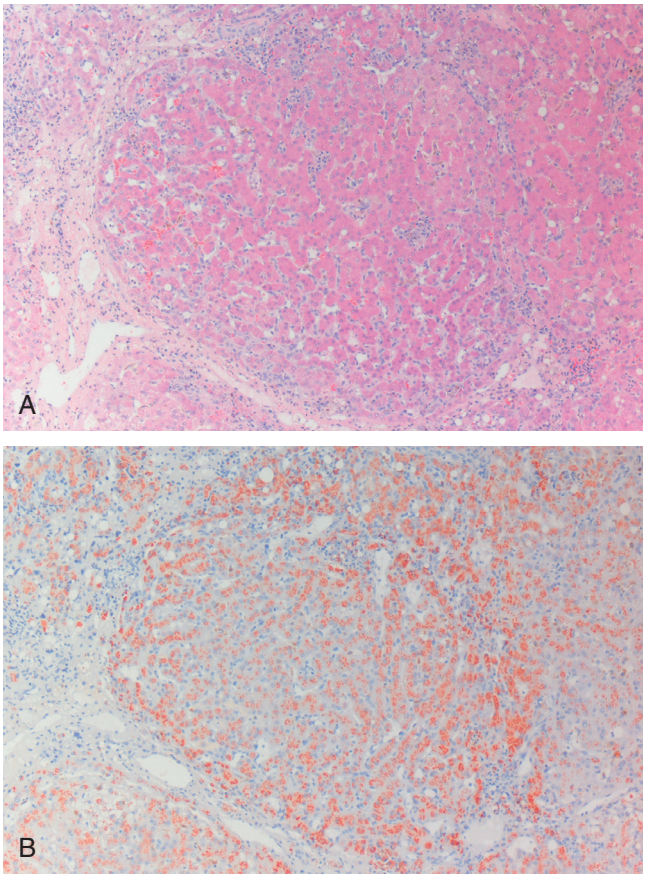

Figure 61-18 Liver biopsy from a 12-year-old Dalmatian/mixed breed dog with copper-associated chronic hepatitis and a quantitative hepatic copper of 8264 µg/g dry weight (normal <400 µg/g). **A,** Periportal hepatitis with portal fibrosis and nodular hepatic regeneration (H&E, 10×). **B,** Rhodanine stain of liver tissue was markedly positive for copper (orange granules, 10×). (Courtesy of Dr. Paul Stromberg.)

hepatitis (acute and chronic) accounted for one-third of all dogs with primary hepatitis.[4] Hepatic copper accumulation and hepatopathy have been described in cats but appears to be rare.[58,59] The severity of hepatic injury correlates with the amount of hepatic copper, but subcellular localization of molecules and the molecular association also plays a role.[54] Serum copper levels do not accurately reflect hepatic copper content and quantitative analysis of copper in the liver is required.[56] Hepatic copper concentration in normal dogs is between 150 and 400 µg/g dry weight (parts per million).[28,57] Inflammatory hepatic injury does not consistently occur until copper concentrations exceed 2000 µg/g dry weight.[60,61] However, there may be breed variations; for example, in Doberman Pinschers hepatic inflammation is present with copper concentrations of less than 2000 µg/g.[27,57] Transient acquired Fanconi syndrome has been described in dogs with excess hepatic copper accumulation.[62,63] Copper granules were demonstrated on renal biopsy in some but not all dogs.

Potential mechanisms for hepatic copper accumulation include primary metabolic defects in hepatic copper metabolism, cholestasis causing impaired biliary excretion of copper, and excess copper absorption.[54,56] A primary defect in hepatic copper metabolism occurs in Bedlington Terriers with a genetic mutation in the gene encoding the copper transport protein, COMMD1 (formerly MURR1), resulting in a defect in biliary copper excretion.[64,65] In

the early stages, copper is sequestered in hepatic lysosomes and hepatic damage is minimal. However, with progressive accumulation of copper, hepatic injury becomes significant. The average copper concentration in Bedlington Terriers with chronic hepatitis is approximately 6000 µg/g dry weight and values up to 12,000 µg/g dry weight have been reported.[23,57] Inherited copper-associated liver disease is also described in the West Highland White Terrier, Skye Terrier, Doberman Pinscher, Dalmatian, and Labrador Retriever, but with the possible exception of Dalmatians, the hepatic copper levels are much lower than in Bedlington Terriers.[25-27,34,35,38] The pathogenesis of copper accumulation and the relationship to chronic liver disease in these breeds is poorly understood. It seems likely that these breeds have a hereditary disorder of copper handling, but it is unlikely to be the same as described for the Bedlington Terrier.

Hepatic copper accumulation in the liver may also be a consequence rather than the cause of chronic hepatitis. Because copper is normally excreted in the bile, chronic cholestasis and impaired bile flow can result in secondary copper accumulation.[57,66] Secondary copper accumulation is predominantly periportal and is usually less than 2000 µg/g dry weight.[57,66] The effect of cholestasis on hepatic copper content was evaluated in three groups of dogs: Bedlington Terriers with copper toxicity, dogs with extrahepatic biliary obstruction (the prototype example of a cholestatic disorder) and chronic hepatitis in breeds not known to be at risk for copper-associated liver disease.[66] Hepatic copper content was evaluated by a semiquantitative method based on copper staining of liver tissue with rubeanic acid, using a scale of 0 (no copper) to 5.[67] Copper staining revealed absent to mild increases (scores of 0 to 2+) in dogs with biliary obstruction and chronic hepatitis when compared with Bedlington Terriers (scores of 5+). It was concluded that copper scores of 3+ or higher were suggestive of a primary copper storage disease.[66] Unfortunately, quantitative copper analysis was not evaluated. Markers of oxidative injury and altered defense mechanisms were similar in the three groups, consistent with the concept that copper, inflammation, and cholestasis can all contribute to oxidative injury.[66]

High dietary copper intake appears to be an unlikely explanation for hepatic copper accumulation and liver disease in dogs.[56] However, the copper content of commercial dog foods ranges from 12 to 16 mg/kg dry matter, which is relatively high compared with recommended minimum daily copper requirements in dogs.[56] There is speculation that the recent increase in pathologically elevated hepatic copper concentrations (specifically evaluated in Labrador Retrievers), may coincide with a pet food industry recommendation to replace cupric/cuprous oxide in feed formulations because of its low bioavailability.[68]

Many dogs with copper-associated chronic hepatitis also have increased hepatic iron concentrations.[69] Hepatic iron accumulation usually correlates with degree of inflammation.[40,69] Whether iron, as an oxidant, interacts with copper to contribute to lesions seen in copper-associated hepatitis remains to be determined.

α_1-Antitrypsin Deficiency. Inherited α_1-antitrypsin deficiency is a well-recognized cause of chronic hepatitis and cirrhosis in humans, and may play a role in the pathogenesis of chronic hepatitis in some dogs.[31] α_1-Antitrypsin is a circulating protease inhibitor that is synthesized and secreted by the liver. α_1-Antitrypsin deficiency in affected humans results in defective formation and impaired hepatic secretion of α_1-antitrypsin, resulting in hepatic accumulation of α_1-antitrypsin and hepatic injury. Serum levels of α_1-antitrypsin are typically low. In a study of 57 dogs with chronic liver disease, α_1-antitrypsin was detected by immunohistochemical staining in the liver of 37 dogs but was not identified in any control samples from healthy livers. None of the dogs had decreased serum levels of α_1-antitrypsin. Positive α_1-antitrypsin staining was a more consistent finding in English and American Cocker Spaniels with chronic liver disease, than in other breeds. The authors concluded that accumulation of α_1-antitrypsin might play a role, but it could not be determined if it was the cause or a result of chronic liver disease.[31]

Drugs and Toxins. Drug or toxin exposure is a potential cause of canine chronic hepatic disease. Drugs that have been incriminated include anticonvulsants (phenobarbital, primidone, phenytoin), oxibendazole-diethylcarbamazine, lomustine, and possibly carprofen.[70-73] Chronic hepatitis and cirrhosis from long-term phenobarbital therapy is most widely recognized.[70,71] Exposure to aflatoxin from contaminated commercial dog food is usually associated with ALF, but low-level long-term exposure in dogs can result in chronic hepatic injury (biliary hyperplasia, fibrosis, nodular regeneration). A breeding colony of German Shepherd dogs developed chronic hepatitis and cirrhosis that was suspected (but never confirmed) to be a result of exposure to a porphyrinogenic substance, based on the finding of aggregates of crystalline pigments with orange birefringence with polarized light.[74] Early recognition of drug- or toxin-induced chronic hepatic injury requires biochemical monitoring of liver enzymes, as dogs are clinically asymptomatic in the early stages.

Autoimmune/Immune Mechanisms. Autoimmune hepatitis has not been documented in dogs. However, some dogs with chronic hepatitis appear to respond to corticosteroid therapy and thus may correspond to autoimmune hepatitis in humans.[41] Autoimmune hepatitis in humans is a progressive chronic hepatitis of unknown cause that is believed to occur when an environmental agent (viruses, medications) triggers a cascade of T-cell–mediated events directed at liver antigens, in a genetically predisposed individual.[43] Women are more commonly affected than men. Hyperglobulinemia is a common finding. An infectious cause is difficult to document, as exposure may have occurred many years prior to the overt autoimmune disease.[43] Certain drugs may induce or unmask an autoimmune hepatitis, or simply cause hepatocellular injury that mimics autoimmune hepatitis.[43] An autoimmune component to Doberman Pinscher hepatitis has been speculated, because of the breed's predisposition, high female predominance, and the finding that expression of MHC class II antigens on hepatocytes of affected dogs correlates with degree of inflammation and decreases after treatment with prednisolone.[75] Dogs with chronic hepatitis may have concurrent disorders associated with immune aberrations (immune hemolytic anemia, hypothyroidism, atopy, glomerulonephritis), but whether this is coincidental or indicative of the presence of multiple immune disorders as seen with autoimmune hepatitis in humans is unknown.[37,76,77]

Autoimmune hepatitis in humans is diagnosed when other causes of acute or chronic hepatitis have been excluded and serum autoantibodies (antinuclear, antismooth muscle, antibody to liver/kidney microsomes type 1, antibody to liver cytosol type 1) are detected.[43] A number of studies have evaluated the role of liver-associated antibodies and cell-mediated response in dogs with chronic hepatitis, but none answers the question of whether the immune response is the primary cause of the hepatitis or a secondary phenomenon. Twenty-four dogs with chronic hepatitis were evaluated for circulating autoantibodies (against cell nuclei, smooth muscle, liver membrane, and mitochondria) by indirect immunofluorescence.[77] Antibodies to cell nuclei and liver membranes were

detected, but were also found in dogs with other types of hepatic disease, suggesting a nonspecific secondary response. Patterns of circulating autoantibodies found in dogs differed significantly from those found in humans with chronic liver disease.[77] In another study, serum anti–liver-membrane-protein antibody-positive dogs (1:40 to >1:1600) had higher ALT activity, total bilirubin concentration, and more severe hepatic lesions than did anti–liver-membrane-protein antibody-negative dogs, but it was not determined whether autoantibodies were primary or secondary.[78] CD3+ lymphocytes are the most common hepatic lymphoid cells in dogs with chronic hepatitis and are associated with hepatic necrosis,[79-81] but also account for 54% of hepatic lymphocytes in normal dogs.[82]

Clinical Examination

Historical and physical examination findings in dogs with chronic hepatitis are indicative of chronic hepatic disease, and are similar regardless of the underlying cause. Signs are often initially vague and nonspecific, such as anorexia, lethargy, vomiting, diarrhea, weight loss, PU, and PD.[4,32] With increased severity of hepatic dysfunction, signs of overt liver failure develop, such as ascites, jaundice, and HE. The presence of ascites and HE suggest that chronic hepatitis has progressed to cirrhosis, and ascites is a negative prognostic indicator.[4,42] Melena associated with gastroduodenal ulceration or coagulopathy is also more likely with advanced liver disease.[32] Because of the large functional reserve capacity of the liver, the onset of signs may appear very recent, initially suggesting an acute rather than chronic hepatic disorder. Clinicopathologic features that support chronicity include poor body condition, ascites, microhepatia, hypoalbuminemia, and histologic evidence of fibrosis.

Diagnosis

In the early (subclinical) stages, dogs are asymptomatic and only identified by biochemical screening for liver enzyme elevations. Increased serum ALT activity, reflecting ongoing hepatic injury, is reported in 75% to 95% of dogs with chronic hepatitis.[4,7] Serum ALT activity may exceed 10 times the upper normal limit.[7] Periods of normal ALT activity may reflect cyclic disease activity and the varying severity of necrosis.[7] Serum ALP activity is also commonly increased, but the magnitude of the increase is generally lower than seen with ALT activity. When chronic hepatitis advances to cirrhosis, liver enzyme activity may be normal, indicating decreased viable parenchymal mass.[7] Abnormalities in biochemical tests such as hyperbilirubinemia, hypoalbuminemia, decreased blood urea nitrogen (BUN), hypoglycemia, and increased SBA indicate hepatic dysfunction and a more advanced stage of disease.[32] Hyperglobulinemia can be seen in dogs with cirrhosis, but it remains to be determined whether this corresponds with increased autoantibodies as occurs in humans with autoimmune hepatitis, or whether it reflects nonspecific systemic antibody production in response to antigens from the portal blood which bypass the liver through acquired PSSs.[83] Mild nonregenerative anemia may be a reflection of chronic disease. Regenerative anemia can occur from blood loss secondary to a coagulopathy or bleeding GI ulcers. Copper-associated hemolytic anemia has only been documented in Bedlington Terriers. Abnormal hemostatic parameters (prolonged aPTT and PT) are indicative of severe hepatic dysfunction or DIC. A prolonged PT and thrombocytopenia may be negative prognostic indicators.[16,37] Analysis of ascitic fluid reveals a transudate or modified transudate.[32,42]

Abdominal radiographs are unremarkable except when advanced stages of disease are accompanied by microhepatia or ascites. In the early stages of chronic hepatitis, ultrasonography of the liver may be normal or reveal nonspecific changes in echogenicity. When chronic hepatitis has advanced to cirrhosis, potential ultrasonographic findings include microhepatia, irregular hepatic margins, focal lesions representing regenerative nodules, increased parenchymal echogenicity associated with increased fibrous tissue, and ascites. Splenomegaly and acquired PSSs may also be detected.

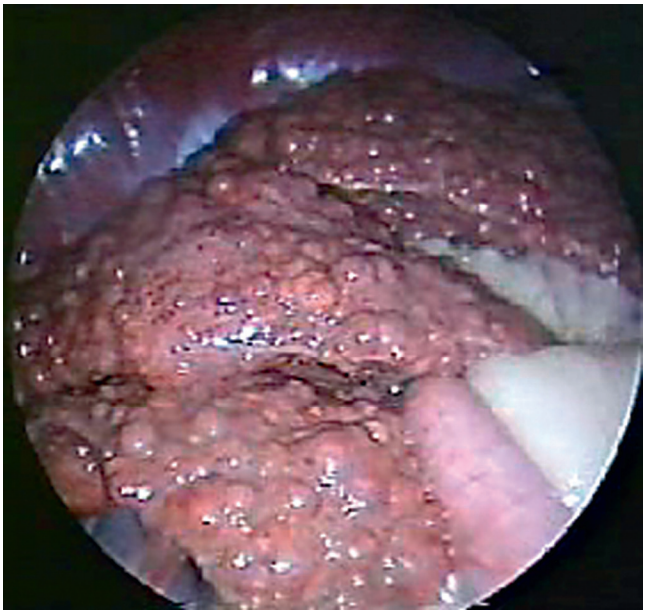

Figure 61-19 Laparoscopic appearance of a cirrhotic liver in a dog with idiopathic epilepsy treated with long-term phenobarbital therapy.

A liver biopsy is essential for the diagnosis of chronic hepatitis. Wedge biopsies are preferred over needle biopsies because they provide more tissue and are more likely to represent pathologic process(es) in the liver. When cirrhosis is present, laparotomy or laparoscopy often provide a better appreciation for the gross nodularity of the liver than can be ascertained from blind percutaneous needle biopsy (Figure 61-19). Chronic hepatitis is characterized histologically by moderate to severe inflammation (usually combinations of lymphocytes and plasma cells) associated with piecemeal necrosis. Piecemeal necrosis, also referred to as interface hepatitis, is necrosis involving the layer of hepatocytes adjacent to the portal tract or "limiting plate."[2] The term *bridging necrosis* is used when necrosis and inflammation dissect across the hepatic lobule from portal areas to central veins or to adjacent hepatic lobules and suggests a severe form of chronic hepatitis.[2] Histopathologic evaluation of the liver should not only consider etiology, but the pathologist should also comment on the activity (amount of inflammation, extent of apoptosis and necrosis) and the stage of disease (extent and pattern of fibrosis; architectural distortion suggestive of cirrhosis).[2]

Biopsies from dogs with chronic hepatitis should routinely be evaluated for copper accumulation. On hematoxylin and eosin staining, excess copper appears as golden brown refractile granules.[28] Histochemical stains, such as rhodanine or rubeanic acid, can be used to semiquantitatively evaluate for copper in the liver (see Figure 61-19). These stains consistently detect copper when amounts exceed 400 μg/g dry weight.[60] Values obtained by quantitative copper analysis have a strong correlation with the number and size of granules seen with histochemical stains within the range of 400 to 1000 μg/g of liver tissue.[60] Zonal distribution of copper

accumulation should be noted, as copper accumulation starting in the centrilobular area is more likely with a primary metabolic defect in copper metabolism.[54] Copper granules can also be detected on cytology of hepatic aspirates or impression smears stained with rhodanine or rubeanic acid. Quantitative analysis for copper, by atomic absorption analysis on fresh hepatic tissue, is the definitive method to document increased hepatic copper content. Needle core biopsy specimens may not be reliable for metal analysis, as copper and iron values are consistently lower in needle core versus wedge biopsy samples.[84] Formalin-fixed tissues should be avoided, because formalin may contain copper or leach copper from the tissue.[57] Hepatic copper can be reliably determined retrospectively on deparaffinized-archived liver biopsy specimens.[84]

Once chronic hepatitis has been confirmed, a careful consideration of known causes of chronic hepatitis is essential (see Table 61-5). Findings that would support a primary metabolic defect in copper metabolism include a previously recognized breed predisposition, copper accumulation that precedes cholestasis or inflammation, centrilobular (zone 3) distribution of copper, histochemical score for copper of 3+ or greater, or quantitative copper measurements that exceed 2000 µg/g dry weight.[54,57,66] Special stains of the liver should be requested to evaluate for infectious agents such as leptospirosis; serum antibody titers for leptospirosis may be indicated. A history of chronic drug therapy should be sought, especially long-term anticonvulsant therapy or other drugs listed in Table 61-5.

Treatment

Recommendations for treatment of chronic hepatitis are empirical at best, because of the lack of controlled therapeutic studies on a well-defined population of dogs with this disorder. If a probable cause or category of injury can be determined, then specific treatment is directed at the primary etiology, for example, discontinuation of phenobarbital, treatment of leptospirosis, or chelation of hepatic copper with penicillamine. In most cases, specific therapy will be unavailable.

Treatment of chronic hepatitis in dogs has traditionally centered on the use of corticosteroids, presuming that, as in humans with the autoimmune form of hepatitis, immunologic mechanisms (inflammatory cells and mediators, local cytokines), contribute to hepatic inflammation and progression to cirrhosis. Corticosteroids have antiinflammatory, immune-modulating, and antifibrotic effects, which may be beneficial in chronic hepatitis. A large retrospective study suggested that corticosteroid therapy at initial immunosuppressive doses (2.2 mg/kg/day; eventually tapered to 0.6 mg/kg/day) improved survival in dogs with chronic hepatitis.[41] However, many concurrent drugs were given and, undoubtedly, a heterogeneous group of disorders were included under the diagnosis of "chronic hepatitis." Corticosteroid therapy appears warranted in dogs with histologic features of active inflammation and persistent increases in serum liver enzyme activity, for which known causes of chronic hepatitis (including infectious causes) have been excluded.[41,85] Glucocorticoid therapy is not indicated for treatment of chronic hepatitis caused by drug therapy, infectious agents, or primary hepatic copper accumulation.

The optimal dose and duration of corticosteroid therapy for treatment of canine chronic hepatitis is unknown, including whether immunosuppressive doses are required, or whether lower, anti-inflammatory levels would suffice.[76] Even in humans with autoimmune hepatitis, immunosuppressive doses of corticosteroids may not be required.[43] Prednisone (or prednisolone) at an initial dose of 1 to 2 mg/kg/day PO and then gradually tapered to 0.5 to 1.0 mg/kg every 48 hours is most often recommended for treatment of canine chronic hepatitis. Complications of corticosteroid therapy include GI bleeding (which may precipitate HE), secondary infections, iatrogenic Cushing disease, and worsening of ascites. Dexamethasone (0.2 mg/kg PO q24h) may be preferred in dogs with ascites or edema, because it lacks mineralocorticoid activity, which could exacerbate these signs.

Prednisone is often used in combination with azathioprine, especially if side effects of prednisone become objectionable. Azathioprine is an antimetabolite with antiinflammatory and immune-modulating effects, and is commonly used in combination with prednisone in humans with autoimmune hepatitis.[43] The dose of azathioprine in dogs is 1 to 2 mg/kg/day PO every 24 hours for 1 to 2 weeks, then tapered to every 48 hours for maintenance therapy. Prednisone (0.5 to 1.0 mg/kg/day) is given on the alternate days. Because azathioprine may cause bone marrow suppression and acute hepatotoxicity, the complete blood count and biochemical profile should be monitored. Antiinflammatory agents and immunosuppressive drugs are discussed in more detail in Chapters 38 and 49, respectively. Because glucocorticoids increase liver enzyme activity (especially serum ALP activity), response to therapy is best evaluated by a followup liver biopsy performed 3 to 6 months after starting therapy. If glucocorticoid therapy is eventually discontinued, clinical and biochemical parameters should be periodically monitored to detect a relapse.

Dogs with hepatic copper concentrations greater than 1500 µg/g, should be treated with the copper chelator penicillamine at a dose of 10 to 15 mg/kg PO every 12 hours.[57] Treatment usually requires months to years to produce significant decreases in hepatic copper. A mean decrease in copper of approximately 1500 µg/g was achieved in Bedlington Terriers treated for 6 months.[86] Dogs with secondary copper accumulation appear to respond more rapidly, possibly because hepatic copper content is lower in these breeds.[86] Doberman Pinschers with subclinical hepatitis treated with penicillamine for 4 months had a mean decrease in copper from 1036 µg/g to 407 µg/g.[87] Penicillamine has additional effects beyond copper chelation, which may be beneficial in dogs with chronic hepatitis, including inhibition of collagen deposition, stimulation of collagenase activity, immunosuppression, and immunomodulation.[54] Common side effects of penicillamine therapy include anorexia, nausea, and vomiting, which can be minimized by giving the medication with a small amount of food. The copper chelator, trientine (10 to 15 mg/kg PO q12h), is also effective for reducing hepatic copper concentrations.[86] It has fewer side effects than penicillamine and is effective in dogs with hemolytic anemia caused by copper release from necrotic hepatocytes. Iatrogenic copper deficiency (microcytosis and hepatic dysfunction) has been described in a dog treated with long-term copper chelation therapy (trientine) and a copper-restricted diet.[88] Decisions on duration of chelator therapy are based on followup liver biopsies with periodic monitoring of quantitative hepatic copper content.

Oral zinc salts can be used for maintenance therapy after copper chelation, or as initial therapy in dogs with hepatic copper concentrations between 400 µg/g dry weight and 1500 µg/g dry weight. Zinc supplementation is typically used in conjunction with dietary copper restriction. Zinc decreases intestinal copper absorption by inducing the intestinal copper-binding protein, metallothionein, within intestinal epithelial cells, which preferentially binds dietary copper and prevents its absorption. Zinc acetate is given at a dose of 100 mg PO BID for 2 to 3 months, then at a maintenance dose of 50 mg PO BID.[89] A minimum of 3 months of zinc therapy is required before copper uptake from the intestinal tract is blocked.[89]

Zinc administration should be separated from meals by at least 1 hour and should theoretically not be prescribed at the same time as a copper chelator.[54] Serum zinc concentrations should be monitored to achieve a level of 200 to 400 µg/dL. Zinc concentrations greater than 500 µg/dL may be toxic (hemolytic anemia).

Low-copper diets are most beneficial for managing early (subclinical) copper accumulation in dogs affected with primary metabolic defects in hepatic copper metabolism.

Feeding a low-copper diet decreases hepatic copper content in Labrador Retrievers with subclinical copper-associated liver disease.[90] Additional treatment with zinc does not appear to increase the copper-lowering effect of dietary management.[90] Foods containing large amounts of copper (liver, other organ meats, shellfish, eggs, bean/legumes, chocolate, nuts, cereals, and copper-containing vitamin supplements) should be avoided.

Because oxidative stress is a significant mechanism for hepatic damage associated with copper accumulation and necroinflammatory hepatic disorders,[66,91] antioxidant therapy with vitamin E (10 to 15 IU/kg/day), or SAMe (20 mg/kg/day) has been advocated.[9] Other cytoprotective agents such as silymarin (milk thistle) and ursodeoxycholic acid may also be beneficial.[9] Chapters 40 and 46 discuss cytoprotective agents used in the treatment of hepatobiliary disease in detail. When end-stage cirrhosis is diagnosed, treatment is mainly supportive, as cirrhosis itself is essentially irreversible. Measures should also be instituted to control the complications of chronic liver failure, such as ascites, HE, gastroduodenal ulcers, and coagulopathy, which are discussed in more detail in "Complications of Liver Disease" section.

Prognosis

The response to treatment of chronic hepatitis is variable, which is not unexpected as it is likely a heterogeneous group of diseases. Some dogs can eventually be taken off medication and remain in remission, but more often, therapy must be continued indefinitely. Other dogs fail to respond, especially those that have advanced disease with cirrhosis.[4,32] In one study, the estimated median survival time in 42 dogs with idiopathic chronic hepatitis was 18 months (range: 0 to 49 months) and in 23 dogs with copper-associated chronic hepatitis it was 17 months (range: 7 to 27 months).[4] Mean survival time in 20 dogs with cirrhosis was 1 week.[32]

Chronic Hepatitis in Specific Breeds

Bedlington Terrier

Bedlington Terriers develop chronic hepatitis and cirrhosis from copper toxicity, as a consequence of an inherited metabolic defect resulting in impaired biliary copper excretion.[23,57,76] The disorder is transmitted by autosomal recessive inheritance. The gene responsible for this metabolic disorder is COMMD1, which is different than that described for copper toxicity (Wilson disease) in humans, in which the gene involved is ATP7B.[64] There is no gender predilection. At one time, it was speculated that as many as 60% of the breed might be affected.[57] Hepatic copper concentration in normal Bedlington Terriers ranges from 91 to 358 µg/g with a mean of 206 ± 56 µg/g dry weight.[23] Bedlington Terrier copper-associated liver disease is associated with progressive, hepatic copper accumulation (copper levels of up to 12,000 µg/g) unless treatment is instituted. The lowest hepatic concentrations of copper are found in the youngest dogs and concentrations increase with age, peaking at around 6 years. Copper content usually declines thereafter in affected dogs, but not to normal. This decline may be a result of replacement of copper-containing hepatocytes by fibrous tissue or regenerative nodules that do not contain copper. The severity of hepatic disease is correlated with the amount of hepatic copper. Hepatic injury is believed to occur when progressive copper accumulation exceeds the storage capacity of the lysosomes; copper is released to the cytoplasm, damaging mitochondria, initiating lipid membrane peroxidation, and eventually causing cell death.

Affected dogs can be asymptomatic (in the early stages) or show signs of acute hepatic necrosis, chronic hepatitis, or cirrhosis.[54] In young dogs, copper accumulates in centrilobular (zone 3) hepatocytes and is sequestered in hepatic lysosomes. During this first stage, copper concentrations are between 400 and 1500 µg/g, dogs are asymptomatic, biochemical testing is within normal limits, and liver biopsy findings are unremarkable. In the second stage, when hepatic copper concentrations are between 1500 and 2000 µg/g, copper granules are also found in midzonal (zone 2) and periportal (zone 1) hepatocytes. Although dogs are still asymptomatic, focal hepatic inflammation (centrilobular mixed cell foci, with necrotic hepatocytes, lymphoplasmacytic inflammation, and copper-laden macrophages) is seen on biopsy, and increased serum ALT activity reflects hepatocellular injury. In the most advanced stage, when hepatic copper concentration exceeds 2000 µg/g, morphologic changes reveal chronic hepatitis that may progress to cirrhosis, and clinical and biochemical evidence of liver disease become apparent. Clinical signs include anorexia, lethargy vomiting, and weight loss. With progression to cirrhosis, findings of jaundice, ascites, and HE may develop. Biochemical findings vary with the stage of disease. Increased serum ALT activity is the most sensitive laboratory indicator, although findings will be normal in young dogs in stage I, because of the lack of hepatic inflammation. Other serum biochemical abnormalities typical of chronic hepatic dysfunction eventually develop. In some cases, acute hepatic necrosis and ALF occur. Hepatocellular necrosis may be associated with release of copper from necrotic hepatocytes, resulting in hemolytic anemia. During episodes of hemolysis, plasma copper levels are increased; other findings include low packed cell volume, hemoglobinemia, and hemoglobinuria. Liver biopsy and quantitative analysis of hepatic copper concentrations is required for definitive diagnosis and staging of the disease. Serum copper or ceruloplasmin concentrations are not helpful to make a diagnosis.[57]

Liver biopsies should be performed in all Bedlington Terriers considered for breeding, in order to identify and remove affected dogs from breeding programs. Screening of asymptomatic dogs with a liver biopsy at 6 months and 15 months of age can determine if an affected dog is homozygous or heterozygous (a carrier).[15] Affected dogs (both homozygous and heterozygous) typically have increased hepatic copper by 6 months of age. However, copper concentrations in dogs who are carriers (heterozygous) return to normal by 1 year of age, whereas copper concentrations in homozygous dogs continues to increase.[57] Selective breeding programs in the Netherlands has decreased the prevalence of Bedlington Terrier copper-associated liver disease from 46% (1976-1986) to 11% (1990-1997).[92]

DNA testing of Bedlington Terriers is available from VetGen (www.vetgen.com). This assay evaluates a linkage-based DNA marker (CO4107, allele 2) that is located in the chromosome close to the gene for copper toxicity.[93] The test can identify normal, affected, and carrier dogs with 90% accuracy. However, the marker can only be relied on for diagnosis of the genetic status of an individual dog when supported by a pedigree study.[93,94] Significant discrepancies were reported in 22 Bedlington Terriers, when comparing results of liver biopsy and the DNA marker.[94] This may be attributed to different subpopulations of Bedlington Terriers with variations in the disease-causing mutation of the COMMD1 gene or a second mutant copper gene could play a role.[94] Liver biopsy for quantitative

copper and morphologic examination remain the best option for diagnosis in the individual dog.[94] A database for certification of Bedlington Terriers is maintained on the Web site (www.caninehealthinfo.org) of the Canine Health Information Center, which is sponsored by the AKC/Canine Health Foundation and the Orthopedic Foundation for Animals.

Affected Bedlington Terriers who are asymptomatic (copper >400 μg/g dry weight but less than 1500 μg/g dry weight) should have dietary copper restriction and zinc supplementation. Bedlington Terriers with copper accumulation (copper >1500 μg/g dry weight) and chronic hepatitis should be treated with a copper chelator such as penicillamine or trientine.[57] Early diagnosis and treatment with either zinc or copper chelators will allow most dogs to lead a normal life.[57] Treatment of hemolytic anemia may require a blood transfusion. Trientine dihydrochloride (but not penicillamine) may be effective in chelating circulating copper during a hemolytic episode. Chapter 43 discusses copper-chelating agents in more detail.

Doberman Pinscher

Doberman Pinschers are at increased risk for the development of severe chronic hepatitis and cirrhosis.[26,28,81,95] Doberman hepatitis accounted for 4% of all deaths in a Dutch population of 340 Dobermans.[96] Middle-aged (4 to 7 years) female dogs are at increased risk, but males also may be affected. Although a hereditary mechanism is suspected, the pathogenesis of this disorder is unclear.[28] Copper accumulation appears to be associated with hepatic damage, but the pathogenesis is different from the Bedlington Terrier disorder.[27] Immune mechanisms may also play a role.[75,81]

Many Doberman Pinschers are diagnosed in the advanced stages of hepatic failure.[26] Evidence of excessive bleeding (gingival bleeding, epistaxis, and melena) are common. Signs of HE often predominate in the terminal stages. Common physical examination findings include ascites, jaundice, and weight loss. Splenomegaly (associated with portal hypertension) is common. Laboratory findings included increased ALT and ALP activity, hyperbilirubinemia, hypoalbuminemia, hyperammonemia, coagulopathy, and thrombocytopenia.[26] Typical histologic lesions include portal inflammation (lymphocytes, plasma cells, and macrophages), piecemeal necrosis, bridging necrosis, bile duct proliferation, and portal fibrosis.

Hepatic copper concentrations are increased in most affected dogs and are typically between 1000 and 2000 μg/g dry weight, although values as high as 4700 μg/g have been reported.[28,81] The significance of the increased hepatic copper concentration in this breed remains controversial. Copper accumulation was originally attributed to secondary mechanisms, as Doberman Pinschers with advanced disease (chronic hepatitis and cirrhosis) have biochemical and histologic evidence of cholestasis. However, evaluation of affected dogs in the early (subclinical) stage, reveals that copper accumulation precedes cholestasis,[27,95] and decreased biliary excretion of radiolabeled copper has been documented.[97] The hepatic distribution of copper and location of inflammation varies with the stage of disease. In the early stages, the copper (and focal inflammation) is centrilobular.[27,81,95] As the disorder progresses, copper accumulation and inflammation are more pronounced in periportal regions and areas of bridging necrosis.[95] Although copper appears to be related to the hepatic inflammatory reaction, copper levels are typically less than 2000 μg/g dry weight, the minimum amount of copper that is believed to cause hepatocellular injury in Bedlington Terriers and West Highland White Terriers.[60,61] A primary copper retention disorder has been proposed,[28] but the genes associated with Bedlington copper toxicity (COMMD1) and Wilson disease in humans (ATP7B) have been excluded.[98]

Recent efforts have focused on identification of affected Doberman Pinschers prior to advanced hepatic disease. In Finland, a survey of 626 randomly selected, clinically healthy Doberman Pinschers, revealed that 8.8% of dogs had increased ALT activity, and 3.4% had hepatitis (parenchymal and portal mononuclear inflammation and positive stains for copper).[99,100] The mean age of dogs with subclinical hepatitis was 3.8 years, compared with clinically affected dogs (5.5 years). The asymptomatic period lasted an average of 19 months. The prevalence of subclinical Doberman hepatitis was investigated in 106 randomly selected 3 year old Doberman Pinschers in the Netherlands.[27] Subclinical hepatitis was identified in 22 dogs (19 females and three males); hepatic copper concentration was higher in dogs with hepatitis (419 ± 414 μg/g dry weight) than those without liver disease (197 ±113 μg/g).[27] Serial liver biopsies over at least a 2-year period, revealed that hepatitis persisted only in dogs with copper levels greater than 400 μg/g dry weight, and copper levels continued to increase in these dogs (939 ± 299 μg/g), supporting a relationship between copper, inflammation and hepatitis.[27]

It has also been proposed that hepatic copper is incidental to chronic hepatitis in this breed, based on the findings that five of 35 Doberman Pinschers with chronic hepatitis had normal copper levels, and histologic changes were similar regardless of copper status.[81,26,75] An immune-mediated mechanism has been suggested, based on the finding that expression of MHC class II antigens on hepatocytes of dogs with Doberman hepatitis was correlated with degree of inflammation.[75] Aberrant MHC class II molecule expression on nonlymphoid cells could be a result of toxins, drugs, viral infection, or autoimmunity, and hepatocytes with MHC class II expression might become a target as an antigen-presenting cell for CD4+ T cells.[75] Dogs treated with low-dose prednisolone (0.1 to 0.5 mg/kg/day) for 4 to 5 months had significantly decreased expression of MHC class II antigens.[75]

Chronic hepatitis should be suspected in any Doberman Pinscher (especially females) with clinical or biochemical evidence of hepatic disease. Definitive diagnosis requires liver biopsy. Other causes of chronic hepatitis should also be considered, since Doberman Pinschers appear to be at risk for drug-induced hepatitis.[71] Early detection of chronic hepatitis provides the best opportunity for treatment. It has been recommended that all Doberman Pinschers older than 1 year of age be screened for ALT activity.[99] Persistent increases in ALT activity suggest further evaluation including liver biopsy is warranted. The magnitude of increased ALT activity is not different between subclinical and clinically affected dogs. Hyperbilirubinemia is suggestive of more advanced disease.[28,99]

Effective treatment for Doberman Pinschers with chronic hepatitis has not been established. However, a preliminary study showed that if diagnosed in the subclinical stage, treatment with penicillamine (200 mg total dose PO BID for 4 months) lowered hepatic copper content and improved hepatic histopathology.[87] Traditionally, antiinflammatory or immunosuppressive drugs such as prednisone with or without azathioprine have been instituted. The efficacy of this treatment remains to be determined but generally, the response is poor if dogs are presented in advanced stages of liver failure. The use of ursodeoxycholic acid (15 mg/kg PO BID) deserves special consideration in this chronic cholestatic disorder, but has not yet been objectively evaluated. Treatment of copper-associated hepatitis in Doberman Pinschers with advanced disease is usually unsuccessful. Most dogs die within weeks to months. The prognosis appears more favorable if the disease is detected in the early stages, but the optimal therapeutic regimen remains to be determined.

West Highland White terrier

West Highland White Terriers are at increased risk to develop chronic hepatitis and cirrhosis.[25,76,101] Males and females are equally affected. The mode of inheritance for the familial copper-associated disorder has not been established.[24] Decreased biliary excretion of radiolabeled copper occurs in affected dogs.[89] Centrilobular (zone 3) copper accumulation occurs during the first year of life, but rarely exceeds 2000 μg/g dry weight.[24] In contrast to the Bedlington Terrier, West Highland White Terriers do not continuously accumulate copper over their lifetime; in fact, copper content may actually decrease with time.[56] In one report of 395 clinically normal West Highland White Terriers, most dogs had hepatic copper levels between 100 and 1500 μg/g dry weight with normal liver biopsies.[25]

In West Highland White Terriers with chronic hepatitis, histologic lesions include multifocal hepatitis, subacute bridging necrosis, massive necrosis, and cirrhosis.[25] The relationship of hepatic copper to chronic hepatitis in the West Highland White Terriers is unclear. There appears to be at least two types of chronic hepatitis.[25] Some dogs have copper-associated hepatitis with elevated copper content (>2000 μg/g) and multifocal centrilobular hepatitis. Copper concentrations do not usually exceed 3500 μg/g dry weight. Lesions of chronic hepatitis can also be seen in the absence of substantial copper accumulation, and have been described as "idiopathic chronic hepatitis."[25] Quantitative copper analysis is necessary to determine if copper accumulation is a significant (>2000 μg/g dry weight) contributing factor.

If chronic hepatitis and cirrhosis are associated with increased hepatic copper content (>2000 μg/g dry weight), treatment for hepatic copper accumulation should be instituted. Mature West Highland White Terriers with chronic hepatitis and less than 2000 μg/g dry weight of copper may not require chelation therapy, as hepatic copper accumulation is not continuous throughout life. Other therapeutic options for treatment of idiopathic chronic hepatitis, such as glucocorticoids, should be considered in these dogs.

Labrador Retriever

Labrador Retrievers are at increased risk for chronic hepatitis.[29,37] Age at presentation ranges from 2.5 to 14 years, with an average age of 7 to 9 years.[36-38] A female predisposition was noted in two studies,[36,38] whereas in another study, males and females were equally affected.[37]

Most affected dogs have increased hepatic copper, which has been described as centrilobular (zone 3) or diffuse.[36-38] Copper concentrations exceeded 2000 μg/g dry weight in 10 of 12 dogs (mean copper: 3369 μg/g; range: 2375 to 4972; reference interval: 120 to 400 μg/g).[38] Most dogs also had elevated iron levels with a mean of 4117 μg/g (reference interval: 350 to 1750).[38] A genetic basis is suspected, based on the finding of increased copper concentrations in asymptomatic related dogs, but the genetic defect remains to be determined.[36] A retrospective survey of hepatic copper content in Labrador Retrievers during two time periods (1980-1997 and 1998-2008), revealed significantly higher copper concentrations in the more recent period both in dogs with chronic hepatitis and in control dogs; no difference in age or gender was noted.[68] It was speculated that increased hepatic copper might reflect increased dietary copper bioavailability, because of pet food industry recommendations to replace cupric/cuprous oxide in feed formulations.[68]

Treatment with penicillamine (15 mg/kg PO q12h) appears to be effective in decreasing hepatic copper content and inflammation.[36,90] However, some dogs appear to respond to immunosuppressive (prednisone, azathioprine), supportive (ursodeoxycholic acid, SAMe, milk thistle), and symptomatic therapies that are not designed to lower hepatic copper.[37] Feeding a low-copper diet to 20 Labrador Retrievers with hepatic copper accumulation (seven of 20 dogs had varying degrees of hepatitis) was effective in decreasing hepatic copper concentrations, but severity of inflammation remained unchanged.[90] Additional treatment with zinc did not appear to increase the copper-lowering effect of dietary management.[90] Long-term survival appears variable.[36,37] Dogs who died within 2 months of diagnosis were more likely to have a prolonged PT and thrombocytopenia.[37]

Dalmatian

Dalmatians are reported to have acute hepatic necrosis, chronic hepatitis, and cirrhosis associated with increased hepatic copper concentrations.[35,102] Cholestasis is not a prominent biochemical or histologic feature until later in the disease, suggesting that hepatic copper accumulation is more likely to be caused by a familial metabolic disorder rather than secondary to altered hepatic biliary copper excretion. Most dogs presented initially with acute GI signs (anorexia, vomiting, and diarrhea). Biochemical findings revealed markedly increased ALT activity with lesser increases in ALP activity. Hyperbilirubinemia and hypoalbuminemia were seen with advanced disease. Glucosuria (in the absence of hyperglycemia) and proteinuria were identified in some dogs. Ultrasound findings were usually unremarkable. Liver biopsy revealed piecemeal necrosis, bridging fibrosis, and inflammation (predominantly lymphocytes or neutrophils). The mean hepatic copper level was 3197 μg/g dry weight (normal <400 μg/g dry weight) with a range of 754 to 8390 μg/g dry weight. In five of nine dogs, copper exceeded 2000 μg/g. Rapid progression of the disease was characteristic. Copper chelation therapy may be beneficial if diagnosed before advanced liver disease occurs.

Skye Terrier

Chronic hepatitis and cirrhosis associated with hepatic copper accumulation (800 to 2200 μg/g dry weight) in genetically related Skye Terriers has been described.[34] In the early stages, copper accumulation is absent, and biopsy findings indicate hepatocellular degeneration with cholestasis and mild inflammation. Chronic lesions are associated with intracanalicular cholestasis, chronic hepatitis, and cirrhosis. Skye Terrier hepatitis is speculated to be a disorder of disturbed bile secretion with subsequent accumulation of copper.

Cocker Spaniel

American and English Cocker Spaniels have an increased incidence of chronic hepatitis and cirrhosis.[29,30] The cause is unknown. Hepatic copper accumulation does not appear to be a consistent feature. It is unclear whether accumulation of α_1-antitrypsin in hepatocytes, a well-recognized cause of cirrhosis in humans, is important in the pathogenesis.[31] Male Cocker Spaniels (average age: 5 years) are at increased risk.[29,30] Despite the chronicity and severity of the underlying hepatic lesions, most affected dogs have a short duration of clinical illness, usually less than 2 weeks. Ascites is the most consistent presenting complaint. Profound hypoalbuminemia (mean: 1.7 g/dL) is a consistent laboratory finding. Total serum bilirubin concentration is normal or only mildly increased, supporting that cholestasis is not a key feature of the disorder. Ascitic fluid analysis is consistent with a transudate or modified transudate. On liver biopsy, hepatic lesions are consistent with chronic hepatitis and cirrhosis. Treatment of Cocker Spaniels with chronic hepatitis consists of general supportive therapy for the complications of liver failure. Corticosteroid therapy prior to progression to cirrhosis may

be beneficial. The prognosis is poor and most dogs die within a month of diagnosis.

A recent report described seven American Cocker Spaniels with histologic features resembling lobular dissecting hepatitis.[33] Males and females were equally affected. In contrast to previous reports of hepatitis in Cocker Spaniels, most dogs in this study improved with corticosteroid therapy.[33]

English Springer Spaniel

A preliminary report has described chronic hepatitis in 34 English Springer Spaniels from Norway and the United Kingdom.[39] Female dogs were overrepresented. Copper does not appear to play a role. The prognosis appears to be poor, with most dogs dying 4 to 7 months after diagnosis.[39]

Hepatic Cirrhosis and Fibrosis

Hepatic cirrhosis (end-stage liver disease), is characterized by fibrosis, regenerative nodules that alter liver architecture and intrahepatic (microscopic) PSSs (see Figure 61-19).[2] Hepatic fibrosis is not synonymous with cirrhosis. Cirrhosis is common in dogs but less so in cats.[2] Cirrhosis can result from postnecrotic scarring after acute massive necrosis or from chronic hepatic injury caused by a variety of insults such as infection (e.g., leptospirosis, CAV-1), hepatotoxins (e.g., copper, phenobarbital, aflatoxin), inflammation (chronic hepatitis), or hypoxia. The common denominator is hepatocyte death, which leads to repair by fibrosis and nodular regeneration. When cirrhosis is fully developed, the histologic features of the original inciting injury often are obscured by the cirrhotic changes.

Substantial hepatic fibrosis (without "cirrhosis") can be seen with long-standing extrahepatic biliary obstruction, noninflammatory fibrosis, congenital hepatic fibrosis (a disorder of biliary system development), and congenital portal vein hypoplasia.[2,103-105] A unique form of macronodular cirrhosis, characterized by noninflammatory regenerative hyperplastic nodules and diffuse vacuolar hepatopathy, is seen in dogs with hepatocutaneous syndrome (superficial necrolytic dermatitis).

Hepatic fibrosis was once considered irreversible, but is now recognized to be a dynamic process, which exists in a balance between synthesis and degradation. A better understanding of the underlying mechanisms may provide potential therapeutic targets.[106] The major fibrogenic cell in the liver is the activated HSC (Ito cell, vitamin A–storing cell), which is normally present in the perisinusoidal space.[106,107] Under the influence of fibrogenic stimuli (inflammation and the immune response, oxidative stress, apoptosis, hypoxia, steatosis), the HSC is activated to a myofibroblast, which produces collagen and other extracellular matrix (ECM) constituents.[106] The cytokine, TGF-β, appears to play a central role in fibrogenesis in humans and dogs.[106,108] Perisinusoidal fibrosis decreases the permeability of normal sinusoids, impairing metabolic exchange between hepatocytes and sinusoidal blood further compromising hepatic function.[106] Excess fibrous tissue also limits the ability of vessels and sinusoids to distend, resulting in increased resistance to hepatic blood flow and portal hypertension. When fibrotic septae become vascularized, these microscopic communications (between portal vein or arterial artery and hepatic vein) lead to portosystemic shunting of blood. Reversal of hepatic fibrosis and improvement in liver function can occur, especially if the underlying cause of injury is treated or removed.[106] Examples in human medicine include antiviral drugs for hepatitis B and hepatitis C and prednisone for autoimmune hepatitis. Although fibrosis is potentially reversible, cirrhosis for all practical purposes is not, because of the accompanying architectural changes and PSSs.[109]

Clinical features of cirrhosis in dogs include ascites (portal hypertension, hypoalbuminemia), HE (intrahepatic and extrahepatic portosystemic shunting of blood), and evidence of decreased hepatic function (hypoalbuminemia, increased SBA, coagulopathy, hyperbilirubinemia). Findings on hepatic ultrasonography (small nodular liver, splenomegaly, and acquired PSSs) are suggestive for cirrhosis, but liver biopsy is required for confirmation. Because cirrhosis is essentially irreversible, treatment is mainly supportive, emphasizing measures that control complications of severe generalized liver failure, such as ascites, encephalopathy, gastric ulcers, coagulopathy, and infection (see "Complications of Liver Disease" section). If clinical signs of liver failure are already present, the prognosis is poor.

Prevention of fibrosis, an important long-term goal, is best achieved by early specific treatment directed at the probable cause of injury (e.g., discontinuing a suspect drug, penicillamine for copper-associated liver disease, antiinflammatory drugs for idiopathic chronic hepatitis, surgical relief of extrahepatic biliary obstruction). Many therapeutic agents used for treatment of liver disease, such as penicillamine, prednisone, azathioprine, milk thistle, ursodeoxycholic acid, and zinc, have potential antifibrotic properties,[9] and are discussed in more detail in other chapters. Colchicine, a microtubule assembly inhibitor which increases collagenase activity, has been recommended for treatment of hepatic fibrosis, but its effectiveness in dogs has not been critically evaluated. The recommended dose in dogs is 0.025 to 0.03 mg/kg/day PO. Reported side effects include nausea, vomiting, and diarrhea. Bone marrow toxicity and myoneuropathy have been reported in humans.

Lobular Dissecting Hepatitis

Lobular dissecting hepatitis is a specific histologic form of cirrhosis seen in neonatal or young adult dogs.[2,4,110-112] It is suggested to be a nonspecific response to a variety of hepatic insults.[111] The age at presentation is younger than for dogs with either acute or chronic hepatitis.[4] In 21 affected dogs, the median age was 11 months, with 12 dogs (54%) being 7 months or younger.[4,110] Females appear to be at increased risk.[4,111] Lobular dissecting hepatitis may occur in an isolated dog or in groups of dogs from the same litter or kennel.[111] Standard Poodles may be at increased risk.[110,112] Clinical features are those of advanced hepatic failure and portal hypertension.[111] The most consistent clinical finding is ascites. Liver enzymes are typically increased and hypoalbuminemia and increased SBA concentrations are common.[4,111]

Liver biopsy is required for diagnosis and to differentiate it from other types of chronic hepatitis and cirrhosis. The lesion is characterized histologically by lobular hepatitis: inflammatory cells (lymphocytes, plasma cells, macrophages, and neutrophils) are scattered throughout the hepatic lobule rather than concentrated in periportal regions. Bands of collagen and reticulin fibers dissect around single or small groups of hepatocytes and disrupt hepatic lobular architecture.[111] Copper stains are negative or moderately positive, consistent with secondary copper accumulation.

Specific treatment has not been reported, but general measures for management of chronic liver failure are appropriate.[4] In a small group of dogs with lobular dissecting hepatitis, the mean survival time was approximately 3 months, which was significantly shorter than for dogs with acute or chronic hepatitis.[4]

Hepatic Infections

Infection of the liver is an important cause of hepatic disease in dogs and cats.[113] The liver may be the primary target of infection (e.g., infectious canine hepatitis, bacterial cholangitis, hepatic abscess) or

it may be one of several organ systems involved in a multisystemic disease process such as feline infectious peritonitis (coronavirus), toxoplasmosis, or histoplasmosis (see Table 61-4). Infectious agents can be associated with widespread invasion of organs with a large mononuclear phagocytic component, including the liver, spleen, lymph nodes, and bone marrow, although clinically significant liver disease is uncommon. Liver biopsy can be diagnostically useful for identification of these organisms in infected animals.

Canine Adenovirus 1

Etiology

Infectious canine hepatitis (ICH) caused by CAV-1 has long been recognized as a cause of acute hepatic necrosis in dogs.[114] This virus is genetically and antigenically distinct from CAV-2, a cause of infectious canine respiratory disease. The incidence of clinical disease caused by CAV-1 is now very low because of effective vaccination procedures. Neutralizing antibodies to CAV-1 are also found in mature, unvaccinated dogs, suggesting that natural exposure to the virus is widespread.

Pathophysiology

CAV-1 has a special tropism for vascular endothelial cells and hepatocytes.[114] Dogs with sufficient immunity (neutralizing antibodies >1:500) do not develop clinical signs of disease. Susceptible dogs (titer <1:4) develop widespread centrilobular to panlobular hepatic necrosis, which is often fatal. Distinctive intranuclear inclusions are present in hepatocytes and the endothelium of other tissues. Experimentally, dogs with an intermediate titer (between 1:16 and 1:500) develop chronic hepatitis that can progress to cirrhosis.[115] Whether CAV-1 is a significant cause of chronic hepatitis under natural conditions is unknown. CAV-1 antigen was demonstrated in formalin-fixed liver sections from five of 53 dogs with various naturally occurring hepatic inflammatory lesions, suggesting that CAV-1 may play a role in spontaneous chronic hepatitis.[46] Other attempts to identify CAV-1 in dogs with chronic hepatitis have been negative.[44,47]

Clinical Examination

ICH is seen most commonly in unvaccinated dogs younger than 1 year of age. Clinical signs vary with the stage of disease. Dogs that are peracutely ill do not have clinical evidence of hepatic disease but simply become depressed and moribund, and die within a few hours. Dogs with a more extended clinical course (5 to 7 days) have signs associated with acute hepatic necrosis that include vomiting, diarrhea, and abdominal pain. A hemorrhagic diathesis may occur during the viremic phase and is manifested by epistaxis, petechial or ecchymotic hemorrhages of the skin, or excessive bleeding from venipunctures. Failure of the liver to clear activated clotting factors and impaired hepatic synthesis of clotting factors probably also contributes to development of DIC. Signs of central nervous system (CNS) dysfunction include depression, disorientation, seizures, and coma and have been attributed to HE or nonsuppurative encephalitis.

Common physical examination findings include fever, enlarged tonsils, pharyngitis, laryngitis, cervical lymphadenopathy, and subcutaneous edema of the head, neck, and trunk. Hepatomegaly, abdominal pain, and abdominal effusion can occur. Jaundice is rare but can develop in dogs that survive the acute fulminant stage of ICH. An uncomplicated clinical course lasts approximately 5 to 7 days before recovery begins. Unilateral or, less frequently, bilateral corneal edema and anterior uveitis ("hepatitis blue eye") are complications that may become evident during the recovery period. These ocular complications occur in approximately 20% of naturally infected dogs, and are caused by corneal endothelial damage and antigen–antibody complexes.

Diagnosis

ICH should be suspected in any young, unvaccinated dog with evidence of ALF. ICH must be differentiated from diseases with similar clinical signs, such as canine distemper, parvoviral enteritis, and hepatotoxicity. Abnormalities on the leukogram are common and vary with the clinical stage of infection. During viremia, neutropenia and lymphopenia are often present. Neutropenia is also a common a feature of canine parvovirus, a much more prevalent disease of puppies. Rebound lymphocytosis and neutrophilia occur in the recovery stages of ICH (7 days after infection). Biochemical findings are characteristic of acute hepatic necrosis and include increased serum ALT and ALP activity, and abnormal liver function tests. Hyperbilirubinemia is a less-consistent finding. Hypoglycemia may complicate the terminal stages of the disease. Coagulation parameters are consistent with DIC. Other potential findings include proteinuria secondary to glomerular damage, abdominal fluid consistent with an exudate, and an increase in protein and mononuclear cells in the cerebrospinal fluid.

The clinical diagnosis of ICH is usually suspected on the basis of age, vaccination history, clinical signs, and laboratory findings, and is confirmed by liver biopsy or necropsy findings. Additional diagnostic tests that are used less frequently include serologic testing, virus isolation, and direct immunofluorescence.

Treatment and Prognosis

Therapy for ALF caused by ICH is primarily supportive care and control of complications that frequently occur such as DIC, HE, and hypoglycemia. The prognosis in dogs with ICH depends on the severity of hepatic necrosis and the incidence of serious complications such as DIC. Hepatic regeneration and recovery is possible unless widespread coagulation necrosis destroys entire lobules. ICH can be effectively prevented by vaccination.

Canine Herpesvirus

Canine herpesvirus causes an acute, afebrile, rapidly fatal disease in neonatal puppies (1 to 3 weeks of age).[116] Hepatic necrosis is one manifestation of the widespread multiorgan necrosis and hemorrhage that occurs in this systemic viral infection. Clinical signs include acute onset of depression, diarrhea, failure to suckle, crying, and abdominal pain in previously healthy puppies. Other findings include petechial hemorrhages and vesicles of the mucous membranes. Jaundice is rare. Seizures and loss of consciousness may be present in the terminal stages, and most pups die within 24 hours of onset of clinical signs. Typical gross pathologic findings include focal areas of necrosis and hemorrhage in the liver, kidneys, lungs, and serosal surfaces of the intestines. Microscopically, these areas are characterized by foci of necrosis with occasional intranuclear inclusions.

Neonates are infected by oronasal exposure to the virus in utero or by secretions from an infected bitch or littermates. Neonates are particularly susceptible, possibly because of their low body temperature and immature mechanisms for temperature regulation. The diagnosis of canine herpesvirus is primarily based on the history, physical examination, and pathologic findings. Laboratory findings are inconsistent but include neutrophilia or neutropenia, and increased serum ALT activity.

Treatment of affected puppies is generally unsuccessful because of the acute fulminant nature of the disease. Maintenance of body

temperature (36.7°C to 37.8°C [98°F to 100°F]) may be helpful. Intraperitoneal infusion of 1 to 2 mL of hyperimmune serum obtained from bitches with previously infected litters may reduce mortality rates. Vaccination for herpesvirus infection is not routinely performed because of the low incidence of disease.

Canine Acidophil Cell Hepatitis

Canine acidophil cell hepatitis, which encompasses a spectrum of hepatic lesions ranging from acute and chronic hepatitis to cirrhosis and liver failure, has been reported in Great Britain.[48,114] It is caused by a transmissible agent, suspected to be a virus that is distinct from CAV-1, although a specific virus has never been identified. The disease is experimentally transmissible by serum or liver extracts from affected dogs. In the experimentally induced disease, acute hepatitis can progress to chronic hepatitis in the absence of clinical signs. Episodic increases in serum ALT activity and fever spikes correspond with histologic evidence of acute hepatitis.

The liver is enlarged and friable in the acute stages, and becomes progressively smaller and nodular with chronicity. The most notable histologic feature, regardless of the stage of disease, is the acidophil cell. Acidophil cells are dying hepatocytes with an angular shape, reduced volume, hyperchromatic nucleus, and strongly acidophilic cytoplasm caused by small acidophilic coalescing granules. End-stage hepatic disease is accompanied by typical findings of cirrhosis.

Most dogs with spontaneous disease are presented with signs of chronic hepatic failure. The duration of clinical signs can exceed 1 year. Based on experimental studies, it is speculated that the early mild stages may go unrecognized until advanced hepatic disease and failure is present. Biochemical findings are consistent with hepatic inflammation and necrosis, evidenced as increased serum ALT activity. With advanced disease, severe hepatic dysfunction is noted. The diagnosis requires liver biopsy. Recommendations for specific therapy of acidophil cell hepatitis await further information on the causative agent. Supportive measures should be instituted as needed.

Feline Infectious Peritonitis (Coronavirus)

Feline infectious peritonitis (FIP) is a highly fatal coronaviral infection of both domestic and wild cats. The liver is one of many organs (kidneys, spleen, pancreas, mesenteric lymph nodes, CNS, uveal tract, omentum, serosal surfaces) that can be affected by widespread immune complex vasculitis and granulomatous or pyogranulomatous inflammation (see Table 61-5).[117]

Clinical findings in cats with hepatic involvement are nonspecific and include lethargy, depression, anorexia, dehydration, weight loss, and fever. Jaundice is a common finding. Extrahepatic findings include nodular renomegaly, abdominal mass (lymph node), and ascites or dyspnea (pleural effusion). Ophthalmoscopic examination may detect chorioretinitis or anterior uveitis, which must be differentiated from similar ocular changes seen with the other systemic disorders that involve the liver such as lymphosarcoma, toxoplasmosis, and the systemic mycoses.

Serum hepatic enzyme (ALP and ALT) activities are usually normal or only mildly increased. Mild to moderate increase in serum bilirubin concentration is common. Other findings indicating hepatic dysfunction include bilirubinuria and increased SBA concentrations. Hyperglobulinemia, neutrophilia, and mild to moderate nonregenerative anemia are other laboratory features of FIP. Abdominal and pleural effusions, when present, are usually pyogranulomatous exudates with greater than 3 g/dL protein. Serologic detection of a high coronaviral antibody titer may support a diagnosis of FIP, but is not definitive because of its lack of specificity. Diagnosis of FIP should be supported by cytologic or histologic evidence of pyogranulomatous inflammation. The currently recommended "gold standard" for FIP diagnosis is immunohistochemistry performed on effusions or lesions containing infected macrophages.[117] The prognosis for recovery is poor.

Leptospirosis

Etiology

Canine leptospirosis is caused by *Leptospira interrogans sensu lato*, with at least 10 serovars appearing to have clinical significance in dogs.[118] Serovars *canicola* and *icterohemorrhagica* have been included in vaccines for more than 30 years and the incidence of clinical disease from these serovars has decreased accordingly. An epidemiologic shift in serovars causing clinical disease has since occurred, with increasing reports of disease associated with serovars *grippotyphosa, pomona*, and *bratislava*.[118] It has been suggested that serovars *icterohemorrhagica* and *pomona* are more likely to be associated with hepatic damage.[118] However, other reports have been unable to correlate serogroups with specific clinical features.[119,120] Chronic hepatitis has been associated with serovar *grippotyphosa*[50] and serogroup *australis*.[51] Reports of clinical leptospirosis in cats are rare, although antibodies to several serovars have been demonstrated.[118]

Pathophysiology

Acute renal failure is the most common clinical disease syndrome in dogs with leptospirosis.[121] The liver can also be a target organ and ALF may occur concurrently in 10% to 20% of dogs with acute renal failure or independent from renal involvement.[121] With acute hepatic involvement, the liver is enlarged, friable, and yellow-brown. Tissues are often markedly jaundiced. Microscopic changes in the liver include intrahepatic cholestasis, liver cell dissociation, and nonspecific reactive hepatitis.[3,122] Hepatic necrosis is an uncommon histologic feature. The liver may not show striking changes, presumably because hepatic dysfunction can be caused by a toxin that produces mainly subcellular damage. Organisms can be identified in tissues with a Warthin-Starry stain.

Clinical Examination

Common clinical signs include anorexia, depression, and vomiting. Hepatocellular involvement is suggested by jaundice. Other findings may include arthralgia or myalgia, PU, PD, fever, and dehydration. Widespread petechial and ecchymotic hemorrhages of the mucous membranes, sclera, and skin are caused by thrombocytopenia and DIC. The terminal stages include signs of cardiovascular collapse, shock, coma, and death.

Diagnosis

A diagnosis of leptospirosis should be considered in dogs with acute cholestatic liver disease, especially when accompanied by acute renal failure. Hematologic findings vary with the stage and severity of disease. Leukocytosis and left shift are frequent, but in the early stages of leptospiremia, leukopenia is more likely. Thrombocytopenia can also be seen. Coagulation parameters are normal unless complicated by DIC. Routine serum chemistry and urinalysis findings reflect involvement of the liver or kidney. Serum liver enzyme activity is usually increased with hepatic involvement, and the magnitude of the increase in serum ALP activity is usually greater than that of serum ALT activity owing to intrahepatic cholestasis. Other findings include hyperbilirubinemia, bilirubinuria, and abnormal liver function tests. An increase in BUN or creatinine may result from renal failure or prerenal uremia. The urinalysis is often compatible with acute nephritis with findings of proteinuria and increased

leukocytes, erythrocytes, and granular casts. Increased serum creatine kinase activity may indicate leptospiral-induced muscle damage. Leptospirosis is most easily diagnosed in the clinical setting by demonstration of a fourfold rise in serum antibody titer (microscopic agglutination test) in paired samples taken at initial presentation and 2 to 4 weeks later. The rise in titer indicates recent or active infection and differentiates a titer from previous exposure or previous vaccination.

Treatment and Prognosis

The optimum treatment regimen for leptospirosis is unknown. Traditionally, intravenous ampicillin, 25 mg/kg every 6 hours (with dose reduction in dogs with renal failure), or penicillin G (25,000 to 40,000 units/kg IV q12h), has been used for initial treatment of leptospirosis.[121] Doxycycline (5 mg/kg PO q12h for 2 to 4 weeks), was recommended as followup therapy to eliminate organisms from renal tubules. However, doxycycline, 5 mg/kg orally or intravenously every 12 hours for 2 weeks, appears to be effective in clearing all phases of leptospiral infection and may be the most effective treatment strategy.[121] Management of fluid, electrolyte, and acid–base imbalances is important supportive therapy. The prognosis generally depends on the degree of renal dysfunction and is poor when oliguria develops.

Clostridium piliforme (Tyzzer Disease)

C. *piliforme* (formerly known as *Bacillus piliformis*), a spore-forming Gram-negative bacteria, is a rare cause of multifocal hepatic necrosis and necrotizing ileitis in dogs and cats.[123] The infection is mainly opportunistic in stressed or immunocompromised animals with a predisposing disorder (e.g., canine distemper, feline panleukopenia, feline leukemia) or familial hyperlipoproteinemia in kittens.[123] Clinical signs include an acute onset of anorexia, lethargy, depression, and abdominal discomfort. Jaundice may be observed, especially in cats. These signs rapidly progress to a moribund state; death occurs within 24 to 48 hours. Marked increases in ALT activity may be detected. Histopathology reveals multifocal periportal hepatic necrosis and necrotic ileitis or colitis. Bacilli are best seen with special staining techniques (Warthin-Starry or Giemsa), or methylene blue–stained impression smears of fresh tissue. Organisms appear as large, slender, intracellular filamentous organisms within hepatocytes surrounding areas of necrosis and in intestinal epithelial cells. Routine culture techniques are ineffective for isolation of this organism. The disease is rapidly fatal, and successful therapy has not been reported.[123]

Sepsis and Endotoxemia

Extrahepatic bacterial infection associated with sepsis and endotoxemia is an important cause of acute functional cholestatic hepatopathy. However, morbidity is generally related to the underlying infection and not overt hepatic failure.[113,124] Studies in humans and experimentally in dogs, suggest that endotoxemia and the subsequent release of cytokines induces functional changes that interrupt the transport and excretion of conjugated bilirubin.[113,125] Microscopically, hepatic lesions are often mild and nonspecific. Intrahepatic cholestasis, characterized by bile canalicular plugs and bile pigment accumulation in hepatocytes, is the most consistent finding.[124] A mild periportal lymphocytic infiltrate can be seen, with scattered foci of macrophages or neutrophils, and occasional individual necrotic hepatocytes. Total serum bilirubin concentrations as high as 30 mg/dL can be seen and are disproportionately high compared to the mild to moderate increases in serum ALP activity. Increased serum ALT activity is a less consistent finding. The serum bile acid concentration can be markedly increased (>200 μmol/L).[124]

Extrahepatic bacterial infection–induced hepatic damage should be considered when evidence of cholestatic hepatopathy is found concurrently with extensive bacterial infection in other organ systems (e.g., pyometra, peritonitis) or with extrahepatic disorders likely to be associated with endotoxemia (e.g., parvoviral enteritis). Associated clinical findings that would be compatible with endotoxemic crisis include shock, fever or hypothermia, hypoglycemia, neutrophilia or neutropenia with left shift, toxic changes of the neutrophils, and hyperbilirubinemia that is disproportionately increased in comparison to serum ALP activity.[124] It is important to recognize that jaundice can occur secondary to extrahepatic infection from a diagnostic standpoint, so that the clinician is not misled into considering that the cause is a primary hepatic or biliary disease. In jaundiced patients with evidence of an inflammatory process, key differential diagnoses include acute pancreatitis, extrahepatic bacterial infections, and primary hepatobiliary disorders such as leptospirosis (dogs only), cholangiohepatitis, cholecystitis, ruptured gallbladder mucocele, and hepatic abscesses. Specific therapy for hepatic disease is not usually required, and hepatic damage is reversible with control of sepsis. Morbidity is related to the underlying disease process and not overt hepatic failure.

Hepatotoxicity

Drug and Toxin-Induced Liver Injury

Etiology

Hepatotoxicity can be caused by a variety of drugs (prescription or over-the-counter), herbal and dietary supplements, or biologic toxins or chemicals (see Box 61-1).[17,126,127] The liver is uniquely susceptible to xenobiotic substances because it is directly exposed to them following absorption from the GI tract. The liver is also vulnerable to toxic injury because it plays a central role in the metabolism of many substances. Hepatic metabolism renders lipophilic substances more hydrophilic, which promotes excretion via the urine or bile.[17] The process is controlled by phase I and phase II reactions. Phase I reactions are catalyzed by the cytochrome P450 enzyme systems, which activate or detoxify (oxidize, reduce, or hydrolyze) a drug or toxin. Phase I reactions may lead to generation of unstable chemically reactive intermediates, which can be toxic. Phase II reactions conjugate drugs or metabolites and produce products that are nontoxic. During biotransformation, the liver can either reduce or enhance the toxicity of the parent compound. For example, after carbon tetrachloride ingestion, the liver converts the nontoxic parent compound into toxic metabolites, which subsequently cause severe hepatocellular damage. Genetic polymorphisms of phases I and II enzymes have the potential to influence drug metabolism in the individual animal.

There has been an increased awareness and recognition that drug-induced liver injury can be a significant cause of liver disease in dogs and cats (see Box 61-1). This information has been gained from isolated case reports, retrospective clinical studies, and experimental studies. Unfortunately, for many of these drug reactions, characterization of the clinical and pathologic features are lacking because only small numbers of affected animals have been described, and liver biopsies are not typically obtained when drug withdrawal results in clinical and biochemical improvement. It is possible that drug-induced liver injury is underrecognized in dogs and cats, as in humans, drug-induced hepatic injury accounts for more than 50% of the cases of ALF in the United States, and is the most frequent reason cited for withdrawal of an approved drug from the market.[17]

The Center for Veterinary Medicine of the Food and Drug Administration, Washington, DC, maintains a registry (http://www.fda.gov/AnimalVeterinary/default.htm) for reporting adverse drug reactions in animals. This service has been useful to accumulate data and to alert veterinarians to suspected drug effects, including hepatotoxicity. However, because reporting of adverse drug reactions is voluntary and the information obtained may be incomplete, only subjective trends can be identified. Furthermore, because mild hepatic injury may not be associated with clinical signs, these cases will not be detected unless biochemical testing is performed while the animal is receiving the drug. Evaluating the incidence of drug-induced liver injury in dogs and cats is further clouded by the fact that there are no pathognomonic clinical, laboratory, or biopsy findings to distinguish drug-induced liver injury from other causes of liver disease. Specific drugs that have been reported to cause drug-induced liver injury are listed in Box 61-1.

Herbal and dietary supplements (herbs or other botanicals, nutraceuticals, vitamins, minerals) have the potential to cause hepatotoxicity, similar to drug-induced injury.[127-129] In humans, herbal and dietary supplements are reported to account for 10% of patients with "drug-induced" liver injury.[127] The incidence of hepatotoxicity may be underrecognized, as only a third of humans taking herbal and dietary supplements reported their use of these products to their health care provider.[127] Causality is difficult to prove, because herbal and dietary supplements are often dispensed without medical supervision, FDA oversight of product quality is minimal, multiple active ingredients contribute to product variability, and product contamination with hepatotoxic substances (toxic herbs or heavy metals) may occur.[127-129] Drug interactions between herbal and dietary supplements and prescription medications may also occur, because medicinal plants can have effects on hepatic P450 enzyme systems.[129] The rate of dietary supplement use in dogs and cats appears to be lower than that reported for humans.[130] However, because these products are often marketed as "all natural," pet owners may assume they are safe and underreport their use to the veterinarian. Reports of herbal and dietary supplement hepatic injury in animals are rare. Pennyroyal oil, a volatile oil derived from plants of the Labiatae family (pennyroyal, squaw mint, or mosquito plant) was associated with fatal acute hepatic necrosis in a dog after topical application for use as a flea repellent. Clinical signs occurred within 1 hour after application and included vomiting, diarrhea, hemorrhage, seizures, and death. Other reports include the finding of increased liver enzyme activity in dogs consuming St. John's wort, and increased ALT activity and hypoglycemia after accidental ingestion of high doses of α-lipoic acid in two dogs.[129,131] The paucity of reported hepatic reactions does not necessarily mean herbal and dietary supplements are safe, and the clinician should maintain a high level of suspicion regarding their potential toxicity. Box 61-1 lists the herbal and dietary supplements that have been incriminated as causing hepatic injury in humans with potential relevance to dogs and cats. For more detailed information on hepatic injury and herbal and dietary supplements, additional sources should be consulted.[127-129]

Hepatic injury may also occur after exposure to a wide variety of industrial chemicals, organic solvents, pesticides, heavy metals, and biologic toxins. Most information on chemical hepatotoxins is derived from experimental studies in dogs or extrapolated from information in other species. Very few clinical case reports are available in the veterinary literature. Isolated reports and case series of clinical liver disease in dogs associated with exposure to biologic toxins, such as aflatoxin, *Amanita* mushrooms, blue-green algae, and Cycads (Sago palms) have been published. Selected hepatotoxins are listed in Box 61-1.

Pathophysiology

Hepatic injury caused by drugs, herbal and dietary supplements, biologic toxins, or chemicals can occur via a number of mechanisms, which influence the histologic pattern of disease.[17,20] Although hepatic necrosis is the most common histologic response, drug- and toxin-induced liver injury can also potentially mimic the full spectrum of acquired hepatic disorders, including acute and chronic hepatitis, granulomatous hepatitis, cholestatic hepatopathy, vacuolar hepatopathy (lipid or glycogen accumulation), hepatic fibrosis and cirrhosis, and venoocclusive disease. Hepatic necrosis occurs when covalent binding of a drug or toxin to intracellular proteins disrupts cellular functions, or formation of drug–enzyme adducts stimulates an immunologic response (antibody- or T-cell–mediated cytotoxicity). Hepatic inflammation (typically lymphoplasmacytic, but may be granulomatous or eosinophilic), suggests an underlying immunoallergic mechanism. Intrahepatic cholestasis occurs when drugs or toxins interfere with hepatic transport proteins at the canalicular membrane, which interrupts bile flow. Hepatic lipid accumulation results when direct damage to mitochondria disrupts fatty acid oxidation and energy production, and is the predominant hepatic lesion seen with stanozolol hepatotoxicity in cats,[132] and tetracycline in dogs and cats.[126] Hepatic vacuolation caused by glycogen accumulation occurs in dogs treated with corticosteroids, and typically does not cause significant clinical evidence of hepatic dysfunction. Damage to the sinusoidal epithelium can result in peliosis hepatic or venoocclusive disease.

Mechanisms of drug-induced liver injury (and also injury from herbal and dietary supplements) can be characterized as either intrinsic (predictable) or idiosyncratic (unpredictable) reactions.[126] Intrinsic hepatotoxic reactions are dose-related and occur shortly after a consistent threshold of toxicity is reached. Because intrinsic hepatotoxins predictably damage the liver in an exposed population, they can be experimentally reproduced and studied. Hepatic injury is caused by a direct toxic effect of the parent compound (or a reliably generated toxic metabolite) on vital cell targets. Acetaminophen is an intrinsic hepatotoxin in dogs and cats and is discussed in more detail later in this section. With intrinsic hepatotoxins, lowering the dose, rather than stopping the drug can be tried. In many cases, most such drugs or chemicals, (e.g., carbon tetrachloride, phosphorus, and chloroform), are no longer used for therapeutic purposes once their intrinsic hepatotoxicity is recognized, but accidental exposures could still occur.

In contrast, idiosyncratic hepatotoxic reactions occur at therapeutic doses in only a small number of individuals in the exposed population. These reactions are unpredictable and infrequent; most individuals treated with the drug do not have a reaction, even at high doses. However, toxicity may be more pronounced at higher doses in susceptible individuals.[72,133] Examples of drugs causing idiosyncratic drug-induced liver injury in dogs and cats that have a dose-related effect are phenobarbital, itraconazole, amiodarone, and lomustine (CCNU).[134] Idiosyncratic reactions are characterized by a variable latency period (5 to 90 days, but may be longer for some drugs, such as phenobarbital, CCNU, amiodarone) from initial drug ingestion to recognition of hepatic injury. Because of the infrequent occurrence (approximately 1 in 100,000), the potential for hepatotoxicity may not be recognized in preclinical screening of a new drug, and cannot usually be reproduced in an experimental setting. Individual differences in susceptibility to idiosyncratic drug-induced liver injury may reflect genetic differences in either (a) alternate metabolic pathways by which a drug is converted to different (potentially hepatotoxic) metabolites, (b) the ability of the individual to detoxify the toxic intermediates, (c) an underlying

immunologic or allergic reaction, or (d) an individual's tolerance or ability to "adapt" to hepatocellular injury (mechanisms unknown) with resolution of injury despite continued medication administration.[20,135] Oral medications with substantial hepatic metabolism are more likely to be associated with adverse hepatic events in humans, presumably because of hepatic generation of reactive toxic metabolites.[136] Because of the unpredictability of an idiosyncratic reaction and the low incidence of occurrence, a cause-and-effect relationship is difficult to establish. If an idiosyncratic reaction occurs, the drug must be discontinued or it could result in death of the patient. An idiosyncratic mechanism is suspected for most of the drugs that cause hepatic injury in dogs and cats.

Susceptibility to hepatotoxicity in humans is influenced by a number of factors such as age, sex, nutritional status, and concurrent drugs.[20] The most important factor may be the effect of genetic polymorphisms on hepatic drug metabolism.[20] In humans, preexisting liver disease does not appear to enhance susceptibility to drug-induced liver injury, but impacts the patient's ability to recover.[20] This may be because drug-metabolizing enzyme systems are remarkably preserved in hepatic disease. Similar information on risk factors for hepatotoxicity in dogs and cats has not been determined. However, because many toxic metabolites are normally detoxified by glutathione, some metabolites may become more toxic when hepatic glutathione stores are depleted (e.g., animals with preexisting chronic necroinflammatory and cholestatic liver disease).[126] A breed predisposition has been suggested for Doberman Pinschers (sulfonamides, amiodarone, diethylcarbamazine/oxibendazole) and Labrador Retrievers (carprofen), which may be a reflection of a genetic predisposition.[21,71,73,134,137]

Clinical Examination

The spectrum of drug- and toxin-induced liver injury and, thus, the associated clinical presentation, can vary from subclinical hepatic injury with only increased serum liver enzyme activity, to severe liver damage manifested as ALF, or chronic end-stage liver disease. Acute rather than chronic liver injury is more likely for most of the drugs and toxins listed in Box 61-1. Clinical features often include acute onset of lethargy, anorexia, vomiting, diarrhea, PU, PD, or jaundice in a previously healthy animal, which corresponds to hepatotoxin exposure. ALF (signs of acute liver disease plus HE and coagulopathy) is most likely with drugs or toxins that cause diffuse hepatic necrosis. Drug- and toxin-induced injury is an important diagnostic consideration in dogs and cats presenting for acute hepatitis and hepatic necrosis.

Drugs or toxins also have the potential to cause chronic hepatic disease, if the initial hepatic injury is mild and goes unrecognized, and exposure to the drug or toxin is continued. For example, phenobarbital, CCNU, or chronic aflatoxicosis can cause chronic liver injury in dogs.[70,72,138]

Diagnosis

There are no pathognomonic clinical, laboratory, or biopsy findings that distinguish drug- or toxin-induced liver injury from other causes of liver disease. The diagnosis of drug-induced liver injury often relies on the clinician maintaining a high level of suspicion, and obtaining an accurate and thorough medication history (including prescription and over-the-counter drugs, and herbal and dietary supplements), in every animal with unexplained increases in liver enzyme activity or clinical liver disease. A definitive diagnosis of hepatic injury caused by biologic toxins or chemicals is rarely possible in a clinical setting, unless the owner specifically observes ingestion of a substance that is a known hepatotoxin.

Increased liver enzyme activity is a common finding with hepatotoxicity. Increased ALT activity (more than three times the upper limit of normal, but can be as high as 100 times the upper limit), suggests hepatocellular injury (often necrosis), and is of more concern than an isolated increase in ALP activity (reflecting cholestasis), although mixed patterns commonly occur. Progressive increases in ALT activity or those accompanied by evidence of hepatic dysfunction (hyperbilirubinemia, increased serum bile acids, coagulopathy, hypoglycemia, hyperammonemia, hypoalbuminemia) are more likely to represent serious hepatic injury.

When drug-induced liver injury is suspected, the diagnostic approach is determined by the clinical presentation. If clinical signs are absent or mild, a minimum database consisting of complete history and physical examination, complete blood cell count, serum chemistry, and urinalysis should be performed. If the only abnormality detected is increased liver enzyme activity, and these increases correspond to the recent administration of a drug (especially those listed in Box 61-1), the drug should be discontinued and serum biochemistries should be repeated in 10 to 14 days. In many instances, clinical and biochemical abnormalities resolve after the suspected hepatotoxic drug is discontinued and a liver biopsy is not performed. Further evaluation of the liver, including SBA concentrations, abdominal radiographs, ultrasonography, and liver biopsy, may be warranted if biochemical abnormalities persist, or if initial clinical and biochemical findings suggest hepatic dysfunction.

With suspected drug- or toxin-induced liver disease, a liver biopsy can be helpful to (a) characterize the histologic changes (are they consistent with previously described lesions caused by this particular drug or toxin?), (b) determine the severity (focal or diffuse necrosis?) or reversibility (is cirrhosis present?) of the lesions for prognostic purposes, and (c) rule out known causes of liver disease. Histologic changes secondary to drug- and toxin-induced hepatic injury are nonspecific and similar to those seen with other non–drug-related causes of acute and chronic liver disease.[126] The most common pathophysiologic response is necrosis without inflammation.[126] Hepatic necrosis may be centrilobular (zone 3) or panlobular (Figure 61-20). Centrilobular hepatocytes have an abundance of P450 enzymes, and are preferentially affected in drug-induced hepatotoxicity when P450 metabolism of the parent drug results in toxic metabolites.[8] Drugs or toxins can also cause a variety of other hepatic lesions, such as cholestasis, lipidosis, or mild inflammation. A chronic response to injury is reflected by findings of biliary

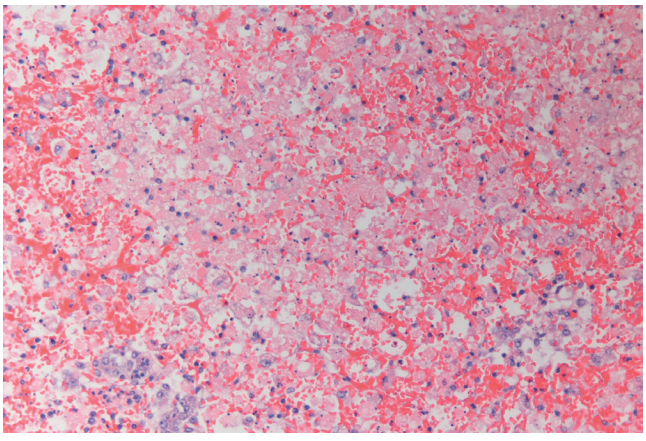

Figure 61-20 Severe panlobular acute hepatic necrosis in a dog with fatal acute liver failure caused by ingestion of *Amanita* mushrooms (20×). (Courtesy of Dr. Paul Stromberg.)

hyperplasia, fibrosis, and cirrhosis. Although individual drugs and drug classes may follow the same pattern, there is often not a consistent reaction for any given drug. For example, hepatic injury in dogs secondary to potentiated sulfonamides may cause hepatic necrosis, primary cholestasis, or marked inflammation.[21] For many of the potentially hepatotoxic drugs listed in Box 61-1, histologic features have not been fully characterized.

It should be emphasized that for most drug-induced disorders, the diagnosis is presumptive and cannot be proved. It can be especially difficult to pinpoint the causative agent when the patient is receiving a combination of drugs. A clinical diagnosis of drug-induced hepatic injury is easier to establish when the hepatotoxicity of the drug has been previously described and the associated clinical and pathologic features have been characterized (see discussion of specific drugs). The diagnosis may be less convincing when the suspected drug has not been previously incriminated as causing liver damage. However, a drug reaction should still be considered, since an idiosyncratic reaction could occur *with any drug*. The clinician should also maintain a level of suspicion regarding the potential for hepatotoxicity in newly marketed drugs that have not yet been used widely in the population, where idiosyncratic reactions are often first detected. The suspected hepatotoxic drug should be discontinued while a complete diagnostic evaluation is pursued for other causes of liver disease, for which a specific treatment might be available.

A diagnosis of drug-induced injury is supported by the following: (a) evidence of liver injury that occurred within the first 3 months of drug therapy (especially if predrug liver enzyme activity was within normal limits); (b) clinical and biochemical improvement when the drug is discontinued; (c) exclusion of other causes of liver disease; (d) reccurrence of hepatic damage after a challenge dose of the same drug (or inadvertent reexposure). It should be emphasized that rechallenge with a suspected hepatotoxic drug is not recommended as a diagnostic consideration, because it is potentially dangerous, especially with a drug that causes acute hepatic necrosis. Rechallenge should only be considered if the association of the drug with hepatic injury is highly questionable and there is no alternative drug available for a significant medical condition. With hypersensitivity or immunologic reactions, the hepatic reaction is more rapid and severe with repeated exposure.[20]

Hepatic injury because of a biologic toxin or chemical is suspected when exposure to a potential hepatotoxin has been documented. A clinical diagnosis of "toxic" hepatic injury is often made when an episode of acute hepatic injury occurs, hepatic biopsy indicates diffuse hepatic degeneration and necrosis, and no other cause for liver disease can be identified. In selected cases, tissues, blood, or food (aflatoxin) samples can be submitted to a toxicology lab to confirm a suspected toxin. Toxin-induced injury should also be considered in the absence of known exposure to toxins, because potential hepatotoxins can be present in contaminated dog food or garbage (aflatoxins), pond water (blue-green algae), and many other unobserved sources.

Treatment

When ingestion of a potential hepatotoxin (e.g., toxic mushrooms, Sago palms) has occurred within the preceding 8 hours, general procedures for GI decontamination are recommended, including induction of emesis or gastric lavage (within first 3 hours), followed by administration of activated charcoal (1 to 3 g/kg).[126,139] Induction of vomiting is contraindicated if the patient is comatose or debilitated in such a way that the gag reflex is diminished, which could predispose to aspiration pneumonia. Whenever possible, the source of toxin exposure should be identified and further exposure prevented. Treatment of drug-induced hepatic disease consists of discontinuing the suspect drug. After a drug is discontinued, clinical (and biochemical) improvement usually occurs within a few weeks, even with chronic drug administration. However, exceptions can occur. For example, in dogs with amiodarone toxicity, liver enzyme elevations can transiently progress despite discontinuation of the drug, and biochemical abnormalities may not resolve for 6 to 8 weeks.[137]

With the exception of NAC for acetaminophen, and silymarin for *Amanita* mushroom toxicity, no specific antidotes are available, and treatment of drug- and toxin-induced liver injury is primarily supportive and symptomatic. However, nonspecific hepatoprotective therapy with antioxidants (vitamin E), glutathione replacement (NAC, SAMe), or milk thistle (silymarin) may be helpful and are discussed in more detail in Chapter 46. Use of NAC (or SAMe) may be beneficial, as glutathione depletion may predispose to hepatotoxicity (e.g., methimazole) or impair metabolism of toxic metabolites to a nontoxic form. NAC has been suggested to be beneficial for treatment of ALF associated with toxicities such as diazepam, methimazole, carprofen, and trimethoprim-sulfa.[9] In addition to treating *Amanita* mushroom hepatotoxicity, silymarin may be beneficial in the treatment of carbon tetrachloride and acetaminophen toxicity.[9,140,141] Corticosteroids are not typically indicated for treatment of drug- and toxin-induced hepatotoxicity.

Prognosis

It is important to consider a drug-induced cause of liver injury because rapid recognition and prompt discontinuation of an hepatotoxic drug can lead to improvement or complete resolution of hepatic disease, depending on the specific drug and the stage of the lesion. When drug- or toxin-induced hepatic injury causes severe or widespread hepatic necrosis, rapid deterioration and death in 3 to 4 days often occur. With less-severe hepatic injury, complete recovery is possible.

Selected Hepatotoxic Drugs

Acetaminophen

Acetaminophen is well known as an intrinsic hepatotoxin in dogs and cats.[142,143] Although acetaminophen is occasionally used as an analgesic in dogs (therapeutic doses up to 15 mg/kg TID), most toxicity occurs because of accidental ingestion of improperly stored medication (dogs) or owner administration without veterinary supervision (dogs and cats).[142] Toxic metabolites of acetaminophen cause oxidative injury to erythrocytes and hepatocytes, resulting in methemoglobinemia, anemia, and hepatic necrosis. In therapeutic doses, acetaminophen is detoxified by a combination of hepatic glucuronidation and sulfation and renal excretion.[144] After acetaminophen overdosage, these pathways become saturated and a greater proportion of acetaminophen is metabolized through the P450 system, leading to production of the toxic metabolite, *N*-acetyl-*p*-benzoquinoneimine (NAPQI). Glutathione detoxifies NAPQI and thus protects hepatic cellular constituents from its direct toxic effect. However, once glutathione levels are depleted by large amounts of NAPQI, centrilobular necrosis occurs. Toxicity occurs in a dose-dependent manner.

There are substantial species differences in both the metabolism of acetaminophen and the toxic manifestations.[142] Cats are uniquely sensitive to acetaminophen because of a deficiency of glucuronyl transferase and limited sulfation capabilities. Clinical signs in cats may develop after administration of as little as 162.5 mg (½ tablet). Signs of methemoglobinemia usually dominate the clinical picture, such as cyanosis, dyspnea, facial edema, depression, hypothermia,

and vomiting. Although increases in serum ALT activity may be detected, centrilobular hepatic necrosis appears to be uncommon. Clinical signs in dogs are more likely when doses exceed 200 mg/kg and can be indicative of methemoglobinemia and/or centrilobular necrosis.[142] Laboratory features include methemoglobinemia, anemia, increased serum ALT activity, and hyperbilirubinemia.

Intravenous NAC is the treatment of choice for acetaminophen toxicity in dogs and cats.[142] NAC increases the synthesis and availability of glutathione, which when conjugated to NAPQI, decreases toxicity. For maximum effectiveness, NAC should be given within 12 hours of acetaminophen exposure; however, there may still be a benefit if given 36 to 80 hours after exposure. NAC (10% solution) is diluted 1:2 or more with saline and given intravenously through a nonpyrogenic 0.25 µm filter at an initial dose of 140 mg/kg over a 20- to 30-minute period. A maintenance dose of 70 mg/kg is given IV or orally every 6 hours for seven treatments. SAMe also serves as a glutathione source and has been shown to have protective effects against acetaminophen-induced oxidative stress on the erythrocytes in cats and dogs.[143,145] In an experimental study in cats, silymarin (30 mg/kg PO) was as effective as NAC for treatment of acetaminophen toxicity when given up to 4 hours after exposure.[140] Vitamin C (30 mg/kg IV q6h) may be helpful in the treatment of acetaminophen toxicity because of its antioxidant effects. Cimetidine (5 mg/kg IV q8h) is also recommended as adjunctive therapy in the early stages (first 16 hours) because it inhibits hepatic P450 enzymes and decreases NAPQI formation.

Amiodarone

The antiarrhythmic drug amiodarone is associated with a reversible hepatotoxicity in dogs.[134,137] Doberman Pinschers may be at increased risk.[134] Toxicity, which appears to be at least partially dose related (doses of 400 mg/day), was identified in 45% of Doberman Pinschers treated with amiodarone in one clinical series.[134] Clinical signs (anorexia, lethargy, vomiting, diarrhea) and biochemical abnormalities (increased ALT and ALP activity ± hyperbilirubinemia) developed 6 days to 8 months after initiation of therapy. Liver biopsy in one dog revealed multifocal hepatocellular necrosis with mild lipidosis and lymphoplasmacytic inflammation.[137] Clinical improvement usually occurs within a few days of stopping the drug, but liver enzyme elevations may not return to normal for 3 months.[134] Transient progression of enzyme abnormalities despite discontinuing the drug has also been noted, which may reflect the long half-life of amiodarone causing a delay in systemic elimination.[137] Biochemical changes precede clinical signs, so monitoring of liver enzymes at least monthly is recommended.

Azole Antifungals

The azole antifungal drugs ketoconazole and itraconazole (and rarely fluconazole) are associated with increased liver enzyme activity and icterus in dogs and cats.[146] Hepatotoxicity is more likely with ketoconazole than with itraconazole. Cats are more sensitive to the hepatotoxic effects than are dogs, but considerable individual variation occurs. Histologic findings are poorly characterized but include bile duct proliferation and infiltration of mononuclear cells. Transient mild subclinical elevations of liver enzymes (ALT and ALP activity) are common, and do not necessarily require a change in therapy. A clinically significant hepatic reaction is suggested by ALT activity that exceeds two to three times the upper limit of normal, especially when accompanied by clinical signs of anorexia and vomiting. Drug therapy should be stopped for 1 to 2 weeks until appetite and liver enzymes return to normal. A rapid recovery usually occurs, and treatment can be restarted at a lower dose (50% of previous dose or given as alternate-day therapy), with careful monitoring of liver parameters every 2 weeks.[146] Hepatotoxicity appears to be at least partly dose related, as dogs receiving higher daily doses of itraconazole (10 mg/kg) are more likely to be affected. Icterus and evidence of hepatic dysfunction suggests a more serious, potentially fatal hepatopathy, requiring discontinuation of medication and symptomatic and supportive care. It is recommended that liver enzymes be monitored on a monthly basis in all animals receiving ketoconazole or itraconazole.

Azathioprine

Azathioprine, a purine analogue commonly used for treatment of immune-mediated disorders in dogs, is commonly listed as a potential hepatotoxin.[126] However, few clinical details regarding the hepatotoxic reaction are available. In a clinical study of 12 dogs with atopic dermatitis treated with azathioprine (2.2 mg/kg daily for 8 weeks) as a single agent, an increase in ALT or ALP activity was noted in the first 2 weeks in 10 (83%) of the dogs.[147] Three dogs had clinical signs suggestive of liver disease, which resolved uneventfully when azathioprine was discontinued. In an experimental study of dogs given azathioprine at a dose of 2 to 4 mg/kg PO daily for 40 days, all dogs had increased liver enzyme activity (ALT >> ALP) within 2 to 7 days of initiating therapy.[148] Values peaked within the first 2 weeks and then declined, but not to normal, despite continued medication administration. Hyperbilirubinemia was absent. Liver biopsies in most dogs revealed centrilobular degeneration and necrosis, with intrahepatic cholestasis but no inflammation.[148] These findings raise the possibility that azathioprine may be an intrinsic (dose related) hepatotoxin in dogs, with possible adaptive tolerance to liver injury. It should be noted that doses used in this study exceeded current clinical recommendations of 1 to 2 mg/kg daily or every other day for maintenance therapy.

Carprofen and Other Nonsteroidal Antiinflammatory Drugs

Hepatotoxicity is considered a class characteristic of NSAIDs, despite the fact that there are many different chemical classes of NSAIDs, and no consistent mechanism of liver injury.[149] With the exception of aspirin, which is an intrinsic (dose-related) hepatotoxin, the mechanism with other NSAIDs is believed to be idiosyncratic (either immune or as a consequence of toxic metabolites).[149,150] Toxicity does not appear to be related to prostaglandin inhibition like the renal or GI side effects.[149] Preexisting hepatic disease has not been shown to be a risk factor for NSAID-induced liver injury.[150] All NSAIDs have the potential to cause idiosyncratic hepatotoxicity in dogs, but hepatic reactions appear to be rare.[150] Carprofen has specifically been reported as a cause of drug-induced liver injury in dogs.[73] Labrador Retrievers were overrepresented in the series, but it is not clear whether this is a true breed predisposition.[150] Clinical signs (anorexia, lethargy, vomiting, PU/PD) occurred within the first 4 weeks of therapy and icterus was a common finding on physical examination. Biochemical evaluation revealed marked increases in liver enzymes (ALT activity usually exceeded ALP activity) and hyperbilirubinemia. Hepatic biopsy findings revealed multifocal to diffuse hepatic necrosis, mild to moderate lymphocytic–plasmacytic inflammation, secondary cholestasis, and variable biliary hyperplasia and bridging fibrosis.[73] Concurrent renal toxicity (glucosuria without hyperglycemia, proteinuria, granular casts) also was noted in some dogs. Most dogs recovered with discontinuation of carprofen and appropriate supportive care, although some dogs died of ALF. General hepatoprotective therapy with SAMe or Silybin has been recommended, although the benefits are unproven. NAC has been suggested for ancillary treatment when carprofen causes ALF.[128]

Early recognition of hepatotoxicity (including periodic monitoring of liver enzymes during the first 3 months) and discontinuation of drug therapy provides the best opportunity for full recovery. Whether dogs with previous carprofen hepatotoxicity can be safely switched to another NSAID without experiencing a hepatic reaction is unknown.

Diazepam

Oral diazepam has been incriminated as a cause of acute idiosyncratic fatal hepatic necrosis in cats.[151,152] Intravenous diazepam, and oral oxazepam, clonazepam, and zolazepam also have been implicated.[126,152] Onset of signs occurs within 5 to 13 days of initiating therapy. Clinical signs and biochemical evaluation are consistent with acute hepatic necrosis and liver failure. Most cats die within 15 days of initial administration of the drug. If treatment of a cat with oral diazepam is unavoidable, liver enzymes should be checked before and within 5 days after starting therapy. If liver enzymes are increased, the drug should be discontinued and symptomatic therapy should be started. Ancillary treatment of ALF with NAC may be beneficial.[9]

Glucocorticoids

Glucocorticoid therapy in dogs is commonly associated with increased serum ALP activity and development of a reversible vacuolar ("steroid") hepatopathy as a result of hepatic glycogen accumulation. These hepatic effects can be seen with virtually any glucocorticoid preparation (including topical ophthalmic and otic preparations) and are influenced by drug preparation (e.g., repositol versus short-acting), dose, duration of therapy, and individual dog susceptibility. In contrast, cats are quite resistant to the hepatic effects of glucocorticoids and only rarely develop these hepatic changes.[153] Increased serum ALP activity can occur within 3 days after initiating glucocorticoid therapy in dogs and is often striking (up to 64 times normal). Glucocorticoids are associated with the induction of a specific corticosteroid-induced isoenzyme of ALP, which may account for 60% to 100% of the total ALP activity.[154] In contrast, serum ALT activity is often normal or only mildly increased. In most dogs, glucocorticoids do not cause significant hepatic dysfunction or clinically relevant hepatic disease and biochemical tests reflecting hepatic function (serum bilirubin, albumin, glucose, blood ammonia concentration, and coagulation tests) are typically normal. Serum bile acid concentrations are normal or only mildly increased (<60 mmol/L). Hepatic glycogen accumulation causes hepatomegaly (which can be detected on abdominal radiographs), and diffuse or multifocal increases in hepatic echogenicity (detected on ultrasonography). The hepatic effects of glucocorticoids are reversible after drug withdrawal. The length of time required for complete resolution is unpredictable, varying from weeks to months.

Lomustine

CCNU [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea] is an oral nitrosourea alkylating agent that is used for chemotherapy of lymphoma, mast cell tumor, histiocytic sarcoma, and brain tumors in dogs. Idiosyncratic dose-related hepatotoxicity was described in 11 of 179 (6.1%) dogs given an oral dose of CCNU (50 to 110 mg/m^2), with a dosing interval of 3 to 6 weeks.[72] The median time to detection of hepatic disease (from the last dose of CCNU) was 11 weeks and ranged from 2 to 49 weeks. Delay in onset was noted, with an inverse relationship between the size of dose, and length of time before abnormal serum ALT was detected.[72] A cumulative dose effect was suspected. Clinical findings of hepatotoxicity included decreased appetite, weight loss, PU/PD, vomiting, ascites, and pleural effusion. Ascites was due to a combination of hypoalbuminemia and portal hypertension. Common biochemical abnormalities included increased liver enzyme activity (ALT, AST, ALP, GGT) and hypoalbuminemia. Other less-consistent findings included hyperbilirubinemia, hypercholesterolemia, and increased serum bile acid concentrations. Glucosuria (without hyperglycemia) and renal failure were noted in some dogs, possibly attributable to CCNU renal toxicity.[72] Liver biopsy findings were nonspecific (hemosiderin-laden Kupffer cells, hepatocellular vacuolization, mild to moderate periportal inflammation, and fibrosis) but suggested chronicity. The majority of affected dogs died from progressive chronic liver disease.

In a recent study, routine monitoring of ALT activity prior to each subsequent dose of CCNU suggested that subclinical elevations of ALT activity (greater than five times the upper limit of normal) are common.[155] Thirty-two of 109 dogs (29%) had increased ALT activity, which developed most commonly after one to three doses of CCNU.[155] Increases in ALT activity were not associated with cumulative dose. The lower incidence of clinical hepatotoxicity in this study (3 of 109 or 2.8%) versus the Kristal study (11 of 179 or 6.1%), was attributed to prompt cessation of CCNU treatment in dogs with significant increases in ALT activity.[155] However, it was noted that chronic administration of CCNU could be associated with chronic irreversible hepatopathy, in the absence of a significant ALT elevation. The mechanism of hepatotoxicity is suspected to be a result of generation of toxic intermediate metabolites (e.g., isocyanates, diazonium hydroxide), and depletion of glutathione may play a role.[126] Preliminary results of a clinical study using Denamarin (SAMe and sylibin; Nutramax Labs, Lancaster, SC) for prevention of CCNU hepatotoxicity, suggested that dogs receiving Denamarin had less-severe liver enzyme elevations.[156]

Methimazole

Methimazole, an antithyroid drug, is associated with hepatic injury in cats with hyperthyroidism. Clinical findings include anorexia, vomiting, lethargy, jaundice, markedly increased serum liver enzyme activity, and hyperbilirubinemia that usually occurs within the first month of therapy.[126] Histologic lesions have not been fully characterized, although biopsy findings in one cat revealed hepatic degeneration and necrosis. Clinical signs resolve within a week of discontinuing therapy but biochemical resolution may take up to 45 days. Reduced hepatic glutathione concentrations, which have been documented in other species with hyperthyroidism, may predispose to hepatic injury.[126] Treatment with SAMe may be beneficial.

Phenobarbital, Primidone, Phenytoin

Phenobarbital is associated with chronic hepatic disease and cirrhosis in dogs.[70] Most dogs have been treated with phenobarbital for more than a year before the liver disease is apparent. The mechanism of hepatic injury is not known but higher doses, higher blood levels (>40 µg/mL), and long duration appear to be important risk factors.[70] Clinical signs in dogs reflect chronic liver disease and include sedation, ataxia, anorexia, weight loss, weakness, ascites, jaundice, coagulopathy, and encephalopathy. Phenobarbital-induced hepatic injury should be suspected in any dog with a history of chronic phenobarbital therapy and clinical and biochemical evidence of hepatic dysfunction.

Routine biochemical screening (every 4 to 6 months) of dogs on long-term phenobarbital therapy is recommended for early detection of hepatic injury. However, mild liver enzyme elevations (especially ALP) are commonly seen in dogs treated with phenobarbital, who do not have clinical or histologic evidence of significant liver

disease.[157] Potential indicators of clinically significant liver injury include increases in ALT and ALP activity that exceed five times the upper limit of normal; ALT activity that exceeds ALP activity; any elevation in AST activity; or enzyme elevations accompanied by evidence of hepatic dysfunction (hyperbilirubinemia, hypoalbuminemia, hypocholesterolemia, increased SBA). Hepatic cirrhosis associated with chronic phenobarbital therapy is characterized grossly by a small, nodular liver and histologically by bridging portal fibrosis, nodular regeneration, biliary hyperplasia, and mild inflammation (see Figure 61-19).[70] These lesions are by no means pathognomonic for phenobarbital-induced hepatic damage; however, in the absence of other known causes of hepatic damage, circumstantial evidence would support drug therapy as a likely cause. Chronic phenobarbital therapy also is associated with superficial necrolytic dermatitis (hepatocutaneous syndrome) in dogs.[158] Liver biopsy changes were typical of those seen with hepatocutaneous syndrome (marked vacuolar change and parenchymal collapse), which are distinct from the characteristic chronic hepatitis and cirrhosis as described above.[158]

Phenobarbital should be decreased or discontinued if possible in dogs with biochemical and histologic evidence of hepatic disease. In dogs with phenobarbital-associated toxicosis, clinical, biochemical, and histologic improvement can occur if the drug is discontinued or used at a reduced dosage prior to severe, end-stage liver disease. Improvement in clinical signs can be noted within days to weeks of decreasing serum phenobarbital levels. Primidone also is associated with chronic liver disease in dogs, likely as a consequence of metabolism of primidone to phenobarbital. Phenytoin can cause acute or chronic hepatitis in dogs, as well as jaundice and death. The risk of hepatotoxicity is increased with combination therapy of phenobarbital, primidone, and phenytoin.[126]

Sulfonamides

Potentiated sulfonamides (trimethoprim-sulfadiazine, trimethoprim-sulfamethoxazole, and ormetoprim-sulfadimethoxine) are associated with the acute idiosyncratic drug-induced liver injury in dogs.[21] Trimethoprim-sulfadiazine was implicated in over 20% of hepatic drug reactions in dogs that were reported to the Center for Veterinary Medicine between 1988 and 1990.[71] Doberman Pinschers are suggested to be at risk for development of polyarthropathy from sulfonamide hypersensitivity, but not necessarily the idiosyncratic hepatic reaction.[159] Onset of clinical signs occurs within 5 to 36 days (mean: 12 days) from starting the drug.[159] Previous exposure to sulfonamides is not required. Doses of potentiated sulfonamides are generally higher in dogs who develop the idiosyncratic hepatic reaction, as compared with other systemic manifestations of sulfonamide hypersensitivity (thrombocytopenia, fever, polyarthropathy, other).[159] Biochemical findings include increased liver enzyme activity (ALT > ALP) and hyperbilirubinemia. Liver biopsy usually reveals marked hepatic necrosis; however, cholestasis and marked lymphocytic–plasmacytic inflammation also have been described.[21] The pathogenesis of the idiosyncratic hepatic reaction is unclear. Dogs in general may be at increased risk for sulfonamide reactions because they lack genes that express the *N*-acetylation enzymes, which is a major metabolic pathway of detoxification of sulfonamides in humans.[21] However, this does not explain individual risks among dogs. Hepatotoxicity may be a result of P450 oxidation of sulfonamides to reactive metabolites, such as hydroxylamine and a nitroso metabolite, which may be associated with hapten formation, T-cell proliferation, or direct cytotoxicity.[21] Impaired detoxification of reactive metabolites via a deficiency in glutathione, cysteine, and ascorbate, may play a role, and theoretically supports the use of glutathione precursors (NAC, SAMe) and vitamin C in treatment.[21] Dogs with hepatopathy are less likely to recover (46%) than are dogs with nonhepatic manifestations of sulfonamide hypersensitivity (89%).[159]

Tetracycline and Doxycycline

Tetracycline can predispose to hepatic lipid accumulation because it inhibits protein synthesis and interferes with hepatic secretion of triglyceride-rich lipoproteins.[126] However, it does not appear that these hepatic effects are clinically significant in most dogs and cats, although clinically significant idiosyncratic hepatic injury has been reported. Increased liver enzyme activity was a common finding in dogs treated with doxycycline (increased ALT activity in 39.4%; increased ALP activity in 36.4%), but the clinical significance remains to be determined.[160]

Other Hepatotoxins

Aflatoxicosis

Aflatoxins are metabolites, produced primarily from strains of the saprophytic fungus, *Aspergillus*, which cause toxic hepatitis in dogs and many other species.[138,161] Exposure in dogs may occur through the inadvertent use of aflatoxin-contaminated corn or peanut meal during the commercial production of dog food, or after ingestion of homemade pet foods, moldy garbage, or improperly stored dog food.[161] Dogs are relatively susceptible to aflatoxins and the liver is the target organ. Clinical cases of aflatoxicosis in cats have not been reported. Aflatoxin B1 is most commonly implicated in hepatotoxicity and toxic effects are seen when levels exceed 60 µg/kg of food.[161] Aflatoxin B1 is readily absorbed from the GI tract and undergoes hepatic metabolism by cytochrome P450 enzymes, to a toxic intermediate (aflatoxin B1 8,9-epoxide), which binds to essential molecules within the cell, leading to hepatocyte necrosis and decreased protein synthesis. Detoxification of aflatoxin B1 8,9-epoxide occurs by conjugation to glutathione.

Depending on the amount consumed, dogs may present with acute, subacute, or chronic liver disease. High-dose exposure is associated with acute hepatic failure, jaundice, DIC, and death. Repeated exposure to low doses can lead to chronic liver disease and cirrhosis. In 2005, an outbreak of aflatoxicosis occurred in at least 100 dogs, as a result of eating a commercially available dog food manufactured with aflatoxin-contaminated corn.[138] Severity of clinical signs varied significantly among dogs. Some dogs died suddenly without preexisting signs of illness. Other dogs were presented with signs of anorexia, lethargy, vomiting, jaundice, diarrhea (including melena and hematochezia), abdominal effusion, HE, and evidence of a bleeding disorder. Common biochemical features included increased liver enzyme activity (especially ALT), hyperbilirubinemia, electrolyte disturbances, hypoalbuminemia, hypocholesterolemia, and prolonged clotting times. Reduced plasma antithrombin III and protein C activities and hypocholesterolemia were suggested to be the most sensitive biomarkers of aflatoxin ingestion in dogs with minimal clinical signs, possibly reflecting an early effect of aflatoxin on biosynthesis of certain proteins and cholesterol.[138]

In dogs with acute or subacute aflatoxicosis, the liver is enlarged and pale yellow with histologic features of diffuse hepatic vacuolation (lipid accumulation), scattered individual hepatocyte necrosis, biliary hyperplasia, and modest inflammation. Collapse of zone 3 hepatocytes around the central vein, associated with perivenular inflammation, may explain clinical features of portal hypertension. With chronic low-level exposure, findings include a small liver with regenerative nodules, acquired PSS, and histologic evidence of marked biliary hyperplasia and periportal fibrosis.

Aflatoxicosis associated with consumption of a commercially manufactured pet food product should be suspected when there is a geographic or temporal cluster of cases in a household, kennel, or region. The history may reveal recent changes in diet or feeding from a new bag. It should be noted that some dogs may consume contaminated food for weeks to months before signs develop. Definitive diagnosis of aflatoxicosis is based on chemical detection of increased levels of aflatoxin (>60 µg/kg) in the food. When aflatoxicosis is suspected, the owner should be advised to retain 1 kg of food in an airtight zippered plastic bag (or four cans of food), for laboratory testing. It is also recommended to save packaging information, including product and date code, to help identify contaminated lots of food. If a sample of food is no longer available, serum or liver samples can be submitted for testing for aflatoxin M1 (aflatoxin metabolite), although usefulness may be limited because of the rapid metabolism and excretion of aflatoxin. Detection of aflatoxin M1 in urine is only useful if the dog is still consuming the contaminated diet, as levels fall below detectable levels within 48 hours.

There is no specific antidote for aflatoxicosis and treatment consists of symptomatic and supportive management of liver failure. Nonspecific hepatoprotective therapy with antioxidants (vitamin E), glutathione replacement (NAC, SAMe), milk thistle (silymarin), and L-carnitine have been recommended.[138] The prognosis is guarded if clinical signs of aflatoxicosis are present, with a reported mortality rate of 64% in a series of 72 dogs that consumed aflatoxin-contaminated dog food.[138] Dogs that survive acute liver injury have the potential to develop chronic liver disease. Consequently, monitoring of liver function is recommended in recovering dogs and treatment with thiol donors such as SAMe for 2 months has been empirically recommended.[138]

Amanita *Mushrooms*

Amanita phalloides (and other varieties such as *Amanita verna* and *Amanita bisporigera*), are poisonous mushrooms found throughout North America that can cause acute hepatic necrosis in dogs and cats.[162,163] Toxicity is attributed to extremely toxic cyclopeptide toxins called amanitins. Ingestion of two *A. phalloides* mushrooms can be lethal to an adult dog.[163] Clinical signs occur within 6 to 24 hours after ingestion and are characterized initially by GI signs such as vomiting, bloody diarrhea, and abdominal pain. The late phase (36 to 84 hours after exposure) is characterized by ALF (hemorrhage, marked hypoglycemia, HE, and terminal coma) caused by severe massive hepatic necrosis (see Figure 61-20). Toxin-induced renal tubular necrosis may also result in renal failure.[162] Biochemical features reflect severe hepatic injury and include increased liver enzyme activity (ALT exceeds ALP), refractory hypoglycemia, and hyperbilirubinemia. Diagnosis is usually made based on positive identification of the suspect mushroom, evidence of its ingestion, and consistent clinical features. Mushroom pieces in gastric contents can confirm exposure, but are difficult to identify. Accurate mushroom identification requires consultation with an experienced mycologist. The suspect mushrooms should be wrapped in paper towels and stored in a paper (not plastic) bag.[162] Definitive confirmation can be established by detecting amanitins in liver or kidney tissue (or serum and urine samples collected during the GI phase) by liquid chromatography-mass spectrometry, through the California Animal Health and Food Safety Laboratory.[163]

GI decontamination procedures are recommended as soon as possible after exposure, as described in the "Treatment" section of "Drug and Toxin-Induced Liver Injury". Symptomatic and supportive treatment for ALF is indicated, including close monitoring and treatment of marked hypoglycemia. Silymarin is believed to reduce hepatocyte uptake of amanitins and has been shown to be protective against experimental *A. phalloides* liver damage in Beagles, when given at a dose of 50 mg/kg IV twice, at 5 and 25 hours after exposure.[141] However, an intravenous form of silymarin is not currently available for clinical use in the United States. Experimental studies suggest that penicillin G may also reduce hepatic uptake of amanitins, even several hours after ingestions.[162] IV NAC may be beneficial as described for treatment of acetaminophen toxicity. Overall mortality rate with *Amanita* mushroom toxicity is high.

Blue-Green Algae

Ingestion of toxin-producing blue-green algae (*Microcystis aeruginosa*) is a rare cause of hepatotoxicity and ALF in dogs.[164] Algae proliferate in shallow, stagnant water, especially in hot, dry weather. Dead or dying algae form a thick blue-green scum on the water's surface, and release the toxic principle, microcystins. Toxicity is caused by ingestion of algae-contaminated water. Signs occur rapidly (within 1 hour of ingestion) and include vomiting, diarrhea, and lethargy, followed by progressive tachypnea and dyspnea, icterus, and coma. Biochemical features reflect hepatocellular injury with increased ALT and AST activity (that typically exceed increases in ALP activity), and hyperbilirubinemia. However, profound or protracted increases in ALT activity may not be detected, because microcystins can interfere with transaminase biosynthesis.[7] Hepatic lesions consist of massive hepatic necrosis of the centrilobular to midzonal hepatocytes. Treatment is symptomatic and supportive. Oxidative injury may play a role,[164] which suggests that glutathione supplementation (NAC or SAMe) may be of benefit. The prognosis is guarded.

Cycads (Sago Palms)

Cycads (Sago palms) are native to tropical and subtropical regions, and are used as houseplants and in residential landscaping. Concentrations of cycasin, the primary toxin in cycads, are highest in the seeds and roots, but present in all parts of the plant.[165] Ingestion of as few as one to two seeds can be fatal in dogs. Following ingestion, cycasin is metabolized by GI bacteria to its active compound, methylazoxymethanol, which causes GI and hepatic toxicity in dogs.[165] Most dogs that ingest cycads develop GI signs, including vomiting, diarrhea, and abdominal pain. Neurologic signs (weakness, ataxia, depression, proprioceptive deficits, seizures, coma) are also common, but it is not clear if they are a result of a neurotoxin or HE.[165] Onset of clinical signs ranges from 15 minutes to 3 days and may last from 24 hours to 9 days.[165] Hepatic injury is suggested by findings of progressive depression, icterus, HE, and excessive bleeding accompanied by increased liver enzyme activity, hyperbilirubinemia, hypoglycemia, and hypoabuminemia.[166,167] Centrilobular hepatic necrosis is found on liver biopsy.[166] No specific treatment is available. Mortality has been reported to vary between 32% and 58%.[165,167]

Xylitol

Xylitol, a 5-carbon sugar alcohol used as a sugar substitute, is associated with hypoglycemia and hepatic necrosis in dogs.[168,169] Xylitol is safe in humans and is commonly used in sugar-free gum and other oral care products, and is available as a granulated powder for baking. Xylitol was first introduced into the United States in 2002, and since that time, reports of toxicity to the ASPCA Animal Poison Control Center have increased from two dogs in 2002 to 2512 dogs in 2008.[168] Ingestion of more than 0.1 g/kg in dogs is associated with a rapid, severe, increase in blood insulin, which results in signs of hypoglycemia within 30 to 60 minutes of ingestion. When amounts exceed 0.5 g/kg, ALF may occur within 9 to

72 hours.[169] ALF is not necessarily preceded by early signs of hypoglycemia. The mechanism of hepatotoxicity is unknown, but has been speculated to be caused by cellular depletion of adenosine triphosphate resulting in hepatocellular necrosis, or production of reactive oxygen species causing oxidative injury.[169] In addition to signs of hypoglycemia, dogs with ALF may have vomiting, icterus, and evidence of excess bleeding. Biochemical findings include markedly increased ALT and AST activity, mild to moderate increased ALP activity, hyperbilirubinemia, hypoglycemia, hyperphosphatemia, prolonged PT and aPTT, and thrombocytopenia.

If ingestion occurred within the last few hours, induction of emesis is recommended (unless showing signs of hypoglycemia). Activated charcoal may be of limited value in adsorbing xylitol, but is still recommended if large amounts have been ingested.[168] Treatment recommendations include hospitalization for observation and monitoring, dextrose supplementation for control of hypoglycemia, and symptomatic and supportive care for complications of liver failure. Hepatoprotective therapy with NAC, SAMe, and silymarin may be beneficial. The prognosis is good for recovery in dogs with uncomplicated hypoglycemia and guarded to poor for dogs in liver failure. However, survival after liver failure does not necessarily correlate with amount of xylitol ingested.[169]

VASCULAR DISORDERS

Viktor Szatmari

Vascular hepatic diseases include congenital and acquired disorders of the *portal vein*. *Congenital* anomalies result from (a) hypoplasia or aplasia of the portal vein, (b) macroscopic communications between the portal vein and a systemic vein, or (c) between the portal vein and an artery. *Acquired* diseases result from conditions that increase the hydrostatic pressure in the portal vein (i.e., portal hypertension).

Macroscopic venous connections between the portal and systemic venous systems result in *portosystemic shunting* (i.e., blood flows from the portal to the systemic veins) via a *congenital portosystemic shunt* (CPSS) or *acquired portosystemic collaterals* (APSC).

Clinical Manifestations

Congenital Portosystemic Shunt

Neurologic Signs

Hepatoencephalopathy (HE) is a reversible central neurologic manifestation of hepatic insufficiency.[1] CPSS causes chronic HE. The following grades of chronic HE are recognized[2]: *grade 1*—depression, behavior changes; *grade 2*—ataxia, compulsive pacing, circling, hypersalivation, head pressing, blindness; *grade 3*—stupor and seizures; and *grade 4*—coma.

Chronic HE is characterized by periods of severe (grades 2 to 3) signs (lasting usually several hours to a few days alternating with longer periods (days to weeks) of no or mild (grade 1) symptoms.[2] Periods of cortical blindness are accompanied by apparent mydriasis. Signs of HE may be triggered by ingestion of protein-rich meals.

Polyuria and Polydipsia

PD means excessive fluid intake (in dogs >100 mL/kg body weight/24 h and in cats >50 mL/kg body weight/24 h). PU may be more difficult to diagnose than PD, as pet owners cannot readily measure urine volume. Repeated low specific gravity of morning urine (<1.025 in dogs and <1.030 in cats) is compatible with PU.

Lower Urinary Tract Disease

Ammonium biurate uroliths are formed most of the time in the bladder (and rarely in the renal pelvis) and can cause dysuria, urethral obstruction in males, and seldom uroabdomen as a result of chronic inflammation and subsequent devitalization of the bladder wall.[3]

Ammonium biurate crystalluria is not pathognomonic for portosystemic shunting, and it may occasionally occur in normal dogs and cats, or be found in certain breeds, such as Dalmatians, because of an inborn error of metabolism.

Anesthesia Intolerance

Prolonged recovery from anesthesia or sedation may occur in seemingly normal dogs and cats that have CPSS. The liver plays a crucial role in the detoxification process of toxins and drugs, including anesthetics. Patients with CPSS or APSC have insufficient functional hepatic mass for detoxification. In addition, dogs with portosystemic shunting have increased endogenous benzodiazepine and GABAergic activities.[4] Therefore administration of diazepam or barbiturate to these animals may have prolonged and exaggerated effects (see "Pathogenesis" section).

Fever

Recurrent fever together with resultant depression and anorexia can be the only clinical manifestation of CPSS in some dogs.[5] Portosystemic shunting should be excluded in dogs experiencing recurrent fever.

Episodic Weakness

This rare manifestation has only been reported in a few dog.[6] Its pathogenesis is not understood.

Hypercortisolism (Pseudo-Cushing Disease)

Elderly dogs with extrahepatic CPSS may present with characteristic signs of hypercortisolism (e.g., PU/PD, polyphagia, thin skin, symmetric alopecia, muscle wasting, pot belly) as primary complaints. These are unusual clinical manifestations of CPSS.

Stunted Growth

Although small body size is observed in cats with CPSS, it infrequently occurs in dogs.

No Symptoms

Many dogs with extrahepatic CPSS show no or only nonspecific clinical signs throughout their lives and the shunt is detected as a coincidental finding at the time of necropsy. It is not understood why certain dogs become clinically ill and others do not with the same type of portal vein anomaly.

No Jaundice, No Ascites, No Hemorrhagic Diathesis

CPSS never cause icterus, ascites, or spontaneous hemorrhages.

In Cats

Portal vein disorders are much less common in cats than in dogs. Most cats are younger than 6 months of age when the first signs appear.[7] Cats with CPSS are often of smaller stature and have unkempt hair coat. Episodic salivation and/or central neurologic signs, for example, compulsive pacing or seizures, as manifestations of HE are the most typical presenting complains.[8,9]

Portal Hypertension

Clinical signs can develop at any age and arise from the presence of increased hydrostatic portal venous pressure, portosystemic

shunting via APSC, and the underlying disease that led to portal hypertension.

Signs from Acquired Portosystemic Collaterals

Clinical signs of portosystemic shunting are similar, regardless whether it is congenital or acquired in origin (see "Clinical Manifestations" section).

Ascites

Accumulation of a large amount of pure transudate or modified transudate (clear, straw-colored, or slightly turbid blood-tinged fluid) in the abdominal cavity is commonly detected with physical examination and abdominocentesis. The absence of free abdominal fluid does not exclude portal hypertension.

Signs from Acquired Underlying Diseases

Depending on the etiology and the anatomic location of the underlying disease that led to the development of portal hypertension various signs may be seen such as jaundice, periodic vomiting, and anorexia.

Signs from Congenital Underlying Diseases

Jaundice and hemorrhagic diathesis are absent in congenital vascular disorders. Congenital arterioportal fistula causes transudative ascites in puppies between 2 and 6 months of age.[10]

Primary hypoplasia of the portal vein (PHPV), also described as "noncirrhotic portal hypertension,"[11] may result in ascites, vague GI signs, and HE, or may be entirely subclinical.[12,13]

When the PHPV is not severe enough to cause portal hypertension, clinical signs may not be obvious. This mild form of PHPV is also known as *hepatic microvascular dysplasia*[14] and may be detected serendipitously if plasma bile acid concentrations are measured for unrelated reasons.

In Cats

Portal hypertension is rare and is not typically associated with ascites in cats.[15] Portosystemic shunting because of CPSS or APSC tend to cause similar signs, for example, periodic salivation and seizures, as manifestations of chronic HE.

The most common acquired disease causing intrahepatic portal hypertension is biliary cirrhosis caused by chronic bile duct obstruction. Jaundice and acholic feces are typical findings in extrahepatic cholestasis.

Pathogenesis

Hepatic Blood Flow

Normal Hepatic Circulation

The liver receives its blood supply from the portal vein (approximately 75%) and the hepatic artery (approximately 25%). With diversion of portal venous blood flow, a compensatory increase of the hepatic arterial flow occurs. Arterial vasodilation is mediated by adenosine from energy-depleted hepatocytes.

The portal vein transports blood from the spleen and the GI tract to the liver.[16] The smallest portal venous and hepatic arterial branches terminate in the capillary system of the liver, the so-called hepatic sinusoids. A large amount of plasma is filtered through the fenestrated walls of the sinusoids to the space of Disse. From here the filtered plasma is taken by lymphatic vessels to the systemic venous system. The remaining sinusoidal blood is then collected by the hepatic veins, which enter the caudal vena cava. Normally no macroscopic connections exist between major vessel systems or their tributaries.

Portosystemic Shunting

Single or multiple portosystemic venous connections allow the portal venous blood to flow directly to the systemic venous system without first flowing through the hepatic sinusoids. This toxin-rich blood will be delivered to all cells of the body through the following route: gut > portal vein > shunt > systemic vein > right heart > lungs > left heart > arteries. A connection through a single, or rarely double, large-bore vein without the presence of portal hypertension is considered to be a CPSS. Single or multiple connections in the presence of portal hypertension are APSC.

Whenever a macroscopic venous connection is present between the portal vein (or one of its tributaries) and a systemic vein, the blood will flow from the portal vein to the systemic vein, because the pressure in the portal vein is higher (8 to 10 mm Hg) than that in the systemic veins (0-5 mm Hg).[8]

Portal Hypertension

Normal portal venous pressure is approximately 8 to 10 mm Hg (10 to 13 cm H_2O). Increased pressure in the portal venous system results in portal hypertension. Anatomically, portal hypertension can be classified[17] as (a) prehepatic (i.e., portal vein), (b) intrahepatic or (c) posthepatic (i.e., hepatic veins, thoracic caudal vena cava, or heart).

Posthepatic (Postsinusoidal) Portal Hypertension. All cardiac diseases that result in right-sided congestive heart failure cause posthepatic portal hypertension. These diseases include the (a) congenital or acquired severe insufficiency of the tricuspid valve (e.g., dysplasia, myxomatous degeneration, annulus dilation caused by dilated or arrhythmogenic cardiomyopathy, pulmonary hypertension of various etiologies), (b) pericardial diseases (pericardial tamponade caused by idiopathic or neoplastic effusion or constrictive pericarditis), (c) various congenital anomalies such as cor triatriatum dexter, atrial septum defect, tricuspid or pulmonic stenosis, (d) intracardiac tumors affecting the right heart, and (e) caval syndrome (caused by *Dirofilaria immitis* heart worms). Kinking or compression of the thoracic caudal vena cava (by diaphragmatic hernia or a mass) occurs rarely. Compression, stenosis or thrombosis of the hepatic veins (the so-called Budd-Chiari syndrome) does not occur in dogs and cats.[13,13A]

In posthepatic portal hypertension the portal and caval pressures increase equally. Therefore no APSC develop, and the blood ammonia concentration remains within reference range. The high postsinusoidal hydrostatic pressure causes a large amount of protein-rich hepatic lymph to be filtered through the hepatic capsule into the abdominal cavity causing accumulation of a modified transudate. Modified transudate has high protein content because the fenestrated endothelial cells of the hepatic sinusoids keep only 10% to 20% of the plasma proteins in the capillary lumen. Marked generalized hepatomegaly and dilated jugular and hepatic veins as well as caudal vena cava are hallmarks of postsinusoidal portal hypertension.

Intrahepatic Portal Hypertension. Chronic *acquired* parenchymal liver diseases lead to hepatocyte necrosis and subsequent collagen deposition. The resultant disorganization of the hepatic architecture and contraction of the connective tissue results in obstruction of the intraparenchymal vessels. The underlying disease processes include viral, bacterial, protozoal, immune-mediated, or copper-associated chronic hepatitis; toxic, drug-induced, or idiosyncratic liver damage; lobular dissecting hepatitis; and chronic bile duct

obstruction. The resultant increase in portal pressure may cause development of APSC as well as ascites.

Modified transudate may accumulate in the abdominal cavity when the disease process predominantly obstructs the postsinusoidal hepatic venules. However, when the disease process affects predominantly the intrahepatic portal vein branches (presinusoidal portal hypertension) pure transudate will accumulate in the abdomen.

Primary hypoplasia of the portal vein is caused by insufficient development of the intrahepatic portal venous branches, the left portal vein branch, or the whole portal venous system.[12,18]

When the hypoplasia is severe enough to cause intra- or prehepatic portal hypertension, APSC develop. This condition is also known as "noncirrhotic portal hypertension."

If hypoplasia is not severe enough to cause portal hypertension, clinical signs will not develop. Because no portosystemic shunting is present, the results of rectal ammonia tolerance test (ATT), portal scintigraphy, and portography are all normal (see "Differential Diagnosis" section). If either macroscopic or microscopic portosystemic communications is present, hyperammonemia or abnormal ATT should also be present, but this has never been documented. The only abnormality that could be detected in these dogs is elevation of serum bile acids levels.[14] The most plausible explanation for this is that the hepatic clearance of bile acids by the hypoperfused liver is probably less effective than that of ammonia. The disease can only be diagnosed by histopathologic examination of liver biopsy specimens. This condition also has been described as "hepatic microvascular dysplasia." Although the terms *noncirrhotic portal hypertension* and *hepatic microvascular dysplasia* have been used to describe this syndrome, both have been replaced by the term PHPV.[13] The occurrence of PHPV in the cat is very rare and poorly documented.

The most common congenital cause of portal hypertension in cats is congenital hepatic fibrosis as a part of polycystic kidney and liver disease complex.[19] This disease may cause clinical signs with or without the presence of macroscopic hepatic or renal cysts.[20]

Prehepatic Portal Hypertension. Narrowing of the portal vein lumen may be caused by (a) extravascular compression by a tumor, enlarged lymph node, cyst, abscess, or hematoma, (b) idiopathic circumscribed stenosis,[21] or (c) intravascular obstruction by a thrombus or parasites, all of which can lead to portal hypertension.[22] Portal vein thrombosis is always secondary either to neoplasia, or to a systemic disorder that causes hypercoagulability such as nephrotic syndrome, immune-mediated hemolytic anemia, hypercortisolism, acute pancreatitis, peritonitis, or sepsis.[23] Parasites in the portal vein also may occur, for example, *Heterobilharzia americana* in North America[24] and *Schistosoma japonicum* in East Asia.[13]

Depending on the degree of portal vein occlusion, increased portal pressure with APSC and ascites may develop. As the hydrostatic pressure in the sinusoids does not increase (in contrast to posthepatic portal hypertension), the resultant ascitic fluid will have a low protein content. The narrowed lumen of the portal vein causes reduced portal flow to the liver, resulting in reduction in the size of the liver. Portal vein thrombosis itself rarely causes clinical signs and is usually a coincidental finding.

Congenital Arterioportal Fistula. Single or multiple macroscopic connections between the main portal vein and hepatic artery are rare congenital anomalies. The high arterial blood pressure causes (a) severe dilation and tortuosity of the affected portal venous branch, (b) hepatofugal flow in the portal vein (i.e., flow away from the liver), (c) development of portal hypertension and APSC, and (d) histologically detectable arterialization of the portal venous wall. The hepatofugal portal flow prevents the splanchnic venous blood from entering the liver and causes the development of alternative pathways and often times accumulation of pure transudate in the abdomen. Interestingly, PHPV always accompanies congenital arterioportal fistulas both in dogs and cats.[5,10,25] When a CPSS and arterioportal fistulas are concomitantly present, APSC and ascites do not develop.[25] Arterioportal fistula can be extrahepatic in cats.

Acquired Portosystemic Collaterals. In healthy mammals, multiple nonfunctional venous connections may exist between the portal and systemic veins. These virtual communications become functional when their lumen becomes sufficiently widened. Gradual dilation takes place when sustained increase of the portal pressure takes place without the simultaneous increase of caval pressure. The resultant APSC are multiple, usually thin, tortuous veins with species-specific anatomical locations; however, large-bore veins may also develop. These latter cases should not be misinterpreted as simultaneous CPSS and APSC. In the presence of an existing CPSS, no APSC would develop even if hepatic cirrhosis develops as there *is* an already existing portosystemic connection that is able to drain 100% of the portal blood without allowing the development of portal hypertension.[26] Splenorenal collaterals are consistently present in almost every dog with APSC.[10,27] These APSC drain the portal venous blood via the splenic vein through acquired connections to the left gonadal vein. The left gonadal vein enters the left renal vein, which later empties into the caudal vena cava. These splenorenal APSC are thought to prevent the spleen from undergoing congestion,[17] which is why splenomegaly is not a feature of canine pre- and intrahepatic portal hypertensive disorders.[10]

Consequences of Hepatic Hypoperfusion for the Liver

Shunting of the portal blood is not only detrimental for the brain, but for the liver as well. Normal hepatic development and function requires sufficient amount of portal venous perfusion of the hepatic sinusoids. Increased arterial perfusion is unable to compensate for portal hypoperfusion. Regardless of whether the insufficient portal venous perfusion is caused by CPSS or by prehepatic portal hypertension, the result is the same: reduced hepatic mass and function. In both cases, histopathologic evaluation of the liver shows stereotypical reaction: small or invisible portal branches, increased number of arterioles and sometimes bile ductules in the portal tracts, hepatocellular atrophy, and periportal sinusoidal dilation.[13] This *secondary* portal vein hypoplasia is reversible and is histologically indistinguishable from PHPV. Because primary and secondary portal vein hypoplasia show identical microscopic features, histopathologic evaluation of liver biopsy specimens is unable to diagnose simultaneous PHPV and CPSS.

Hepatic Encephalopathy

Glycine and GABA are the most important inhibitory neurotransmitters, whereas glutamate and dopamine are the most abundant excitatory neurotransmitters in the brain. In HE, a net increase in inhibitory transmission occurs.[28] This results from upregulation of GABA receptors and downregulation of dopamine receptors.[29] Activation of GABA receptors causes opening of chloride channels, which leads to hyperpolarization of the postsynaptic membrane. In the presence of GABA, benzodiazepines increase the frequency, whereas barbiturates increase the duration of the chloride channel opening.[30] The cause of increased GABAergic tone in HE is thought to be gut derived,[4] but GABA may also be formed in the neurons from glutamate.[28] Endogenous benzodiazepines are proven to be

produced in the intestines of dogs with CPSS.[4] Their source is not quite clear: they could arise from the diet, intestinal flora, or by endogenous modification of inactive gut precursors.[4]

In all forms of portosystemic shunting the concentration of aromatic amino acids are increased in the systemic circulation whereas the concentration of branched-chain amino acids is decreased. High aromatic amino acid concentration results from impaired hepatic clearance and increased production caused by muscular breakdown exacerbated by hyperglucagonemia.[28,31] The decreased concentration of branched-chain amino acids results from their increased utilization for gluconeogenesis.[28,32] This amino acid imbalance may result in the development of "false" neurotransmitters (e.g., octopamine) in the brain, which contribute to the development of HE.[33,34] These "false" neurotransmitters have a fraction of dopamine's excitatory effect on dopamine receptors. Drugs with dopaminergic effect, such as bromocriptine, may improve signs of HE.[35-37]

Intestinal toxins such as ammonia and bacteria are normally inactivated by hepatocytes and hepatic macrophages (Kupffer cells), respectively, so that toxin- and bacterium-free blood can enter the systemic circulation. In patients with portosystemic shunting the majority of the portal venous blood bypasses the liver, allowing the toxin- and bacterium-rich blood to enter the systemic circulation. Ammonia is a neurotoxin and can contribute to the clinical signs of HE.[38] Other toxins that are believed to play a role in HE are endogenous benzodiazepines, GABA, tryptophan, glutamine, serotonin, mercaptans, indoles, and skatoles.[4,34,39]

Hyperammonemia also leads to impaired glial function through increased intracellular glutamine concentration.[38] Glutamine is made in the glial cells through incorporation of ammonia into glutamate by glutamine synthetase.[28] Chronically increased glutamine concentrations cause swelling of the glial cells resulting in so-called Alzheimer II type degeneration of astroglias.[40,41] Glial dysfunction contributes to the development of HE.[40,42] During hyperammonemia, the reserve capacity of the astrocytic glutamine synthetase is exceeded, and ammonia can enter the neurons.[42] In the neurons ammonia inhibits glutaminase, an enzyme that converts glutamine to glutamate. Because glutamate is an excitatory neurotransmitter, its reduced level contributes to the development of HE.[28]

Portosystemic shunting or an atrophic liver alone is insufficient to allow HE to develop; HE can only develop when they are both simultaneously present.[28] The reserve capacity of the liver ensures that even in advanced chronic parenchymal liver diseases, HE does not develop in the absence of portosystemic shunting. Alkalosis and hypokalemia can worsen the signs of HE. In alkalosis the $NH_3 + H^+ = NH_4^+$ reaction shifts to the left, causing the more lipophilic ammonia to enter the cells of the CNS. During hypokalemia, potassium ions (K^+) move from the cells to the extracellular space in exchange for H^+, which later results in extracellular alkalosis and intracellular acidosis. Intracellular acidosis causes NH_4^+ trapping within the cells. This might explain why the blood concentration of ammonia is not closely related to the severity of the clinical signs of HE in individual cases.

Polyuria and Polydipsia

Several mechanisms contribute to the development of PU/PD in portosystemic shunting.

Hypoosmotic Renal Medulla

High concentrations of sodium and urea in the renal medullary interstitium are essential for the production of concentrated urine. These create a high osmotic gradient between the renal tubular lumen and interstitium, which is necessary for water reabsorption. When the liver receives little portal venous blood, an insufficient amount of substrate (i.e., ammonia) is available for hepatic urea production. This theoretically results not only in a low plasma urea concentration, but also in a lower renal medullary urea concentration, which impairs renal concentrating ability and causes PU.

Hypercortisolism

Increased basal plasma concentrations of ACTH and cortisol as well as increased urinary cortisol-to-creatinine ratios are invariably present in dogs with portosystemic shunting.[43-46] Cortisol interferes with the action of arginine-vasopressin at the renal tubule, causing a *nephrogenic-type* diabetes insipidus.[47] Hypersecretion of ACTH (and α-melanocyte stimulating hormone [α-MSH]) has been shown to arise predominantly from the intermediate lobe of the pituitary.[43,48] The hormone secretion of this lobe is regulated by tonic dopaminergic inhibition. ACTH-hypersecretion can be explained by the production of "false" neurotransmitters (e.g., octopamine), whose effect is about one-fiftieth that of dopamine on the dopamine receptors.[35]

Diabetes Insipidus

Central diabetes insipidus also contributes to PU in dogs with HE. Impaired release of arginine-vasopressin from the posterior lobe of the pituitary is caused by a reduced magnitude of response and a highly increased threshold to increased plasma osmolality.[45] Release of arginine-vasopressin is inhibited by the GABA inhibitory neurotransmitter system, whose activity is increased in HE.[29,45]

Pseudohyperaldosteronism

Cortisol and aldosterone have similar affinities to bind aldosterone receptors. However, cortisol is normally inactivated by 11β-hydroxysteroid dehydrogenase in tissues where aldosterone action is required.[49] High serum bile acids concentrations inhibit this enzyme, and cortisol can bind to aldosterone receptors resulting in increased mineralocorticoid effect.[45] Plasma cortisol concentrations are 10-fold those of aldosterone, causing constant and inappropriate pseudohyperaldosteronism. The resultant sodium retention causes secondary water retention and subsequent PU by pressure diuresis. Hypokalemia caused by hyperaldosteronism also contributes to PU[50,51] according to the following mechanism. The presence of aquaporin-2 channels in the renal collecting ducts' cell membranes is necessary for water reabsorption. Intracellular signaling pathways through cyclic adenosine monophosphate regulate the insertion of these channels. Hypokalemia decreases the sensitivity of cyclic adenosine monophosphate to arginine-vasopressin, which results in decreased insertion of aquaporin-2 channels into the cell membrane.[50] This leads to nephrogenic diabetes insipidus and PU.

Renomegaly and Renal Hyperfunction

Congenital portal venous anomalies in dogs are typically associated with enlarged kidney volume. Increased renal gluconeogenesis as a compensation of insufficient hepatic gluconeogenesis may cause the kidneys to enlarge.[52] In addition, increased systemic circulating growth factor concentrations released from the pancreas may play a role in this increased volume.[53] Normally, these growth factors act only in the liver, as they do not reach the systemic circulation in high concentrations.

Primary Polydipsia

Behavior changes and abnormalities in the thirst center due to HE may contribute to PD; however this is difficult to prove in individual patients.

Urolithiasis

Hyperammonemia results in a higher filtered load and urinary concentration of ammonia. High urinary concentration of uric acid results from decreased hepatic conversion of uric acid to allantoin because of reduced hepatic mass and function.

Fever

The portal vein carries bacteria and endotoxins from the intestines, which are normally phagocytized by the Kupffer cells. Shunting of portal blood permits these bacteria and/or endotoxins to enter the systemic circulation causing bacteremia and/or endotoxemia, and subsequent fever.[5] Systemic antibiotics may result in temporary resolution of clinical signs, however give no definitive cure.

Etiologies

Congenital Portosystemic Shunt

If a CPSS occurs within hepatic parenchyma it is called *intrahepatic*.[54,55] Intrahepatic CPSS arise either from the left or right portal branches. A left divisional intrahepatic CPSS results from the failure of postnatal closure of the embryological *ductus venosus*.[56,57] The intrahepatic CPSS arising from the right portal branch and all *extrahepatic* CPSS are thought to be developmental anomalies with poorly understood etiology. In dogs the anatomy of the shunting vessel is fairly consistent: intrahepatic CPSS may be left, right, or central divisional, whereas extrahepatic shunts drain the portal venous blood via the splenic or the right gastric vein to the caudal vena cava or the azygos vein.[15] The course of the shunting vein in the cat is much more variable.[15,58]

The inherited nature of the disease has been established in several breeds including the Irish Wolfhound and the Yorkshire and Cairn Terriers.[59-61]

Portal Hypertension

PHPV is a congenital anomaly of unknown etiology. Although arterioportal fistulas may develop as a result of neoplasm or trauma (e.g., shot wound or liver biopsy), only the congenital form has been reported in dogs and cats. Congenital hepatic fibrosis caused by polycystic kidney disease (PKD) is a genetic disease.[19,62]

Differential Diagnosis

Hyperammonemia

High fasting venous blood ammonia concentrations together with high fasting serum bile acid concentrations are very specific and sensitive tests for diagnosing portosystemic shunting.[63]

Urea Cycle Enzyme Deficiency

Reduced activity of one or more enzymes of the urea cycle may result in elevated blood ammonia concentration.[10,64] Serum bile acids levels should remain within reference range in patients with urea cycle enzyme deficiencies. Normal hepatic scintigraphy and liver histopathology results should confirm the absence of portosystemic shunting. Certain metabolites (e.g., citrulline) and enzyme activities (e.g., argininosuccinic acid synthetase) can be measured in urine and liver biopsy specimens, respectively, to establish a definitive diagnosis. The condition is rare in dogs. One suspected feline case has been reported.[65]

Irish Wolfhound Puppies

Healthy Irish Wolfhounds commonly have moderate hyperammonemia (<120 µmol/L) at the age of 6 to 7 weeks.[66] This is thought to result from reduced enzyme activity of the urea cycle. As no clinical signs are present, no treatment is required. The condition resolves spontaneously with age because these pups develop enhanced incorporation of ammonia into glutamine.

Uroabdomen

Peritoneal absorption of ammonia-containing urine can cause hyperammonemia, however in such a patient the clinical signs of acute uremia predominate. The presence of urease-producing bacteria (e.g., *Staphylococci*) are necessary to split urinary urea to ammonia.[67]

Fulminant Hepatic Failure

Ingestion of blue algae or certain mushrooms (e.g., *A. phalloides*) results in peracute insufficiency of hepatic function. These animals develop hepatic coma and die shortly thereafter. Hyperammonemia, DIC, and icterus are present in most affected patients.

Arginine Deficiency in Cats

Arginine is an essential amino acid in the cat. In anorectic cats hyperammonemia may develop along with hepatic lipidosis because of the insufficient amount of hepatic arginine needed for urea synthesis in the urea cycle.[68] Thus, in contrast to adult dogs, hyperammonemia in cats can occur without portosystemic shunting.

Miscellaneous Disorders

Methylmalonic acidemia associated with cobalamin deficiency in dogs,[69a] a cat[69] and a suspected "transient hyperammonemic syndrome" in a German Shepherd dog[70] have been reported as extraordinarily rare causes of hyperammonemia.

Sampling or Laboratory Error

If blood sampling and sample processing are performed inappropriately hyperammonia may be erroneously diagnosed.

High Serum Bile Acid Concentrations

High bile acids concentration may result not only from portosystemic shunting, but also from any primary or secondary hepatic diseases associated with intra- or extrahepatic cholestasis.[71] Therefore, the presence of high serum bile acids concentrations is very sensitive, but not a specific indicator of portal venous disorders.[63]

Ascites

The simultaneous presence of hyperammonemia and a large amount of pure or modified transudate in the abdominal cavity indicates the presence of severe pre- or intrahepatic portal hypertension with APSC. Biochemical and cytologic analysis of the peritoneal effusion, ultrasonography and central venous pressure measurement may be useful in identifying the source of ascites.[17] Suspected intrahepatic causes should be further evaluated by histopathologic examination of liver tissue.

Central Neurologic Signs

In any animal that is presented with central neurologic signs, portosystemic shunting (along with other metabolic encephalopathies, e.g., hypoglycemia or electrolyte imbalance) should be investigated with appropriate blood tests.

Diagnosis

No single finding is pathognomonic for diagnosing vascular liver disorders. Therefore, a combination of history, physical

examination, laboratory tests, diagnostic imaging results and histopathology of liver biopsy specimens are often required to establish a specific diagnosis.

Signalment

Intrahepatic CPSS tend to cause clinical signs between 2 months and 1 year of age in large-breed dogs. Bernese mountain dogs, Irish Wolfhounds, Hovawarts, and Retrievers are predisposed. Australian Cattle dogs and male dogs more likely have right-sided than left-sided intrahepatic CPSS,[72] whereas Irish Wolfhounds tend to have left-sided intrahepatic CPSS. Intrahepatic CPSS in small-breed dogs are very rare. Extrahepatic CPSS usually occur in small breeds and can cause clinical signs at any age.[73] The most commonly affected breeds are Maltese dogs, Miniature Schnauzers, Dachshund, Yorkshire Terrier, Jack Russell Terrier, and Cairn Terrier. No clear sex predisposition is known.

History

The waxing–waning nature of central neurologic signs is suggestive of chronic HE. Symptoms may improve spontaneously.

Physical Examination

Congenital Portosystemic Shunts

Physical examination often fails to detect abnormalities. Occasionally an enlarged left kidney is palpated. The presence of ascites detectable with physical examination should exclude CPSS. Some authors suggest that copper-colored iris is a typical finding in cats with CPSS; however, no reports exist about its positive or negative predictive value.

Portal Hypertension

Dogs with intra- or prehepatic portal hypertension may have ascites. Jaundice is absent in congenital portal hypertensive disorders, but may be present in acquired parenchymal liver diseases.

Laboratory Examination

Blood Ammonia Concentration

Fasting (12-hour) venous hyperammonemia is a very specific and sensitive indicator of portosystemic shunting. As a single test, increased blood ammonia level is usually sufficient to diagnose portosystemic shunting. Because ammonia is formed from amino groups of proteins and urea also in the collection tube, the blood sample (in an ethylenediaminetetraacetic acid tube) should be placed directly on ice after sampling and the measurement should be performed within 30 minutes. Because contamination of the sample with airborne ammonia may cause false-positive results, samples should be taken using a closed system (needle on a syringe or directly into a Vacutainer). Hemolysis may artificially increase ammonia concentration because erythrocytes contain two to three times more ammonia than the plasma. Measurement of ammonia should be performed in a clean location, where the air is not contaminated with ammonia from congested urine or cigarette smoke. A number of analyzers offer the possibility of ammonia measurement; however some of them are unreliable.[46] Measuring *arterial* ammonia concentration provides no additional value.

Ammonia Tolerance Test

If venous ammonia concentration is within the reference range or only slightly elevated, rectal ATT is the cheapest and quickest test to exclude or justify the presence of portosystemic shunting in patients with a suggestive history. Most of these "suspicious" animals also have high fasting plasma bile acids levels.[74] A 5% ammonium chloride (NH_4Cl) solution is administered at a dose of 2 mL/kg fifteen cm into the descending colon using a soft feeding tube.[75] Venous blood ammonia is measured before and 20 and 40 minutes thereafter. In the presence of portosystemic shunting the ammonia concentration will increase at least twofold by the 20- and/or 40-minute sampling times. Normal ATT result excludes portosystemic shunting (Figure 61-21). The degree of increment is a semiquantitative measure of the degree of portosystemic shunting. It should be noted that rectal administration of NH_4Cl solution may cause transient irritation of the colonic mucosa during the first 10 minutes. Rectal ATT is a safe procedure, with signs of HE rarely occurring. ATT should not be performed in patients with very high (>150 µmol/L) blood ammonia levels.

Postprandial measurement of ammonia is thought to decrease the possibility of iatrogenic hyperammonemic HE; however the increase of blood ammonia concentration after feeding takes longer and its peak time point is poorly predictable.[76]

Serum Bile Acid Concentrations: Pre- and Postprandial

Markedly increased preprandial (i.e., 12-hour fasting) bile acids concentrations are often found with portosystemic shunting as a result of interrupted enterohepatic circulation of the bile acids.[77,78] High 12-hour fasting plasma bile acid concentration is a sensitive, but nonspecific indicator of portosystemic shunting. Specificity can be increased by simultaneous measurement of venous ammonia. As plasma bile acids concentrations are invariably high in icteric animals, the presence of APSC can only be justified or excluded by measuring blood ammonia concentration or performing an ATT.

In case of a normal 12-hour fasting bile acids concentration (<15 µmol/L), measuring an increased postprandial plasma bile acids concentration (>25 µmol/L) 2 hours after a meal increases the sensitivity, but not the specificity of the bile acids test in diagnosing portosystemic shunting.[79] Meal-induced gallbladder contraction causes an endogenous bile acid load, which will be absorbed in the ileum and will substantially increase the plasma bile acids concentration when portosystemic shunting is present. An abnormal postprandial increase of bile acids occurs also in cholestatic liver diseases.

Miscellaneous Biochemical Alterations

In patients with portosystemic shunting hypoalbuminemia, hypoproteinemia, hypocholesterolemia, and low plasma urea concentration may be found as a result of their reduced hepatic synthesis. Hypoglycemia may be seen as a result of reduced hepatic glyconeogenesis and glycogen storage. Low creatinine concentration reflects the increased glomerular filtration rate.[53] None of these biochemical changes is specific for portosystemic shunting; furthermore, many of these findings may be present in healthy pups. The cause of mild increase of plasma ALT and alkaline phosphatase activities is not fully understood.

Hematologic Alterations

Microcytosis with or without mild nonregenerative anemia and leukocytosis may accompany portosystemic shunting. Relative iron deficiency is thought to cause the microcytosis[80-82] and bacteremia may induce the leukocytosis.

Coagulation Abnormalities

Although aPTT is often moderately prolonged in dogs with CPSS, no spontaneous bleeding tendency or hemorrhage occurs.[83,84] Hemoabdomen is a possible postoperative complication after surgical attenuation of a CPSS. Acquired parenchymal hepatic diseases

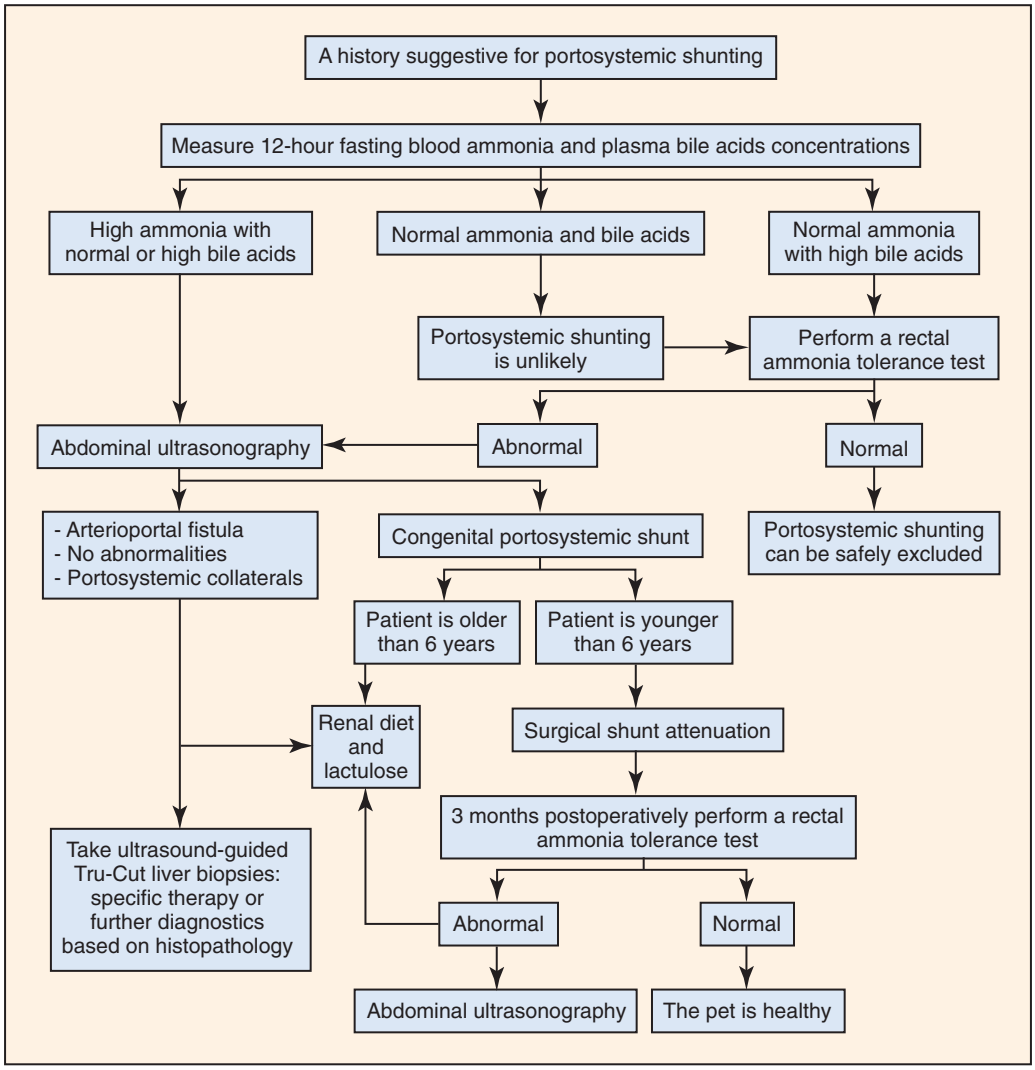

Figure 61-21 Algorithm of diagnostic steps for vascular liver disorders.

may lead to spontaneous hemorrhages because of the presence of DIC.

Diagnostic Imaging

Radiography

Plain radiographs give very limited additional information (such as small liver, possibly enlarged kidneys, and sometimes visible uroliths) to the history and laboratory results, so they don't have to be part of the routine diagnostic workup of vascular liver diseases. Pure ammonium biurate uroliths are radiolucent.

Ultrasonography

Abdominal ultrasonography is the first choice of diagnostic imaging modality, once the presence of portosystemic shunting has been established by fasting hyperammonemia or abnormal rectal ATT (see Figure 61-21). The major advantage is that ultrasonography requires no sedation or anesthesia. Its drawback is in the operator dependence. By using a systematic examination protocol one can accurately differentiate CPSS from APSC, and intrahepatic from extrahepatic CPSS, as well as readily diagnose arterioportal fistulas and prehepatic portal hypertensive disorders.[15,85,86] Although color-flow Doppler highly facilitates evaluation of portal vascular anomalies, a high-resolution grayscale ultrasound is often sufficient to establish a definitive diagnosis.[10]

Although certain secondary changes (such as small liver, large kidneys with hyperechoic medulla, and sediment or stones in the urinary bladder) suggest the presence of CPSS, diagnosing a CPSS requires the visualization of the anomalous vein from its origin to its termination. The urinary bladder should always be evaluated for the presence of urinary calculi. Ultrasonography can be used not only during the initial diagnostic workup, but also during the surgical treatment and postoperative followup of CPSS.[86] Intraoperative grayscale ultrasonography facilitates localizing the course of an intrahepatic CPSS,[26] whereas intraoperative color and spectral Doppler examination helps to determine the optimal degree of shunt attenuation.[87]

Scintigraphy

Free ^{99m}Tc-pertechnetate administered into the colon is absorbed into the portal vein and appears in the liver first in animals without portosystemic shunting, or in the heart in patients with portosystemic shunting.[88] Isotopically labeled albumin macroaggregates may be directly administered into a splenic vein with ultrasound guidance.[89] The macroaggregates are trapped in the first capillary bed, which is normally the liver. The fraction of portal

blood that bypasses the liver will be trapped in the pulmonary capillaries. Splenic venous injection of isotopes makes exact calculation of the shunting fraction possible (i.e., activity in lungs/activity in liver + lungs); however, the procedure requires anesthesia.

Scintigraphy is the gold standard diagnostic imaging method to justify or exclude the presence of portosystemic shunting. However, differentiating congenital from acquired portosystemic shunting or intrahepatic from extrahepatic CPSS is not possible. Information provided by a scintigram is a "yes" or "no" answer to the question: Does the patient have portosystemic shunting? This answer can be reached much more easily and without using radiopharmaceuticals by documenting a single high blood ammonia concentration or by rectal ATT.

Angiography

Injection of iodinated contrast agent into the portal vein or into one of its tributaries under fluoroscopy used to be the only form of diagnostic imaging available to diagnose anomalous vessels. Angiography allows identification of extra- and intrahepatic CPSS as well as APSC.[90] The major drawback of selective portography is its invasiveness and the need for general anesthesia. Whereas *mesenteric* portography requires catheterization of a mesenteric vein during laparotomy,[91] *splenic* portography can be performed by ultrasound-guided percutaneous injection of contrast material into a parenchymal splenic vein. Visualizing portal vein segments with hepatofugal flow or shunt segments with hepatopetal flow (Figure 61-22) is problematic, as no contrast reaches these parts of the vessels.

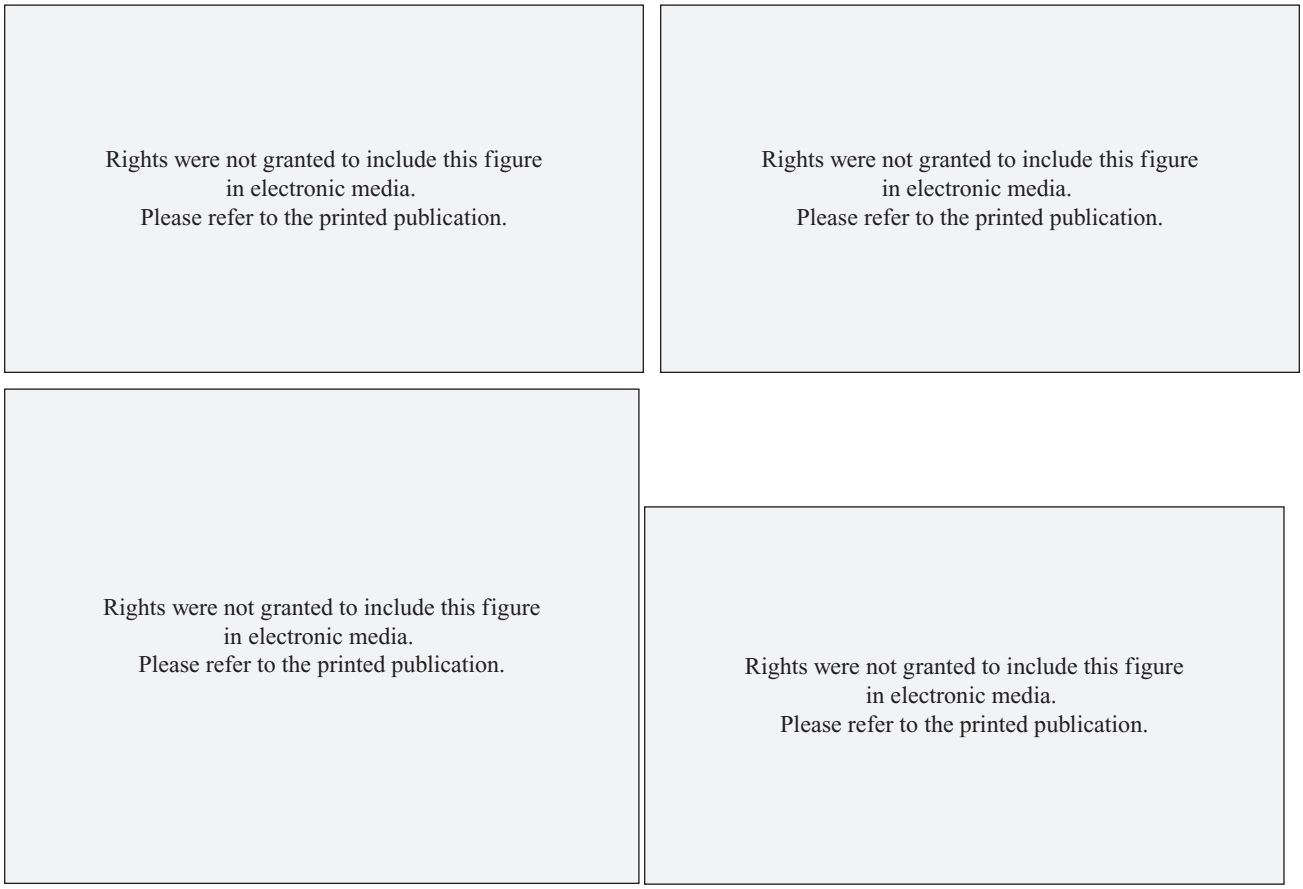

Figure 61-22 Hemodynamic features of the portal flow in the most common type of congenital extrahepatic portosystemic shunt in a dog (**A, C**), and the effect of shunt attenuation (**B, D**). The vascular structures on the schematic pictures (**C, D**) within the rectangle correspond to the vessels shown on the ultrasound images (**A, B**). *Arrows* indicate the direction of blood flow. **A** and **B,** Intraoperative color-flow Doppler ultrasound images of the portal vein at the point where the congenital splenocaval shunt originates. Note that the diameter of the shunt (*SH*) is larger than that of the portal vein (*PVcaudSH*). The portal vein cranial to the origin of the shunt (*PVcrSH*) becomes narrower than the portal vein caudal to the shunt origin (*PVcaudSH*) because of hypoperfusion. **A** and **C,** Because blood always flows toward the lowest resistance, 100% of the portal venous blood flows through the shunt (*SH*) to the caudal vena cava (*CVC*). Note that the blood from the gastroduodenal vein (*GDV*) finds lower resistance to flow caudally toward the shunt, than toward the liver. This creates a hepatofugal flow (i.e., flow away from the liver) in the portal vein segment between the entering point of the *GDV* and the origin of the shunt (*PVcrSH*). Note that the portal vein becomes even narrower cranial to the point where the *GDV* enters it (*PVcrGDV*). The diameter of the various portal vein segments varies because of the varying amount of blood that flows through a certain segment. **B** and **D,** Note that *partial* occlusion of the shunt increases the resistance in the shunt to such an extent that the blood from the splenic vein (*SPLV*) finds lower resistance to flow through the shunting vessel toward the portal vein. This reversed flow in the shunting vessel (*) prevents the portal venous blood from shunting. Thus, even though the shunt is only partially closed, it is *nonfunctional*. Also note that the blood in the whole length of the portal vein is forced to flow toward the liver (i.e., hepatopetal flow direction), establishing normal perfusion of the sinusoids. A portion of the splenic venous blood will continue to flow through the attenuated shunt to the *CVC*, but the splenic blood contains no more toxins than any systemic vein, so no hyperammonemia develops. "Blue," flow from top to bottom, "red," flow from bottom to top; Caud, caudal; Cr, cranial; D, dorsal; PVbrR and PVbrL, right and left portal vein branches; V = ventral. (Reproduced from Szatmári V, van Sluijs FJ, Rothuizen J, Voorhout G: Ultrasonographic assessment of hemodynamic changes in the portal vein during surgical attenuation of congenital extrahepatic portosystemic shunts in dogs. *J Am Vet Med Assoc* 224(3):395, 2004, with permission.)

Computed Tomography

Helical CT, especially the multiscale versions, makes excellent images of the abdominal vasculature (including CPSS) relatively quickly after intravenous injection of iodinated contrast agents.[92] Three-dimensional reconstruction provides impressive anatomic details of shunting vessels.[93] The drawback is the limited availability of the new-generation scanners and the need for patient anesthesia.

Magnetic Resonance Imaging

Although magnetic resonance angiography can provide high-quality images of the abdominal vessels,[94] it has not become popular because of its limited availability and high costs. Patents require general anesthesia and the examination lasts longer than CT angiography.

Characteristic MRI changes have been described in the brains of dogs and cats with CPSS,[95] but these findings are more of research than of clinical interest.

Histopathology

Histopathologic examination of liver biopsy specimens is essential in identifying the underlying disease process in intrahepatic portal hypertensive disorders. In the routine diagnostic workup of canine CPSS liver histopathology gives no additional information.

Currently, the presence of coexistent PHPV cannot be diagnosed preoperatively in dogs with CPSS.[18] This is because the histologic findings of liver biopsies are identical in the following conditions: CPSS, PHPV, CPSS with PHPV, congenital arterioportal fistula, any prehepatic portal hypertensive disorder (e.g., portal vein thrombosis).[13] However, taking liver biopsies for histopathologic examination is recommended in cats before deciding about surgical closure of a CPSS. This is because congenital hepatic fibrosis as part of PKD can be present simultaneously, especially in Persian and Persian crossbreeds.[20] Surgical shunt attenuation is not recommended when congenital hepatic fibrosis and a CPSS are simultaneously present in a cat.

Treatment

Emergency Treatment of Hepatic Encephalopathy

Patients with grades 2 to 4 HE may present on an emergency basis. The source of ammonia and other protein breakdown products, which cause the clinical signs of HE, is the colon. The purposes of the treatment are (a) to reduce the production and amount of ammonia in the colon by removing its content with warm water enema, and (b) to inhibit the absorption of ammonia by administering lactulose syrup into the emptied colon. Lactulose may be given as a retention enema with a soft feeding tube 0.5 to 1.0 mL/kg deep rectally). Lactulose is a nonabsorbable disaccharide, which is metabolized by the colonic bacteria into short-chain fatty acids. These fatty acids acidify the intraluminal content causing to form ammonium ion (NH_4^+) from ammonia (NH_3). Because of its polarity, NH_4^+ will not pass the enterocystic membrane, and is instead trapped in the colonic lumen.[96] It is also essential (c) to correct the acid–base and electrolytic disorders of the patient with IV fluid therapy as alkalosis and hypokalemia can worsen the signs of HE.

Seizures caused by HE can be controlled with intravenous propofol. Administration of benzodiazepines (e.g., diazepam) and barbiturates should be avoided as they can worsen the clinical signs.[97] Binding of a benzodiazepine molecule to its neuronal benzodiazepine receptor increases the effect of GABA on its $GABA_A$-receptor.[4]

Conservative Maintenance Therapy for Hepatic Encephalopathy

The cornerstone of therapy in cases of CPSS (until definitive surgical therapy) and in all cases of APSC is a high-quality, low-protein diet.[32,33,98,99] Commercially available renal prescription diets are ideal and preferable to most hepatic diets, as the protein content is higher in the latter.[28] To decrease colonic transit time and reduce the production of ammonia, oral administration of lactulose is recommended. The dosage of lactulose should be titrated in individual patients to yield soft feces, but not diarrhea. An initial dosage 0.5 mL/kg q12h is recommended. If diarrhea results the dosage should be reduced. Lactulose also stimulates the growth of colonic bacteria that can incorporate ammonia into bacterial protein.

Additional use of antibiotics (e.g., neomycin, metronidazole) has been recommended by some to diminish ammonia-producing colonic flora. Antibiotic administration is usually not necessary to control clinical signs of HE; moreover, neomycin is thought to antagonize the action of lactulose and may cause the release of endotoxins.[100] Furthermore, chronic antibiotic use may contribute to the development of antibiotic resistance.

Surgery of Congenital Portosystemic Shunts

Surgical closure of APSC is generally impossible because of their multiple nature, but also contraindicated, as APSC are usually a compensatory mechanism to resolve high portal venous pressures. Surgical narrowing (i.e., banding) of the caudal vena cava to reduce shunting by increasing the caval pressure is currently not recommended.

In cases of CPSSs, attenuation or complete closure of the shunting vein is the choice of treatment. The goals of shunt occlusion are (a) reducing portal flow via the CPSS and (b) simultaneously increasing portal venous perfusion of the liver.[86] The shunt should be attenuated as close as possible to the point where it enters the systemic circulation. Attenuating intrahepatic CPSS is more risky because of the possibility of major bleeding because of excessive dissection of hepatic parenchyma. There are several techniques described, of which none are perfect.

The major problem with shunt reduction is that blood from the portal vein will be redirected to newly perfuse the liver and its vasculature. Poorly developed portal branches have insufficient capacity to accept even normal amounts of portal venous blood, and in the case of complete shunt occlusion, splanchnic congestion will develop. The goal of surgery is to sufficiently reduce the amount of shunting blood without causing portal hypertension to develop.[86]

Although complete shunt occlusion would theoretically be ideal, partial shunt attenuation is often sufficient because (a) partial attenuation often results in *functional* closure[18] (see Figure 61-22), and moreover (b) complete anatomic occlusion may follow in many cases.[101] Partial occlusion of extrahepatic CPSSs often results in a better outcome because the chance for development of postligation portal hypertension is much smaller compared to complete occlusion.[86]

The liver, which may be less than 30% of its normal size, grows very quickly following successful surgery. Regeneration may take 2 to 3 weeks to complete in uncomplicated cases. The regenerating liver receives progressively more portal blood flow usually resulting in spontaneous complete closure of the CPSS.

Gradual shunt attenuation is believed to reduce the risk of portal hypertension by allowing the portal venous branches to adapt gradually to the increased flow.[102,103] APSC do develop with gradual shunt attenuation techniques (e.g., cellophane banding and ameroid constrictor ring).

Cystic calculi should be removed during the same surgical procedure, because resolution of ammonium biurate uroliths with dietary management can only be achieved in 30% of cases.

Surgical Ligation

The shunting vessel is identified by midline laparotomy and narrowed or completely occluded by a nonabsorbable ligature.[104] Portal hypertension is determined by direct measurement or estimated based on subjective criteria including severe intestinal cyanosis, increased intestinal peristalsis, reduction in arterial blood pressure, and compensatory tachycardia as a result of stasis in the splanchnic circulation.[105] Intraoperative Doppler ultrasonography will greatly facilitate determination of the optimal degree of shunt attenuation in extrahepatic CPSS, helping to prevent severe portal hypertension.[86,87]

Cellophane Banding

Instead of a ligature, a 3-mm–thin, three-layer–thick cellophane band is placed around the shunting vessel with or without narrowing of the shunt diameter.[106] Gradual shunt occlusion takes place as a result of inflammation induced by the cellophane.[107,108]

Ameroid Constrictor Ring

A metal ring filled with a thick layer of casein is placed around the shunting vessel. Swelling of the casein occurs as it absorbs fluid. Because of the outer metal ring, the casein can only expand centripetally causing gradual occlusion of the shunt over 1 to 3 months.[102,109,110] The major drawback of this simple technique is that the rate and magnitude of occlusion is uncontrollable and kinking caused by the weight of the device may cause acute fatal portal hypertension. Moreover, because of its relatively large size it can only be easily applied on extrahepatic CPSS.[111,112]

Laparoscopy

Laparoscopic shunt narrowing (by clips) is a less-invasive alternative for shunt ligation (see Chapter 28).[113]

Coil Embolization

With this minimally invasive intravascular technique metal spirals with thrombogenic fibers are placed in the shunting vessel. The coils are delivered via a catheter, which is inserted through the jugular vein into the shunt under fluoroscopic guidance in anesthetized animals.[114,115] The coils cause thrombus formation in the shunt resulting in its partial or complete occlusion. To prevent coil dislodgment from the shunt, an intravascular stent is often placed in the caudal vena cava to cover the point where the shunt enters the caudal vena cava. Coiling is an especially attractive method for treating intrahepatic shunts because liver dissection is thereby avoided.

In my opinion, the safest and most effective way for attenuation of extrahepatic CPSS is Doppler ultrasound-guided *partial* attenuation via surgical ligature.[86,87] For intrahepatic CPSS, coil embolization appears to be an excellent method. Ameroid constrictor and cellophane banding would be ideal in extrahepatic CPSS, when the portal vein cranial to the shunt origin is severely hypoplastic and the patient is at risk for severe portal hypertension.

Postoperative Complications of Shunt Occlusion

Portal Hypertension

Acute Portal Hypertension. Variable degrees of portal hypertension necessarily develop subsequent to any CPSS attenuation. If portal hypertension is severe, circulatory collapse may develop because of sequestration of blood in the splanchnic veins.[116] Slight abdominal enlargement as a result of ascites requires no intervention. This usually resolves spontaneously within 1 week of surgery. Severe ascites with signs of shock (e.g., depression, tachycardia, hypotension, prolonged capillary refill time, hemorrhagic diarrhea) necessitates emergency surgery and removal of the ligature or constrictor ring from around the shunt. These signs usually develop within 24 hours postoperatively. Unfortunately, the prognosis following emergency surgery is poor.

Portal Vein Thrombosis. This rare postoperative complication causes sudden onset of shock usually within several days of shunt attenuation.[87,117] Exaggerated shunt occlusion, severe portal hypertension, and stasis of portal venous blood are believed to be the cause of the thrombosis. No survivals have been reported.

Chronic Portal Hypertension. When the growth of the liver and development of portal branches is insufficient following partial shunt closure, chronic portal hypertension may induce formation of APSC. This may occur as late as 4 to 8 weeks postoperatively. In such cases, evaluation usually reveals partially patent shunt and increased portosystemic shunting as a result of newly formed APSC. The development of APSC has been documented with all surgical methods. APSC can develop as a result of underdeveloped portal branches or exaggerated shunt attenuation.[18] In most dogs, these APSC remain clinically silent because the hepatic mass has substantially increased following shunt attentuation.[18]

All patients should be re-evaluated at 1 month after shunt attenuation by measuring fasting blood ammonia concentration and performing an abdominal ultrasonography. If fasting blood ammonia concentration is within the reference range, rectal ATT should be performed. If fasting hyperammonemia is present or the ATT is abnormal, ultrasonography should identify whether shunting occurs via the narrowed CPSS, APSC, or both. In the latter cases, lifelong conservative therapy with dietary modifications and lactulose is recommended. A normal ATT result implies complete shunt occlusion and the pet will generally have a very favorable prognosis. A second surgery to reach further shunt attenuation should only be attempted in patients with persistent clinical signs that have high fasting blood ammonia concentration or abnormal rectal ATT result 3 months after the first surgery.[18,86] Spontaneous gradual shunt closure would not occur beyond 3 months after surgical ligation.

Postligation Seizures: Cerebrocortical Necrosis

This rare and usually fatal complication of shunt attenuation causes generalized seizures 1 to 3 days postoperatively, almost exclusively in cats and small-breed dogs (often in Maltese dogs).[31,100,118,119] Its occurrence is unpredictable. The pathogenesis is unknown, but the sudden decrease of endogenous benzodiazepine ligands is thought to play a role (i.e., "benzodiazepine withdrawal syndrome").[4] Because cerebral edema is suspected to be the initial disorder, intravenous mannitol (0.5 to 1.0 g/kg IV, during 20 minutes) may be administered when a patient shows subtle central neurologic signs during the first three postoperative days. Once seizures have developed, the prognosis is usually very poor. Most patients are euthanized because of uncontrollable seizures or persistent neurologic defects. Propofol

(1 to 5 mg/kg, IV) followed by constant rate infusion [CRI]) may be used to control seizures.[120] Prophylactic treatment with phenobarbital does not reduce the risk of development of postligation neurologic complications,[118] but may be used in long-term seizure management. Preventive use of potassium bromide has not been shown to reduce the possibility of postoperative seizures. In every patient with central neurologic signs in the early postoperative period, hypoglycemia and HE should be excluded by measuring venous glucose and ammonia concentrations, respectively.

Blindness and other types of central neurologic signs may develop in cats shortly after surgery. The pathogenesis of these changes is unknown.

Hemoabdomen

Hemorrhage from liver biopsy sites or shunt dissection in cases of intrahepatic CPSS can lead to hypovolemic shock and death in the early postoperative period. Dogs with hepatic insufficiency are prone to develop hemorrhagic complications because of decreased concentrations or abnormal synthesis of coagulation factors.[84] Postoperative portal hypertension may also contribute to bleeding tendency from hepatic parenchymal dissection. Hemoabdomen should be carefully differentiated from severe portal hypertension and septic peritonitis as they all can cause shock and variable degrees of abdominal distention. Coagulation parameters (e.g., PT, aPTT) should be routinely monitored after surgery and if they are abnormal or if there is evidence of clinical bleeding, fresh-frozen plasma transfusion should be administered (10 to 20 mL/kg, IV).

Hypoglycemia

Toy-breed dogs are prone to develop hypoglycemia during or shortly after surgery.[100] Blood glucose concentrations should be regularly monitored and hypoglycemia should be treated with glucose-containing infusions.

Portal Hypertensive Disorders

Specific treatment should address the underlying parenchymal liver disease based on the histologic results of liver biopsy. HE can often be controlled with dietary modification and lactulose.

Primary Hypoplasia of the Portal Vein

Currently, no specific treatment exists for portal venous hypoplasias. Renal or hepatic prescription diets and lactulose may alleviate clinical signs of HE in case of APSC. Diuretic agents may be useful in dogs with ascites.

Congenital Arterioportal Fistulas

Liver lobe resections have been reported in animals with congenital arterioportal fistulas. However, simultaneous presence of PHPV in the whole liver prevents postoperative resolution of portal hypertension and the portosystemic shunting via APSC.[10,25] Therefore, the pet owner should be educated that partial hepatectomy may not result in complete recovery of portosystemic shunting, and that lifelong dietary support and/or lactulose will likely be required.

Ascites

Severe abdominal effusions should be treated with diuretic agents.[121] The first choice is spironolactone (1 to 2 mg/kg q12h), an aldosterone receptor antagonist as (a) chronic hepatic insufficiency is associated with hyperaldosteronism, (b) concurrent hypercortisolemia is associated with cortisol binding to the mineralocorticoid receptor, and (c) potassium-sparing diuretics prevent development of hypokalemia and alkalosis, both of which would worsen signs of HE. If spironolactone alone does not resolve ascites, furosemide may be added to the therapy. Abdominocentesis for removal of abdominal effusion is not recommended because of loss of protein and exacerbation of Starling forces permitting further fluid accumulation.

Prognosis

Congenital Portosystemic Shunts

Conservative Treatment

Dietary and medical management relieves clinical signs only temporarily in most symptomatic young dogs.[99] Without surgical shunt attenuation, gradual deterioration of liver function occurs; consequently, conservative therapy alone offers a guarded prognosis in young dogs. In older animals (>6 years) with newly reported signs of CPSS, lifelong conservative treatment may be recommended because of the significantly higher complication rates of surgical therapy in these patients.[105]

Surgical Treatment

The prognosis depends on (a) whether the CPSS is intra- or extrahepatic, (b) the coexistence of PHPV, (c) the extent of shunt attenuation, (d) experience of the surgeon, and (e) the age of the dog at the time of diagnosis. Intrahepatic CPSS generally has poorer prognosis. Complete resolution of clinical signs can be expected in approximately 60% to 80% of dogs with extrahepatic CPSS in the hands of an experienced surgeon. This same parameter is approximately 50% to 70% for intrahepatic CPSS.[102-105] With extrahepatic CPSS, an excellent prognosis can be expected when blood flow is hepatopetal (i.e., toward the liver) in the portal vein segment, which is cranial to the shunt origin. This can be established preoperatively with Doppler ultrasound.[86] In cats, regardless of the shunt type and the surgical method, the success rate is approximately 30% to 50% because of the development of postoperative central neurologic signs.[105,122,123]

Portal Hypertension

The prognosis of portal hypertensive disorders depends on the underlying disease. In the majority of acquired diseases the underlying disorder is chronic and so severe that even stopping the disease process will not cause regression of APSC.

NEOPLASTIC DISORDERS

Josep Pastor and Marta Planellas

Hepatobiliary Neoplasia

Primary liver neoplasms are infrequent in the dog and cat, with an estimated prevalence in necropsy studies of 0.6% to 2.6% in the dog and 1.5% to 2.3% in the cat. Liver metastases are more frequent than primary hepatic tumors in the dog, and tend to originate from the spleen, pancreas, and GI tract. Primary hepatobiliary tumors are more common than metastatic disease in the cat.[1-5]

Etiology

The etiology of liver cancer in dogs and cats is incompletely understood. Potential causes such as aflatoxins, nitrosamines, food additives, parasites, and radioactive compounds have been reported.[6-8] Liver cancer in the dog has many clinical, pathologic, and histologic homologies with liver cancer in humans.[9-12]

In human medicine, chronic diseases of the liver, such as hepatitis B or C infection, as well as cirrhosis, are often associated with

hepatocellular tumors; however, there is no established association between hepatic tumors and viral infections in the dog or cat. Moreover, canine hepatic cirrhosis does not appear to predispose to hepatocellular carcinoma (HCC).[1] A possible association between hookworm or whipworm infection and liver cancer has been reported, and cats with chronic cholangitis may have an increased predisposition to biliary carcinoma.[7,13]

Several liver mitogens and tumor suppressor genes such as epidermal growth factor, TGF-α, vascular endothelial growth factor (VEGF), p53, and TGF-β and its receptors (TGF-β-r) have been associated with liver cancers in humans and these may play a similar role in the dog.[14-17]

In human medicine a small percentage of HCCs or cholangiocarcinomas originate from hepatic progenitor stem cells. Dogs diagnosed with HCC or cholangiocarcinoma do demonstrate activation of hepatic progenitor stem cells in response to liver injury, but hepatic progenitor stem cell expression in liver tumors is relatively low.[18]

Pathophysiology

The hypothesis of cancer development as a multistep process applies to liver tumors in the dog and the cat, as well.[7,19] Precancerous lesions, such as dysplastic nodules, can be identified before the development of overt malignancy in humans. Dysplastic nodules are characterized by cell atypia, cellular crowding, trabecular thickness, microacini, and histochemical markers.[20] Dysplastic nodules have not been reported in the dog or cat and further studies are needed to understand the chronology of hepatic malignancy in domestic animals. Preliminary reports of histochemical markers have been reported in dogs with hyperplastic hepatic lesions and hepatocellular and biliary neoplasms.[11]

Liver tumors cause damage to the liver by several mechanisms: inflammatory effects, obstruction of the biliary system, obstruction of the vascular compartment or adjacent organs, and spontaneous rupture with hemoabdomen.[21]

Hepatic tumors are usually resistant to chemotherapy.[7,19] In one recent study, P-glycoprotein was more highly expressed in HCC than in cirrhosis, which is consistent with the known resistance of HCC to chemotherapy. P-glycoprotein, which is encoded by the multidrug resistance gene (MDR-1), is normally expressed in tissues with excretory function, including the jejunum, kidney, liver, and adrenal gland.[22]

Primary liver neoplasms are usually classified according to their cellular origin and macroscopic appearance. With respect to cellular origin, these tumors may be hepatobiliary, hematopoietic, sarcomas, or metastases of other tumors (Box 61-2). In relation to macroscopic appearance, they can be classified as lobular, multiple nodular, or diffuse (). The combination of histopathologic and morphologic classification has consequences for prognosis and treatment strategy in these animals (Tables 61-6 and 61-7), and the clinician must always address these factors to arrive at correct management decisions. In dogs, malignant tumors are more common than benign lesions. In cats, biliary neoplasms are the most common presentation, particularly intrahepatic benign forms.[6,7]

Clinical Examination

Most animals with liver neoplasia present with nonspecific clinical signs such as anorexia and weight loss. Less-frequent clinical signs include vomiting and diarrhea, PD and PU, pale mucosal membranes, and acute weakness because of anemia and hypovolemia coincident with tumor rupture.[7,19] Up to 25% of affected animals show no clinical signs, and liver cancer is suspected only with increases in serum liver enzyme activities.[3,5,7,23]

Box 61-2 Classification of Liver Neoplasms According to Cellular Origin

Primary

- Hepatobiliary neoplasm
  - Hepatocellular carcinoma
  - Biliary carcinoma (cholangiocarcinoma, biliary adenocarcinoma)
  - Hepatocellular adenoma (hepatoma)
  - Biliary duct adenoma (cystadenoma)
  - Carcinoid tumor (neuroectodermal neoplasm)
- Hematopoietic neoplasm
  - Lymphoma
  - Leukemia
- Sarcomas
  - Hemangiosarcoma
  - Sarcoma
  - Leiomyosarcoma
  - Rhabdomyosarcoma
  - Osteosarcoma
  - Chondrosarcoma

Metastatic

- Gastrointestinal tract
- Spleen
- Pancreas
- Kidneys
- Mammary tissue
- Prostate

The most common physical examination findings are cranial abdominal mass (35%), abdominal bloating (30%), and jaundice (18%). Jaundice is a less common finding in cases of metastatic cancer. Other manifestations include neurologic signs as a result of HE; paraneoplastic syndromes such as hypoglycemia and myasthenia gravis; and skin alterations consistent with hepatocutaneous syndrome.[7,24]

No clear breed predisposition is observed with canine liver cancer, although Poodles, Fox Terriers, Miniature Schnauzers, Labrador Retrievers, and male dogs are overrepresented in some reports of hepatocellular carcinoma.[1,12,19,25,26] Labrador Retrievers and female dogs are overrepresented in reports of bile duct carcinoma.[1,26-28] Whether male and female cats are equally at risk for bile duct carcinomas is unsettled,[28,29] but male cats appear to be at greater risk for bile duct adenomas.[30,31] Neuroendocrine tumors are generally observed in younger animals.[1,32]

Diagnosis

Definitive diagnosis of liver cancer can be made only by liver biopsy. Asymmetrical enlargements of the liver detected on physical examination, abdominal ultrasonography, or survey radiography should not be assumed to be neoplastic in origin. Similarly, laboratory data do not distinguish hepatic neoplasia from other liver pathologies.[33]

The clinical approach to an animal with suspected liver cancer should include basic information such as a complete blood cell count, serum biochemistry, coagulation tests, urinalysis, thoracic and abdominal radiographs, abdominal ultrasound (Figures 61-23 and 61-24), and fine-needle or core biopsy of the liver.

A TNM tumor classification system has been used for staging of liver cancer where T represents tumor (T0, no evidence of tumor;

Table 61-6 Incidence of Histologic Liver Tumor Types, Morphologic Patterns, Metastatic Rates, and Treatment in Dogs

Histologic Tumor Type	Morphologic Pattern	Incidence	Metastatic Rate	Treatment	Reference
Hepatocellular carcinoma		50%			1
	Lobular or massive	53% to 84%	4.8% to 6.6%	Lobectomy	26, 27
	Multiple nodular	16% to 25%	93%	Chemoembolization Metronomic therapy (human medicine)	27, 52
	Diffuse or infiltrating	0% to 19%	100%	Chemoembolization Metronomic therapy (human medicine)	27, 51, 52
Biliary carcinoma		22%	27% to 88%		1, 13, 26, 27
	Lobular or massive	37% to 46%		Lobectomy	26
	Multiple nodular	0% to 21%		Chemotherapy	26
	Diffuse or infiltrating	17% to 54%		Chemotherapy	26
Sarcoma		13%	86%	Lobectomy Metronomic therapy (soft-tissue sarcomas)	1, 7, 19
	Lobular or massive	36%			1
	Multiple nodular	64%			1
	Diffuse or infiltrating	67%			1
Carcinoid tumors		14%	93%		1, 56, 64
	Lobular or massive	0%			1, 56, 64
	Multiple nodular	33%		Cholecystectomy Chemoembolization	64, 66
	Diffuse or infiltrating	0%			1, 56

Diffuse or infiltrating, multiple coalescent nodules in all the lobes, or diffuse disappearance of the liver parenchyma; *lobular*, nodule or large mass in a single liver lobe; *nodular*, several nodules throughout the liver parenchyma, or several affected liver lobes.

Table 61-7 Incidence of Histologic Liver Tumor Types, Metastatic Rates, and Treatment in Cats

Histologic Tumor Type	Morphologic Pattern	Incidence	Metastatic Rate	Treatment	Reference
Hepatocellular adenoma		8% to 22%	25%	Lobectomy	29
Hepatocellular carcinoma		2%	NR		28
Biliary carcinoma		24% to 28%	77%		73
Hepatobiliary cystadenoma		12%	0%	Lobectomy	29, 62
Biliary adenoma		32% to 52%	0%	Lobectomy	29
Sarcoma		2% to 13%	60% to 100%	Lobectomy	29
Carcinoid tumors		4%	100%	Lobectomy, cholecystectomy	64, 65

T1, tumor involving one lobe; T2, tumor involving more than one lobe; and T3, tumor invading neighboring structures); N represents regional lymph nodes (RLNs) (N0, no evidence of RLN involvement; N1, RLN involved; N2, distant LN involved); and M represents distant metastasis (M0, no evidence of metastasis; M1, distant metastasis detected). Although recommended, this system has not been universally adopted.[34]

Table 61-8 illustrates the most typical hematologic and biochemical findings in dogs and cats affected with liver neoplasia. Leukocytosis is a result of inflammation and necrosis of large tumors; anemia tends to be moderate and nonregenerative and is thought to be caused by chronic illness, inflammation, or iron deficiency[19]; thrombocytosis is attributable to a paraneoplastic syndrome characterized by thrombopoietin production, iron deficiency, or anemia.[7]

Serum liver enzyme elevation is a frequent, but not universal, finding in animals with liver neoplasia. It should be noted, however, that the degree of serum enzyme elevation does not correlate with the degree of liver involvement or severity of disease. In one survey, animals with primary liver tumors tended to have greater elevations in serum ALT and ALP activities than animals with metastatic disease, while the latter tended to have greater elevations in serum bilirubin and AST.[35] It also has been suggested that an AST-to-ALT ratio of less than 1 is more compatible with carcinoma, while a ratio of greater than 1 is more indicative of a sarcoma or carcinoid.[1] Other reported biochemical changes include hypoglycemia, hypo- or hyperalbuminemia, and increased serum bile acids. Hypoglycemia as a paraneoplastic syndrome associated with hepatocellular carcinoma is attributed to the secretion of insulin-like growth factor II.[36] Unlike dogs, cats usually present with a high incidence of serum creatinine and BUN elevations.[28,29]

Coagulation factor abnormalities are more commonly associated with hemangiosarcoma, although DIC may be evident in end-stage liver cancer or in decompensated patients. Coagulation studies should always be performed before undertaking invasive diagnostic procedures.[23]

Serum α-fetoprotein has been evaluated in the dog, and increases are reported in 75% of animals with hepatocellular carcinoma, and in 55% of those with biliary carcinomas. The use of this biomarker is limited by the fact that it is increased in cases of hepatic

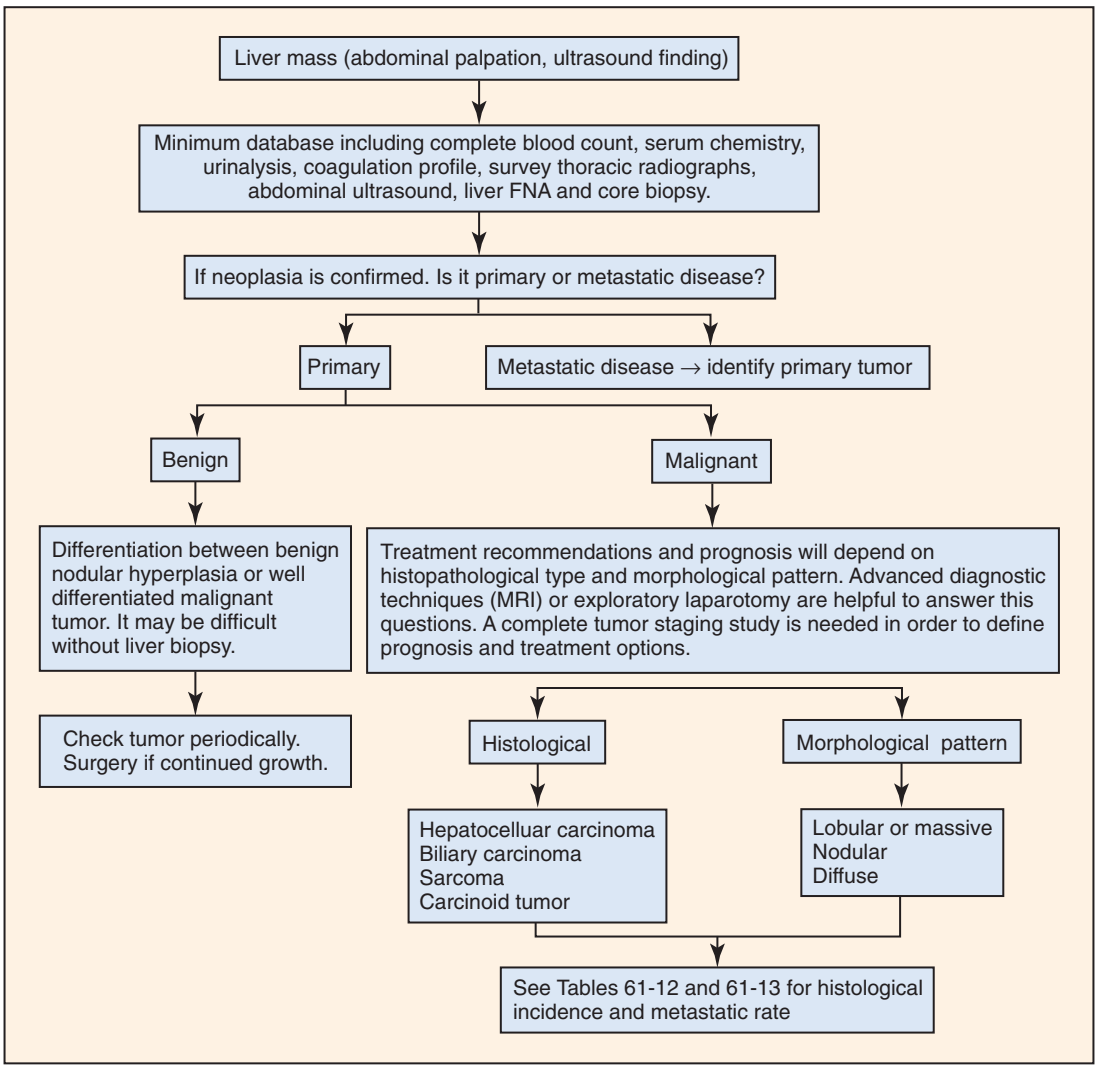

Figure 61-23 Diagnostic decision tree in an animal with a liver mass.

Table 61-8 Hematologic and Biochemical Changes Observed in Dogs and Cats with Liver Neoplasms

Parameter	Change	Incidence in Dog	Incidence in Cat
Hematocrit	Decrease	27% to 50%	ND
Leukocytes	Increase	54% to 73%	ND
Platelets	Increase	50% hepatocellular carcinoma	ND
Alkaline phosphatase	Increase	61% to 100%	10% to 64%
Alanine aminotransferase	Increase	44% to 75%	10% to 78%
γ-Glutamyltransferase	Increase	39%	78%
Total bilirubin	Increase	18% to 33%	33% to 78%
Bile acids	Increase	50% to 75%	67%
Albumin	Decrease	52% to 83%	ND
	Increase	Occasionally	ND
Glucose	Decrease	Occasionally	ND

ND, Indicates percentage not described.
From Thamm DH: Hepatobiliary tumors. In: Withrow SJ, MacEwen EG, editors: *Small Animal Clinical Oncology*, ed 3, Philadelphia, 2001, Saunders; Liptak J: Hepatobiliary tumors. In: Withrow S, Vail D, editors: *Withrow and MacEwen's Small Animal Clinical Oncology*, St. Louis, 2006, Elsevier.

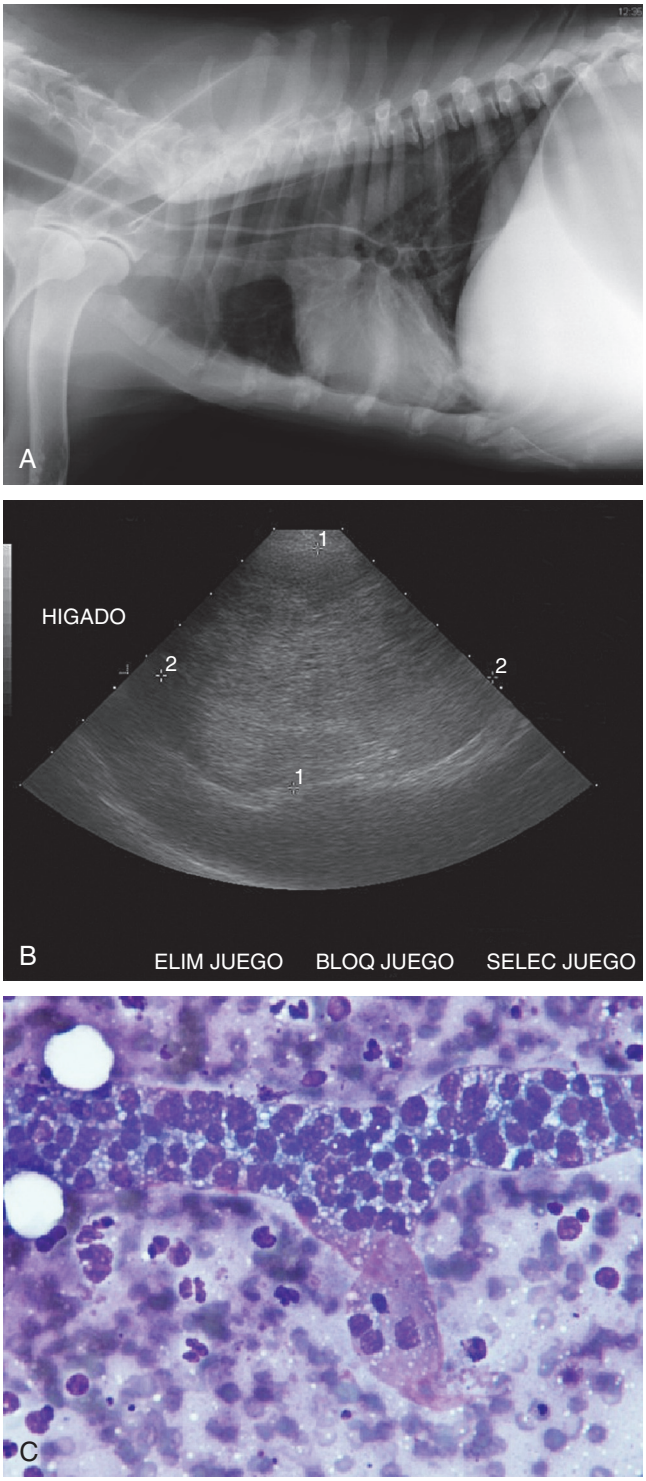

Figure 61-24 **A,** Thoracic radiography of a 10-year-old mixed-breed dog with a large abdominal mass showing megaesophagus. **B,** Abdominal ultrasound of the same dog. Hyperechoic mass with hypoechoic areas in the liver. **C,** Fine-needle cytology of the liver mass, a dense aggregate of large epithelial cells with vacuolated cytoplasm without obvious cytologic criteria of malignancy. A diagnosis of cholangioma or well-differentiated cholangiocarcinoma was made. Histopathologic study of the mass confirmed a cholangioma in this dog.

Box 61-3 Basic Ultrasound Patterns in Liver Neoplasia

Diffuse or multifocal

- Diffuse or multifocal liver neoplasms tend to present with hepatomegaly, but this depends on the degree of infiltration. Liver carcinomas can be diffuse or affect multiple lobes, with variable ultrasound characteristics depending on the presence of necrosis, inflammation, hemorrhage, or cavitation. In these malignant tumors it is common to observe a mixed echogenicity pattern. Lymphoma can affect the liver without detectable ultrasound changes, or cause diffuse hypoechogenicity, hyperechogenicity, or mixed echogenicity with or without hypoechoic nodules. Consequently, if lymphoma is suspected, even if the liver ultrasound findings appear normal, fine-needle aspiration cytology is recommended. Histiocytic neoplasms are more often associated with multiple nodules and hypoechoic masses, although diffuse liver hypoechogenicity has also been described. Mast cell infiltration of the liver tends to produce diffuse hyperechogenicity.
- Nodular patterns
  - Benign nodular hyperplasia is common, particularly in dogs, and accounts for many of the focal liver lesions identified at ultrasound exploration. It has been estimated that 25% to 36% of all nodular masses detected in the liver are nodular hyperplasia.
  - Benign liver adenomas or hepatomas can manifest as a focal mass of variable size and of normally hyperechoic characteristics.
  - The liver is a frequent location of metastatic spread, fundamentally through the portal system that drains most of the abdominal structures.
  - Primary liver neoplasms such as hepatocellular carcinoma can present as focal or multifocal masses, although less often so than in the case of metastases. Focal hypoechoic lesions with a hyperechoic center or core (referred to as target or bull's-eye lesions) are usually associated with metastases, although some benign processes, such as nodular hyperplasia, can generate similar patterns.
- Biliary obstruction: Ultrasound has become an important tool for evaluating biliary obstruction in icteric dogs and cats. Primary tumors of the liver, biliary tract, duodenum, or pancreas are capable of causing biliary obstruction.

lymphoma and other liver pathologies, and only very dramatic elevations in α-fetoprotein may be taken to indicate hepatocellular carcinoma.[37-39]

Abdominal radiographs often reveal a mass effect in the cranial abdomen, although this finding will depend upon the size of the neoplasm and the number and size of metastatic tumors. Other reported findings include dorsal displacement of the stomach, hepatomegaly, loss of abdominal detail (because of the presence of free abdominal fluid), and, occasionally, biliary tract calcification. Thoracic radiography should be considered as part of the staging procedure for animals with metastatic disease.[25,40]

Changes in the ultrasound density of the liver may take a variety of forms (Box 61-3). Most changes are not pathognomonic for a given disease process, and the final diagnosis is established only on the basis of clinical findings, laboratory testing, and results of cytology or histopathology (see Figures 61-24 and 61-25). Ultrasound is also very useful for evaluating other abdominal structures, and for the staging of cancer.[40-42]

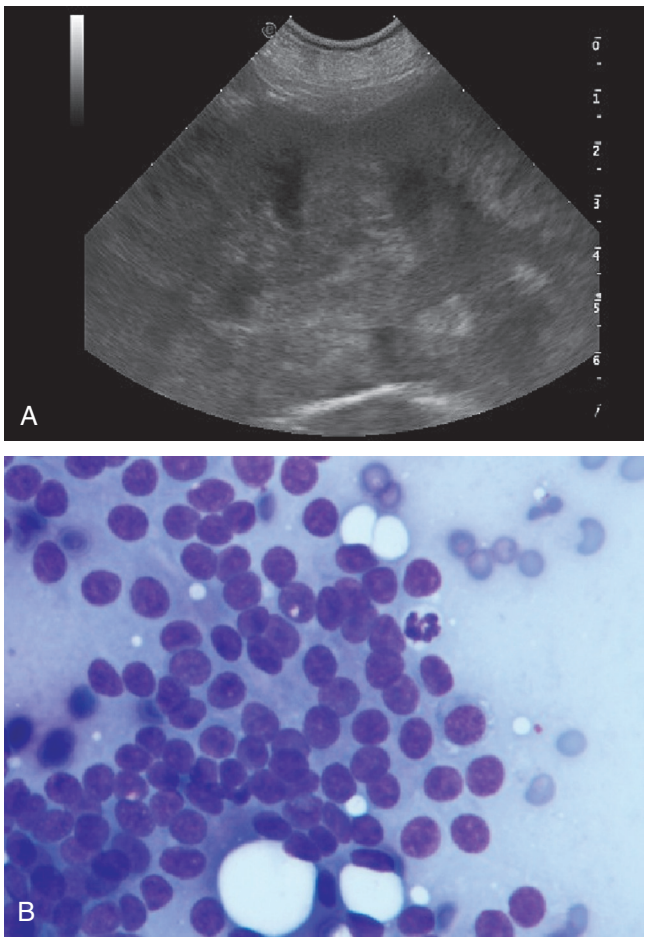

Figure 61-25 **A,** Ultrasonography of a large abdominal mass in a 12-year-old spayed Golden Retriever dog. **B,** Fine-needle aspiration showing round to oval cells that have features consistent with hepatic carcinoid, a neuroendocrine tumor.

High-field MRI scanning has an accuracy of 94% in differentiating malignant from benign lesions with a sensitivity and specificity of 100% and 90%, respectively. MRI classified malignant hepatic lesions as HCC in all confirmed cases and correctly predicted the histologic grade of five HCC lesions. These results suggested that MRI is a useful modality for abdominal imaging in veterinary patients, and that MRI accurately differentiates benign from malignant focal hepatic lesions.[43]

Liver cytology is useful in the initial evaluation of hepatomegaly and usually permits differentiation between primary tumors, metastatic disease, and focal infection (see Figures 61-24 and 61-25). However, cytology does not distinguish between benign focal inflammatory disease and progressive chronic liver disease, and it cannot establish the extent and distribution of disease. Likewise, a definitive diagnosis of regenerative nodular hyperplasia cannot be established, and the technique is unable to differentiate a benign inflammatory reaction from cell changes associated with other pathologies. Contraindications to ultrasound-guided cytology include the following:

- Coagulation abnormalities—If one or more coagulation test parameters are altered, it is advisable to administer vitamin K_1 via the subcutaneous route 12 hours before cytology.
- Cavitary masses—The ultrasound detection of a large cavitary lesion in an elderly dog usually contraindicates cytology, particularly in male German Shepherds or Golden Retrievers, because of the high probability that such lesions correspond to hemangiosarcoma.

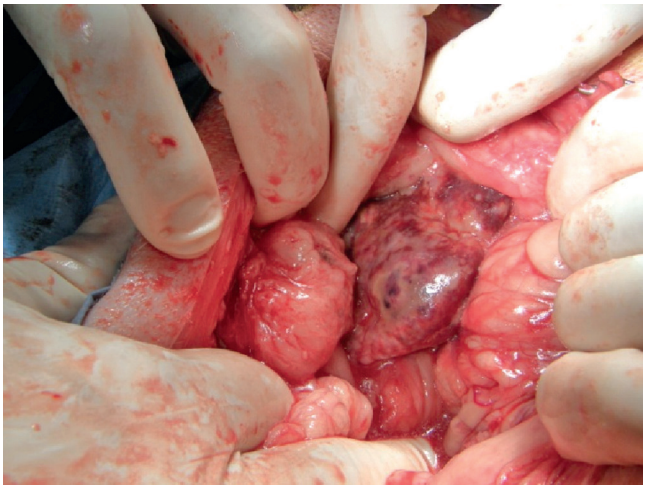

Figure 61-26 Macroscopic appearance of diffuse hepatocellular carcinoma during exploratory laparotomy in a dog. (Courtesy of Félix Gracia.)

Liver cytology has obvious limitations in that it cannot distinguish between liver adenomas and regenerative nodules, and even some hepatocellular carcinoma aspirates may be composed entirely of normal-appearing hepatocytes. In many cases it may prove necessary to resort to ultrasound-guided biopsy, laparoscopy, or exploratory laparotomy. However, cytology may prove useful in determining the presence of lymphoma, mastocytoma, and histiocytic sarcoma, as well as contribute to the initial classification of tumor type. Concordance rates between cytology and histopathology findings may be good for some disease processes, but the reported concordance rate varies from 14% to 86%.[44,45]

Treatment and Prognosis

The treatment to be provided and the prognosis of animals with primary liver cancer depend on the cell of origin, degree of malignancy, and clinical presentation. The clinician should quickly determine if surgery, chemotherapy, radiation therapy, or palliative care is the treatment of choice in individual patients. Palliative treatment is the option for animals that are not surgical candidates, for example, tumors with poor response to systemic chemotherapy, and for whom pain management and general liver failure treatment are the best recommendations.

The success of newer options such as chemoembolization, metronomic therapy, antiangiogenic drugs, and tyrosine kinase inhibitors in the treatment of these patients has not been clearly established.[41-53]

Hepatocellular Carcinomas

The macroscopic presentation is clinically very important (see Figure 61-26), as 100% of the diffuse forms have metastasis at the time of diagnosis, versus 37% of the isolated (massive or nodular) clinical presentations.[27] It should be noted, however, that some dogs with massive HCC present without metastasis, and deaths in these cases may be unrelated to HCC.[1,26,54] Histopathologic subtype and anaplastic characteristics in general influence the prognosis and predictability of metastasis.[1,26,55] Metastatic spread usually affects the regional lymph nodes, lungs, and peritoneum.[26,56]

Prognostic factors in dogs with massive HCC include need for surgery, liver lobe involvement, serum ALT and AST activities, and ratios of ALP to AST and ALT to AST.[26] Liver lobectomy is recommended for cats and dogs with hepatic tumors that have a massive morphologic appearance without metastases. However surgical complications are reported in more than 28% of cases, with a mortality rate of almost 12%.[26] The predilection of massive HCC for left-sided liver lobes has been reported.[27,54] Advanced imaging and intraoperative ultrasonography may provide useful information on the relationship of right-sided and central liver tumors to the caudal vena cava prior to liver lobectomy.[40,41,43] Even though right-sided liver tumors have a poorer prognosis because of intraoperative death, there is no difference in the survival time after successful surgery.[26] The considerable regenerative capacity of the liver can permit successful resection of up to 80% of hepatic mass if the remaining tissue is functionally normal and critical supportive care is provided.[56] The median survival time for dogs with massive HCC following liver lobectomy is greater than 4 years. Without surgery the average life expectancy is 270 days and the prognosis is generally considered poor.[26] Tumor recurrence in dogs with massive HCC is rare and reported to be 0% to 13% after lobectomy.[26,54]

The prognosis for dogs with nodular and diffuse HCC is poor. Surgical resection is usually not possible because of involvement of multiple liver lobes.

No effective systemic chemotherapy or radiation therapy protocols have been described for HCC treatment. HCC is considered chemoresistant in humans although mitoxantrone has been reported to be helpful in some cases.[7,56,57] The most likely reason for the poor response to systemic chemotherapy is the expression of P-glycoprotein in hepatocytes.[22] Treatment options for nodular and diffuse HCC in humans include liver transplantation and minimally invasive procedures for regional control, such as ablation, chemoembolization, immunotherapy, hormonal therapy, and low-dose metronomic chemotherapy.[56,58] A recent report recommends therapy with sorafenib, a multikinase inhibitor and antiangiogenic agent.[47,48]

Chemoembolization is a procedure commonly used in the treatment of diffuse hepatocellular carcinoma in humans with median survival times of 1 to 2 years compared with 3 to 6 months with systemic chemotherapy.[52,51] In veterinary medicine, chemoembolization has been reported with moderate success in the palliation of four dogs with HCC.[52,53] In cats, hepatocellular carcinoma is less frequent, and less data are available.[28,59]

Hepatocellular Adenomas

These tumors are also known as hepatomas and are more common in cats than in dogs. In the dog it is sometimes very difficult to distinguish adenoma from reactive nodular hyperplasia, and biopsy is needed to clarify the diagnosis. The prognosis for adenomas is usually good, but it is advisable to remove focal mass lesions because they can grow and spontaneously rupture with severe bleeding.[19]

Bile Duct Carcinoma (Adenocarcinoma and Cholangiocarcinoma)

Bile duct carcinoma is the most common liver malignancy in the cat, and the second most common liver malignancy in the dog (see Figure 61-27). Tumor behavior is very aggressive in both species, and metastases are present at the time of diagnosis in 60% to 88% of cases. Bile duct carcinomas usually metastasize to the regional lymph nodes, lungs and peritoneum, kidneys, heart, adrenal glands, eye, and bone.[1,60] Bile duct carcinoma can be intrahepatic or extrahepatic, but rarely occurs within the gallbladder. Intrahepatic bile duct tumors are more common in dogs, and extrahepatic bile duct tumors are more common in cats.[1,13, 27,61] Three morphologic forms or presentations have been described: lobular, multifocal, and diffuse. In general, only the lobular form should be considered for surgical removal as long as there is no evidence of metastasis. The prognosis for multifocal and diffuse bile duct carcinomas is very poor, surgery is usually not feasible, and most animals die within 6 months of surgery.[29] No effective chemotherapeutic options have been described for these malignancies in dogs or cats.

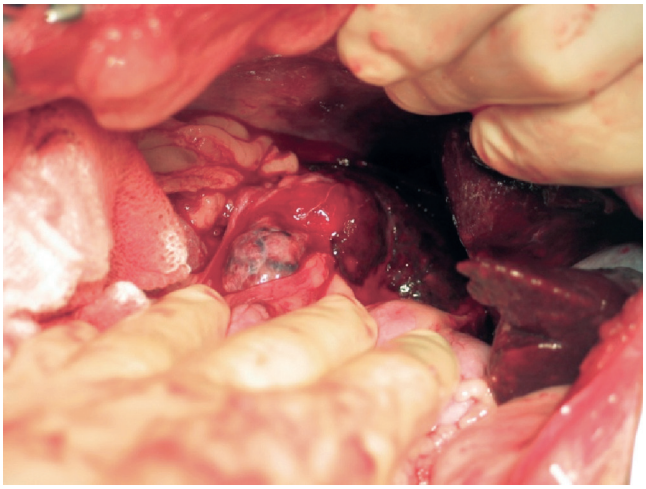

Figure 61-27 Macroscopic appearance of intrahepatic biliary carcinoma during exploratory laparotomy in a dog. (Courtesy of Félix Gracia.)

Bile Duct Adenomas

Also known as biliary cystadenomas, biliary adenomas, cholangiocellular adenomas, and cholangiomas, these tumors are common findings in aging cats. Males appear to be more frequently affected than females (Figure 61-28). In cats, 50% of these lesions are isolated or lobular and 50% are multifocal.[59] Biliary duct adenomas usually do not cause clinical signs unless they grow and compress other structures.[62] Despite the benign nature of these tumors, surgical removal is usually recommended because malignant transformation is always possible and because expansion into the porta hepatis may cause life-threatening consequences.[63] Liver lobectomy is recommended for cats with single bile duct adenoma or multifocal tumors confined to one or two lobes. In cats, surgical resection of biliary adenomas may provide cure or tumor-free survival of several years.[28,29,31,59,62,63]

Carcinoid Tumors

Neuroendocrine (carcinoid) tumors are infrequent in the dog and cat. In dogs, carcinoids have an aggressive biologic behavior and are usually not amenable to surgical resection as they tend to present as diffuse lesions (see Figure 61-25).[1,27] Carcinoid tumors in dogs have also been described in the gallbladder, and these have been managed successfully with cholecystectomy.[64,65]

Carcinoid tumors in cats can be intrahepatic or extrahepatic involving the bile duct and occasionally the gallbladder.[65] The extrahepatic form of carcinoid tumors may cause biliary tract obstruction, icterus, and increases in serum hepatic enzyme activities. Biliary tract diversion procedures should be considered for obstructive lesions involving the extrahepatic biliary tract. Unlike the circumstance in dogs and humans, female cats are more often affected by these tumors than males (female-to-male ratio of 5:1).[66]

The prognosis of carcinoid liver tumors in dogs and cats is generally poor, and metastatic disease is present in 90% of the cases at

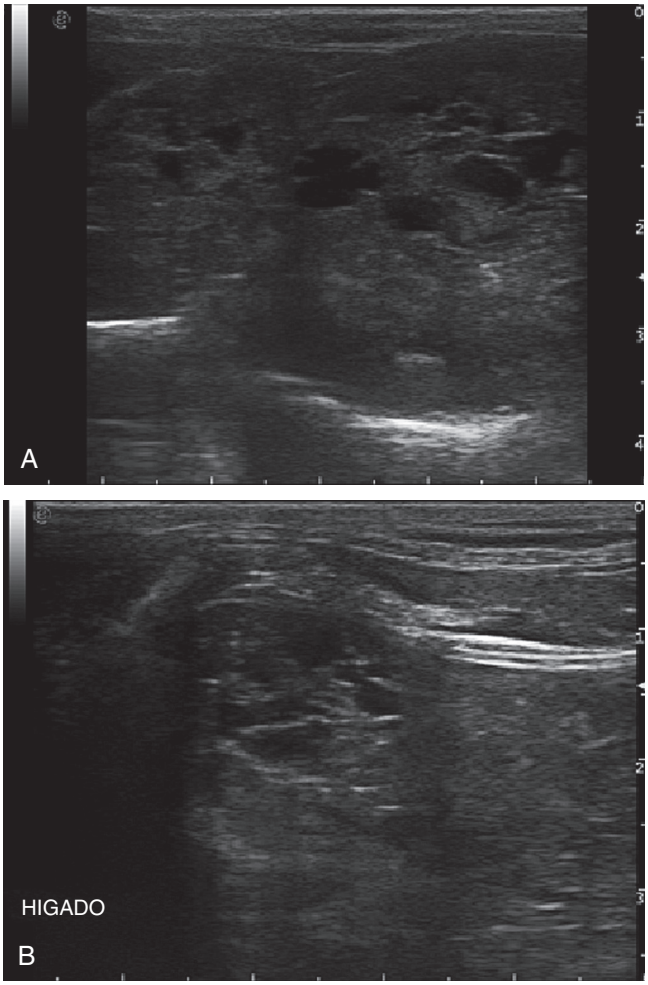

Figure 61-28 **A** and **B,** Liver mass in two cats with multiple anechoic cavities consistent with biliary cystadenomas.

the time of diagnosis.[7] A better prognosis is observed with extrahepatic carcinoids with a life expectancy of more than a year.[64,65]

Liver Sarcomas

Primary liver sarcomas are rare in the dog and cat. Hemangiosarcoma is the most frequent primary hepatic sarcoma in cats and leiomyosarcoma the most common in dogs.[7,19] There also have been reports of hepatic fibrosarcoma, rhabdomyosarcoma, osteosarcoma, liposarcoma, and histiocytic sarcomas in both animal species.[1,28,29,55] These are usually very aggressive tumors, metastasizing in 86% to 100% of cases to the spleen and lungs, or spreading diffusely within the liver.[5] Chemotherapy has not been studied in the treatment of primary hepatic sarcomas, although, similar to other solid sarcomas, response rates are likely to be poor. Histiocytic sarcomas respond partially to CCNU, with a mean duration of remission of 85 days and a survival of 172 days.[67] Continuous low-dose oral chemotherapy may be an effective alternative to conventional high-dose chemotherapy for adjuvant therapy of dogs with hemangiosarcoma.[50] Mass resection may offer some palliation in the circumstance of tumor hemorrhage despite irrefutable evidence of metastasis. A cat with a primary extraskeletal hepatic osteosarcoma was treated with surgery and carboplatin and was alive 42 months after diagnosis with no clinical evidence of disease.[68] On the other hand, metastases must always be considered as a possibility when a hepatic tumor is diagnosed.

Benign mesenchymal neoplasms, such as fibroma and hemangioma, have been described but are quite rare.[6,1,29,59]

Lymphoma

In dogs the liver can be involved in variable forms of lymphoma, including multicentric, alimentary, and hepatosplenic forms. A study in cats documented that abdominal lymphoma is currently the most common anatomic location and the liver occasionally is the only organ involved.[69,70] Many protocols are recommended for treatment of lymphoma in dogs and cats; most include vincristine, cyclophosphamide, and prednisone, with variable combinations of L-asparaginase, methotrexate, and doxorubicin. Careful evaluation of liver function is necessary before starting chemotherapy because many drugs undergo hepatic metabolism and altered hepatic clearance may lead to unpredictable and potentially increased toxicity.[5]

Other Neoplasms

Surgical resection with liver lobectomy is recommended for cats with primary hepatic myelolipoma and the prognosis is excellent with prolonged survival time and no reports of local recurrence.[7]

In dogs with advanced disease, mast cell tumors can metastasize to the liver. Primary visceral mast cell tumors are more common in cats than dogs. The spleen is usually the primary site with metastasis to the liver and bone marrow, and the survival time with splenectomy alone can be a year or more.[71] The overall prognosis for disseminated mast cell tumor in the dog is grave. The median survival time reported in one study was 43 days despite therapy with various chemotherapy agents.[72] Canine mastocytoma involving the liver can be controlled with cyclophosphamide, vinblastine, and prednisone.[73] Recently, tyrosine kinase inhibitors have shown some promise and CCNU has been shown to be active against feline mast cell tumors.[49,74]

Hepatic Nodular Hyperplasia

Hepatic nodular hyperplasia is a common benign lesion observed in the liver of older dogs that can occasionally be observed in some cats. It is characterized by a discrete accumulation of hyperplastic hepatocytes arising as either macroscopic or microscopic hepatic nodules. It reportedly occurs in 70% of dogs older than 6 years and 100% of dogs over 14 years.[75-77] The WSAVA standards for clinical and histologic diagnosis of canine and feline liver diseases include hepatic nodular hyperplasia in its classification system of hepatocellular neoplasia so that it may be differentiated from true neoplasia.[78]

Etiology

The etiology of hepatic nodular hyperplasia is unknown. It has been suggested to be a preneoplastic lesion,[76] but this has not yet been reported in the dog.[77] Because of hepatocyte microscopic changes, it is suggested that nutritional and metabolic disorders play a role in the pathogenesis of this lesion.[77]

Pathophysiology

Hepatic nodular hyperplasia is characterized microscopically by well-differentiated hyperplastic hepatocytes with increased mitotic activity.[75-77] Hyperplastic nodules may be accompanied by concurrent focal intrahepatic cholestasis, mechanical compression on

surrounding hepatic parenchyma, as well as alterations in the microvascular circulation. Vacuolar changes are seen frequently, suggesting a reactive or metabolic condition such us hyperadrenocorticism, lipidosis, or hypothyroidism.[77]

Clinical Examination

Nodular hyperplasia affects older dogs with a mean age of 11 years without gender or breed predisposition. Hepatic nodular hyperplasia does not appear to cause clinical signs or illness.[77]

Laboratory findings may include mild to marked increases in serum alkaline phosphatase activity and, less commonly, increases in serum ALT activity. Liver function tests are usually normal with hepatic nodular hyperplasia.[77]

Diagnosis

Hepatic nodular hyperplasia is usually discovered as an incidental finding during a diagnostic workup for other medical problems. Nodular hyperplasia is clinically important because it may easily be confused with primary or metastatic hepatic neoplasia during abdominal ultrasound or at surgery. Even microscopically, it may be impossible to differentiate hepatic nodular hyperplasia from hepatocellular adenomas, and a large sample (wedge rather than needle biopsy) may be required to confirm hyperplasia from well-differentiated HCCs.[75-77]

Routine abdominal radiographs are generally unremarkable and ultrasonographic features are inconsistent because of the varied hepatocellular morphologic characteristics and size of the nodules.[79] Multiple nodules varying in size, distributed randomly among the liver lobes, being superficial or deep within the parenchyma are found in most cases.[77]

Hyperplasic nodules of hepatocytes need to be differentiated from regenerative nodules. Hyperplasic nodules develop in livers of normal mass, whereas regenerative nodules arise as a result of compensatory hyperplasia of surviving hepatocytes in a background of hepatic injury, atrophy, and fibrosis.

Treatment

No treatment is usually required. Rupture of large nodules may require emergency mass removal and blood transfusion (rare).

Prognosis

Hepatic nodular hyperplasia has no significance in the morbidity of affected patients.[77]

METABOLIC DISORDERS

Deborah S. Greco

Metabolic disorders of the liver are commonly encountered in companion animal practice. This section focuses on the metabolic liver disease induced by concurrent endocrinopathies (hyperthyroidism, hypothyroidism, diabetes mellitus, and hyperadrenocorticism), lipid disturbances (lipoproteinemias, feline hepatic lipidosis, and hyperlipidemias), and metabolic infiltration (amyloidosis). Hepatic lipidosis and hyperthyroid hepatopathy are the primary metabolic hepatopathies in cats. In dogs, steroid (or glycogen vacuolar) hepatopathy is the most frequent metabolic liver disorder; diabetic hepatopathy and hyperlipidemic hepatopathies (lipoproteinemias, hypothyroidism) occur less commonly.

Box 61-4 Factors Predisposing the Domestic Cat, an Obligate Carnivore, to Fat Mobilization and Hepatic Lipidosis

- Essentiality of dietary arginine[6]
- Low levels of hepatic ornithine[7]
- High dietary protein requirements[7]
- Lack of hepatic enzyme adaptation to low protein[8]
- Insufficiency of hepatic glutamate reductase[7]
- Insufficiency of intestinal ornithine transcarbamylase[7]
- Diversion to orotic acid metabolism[9]
- Differences in lipoprotein metabolism (HDLs)[10,11]

Feline Hepatic Lipidosis

Etiology

Feline hepatic lipidosis (HL) is a metabolic syndrome found in obese, middle-aged cats that undergo a period of acute anorexia and catabolism. Morbidly obese cats are at increased risk and more than 85% of cats with HL suffer from an underlying disorder that contributes to the initial anorectic event. [1-5]

Pathophysiology

Although the underlying pathogenesis of hepatic lipid accumulation in cats has not yet been completely elucidated, several unique biochemical and nutritional features place this obligate carnivore at risk for fat mobilization and fatty infiltration of the liver during periods of anorexia or starvation (Box 61-4).[2,6-11] There is a general consensus that reduced caloric intake and protein-calorie malnutrition are important predisposing factors. The result is a rapid mobilization of peripheral fat culminating in fatty accumulation in the liver.[1] Intracellular processing of fats is an important function of the hepatocyte. During fasting or starvation, fatty acid metabolism becomes deranged in an obligate carnivore as a result of obesity, catabolism, chronic overnutrition, impaired fatty acid oxidation or VLDL secretion, and enhanced hepatic fatty acid synthesis (Figure 61-29).[1,6-11]

Clinical Examination

HL is a disorder of middle-aged to older cats; domestic short-haired cats are more commonly affected. Cats with HL often present with a history of acute stress and/or near-complete anorexia of several days duration.[1-3] Icterus is a variable feature of HL. When serum bilirubin concentrations exceed 1.5 mg/dL, clinical icterus can be observed on the pinnae, mucous membranes, sclera, and hard palate in the cat. In general, most cats with HL are obese at the time of presentation, with many cats being 20% to 30% over ideal body weight prior to an episode of HL. Other physical features of HL include hepatomegaly, dehydration, vomiting, and weakness. If HE develops as a consequence of HL, neurologic abnormalities such as ptyalism, stupor, coma, ataxia, and seizures may be observed.[1-3]

A minimum database, including complete blood cell count, serum chemistry, and urinalysis, almost always reveals severe liver enzyme elevation and other abnormalities such as nonregenerative anemia, stress leukogram, poikilocytosis, and bilirubin crystalluria.[1] The pattern of liver enzyme elevation is typically cholestatic in nature and characterized by marked increases in serum ALP activity, followed by smaller increases in serum ALT and serum AST activities. Serum GGT activity is often normal in affected cats. Increased serum bile acids and bilirubin are often observed in cats with HL, and electrolyte abnormalities, such as hypophosphatemia and

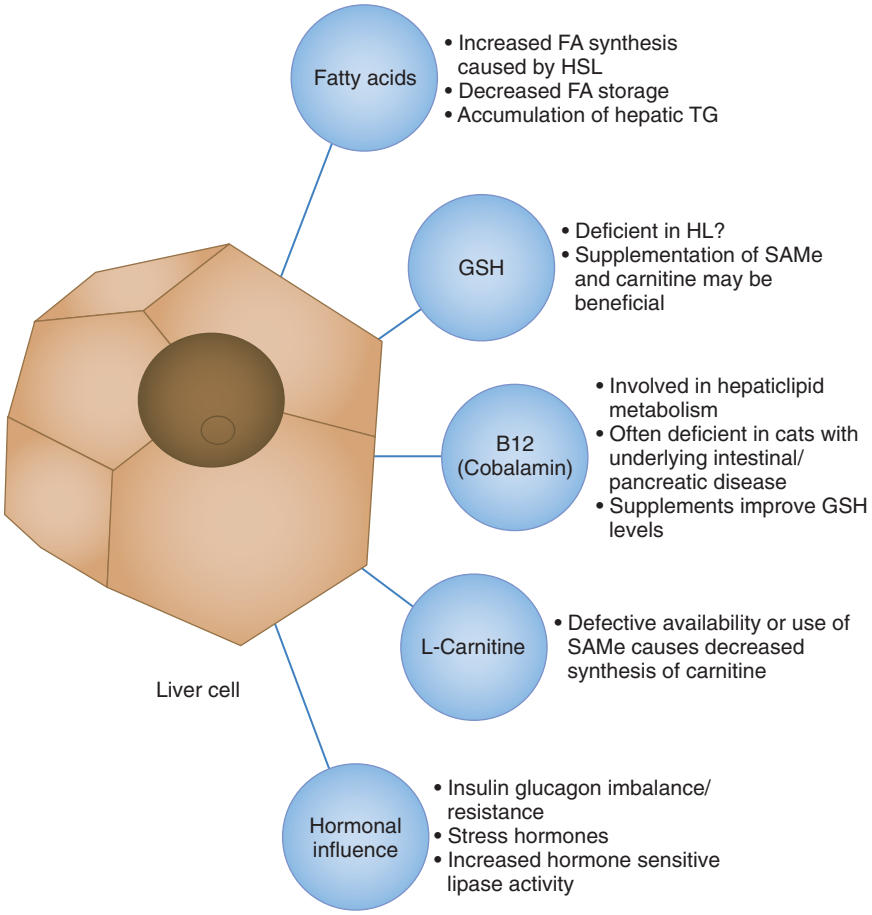

Figure 61-29 Fat metabolism in the feline hepatocyte during hepatic lipidosis.

hypokalemia, may be frequently observed. In particular, the presence of hypophosphatemia should alert the clinician to the possibility of refeeding syndrome.[12]

Diagnosis

Presumptive diagnosis of feline HL can be made on the basis of clinical history, physical examination, clinicopathologic features, ultrasound examination, and liver aspirates.[1,13-15] Ultrasound examination of the liver often reveals hepatic parenchyma that is hyperechoic to that of falciform fat, but a thorough ultrasound evaluation of the gallbladder, pancreas, intestines, kidneys, bladder, and other abdominal structures is essential to rule out other primary disorders, such as acute pancreatic necrosis, which may be the basis of the anorectic event precipitating an episode of HL. Definitive diagnosis is best achieved through liver biopsy[16]; however, anesthesia and biopsy may not be possible in acutely ill patients because of the presence of coagulopathies from vitamin K deficiency.[17] A liver aspirate that reveals more than 80% fatty infiltration of the hepatocytes may be used for presumptive diagnosis of HL. If there is no response to treatment after 3 to 5 days, liver biopsy may be necessary to rule out other underlying hepatobiliary conditions such as cholangitis.

Treatment

A catabolic state develops quickly in the anorexic cat and prompt measures should be taken to place an enteral feeding tube (Table 61-9). Nasoesophageal, esophageal, and gastrostomy tubes can be used for this purpose. The caloric needs should be approximately 60 to 90 kcal/kg body weight in most cats.[1,18] Unless HE is present, dietary protein should not be restricted (ideal is 35% to 45% protein on a dry matter basis) and even then protein restriction is controversial as protein is needed to support hepatic regeneration. Feeding multiple small frequent meals may help to maintain euglycemia and lessen the metabolic impact on the liver. The protein content of the diet should be considered when HE is present (see Chapter 32). Dairy and vegetable-based proteins are higher sources of branched-chain amino acids than meat-derived proteins and may lessen the signs of HE. Diets high in fiber generally should be avoided because they decrease the nutrient density of the diet.

Cats with HL occasionally may experience a refeeding syndrome, a condition that results in metabolic and electrolyte disturbances.[12] With the reintroduction of food, insulin secretion promotes intracellular uptake of phosphorus, potassium, and magnesium. Hypophosphatemia can result in muscle weakness and hemolytic anemia. Gradual reintroduction of food and correction of electrolytes diminishes the risk of refeeding syndrome.

Glucose intolerance and hyperglycemia are common in cats with HL and can be addressed by decreasing the carbohydrate content of the diet. Canned low-carbohydrate, high-protein formulations without added fiber are ideal for the treatment of feline HL as they provide amino acids, limited carbohydrates, and water, and are easily administered through a feeding tube. Small amounts of food should be administered via the feeding tube after residual gastric fluid contents have been removed. Trickle feeding can be performed by placing liquefied food into an empty fluid bag and allowing gravity to force flow into the feeding tube. Alternatively, a large-bore

Table 61-9 Nutritional and Therapeutic Support of Cats with Hepatic Lipidosis

Feeding Tubes	General Tips	Diet to Feed	Fluid Therapy and Supplements	Drugs and Supplements
Nasogastric Nasoesophageal	Trickle feed 60 kcal/kg/day	Liquid high-protein, low-carbohydrate diet	Crystalloid fluids Avoid dextrose and lactate KCl, KPO_4 B vitamins	Maropitant 1-2 mg/kg SC, PO, IV q24h Ondansetron 0.1 to 1.0 mg/kg q12-24 h Vitamin K_1 0.5 to 1 mg/kg SC q12h for 3 transfusions
Esophagostomy or gastrostomy	Multiple times daily 60 to 90 kcal/kg/day	Canned high-protein diet*		L-Carnitine 250 to 500 mg/day Taurine 250 to 500 mg/day SAMe 20 to 40 mg/kg/day Vitamin E 10 IU/kg/day PO

*Less than 10% carbohydrate, >40% protein on dry matter basis.

syringe attached to a syringe pump may be useful in delivering the food through the feeding tube.

Crystalloid fluids supplemented with fortified B vitamins, including thiamine, riboflavin, niacinamide, D-panthenol, pyridoxine, and cyanocobalamin, should be used.[1] Nutritional supplements to enhance antioxidant function, such as vitamin E and glutathione precursors (e.g., SAMe) may also be beneficial. Amino acid supplements that support hepatic regeneration and metabolism include carnitine and taurine.[1,18-20] Carnitine functions in the transport of fatty acids into hepatic mitochondria for energy production. Taurine is an essential nutrient for cats and is involved in CNS, cardiac, and biliary functions. Signs of taurine deficiency may be similar to those associated with HE.

Antiemetic therapy is necessary to control vomiting and facilitate feeding of an appropriate type and quantity of diet (see Chapters 23 and 35). Injectable antiemetics, such as maropitant (Cerenia, Pfizer Animal Health, Kalamazoo, MI), a selective NK-1 receptor antagonist, at a dosage of 1 mg/kg SC or IV on a daily basis, is preferred.[21] Oral maropitant at the same dosage or oral ondansetron, a 5-HT_3 receptor antagonist at a dosage of 0.1 to 1.0 mg/kg q12-24 h may be used in cats with larger-bore feeding tubes. Persistent vomiting should be investigated to identify feeding tube occlusion or other undiagnosed disease.

Prognosis

The prognosis depends upon duration of illness, and the time frame of resolution of hepatic enzyme elevation, hyperbilirubinemia, and other biochemical changes. Cats that survive an episode of HL have a greater than 50% reduction in liver enzyme and bilirubin concentrations within 10 days of therapy, whereas cats that die usually do so within 7 days of hospitalization.[1] Long-term prognosis for recovery is good with the majority of cats having resolution of HL as long as the underlying disease process (e.g., pancreatitis) is identified and treated.

Hyperthyroid Hepatopathy

Etiology

Hyperthyroidism in cats is caused by adenomatous hyperplasia of the thyroid gland resulting in increased circulating concentrations of thyroxine and triiodothyronine.[22,23] Hyperthyroxinemia increases hepatic metabolism without proportionate increases in hepatic blood flow with the overall consequence of reduced oxygen delivery to hepatocytes.[24]

Pathophysiology

Increases in serum AST and ALT activities have been reported in approximately 80% of hyperthyroid cats.[22,23] Liver enzyme elevation has been attributed to increased liver metabolic activity compared to blood flow. Long-term untreated hyperthyroidism in human beings can ultimately lead to cirrhosis.[24-26]

Clinical Examination

Middle-aged to older cats are typically affected, and there is no breed or sex predilection. Because hyperthyroidism is characterized by hypermetabolism, polyphagia, weight loss, PD, and PU are prominent features of the disease.[22,23] Hyperactivity, tachycardia, pupillary dilation, and behavioral changes are also characteristic of the disease and are associated with activation of the sympathetic nervous system. Long-standing hyperthyroidism leads to hypertrophic cardiomyopathy, high-output heart failure, and cachexia. Long nails, dermatologic conditions, panting, elevated body temperature, and poor grooming or overgrooming are additional clinical signs of feline hyperthyroidism.

Clinicopathologic features of hyperthyroidism include erythrocytosis and stress leukogram (neutrophilia, lymphocytosis) caused by increased circulating catecholamine concentrations. Increased catabolism of muscle tissue in hyperthyroid cats may result in increased BUN, but not serum creatinine. Most cats will have decreased urine specific gravity, particularly if they are exhibiting PU as a clinical sign. Increased metabolic rate results in liver hypermetabolism, therefore serum activities of liver enzymes (ALT, AST) are increased in more than 80% of hyperthyroid cats.[22,23]

Diagnosis

Diagnosis of feline hyperthyroidism is achieved by measurement of serum total thyroxine (TT_4) concentration. Serum thyroxine concentrations are elevated in more than 90% of hyperthyroid cats, making this a very sensitive test of thyroxine-induced hypermetabolism.[22,23] False-positive test results are rare to nonexistent, suggesting that hyperthyroxinemia is a specific test for feline hyperthyroidism.

In a clinically hyperthyroid cat, thyroid hormones still fluctuate on a daily (and hourly) basis with hormone concentration intermittently decreasing into the normal range.[27] To avoid this type of diagnostic error the clinician should repeat blood sampling 1 to 2 weeks after the first test. Nonthyroidal disease can have a significant effect on circulating thyroid hormone concentrations.[28-30] In the case of persisting nonthyroidal illness (e.g., renal disease), the

measurement of unbound thyroxine (T_4) or free T_4 may be preferable to repeated TT_4 measurements.

Free T_4 concentrations are a very sensitive test for the diagnosis of hyperthyroidism with 98% of hyperthyroid cats exhibiting elevated serum free T_4 concentrations. The specificity of free T_4 is not as good as its sensitivity; as many as 12% of euthyroid cats with concurrent illness will have high free T_4 concentrations for reasons that remain unclear.[29] As a result, free T_4 should not be used as a screening test, and free T_4 values should be interpreted in light of the TT_4 concentrations. The combination of a high free T_4 with a low TT_4 is indicative of nonthyroidal illness; however a high free T_4 with a high-normal TT_4 is suggestive of hyperthyroidisim.[31]

Treatment

Methimazole (Tapazole) is the antithyroid drug most often recommended (2.5 to 5 mg q12h). It is available as a transdermal gel or as an oral tablet. Methimazole is often used to prepare the patient for surgical thyroidectomy or radioiodine therapy. Antithyroid drugs have several side effects. Anorexia and vomiting are common side effects of methimazole, whereas rare side effects include self-induced excoriation of the face, thrombocytopenia, bleeding diathesis, agranulocytosis, development of serum antinuclear antibodies, and cholangitis. Bleeding, jaundice, and agranulocytosis necessitate immediate withdrawal of the drug. Hepatic injury related to antithyroid therapy such as methimazole is well documented in humans and reported in the cat.[22,32] Mild histologic changes are common, but cases of fulminant hepatic failure with central lobular necrosis have been described.[33]

Prognosis

Prognosis is excellent with definitive therapy of the hyperthyroidism (surgery or radioactive iodine). Hepatic reactions to methimazole will necessitate discontinuation of therapy.

Diabetic Hepatopathy (Hepatocutaneous Syndrome, Superficial Necrolytic Dermatitis)

Etiology

The etiology is unknown, but hypoaminoacidemia may play a role in the development of diabetic hepatopathy.[34,35] Fatty acid, niacin, and zinc deficiencies also may be involved in the pathogenesis. Increased serum glucagon, originally thought to be the cause of diabetic hepatopathy, is found in only one-third of the reported cases. A much stronger association between the skin lesions of superficial necrolytic dermatitis and glucagonoma, hyperglucagonemia, and poorly regulated diabetes mellitus have been observed in both humans and dogs.[34,35]

Pathophysiology

Hepatopathy is thought to occur secondary to the metabolic abnormalities associated with diabetes mellitus, glucagonoma, or nutritional deficiencies.[34-37] Hepatic features include vacuolar hepatocyte degeneration, hepatic parenchymal collapse, and hepatic nodularity.

Clinical Examination

The disorder is seen most frequently in middle-aged male dogs, and has been reported in one cat.[34-37] Acute presentations may include clinical signs such as vomiting, diarrhea, lethargy, weight loss, PD, PU, icterus, and lameness because of dermatopathy of the footpads. In some cases clinical signs are mild or nonexistent. Physical examination may reveal poor body condition, lethargy, and characteristic lesions of superficial necrolytic dermatitis (hard, cracked foot pads and elbows). Painful feet caused by footpad lesions are common.

Clinicopathologic features include mild nonregenerative anemia, microcytosis (with advanced liver dysfunction), increased serum liver enzyme (ALP and ALT) activities, hypoproteinemia, hypoalbuminemia, and fasting hyperglycemia. Serum bile acids are usually increased. Serum glucagon is inconsistently elevated, but plasma amino acid concentrations are often less than 50% of normal.[34,37]

Diagnosis

Abdominal ultrasonography may reveal small, normal or increased liver size; however, there usually is a characteristic "Swiss cheese" appearance of the hepatic parenchyma as a result of hepatic degeneration, nodularity, and collapse.[36,38] Pancreatic imaging and biopsy are indicated if a glucagonoma is suspected.

Treatment

Symptomatic palliative therapies may be beneficial and include high-protein diets with egg white (approximately 2 to 4 egg whites/day for a 25-kg dog), zinc (2 mg/kg q24h PO) niacinamide (250 to 500 mg/dog q24h PO), ursodeoxycholic acid (10 to 15 mg/kg/day PO), vitamin E (10 IU/kg daily PO), SAMe (20 mg/kg/day PO 2 hours before feeding), and fatty acid supplementation. Some patients will respond to 10% parenteral amino acid solutions (Aminosyn, Abbott Laboratories, Chicago) given at a dose of 500 mL over 8 to 12 hours intravenously through a large-bore central venous catheter. If no response is observed following the initial amino acid infusion, therapy should be repeated every 7 to 10 days for a total of four treatments.

Prognosis

Prognosis is poor for most cases; however, remissions of longer than 2 years have been reported with intensive amino acid and hepatic support therapy.[37]

Steroid Hepatopathy

Etiology

Steroid hepatopathy develops following exogenous corticosteroid therapy, or from endogenous hyperadrenocorticism of pituitary or adrenal origin. The dog liver is uniquely susceptible to both glucocorticoid- and sex steroid–induced liver enzyme elevation, glycogen accumulation, and vacuolar degeneration.[39,40]

Pathophysiology

In healthy dogs, glucocorticoid administration results in significant liver enzyme (ALP and ALT) elevation in 2 to 3 days. Increased ALP and GGT activities develop in parallel as the enzymes undergo induction and release from sinusoidal and canalicular membranes. Within 7 days of glucocorticoid administration, the glucocorticoid-induced ALP isoenzyme increases significantly. Glycogen accumulates within the hepatocyte resulting in a vacuolar degeneration typical of the syndrome.[1,39,40]

Clinical Examination

Steroid hepatopathy occurs primarily in the dog. There is only one reported case of steroid hepatopathy in the cat.[41] A history of corticosteroid administration or signs consistent with endogenous steroid overproduction (Cushing syndrome) are usually evident, for example, PD, PU, panting, potbellied appearance, bilaterally symmetric alopecia on the trunk, and polyphagia. In dogs affected with

atypical hyperadrenocorticism caused by sex steroid overproduction, dermatologic changes (alopecia, poor hair coat) and reproductive manifestations (perianal adenoma in a castrated male or female dog) are often the only signs suggestive of sex steroid imbalance. Atypical hyperadrenocorticism with sex steroid excess may present with no clinical signs except increased serum liver enzyme activities.[1]

Diagnosis

Diagnosis of steroid hepatopathy should be based on a history of exogenous steroid administration or endocrine function testing with or without liver biopsy. Classically, liver enzyme elevations consist of moderate to marked increases in ALP and GGT, and mild to moderate increases in ALT and AST. Bile acids may also be increased.[40]

The low-dose dexamethasone suppression (LDDS) test is considered the screening test of choice for endogenous canine hyperadrenocorticism.[42,43] The LDDS test has a high sensitivity at 92% to 95%. Only 5% to 8% of dogs with PDH will exhibit suppressed cortisol concentrations at 8 hours. In addition, 30% of dogs with PDH will exhibit suppression at 3 or 4 hours followed by "escape" of suppression at 8 hours. This pattern is considered diagnostic for PDH, making further testing unnecessary.[43] The major disadvantage of the LDDS test is the lack of specificity in dogs with nonadrenal illness.[44]

The corticotropin (ACTH) stimulation test is used to diagnose a variety of adrenopathic conditions, including endogenous or iatrogenic hyperadrenocorticism, as well as spontaneous hypoadrenocorticism.[42,45,46] As a screening test for the diagnosis of naturally occurring hyperadrenocorticism, the ACTH response test has a diagnostic sensitivity of approximately 80% to 85% and a higher specificity than the LDDS test.[45,46] In a study by Kaplan and Peterson, only 15% of dogs with nonadrenal disease exhibited exaggerated response to ACTH stimulation.[44] I prefer the ACTH response test over the LDDS test as the ACTH response test is more accurate for the diagnosis of iatrogenic hyperadrenocorticism (if the history is incomplete) and sex steroid imbalance in addition to PDH or adrenal-dependent hyperadrenocorticism.

The urine cortisol-to-creatinine ratio (UCCR) is highly sensitive in separating normal dogs from those with hyperadrenocorticism; however, the test is not highly specific for hyperadrenocorticism because dogs with moderate to severe nonadrenal illness also exhibit elevated ratios.[47-49] An elevated UCCR should always be confirmed with an LDDS test. In the UCCR test, urine is collected for 2 days for a baseline UCCR. The animal then is given three doses of dexamethasone (0.1 mg/kg, PO q6-8 h) and the final UCCR is collected 24 hours after the first dose of dexamethasone. Failure of the UCCR to suppress into the normal range is diagnostic for hyperadrenocorticism.

Treatment

Treatment for exogenous hyperadrenocorticism consists of discontinuation of exogenous steroids by slowly weaning the patient to prevent the development of addisonian crisis. Treatment for endogenous hyperadrenocorticism can be achieved with chemotherapy (o,p′-DDD, or trilostane) or surgery (hypophysectomy or adrenalectomy). Treatment of sex steroid imbalance can be achieved with mitotane or trilostane.

Prognosis

Prognosis for steroid hepatopathy is good to excellent if diagnosed early and if corticosteroid injury can be abated by discontinuation of steroid therapy or treatment of the underlying disorder.

Miscellaneous Metabolic Hepatopathies

Lipoproteinemias

Etiology

Genetic abnormalities in lipid metabolism lead to diffuse vacuolar hepatopathy and biliary mucoceles.[1]

Pathophysiology

Increased circulating cholesterol and triglyceride cause a vacuolar hepatopathy associated with excess lipid accumulation and/or hepatocyte glycogen synthesis and storage. Chronic hypercholesterolemia increases biliary cholesterol content and predisposes to cystic hyperplasia, dysmotility of gallbladder smooth muscle, and biliary mucocele.[1]

Clinical Examination

Familial hypercholesterolemia and other hyperlipidemias are found in certain breeds of dogs including the Miniature Schnauzer, Shetland Sheepdog, Briard, West Highland White Terrier, Scottish Terrier, Cairn Terrier, and Beagle. Mixed-breed dogs may also be affected. Clinical signs are usually associated with necrotizing cholecystitis and may include icterus and cranial abdominal pain. More often, dogs are asymptomatic and biliary mucoceles are identified serendipitously during ultrasound evaluation for some other medical problem (such as pancreatitis). Clinical pathology findings usually include hypercholesterolemia or hypertriglyceridemia, and elevated liver enzyme activities, particularly ALP. Necrotizing cholecystitis may be accompanied by leukocytosis, neutrophilia, and hyperbilirubinemia.

Diagnosis

Diagnosis may be made by characteristic ultrasound findings of nongravitational gallbladder sludge, increased gallbladder wall thickening, "kiwi"-shaped mucosal image, and bi- or trilaminar appearance of the gallbladder wall. The hepatic parenchyma may have a pattern of multifocal hyperechogenicity and hypoechoic nodules.[1]

Treatment

The best treatment for biliary mucoceles is surgical removal of the mucocele and/or cholecystectomy and may become an emergency procedure if the clinical signs of necrotizing cholecystitis are severe. Medical therapy following surgical removal is usually necessary and includes a fat-restricted diet and lifelong treatment with ursodeoxycholic acid (15 mg/kg PO q24h).

Prognosis

Prognosis is good for patients undergoing successful removal of the mucocele as long as lifelong medical therapy is continued.

Amyloidosis

Etiology

In dogs and cats, amyloid deposition is usually secondary to sustained systemic inflammatory response, for example, chronic infection, chronic inflammation, immune disorders, and malignancy.[50] Amyloidosis is a familial disorder in the Chinese Shar-Pei dog, and in Abyssinian, Oriental, and Siamese cats.[51-54] Hepatic amyloidosis has also been reported secondary to vitamin A toxicity in cats.[55]

Pathophysiology

Deposition of amyloid fibrils within and between hepatic sinusoids results in progressive organ dysfunction. Light deposits are found in the space of Disse and heavier deposits are often found in the

sinusoidal lumen. Amyloid fibrils are readily detected on routine hematoxylin and eosin or Diff-Quik staining. Amyloidosis is confirmed on examination of Congo red–stained aspirates or biopsies under polarized light where the extracellular material shows characteristic green birefringence.[50] Concurrent amyloid deposition in the kidneys, liver, spleen, and adrenal glands can occur, but clinical manifestations of liver failure are most common.

Clinical Examination

Chronic progressive liver failure with clinical signs of anorexia, weight loss, and lethargy, is the typical clinical course in many cases. Some animals may instead present with acute collapse following hepatic rupture and intraabdominal hemorrhage.[50] Pallor of mucous membranes, hypothermia, and hepatomegaly are the most frequently recognized physical examination findings. Typical laboratory findings include regenerative anemia, leukocytosis, thrombocytopenia, marked elevations in serum ALT and AST, and marked prolongations in aPTT and PT times.

Diagnosis

Radiography is useful in detecting free peritoneal fluid, hepatomegaly, and irregular hepatic borders. Ultrasonography reveals a diffuse, heterogeneous echogenicity with highly echogenic ("sparkling") areas and hypoechoic foci.[50] Definitive diagnosis requires tissue biopsy and Congo red staining.

Treatment

There are no specific treatments for this disorder. Colchicine has been recommended because it may block formation of amyloid in the early stages of the disease, but it is of unproven benefit and has been associated with significant side effects. Dimethyl sulfoxide has been recommended because it may promote resorption of amyloid. As there are no specific therapies for this disease, treatment is instead largely symptomatic and supportive.

Prognosis

With progressive amyloidosis lesions, the prognosis for long-term survival is poor.

Lipoprotein Lipase Deficiency

A familial hyperlipoproteinemia has been reported in cats that is characterized by fasting hyperchylomicronemia, elevated circulating concentrations of VLDLs, and hypertriglyceridemia.[56,57] Serum cholesterol is only minimally elevated. The underlying biochemical lesion is a reduction in the activity of lipoprotein lipase, and the disorder is transmitted as an autosomal recessive gene. Xanthomas accumulate in the soft tissues, including the liver, but clinical signs are more often related to involvement of the peripheral nerves. Dietary fat restriction improves clinical signs in some affected animals.[58]

Hypothyroid Hepatopathy

Etiology

Decreased circulating thyroid hormone concentration affect hepatic metabolism and cholesterol turnover in the liver. Liver function tests are mildly disturbed in almost 50% of patients with hypothyroidism despite normal histologic findings.[59]

Pathophysiology

Decreased hepatic metabolism in hypothyroidism is reflected by reduced oxygen consumption.[33,59,60] Patients with a common bile duct stone and gallbladder stone have, respectively, sevenfold and threefold increases in the frequency of hypothyroidism.[61] The pathogenesis of stone formation in hypothyroidism is believed to involve hypercholesterolemia, gallbladder dysmotility, and bilirubin retention.[61]

Clinical Examination

The most common clinical symptoms of hypothyroidism are lethargy, weight gain, depression, hypothermia, and bradycardia. GI signs such as reflux esophagitis, gastric atony, constipation, diarrhea, and hepatopathy with mucocele formation are rare clinical signs of hypothyroidism in dogs.[62] Symmetric truncal or tail-head alopecia are a classic findings in hypothyroid animals.[62] Hyperkeratosis, hyperpigmentation, secondary pyodermas, and demodicosis are also observed.

Clinicopathologic findings such as normocytic normochromic anemia, hypertriglyceridemia, and hypercholesterolemia are seen in the majority of hypothyroid animals because of altered lipid metabolism and binding proteins (increased HDLs), decreased fecal excretion of cholesterol, and decreased conversion of lipids to bile acids.[63]

Diagnosis

Total serum T_4 concentration and endogenous thyroid-stimulating hormone (TSH) may be used to confirm the diagnosis of hypothyroidism. This combination of tests has been shown to have the highest specificity, sensitivity, and lowest overall cost. If the TT_4 is in the low normal or below normal range and the TSH is high, the animal is suffering from primary hypothyroidism.[64,65] If the TT_4 and TSH are both low, free T_4 by dialysis should be determined to distinguish euthyroid sick syndrome (normal free T_4) from true secondary hypothyroidism (low canine thyroid stimulating hormone [cTSH] resulting from pituitary TSH deficiency).[66]

Treatment

Synthetic thyroid hormone supplementation is the treatment of choice for hypothyroidism. Levothyroxine sodium therapy is started at a dosage of 0.02 mg/kg given orally twice daily.[66] Thyroid function should be monitored every 6 to 8 weeks for the first 6 to 8 months of treatment and then once or twice yearly thereafter. In stable well-controlled animals, the total treatment may be given once daily with excellent clinical results, as long as adequate peak hormone concentrations are achieved.[67]

Prognosis

With thyroid hormone replacement therapy in hypothyroid dogs, the prognosis is excellent.

INTRAHEPATIC BILIARY DISORDERS

Mark P. Rondeau

Cholangitis

Inflammatory disease involving the intrahepatic bile ducts is commonly encountered in veterinary practice. Cholangitis is recognized more commonly in cats than in dogs, but both species can be affected. The WSAVA Liver Standardization Group suggests that cholangitis be considered in the following four groups: neutrophilic cholangitis (NC), lymphocytic cholangitis (LC), chronic cholangitis associated with liver fluke infestation, and destructive cholangitis.[1]

Feline Cholangitis Complex

Cholangitis is a common hepatobiliary disorder of cats, second only to HL.[2] Although varying terminology has created some confusion regarding this syndrome, it is clear that feline cholangitis includes a spectrum of disease processes, including forms displaying neutrophilic inflammation and those lacking neutrophilic inflammation.

Neutrophilic Cholangitis

Histologically, NC is characterized by the presence of neutrophils in the lumen and/or epithelium of the bile ducts.[1] The disease is recognized to occur in acute and chronic forms. In acute neutrophilic cholangitis (ANC) edema and neutrophilic inflammation are seen in the portal areas, with occasional extension of inflammation to the hepatic parenchyma. In chronic neutrophilic cholangitis (CNC) there is a mixed inflammatory infiltrate consisting of neutrophils, lymphocytes, and plasma cells. Varying degrees of bile duct hyperplasia and fibrosis will be present depending on the chronicity of disease.

Etiology. Although the true etiology remains unknown, NC is largely suspected to be caused by ascending bacterial infection from the intestine.[1-3] Rates of bacterial isolation using traditional methods have varied greatly, from less than 20% to more than 60% in affected cats.[3,4] Recently, fluorescence in-situ hybridization (FISH) with a 16S rDNA probe that recognizes bacteria in general has been used to identify and localize bacteria in cats with cholangitis.[5] Combining traditional culture and FISH, bacteria were isolated in three of three (100%) cats with ANC and eight of 13 (61%) with CNC. The localization of the bacteria identified using FISH supports translocation of enteric bacteria as the cause of infection. Although it appears that bacteria play an important role in the etiology of NC in many cases, it is important to note that they are not identified in all affected cats. Some authors theorize that NC, and CNC in particular, may have an immune-mediated etiology with persistent inflammation following an initial bacterial infection or other unknown initiating factor.[3,6]

Pathophysiology. NC in cats is commonly associated with inflammatory bowel disease and pancreatitis.[3,4,7] The pathophysiology underlying the relationship of these diseases is unknown, but rational theories revolve around the unique anatomy of the feline biliary and pancreatic duct systems. In the cat, the common bile duct and pancreatic duct merge prior to entering the duodenum at the major duodenal papilla.[7] Cholangitis may develop secondary to reflux of ascending bacteria from the duodenum during vomiting. Pancreatitis may result from bacterial reflux into the pancreatic duct, or from pancreatic duct obstruction secondary to cholangitis.[7] In most reported cases, the inflammatory bowel disease associated with cholangitis is moderate or severe, whereas the pancreatitis tends to be mild chronic interstitial disease.[7]

NC is also commonly associated with extrahepatic bile duct obstruction (EHBDO). EHBDO has been identified in 40% of cats with ANC and 76% of cats with CNC.[4] Cholangitis and/or pancreatitis are the most common cause of EHBDO in the cat.[8] In one study, 64% of cats with EHBDO had cholangitis, representing 93% of cats that did not have a neoplastic cause.[8] It is unknown whether cholangitis is the cause or the result of EHBDO. Histologic changes consistent with CNC have been seen in the livers of cats with EHBDO secondary to pancreatic carcinoma, cholelithiasis and surgical occlusion of the common bile duct.[8] In contrast, cholangitis has been implicated as the sole cause of EHBDO resulting from proliferation of mucosa within the common bile duct.[8] Bacterial infection of bile has been commonly identified in cats with EHBDO,[4,8] but whether that infection is a cause or effect of EHBDO remains unknown.

Clinical Examination. Previous literature has highlighted differences in clinical presentation between cats with different forms of cholangitis. However, we have recognized few differences between the various forms[4,9] and suggest that any statistically significant differences cited previously hold little clinical relevance given the large degree of overlap within data ranges. NC can occur in cats of any age, breed, or sex. Clinical signs are nonspecific and include anorexia, lethargy, vomiting, and weight loss. The duration of these clinical signs ranges from a few days to a few months and may be shorter in cats with ANC than in those with CNC,[3] but this is not a consistent finding.[4,9] Physical examination findings commonly include dehydration and icterus. Fever is present in 19% to 37.5% of cases.[4,10] Some reports suggest that fever is more commonly associated with ANC than CNC,[10] while others recognize no difference.[4,9] Hepatomegaly is seen in fewer than half of the cases. Abdominal pain is noted occasionally.[3,4,9]

Diagnosis. Definitive diagnosis is made by examination of liver biopsy specimens, with ancillary diagnostics providing supportive information. Hematologic findings are variable and may include poikilocytosis, neutrophilia, and left shift, although these abnormalities are present in fewer than one-third of cases.[3,4,9,10] Biochemical analysis commonly reveals increased activity of ALT, AST, ALP, and GGT ranging in severity from mild to severe. However, increased liver enzyme activity may be absent in some cases. Serum total bilirubin is increased in most cases. Serum cholesterol may become increased in cases with EHBDO. Imaging findings are nonspecific for cholangitis, but may provide useful information regarding concurrent disease. Abdominal radiographs are rarely helpful. Ultrasonographic appearance of the liver in cats with NC can vary greatly, with the most common abnormality being a diffuse change in echogenicity ranging from hypo- to hyperechoic.[11] Dilation of intra- and/or extrahepatic bile ducts, gallbladder distention, increased gallbladder sediment, and thickening of the gallbladder or bile duct walls may be seen. Gallbladder distention and bile duct dilation may indicate EHBDO, but these changes may occur in cats with cholangitis lacking obstruction. Ultrasonography will also provide information regarding the presence of concurrent disease, such as pancreatitis and inflammatory bowel disease.

Wedge liver biopsy during laparotomy is the optimal method for obtaining a definitive diagnosis. Other biopsy techniques that may be considered include laparoscopic and ultrasound-guided Tru-Cut needle approaches. Tru-Cut needle biopsy diagnoses correlate with wedge biopsies in fewer than 50% of cases.[12] Diagnostic accuracy of laparoscopic liver biopsies compared with wedge biopsies have not been evaluated. Laparotomy and laparoscopy provide the additional benefit of evaluation and sampling of extrahepatic structures. Laparotomy should be performed in any cat suspected of having EHBDO. While the optimal sampling strategy is unknown, biopsies should be obtained from multiple liver lobes, as we have recognized wide ranges of severity between different lobes in the same cat. In patients that are not stable enough for liver biopsy, such as those with hypotension, coagulopathy or HE, fine-needle aspiration with cytology offers a less-invasive diagnostic approach as it can usually be performed quickly with light sedation. However, liver cytology correlates with biopsy results in only 39% to 60% of cases.[13,14] Cytology is sensitive for identifying the presence of HL, however this is the most common misdiagnosis when using cytology.[14] Cytology is

insensitive for identifying cholangitis in cats, diagnosing fewer than 30% of cases.[14] Cytologic examination of bile may prove more sensitive for the diagnosis of NC in cats. In five of seven cats with CNC evaluated at this institution, bile cytology revealed neutrophilic inflammation, presence of bacteria, or both. Techniques have been described for safely obtaining bile via ultrasound-guided percutaneous cholecystocentesis in lightly sedated cats.[15]

Samples for aerobic and anaerobic bacterial cultures should be obtained in any cat suspected of having cholangitis. Gallbladder bile is preferred to liver tissue as the culture source. In a group of 58 cats suspected of having hepatobiliary disease, bile cultures isolated pathogens in 36% compared with only 14% of liver cultures.[16] In the same study, 22 dogs and cats had both liver and bile cultured and none had a positive liver culture in the absence of a positive bile culture.[16] In a group of cats with cholangitis, bile cultures were more likely to isolate pathogens (75% vs. 33%) and less likely to yield contaminants (4% vs. 29%) than liver cultures.[4] In a small study comparing bile versus liver cultures in 22 cats with various hepatobiliary diseases, bile culture was positive in five (four had CNC) while liver culture was positive in only two. In the two cats with positive liver cultures, the same organism was isolated from bile.[17]

It is important to recognize that many cats with NC (and other hepatobiliary diseases that mimic it clinically) are not stable enough to tolerate diagnostic testing. In such patients, the risk of aggressive diagnostics may outweigh the benefits of obtaining a definitive diagnosis. In these cases, the diagnosis may be suspected based on clinical response to supportive care, including broad-spectrum antibiotic therapy.

Treatment. Optimal treatment protocols for cats with NC are unknown and the recommendations herein are based solely on anecdotal clinical experience. Antibiotics are the mainstay of treatment. Drug selection is ideally based on results of bacterial culture and susceptibility testing. In cases where cultures are not performed, or while results are pending, broad-spectrum coverage should be provided. The most commonly isolated pathogens are aerobic and anaerobic bacteria of enteric origin,[16] including *E. coli*, *Enterococcus* spp., and *Clostridium* spp., among others.[3,4,16] Effective empiric antibiotic combinations would include a penicillin, a fluoroquinolone, and metronidazole. The optimal duration of antibiotic therapy is unknown, but we recommend a 4- to 6-week course for initial treatment.

Supportive care and treatment of specific sequelae of liver disease should be included as indicated. Nutritional support is required in many cats and is best accomplished by use of enteral feeding tubes. We recommend placement of esophageal feeding tubes in cats with cholangitis if they are anorexic and stable enough for general anesthesia. In unstable patients, nasoesophageal feeding tubes offer a less-invasive method of providing short-term support.

Several medications and nutritional supplements (including ursodeoxycholic acid [UDCA], SAMe, milk thistle, vitamin E, vitamin C, carnitine, taurine, and phosphatidylcholine) have been suggested for treating cats with cholangitis. While most of these compounds have theoretical benefits, a clinical benefit has not been proven. To optimize client compliance and avoid adverse drug reactions, I prefer to minimize the number of medications given to feline patients. Because most cats with ANC respond well to antibiotic therapy, I rarely include other medications in our treatment protocol. However, in cats with ANC that do not quickly respond to antibiotics and in many cats with CNC, I like to use UDCA. Among its theoretical benefits, UDCA has immunomodulatory and choleretic properties that make it a rational choice for treating cholangitis.

Because of the possibility of immune-mediated mechanisms in the perpetuation of NC, particularly with CNC, corticosteroids may be appropriate in some cases. Initial treatment should always involve antibiotics in cats with NC. Failure to improve within 2 weeks of antibiotic therapy, or clinical deterioration prior to that time, warrants initiation of corticosteroid therapy. Prednisolone at 1 to 2 mg/kg twice daily is given initially and gradually tapered to the lowest effective dose. Antibiotics should be continued concurrently with corticosteroids for a minimum of 4 weeks. The duration of corticosteroid therapy varies between individual patients. Many cases can be gradually tapered off of corticosteroids over 4 to 6 months, while others require lifelong therapy.

Surgical intervention is required in cats with EHBDO; however, the optimal surgical procedure is unknown. Biliary diversion (cholecystocholedochostomy, choledochoduodenostomy, or cholecystojejunostomy) and choledochal stenting are the most common procedures. Surgery in cats with EHBDO is associated with significant perioperative morbidity. In many cases, profound hypotension develops intraoperatively after 45 to 60 minutes as a result of decreased vascular responsiveness and decreased myocardial contractility and is often refractory to interventions such as fluid or vasopressor therapy.[8,18,19] Whichever surgical procedure is chosen, it is clear that anesthesia time should be minimized and long-term medical management will be necessary. Biliary diversion is associated with short-term mortality rates of 36% to 57%[8,18] and is associated with long-term complications.[8,19] In a small case series describing choledochal stenting in cats with pancreatitis and cholangitis, five of seven experienced long-term survival (≥7 months), but reobstruction occurred in two of seven and chronic vomiting and recurrent cholangitis were reported.[19]

Prognosis. The prognosis for cats with NC is typically good.[3,4,10] Survival to discharge was reported in 72% of all cats with cholangitis in one study.[4] Median survival time of 29.3 months has been reported in cats with NC, with no difference between ANC and CNC.[10] Prognostic factors have not been identified. Given the high rate of perioperative morbidity and mortality, it seems likely that cats with EHBDO have a worse prognosis than those without EHBDO. Thirty percent to 40% of cats with EHBDO secondary to inflammatory disease die within a week of surgery.[8,18] However, in those that survive to discharge, long-term survival has been reported.[18]

Lymphocytic Cholangitis

The WSAVA Liver Standardization Group describes LC as a common, slowly progressive, chronic disease of cats characterized histologically by infiltration of small lymphocytes (and occasionally plasma cells or eosinophils) restricted to the portal areas associated with varying degrees of fibrosis and bile duct hyperplasia.[1] They remark that inflammation centered on the bile ducts may be present, but is not a hallmark of the disease. It is also stated that well-differentiated lymphoma may be difficult to differentiate from LC. Based on the existing literature regarding LC in cats, the description from the WSAVA group includes several clinically and histopathologically different subsets that may or may not revolve around a common pathogenesis. Recognition of these different subsets within the umbrella of LC may have therapeutic and prognostic ramifications.

Several investigators describe a group of cats with LC where inflammation is confined to portal regions and there is a lack of targeting of bile ductules or biliary epithelium.[2,20,21] This has been

referred to as *lymphocytic portal hepatitis*.[2,21] The connection between this histopathologic finding and clinical disease in cats is unknown, as it may represent a common change associated with aging. It was identified in 82% of cats older than 10 years of age and 96% of cats older than 15 years of age from a necropsy population that did not have primary liver disease.[21] It is also possible that this lesion represents a response to inflammation at a distant site, as it is similar to the lesion of nonspecific reactive hepatitis associated with chronic extrahepatic disease.[1] Although clinical signs have been described in cats with lymphocytic portal hepatitis,[10] the common occurrence of concurrent disease in these cats makes it difficult to know if the clinical signs are attributable to the lesions in the liver.

In another subset of cats, LC is marked by inflammation targeting bile ductules and infiltrating biliary epithelium, leading to progressive ductopenia.[20,22,23] These cases seem more likely to have clinical disease attributable to their liver pathology, although side-by-side comparisons of cases with and without bile duct targeting have not been performed. Cats with this form of LC in the United States have a similar clinical picture to cats with NC.[9] In the United Kingdom, this lesion has been associated with ascites, icterus, and hyperglobulinemia in young cats and termed *progressive lymphocytic cholangitis*.[22,23]

Etiology. Although the etiology of LC is unknown, theories suggest that it is an immune-mediated or infectious phenomenon. Genetic factors may also play a role, as Persian cats are overrepresented in the United Kindgom.[22,23] Immunohistochemistry in affected cats has provided evidence for an immune-mediated pathogenesis, although the inciting antigen is unknown.[20,23] Bacteria have been identified in the liver or bile of fewer than 20% of cats with LC.[3-5,17,20] Although *Helicobacter pylori* has been isolated from the liver and bile of cats with cholangitis, the evidence for this organism playing an important role in feline cholangitis is not compelling at this time.[24,25]

Pathophysiology. As with NC, concurrent inflammatory bowel disease and pancreatitis appear to be common in cats with LC,[3,4] although some authors report it to be uncommon.[23] The theory that reflux of duodenal bacteria into the biliary and pancreatic ducts incites inflammation may hold true for cats with LC, although a common immune mechanism must be considered.

Clinical Examination. The clinical picture of cats with LC varies widely and has significant overlap with other forms of hepatobiliary disease in cats, including NC.[4,9] Although some studies describe a predominance of older cats,[3] others describe more younger cats.[22,23] Nonspecific clinical signs, including anorexia, lethargy, vomiting, and weight loss, may be chronic and intermittent.[3,6,22] Physical examination findings may include icterus, hepatomegaly, or ascites, but none are consistent findings. Signs of HE (dullness, ptyalism, seizure) may develop in severely affected cats.

Diagnosis. Definitive diagnosis is made by liver biopsy. As discussed for NC, ancillary diagnostics will provide information to support hepatobiliary disease, but are not specific for LC. Hematology results may be unremarkable, even though marked lymphocytosis has been described in some cases.[3] Activity of serum liver enzymes is increased in many, but not all cases and varies in severity. Hyperglobulinemia has been described.[3,6,22] Abdominal radiographic and ultrasonographic findings are nonspecific, but may aid in the recognition of concurrent disease.

Distinction between LC and well-differentiated (small cell) lymphoma can be a challenge even for experienced pathologists. Preliminary data using immunohistochemistry and PCR for T-cell receptor clonality has not proven useful in differentiating between the two conditions. Surprisingly, cats with both LC and lymphoma had monoclonal T-cell receptors, oligoclonal T-cell receptors, and polyclonal T-cell receptors.[20] Using light microscopy, the following features were unique to LC and not present in cats with lymphoma: ductopenia, bile duct targeting by lymphocytes, and the presence of lipogranulomas within portal regions (representing a residual marker of cell death).[20] Until more studies are done evaluating molecular techniques, these features may prove useful in differentiating the two conditions. Interestingly, bile duct hyperplasia and fibrosis were present in cats with LC and those with lymphoma. This may suggest that an inflammatory state precedes the development of lymphoma,[20] which has been reported anecdotally.

Treatment. The therapeutic approach to cats with LC should be similar to that described for cats with NC in regards to supportive care and symptomatic treatment of the sequelae of liver disease. Because bacteria have been isolated from some cats with LC, I recommend treatment with broad-spectrum antibiotics while awaiting results of bacterial cultures. In contrast to NC, long-term treatment of culture negative cats with antibiotics is not warranted. Immunomodulation and immune suppression are the major components of treatment based on a presumed immune-mediated etiology. Cats that are culture negative, or that have failed to respond to antibiotics within a few days, should be treated with prednisolone at 1 to 2 mg/kg twice daily. Responders should be tapered gradually over 4 to 6 months to the lowest effective dose. Other drugs that are useful for immunomodulation include metronidazole and UDCA. Cats that fail to respond completely to corticosteroids and/or other immunomodulators, or who relapse while being treated, may require additional immunosuppressive drugs. Although these drugs have not been well evaluated in cats with LC, chlorambucil and methotrexate are suggested by some authors.[3] Cats with small cell lymphoma often respond to combination therapy with prednisolone and chlorambucil, but they may require a multidrug weekly sequential chemotherapy protocol.

Prognosis. Cats with LC have a variable prognosis,[3,6] likely a result of being diagnosed at different stages of a chronic disease process. Survival of greater than 5 years has been reported, and many cats that die appear to succumb to disease unrelated to the liver.[22] Other cases have been reported that fail to respond to treatment and die more acutely,[6] though this is uncommon in my experience. This is a disease that likely requires lifelong management and monitoring with relapse of illness possible as medication doses taper.

Chronic Cholangitis Associated with Liver Fluke Infestation

Trematode parasites of the families *Dicrocoeliidae* and *Opisthorchiidae* may inhabit the gallbladder and bile ducts of cats and rarely dogs.[26] There are multiple species with worldwide distribution. The most commonly identified species include the dicrocoelid *Platynosomum concinnum* and the opisthorchid *Amphimerus pseudofelineus*.[27-29] *P. concinnum* is mainly found in tropical and subtropical areas, including the southeastern United States.[26] *A. pseudofelineus* has a wider area of distribution throughout North and South America.[26] The life cycle is similar for both dicrocoelids and opisthorchids.[26] Parasite eggs are ingested by a land snail (*Subulina octona* or *Eulota* [*Bradybaena*] *similaris*), develop into cercariae and enter a second

intermediate host.[26,27,29] The dicrocoelids tend to use an arthropod, while opisthorchids utilize fish. Typically cats acquire infection by ingesting the second intermediate host. In the case of *P. concinnum*, the sporocysts leaving the snail intermediate host may be eaten by a paratenic terrestrial isopod host (pill, sow, or dung bugs).[26,29] Cats are infected by ingesting this form in a variety of lizard or amphibian intermediate hosts. Cercariae migrate from the cat intestine to the gallbladder and bile ducts, where they develop into adults. Eight weeks or more after infection eggs are passed in the feces to complete the life cycle.[27]

Clinical signs are proportional to parasite burden. Cats with light infections are often asymptomatic.[27,28] Clinically ill cats may present with nonspecific signs such as anorexia, lethargy, vomiting, or diarrhea. Severely affected cats present with signs of EHBDO such as icterus and acholic feces.[27-30] Preliminary diagnostics are nonspecific for fluke infestation. Eosinophilia proportional to the parasite burden may be present.[29] Serum liver enzyme activity may be mildly to moderately increased, although it is normal in many cases.[28-30] Abdominal ultrasound often reveals evidence of EHBDO.[28-30] Definitive diagnosis by identification of flukes or fluke eggs in the feces is difficult as small numbers of eggs are shed daily, the eggs have varying morphology at different stages of development, and the eggs are quite small.[27] Fecal concentration-sedimentation using the formalin-ether technique is the most reliable method of identifying eggs in stool.[27,29] Eggs may also be identified in cytologic preparations of bile.[28,30] Eggs or adults may be seen in liver biopsy specimens, but they are inconsistently identified.[1,27,28,30] Histopathologic changes seen in the liver are characterized by dilation of larger intrahepatic bile ducts associated with papillary projections and marked periductal and portal fibrosis. Mild to moderate inflammation may be present within the ducts (neutrophils and macrophages) and in the portal areas (neutrophils, lymphocytes, plasma cells). Eosinophils may be present in limited numbers.[1] Rarely, chronic cholangitis associated with liver flukes can result in the development of cholangiocarcinoma.[1,29]

Optimal treatment protocols have not been established, but praziquantel at 10 to 20 mg/kg daily for 3 days appears to be the most effective.[26-30] Doses as high as 40 mg/kg daily have been used successfully,[28] but this dose has also been fatal in cats.[26] Sporadic resumption of egg shedding following praziquantel has been reported, suggesting that it does not completely eliminate infection.[26,27] For this reason, continued treatment at 12-week intervals has been recommended.[29] Symptomatic and supportive therapy should be tailored to the individual patient. Cats with EHBDO require surgical decompression. Glucocorticoids and UDCA may have some benefit in controlling inflammation and providing choleresis. Although infected cats may remain asymptomatic, patients with EHBDO appear to have a grave prognosis. Long-term survival has only been reported in rare cases.[28,30]

Canine Cholangitis

Cholangitis is rarely reported in dogs. Reports of cholangitis in dogs include two distinct entities: destructive cholangitis and NC.

Destructive cholangitis is characterized histopathologically by a loss of bile ducts (ductopenia) within the smaller portal areas, associated with cholestasis, portal inflammation consisting primarily of macrophages, neutrophils, and occasionally eosinophils, and progressive portal fibrosis.[1,31,32] This is a rarely reported lesion of unknown etiology. It has been postulated that the lesion represents an idiosyncratic drug toxicity. However, of the eight cases reported in the literature only three had a prior drug history: two having received potentiated sulfonamides and the other having received amoxicillin-clavulanate, milbemycin oxime, and amitraz prior to the onset of clinical signs.[31,32] The proposed toxic etiology is based on these case histories and histopathologic similarity to humans with idiosyncratic drug toxicity. Other toxic insults and viral infection, such as canine distemper, can also result in destructive cholangitis.[1] Affected dogs typically present for signs referable to cholestasis, including anorexia, icterus, vomiting, and acholic feces.[31,32] Activities of serum liver enzymes and total bilirubin are moderately to markedly increased. Abdominal ultrasound may be unremarkable, showing only mild dilation of intrahepatic bile ducts.[32] Definitive diagnosis is made by liver biopsy. Optimal treatment options are unknown, but should undoubtedly involve discontinuation of any medications that preceded illness. Corticosteroids for immune suppressive and antiinflammatory effects and UDCA for immunomodulatory and choleretic effects would be rational treatments options, but their efficacy has not been documented. The prognosis appears to be poor, as six of the eight reported cases were euthanized within 6 weeks and the remaining two had only short-term followup (<6 months).[31,32]

NC, as described in the cat, has been rarely reported in dogs.[33-37] Bacteria have been isolated from the majority of cases reported, including *E. coli*, *Klebsiella* spp., *Proteus mirabilis*, *Streptococcus* spp., and *Clostridium* spp.[34-37] Although the bacterial species would support ascending infection from the intestine, the literature is too sparse to make conclusions about the pathophysiology of this disease in dogs. Bacterial infection could also spread hematogenously or via translocation from the portal circulation. The clinical presentation and diagnostic findings are similar to those reported for cats with NC. Affected dogs present with lethargy, anorexia, vomiting, and icterus usually of acute onset. Fever is reported in approximately half of the cases.[33-37] Neutrophilia with or without a left shift is common. Activity of serum liver enzymes is typically increased and most dogs have mild to moderate elevation of serum total bilirubin. Ultrasonography is nonspecific, with the liver varying from normal to hyperechoic with some heterogeneity.[34,36] Thickening and hyperechogenicity of the gallbladder wall is common.[36] Definitive diagnosis is made by liver biopsy with changes similar to those reported in cats with NC. Most dogs have some degree of mixed inflammatory infiltrate, similar to CNC.[33-37] This infiltrate extends into the hepatic parenchyma in the majority of cases. Aerobic and anaerobic culture of bile or hepatic tissue should be performed, though bile has been the source of bacterial isolation in most cases. Treatment involves antibiotic therapy guided by culture and susceptibility results. Duration of treatment should be prolonged, as antibiotic courses of 8 to 12 weeks or greater have been required to completely eliminate bile infections. Clinical improvement precedes bacterial eradication.[36] The prognosis appears to be good in most cases, although dogs with concurrent disease may have a worse prognosis.

Congenital Disorders

Cystic Disease

Liver cysts arising from the intrahepatic bile ducts are rarely encountered in veterinary practice. Although cysts may be acquired secondary to trauma, neoplasia, inflammation, or biliary obstruction,[1,38] the vast majority of cases described in the literature are congenital in origin. Congenital cystic liver diseases result in dilation of various segments of the intrahepatic bile ducts, and they are associated with varying degrees of hepatic fibrosis and cysts in other organs (most commonly the kidneys). Little is known about inheritance patterns of cystic disease in dogs and cats. The various morphologic patterns of cystic disease likely represent abnormalities of bile duct

development at different stages of their formation. The WSAVA Liver Standardization group suggests that cystic disorders be classified into one of the following groups: congenital dilation of the large and segmental bile ducts; juvenile polycystic disease/congenital hepatic fibrosis; and adult polycystic disease.[1]

Congenital dilation of the large intrahepatic bile ducts (i.e., the hepatic ducts and segmental ducts) has been described in dogs.[39-41] The lesion is similar to that of Caroli disease in humans and it is thought to represent an early defect in the formation of the intrahepatic bile ducts.[1,40] The disease is marked by extreme, diffuse, grossly evident dilation of the extrahepatic portion of the large intrahepatic bile ducts containing pale-yellow viscous fluid. The gallbladder and common bile duct are normal, as these have a separate embryologic origin from the intrahepatic bile ducts. The liver is normal to mildly increased in size, with diffuse cysts of varying sizes throughout. Histologically there are areas of marked bridging portal fibrosis containing multiple dilated bile ducts. The lobular architecture of the liver is normal. Although concurrent ascending cholangitis commonly occurs in humans, this is rarely reported in dogs.[39-41] In addition to the hepatic lesions, affected dogs have fusiform, radially arranged renal cysts with moderate to marked fibrosis throughout the renal cortex and medulla.[39-41] Affected dogs are presented early in life, ranging from 13 weeks to 3.5 years.[39-41] Clinical signs include vomiting, weight loss/failure to thrive, decreased appetite, lethargy, ascites, and rarely icterus and neurologic signs. The clinical signs are typically chronic in nature. GI signs, neurologic signs, and ascites are likely a result of portal hypertension caused by pressure of the cysts on the portal vein. Hepatomegaly may be noted on physical examination. Activity of serum liver enzymes is typically normal to mildly increased, although marked increases in activity of ALT and ALP have been reported.[40] Renal azotemia is present in some patients. Ultrasonographically, cystic dilations of the intrahepatic ducts (most with associated calcification) are easily recognized, even though the renal cysts are not always apparent. Definitive diagnosis is made on the basis of the gross and histologic findings described above. Rational treatment options and prognosis are unknown, as only one dog in the literature has been treated. This dog was doing well on a low-protein diet at 5 months of followup.[40] Most of the affected dogs have been fairly stable, and supportive care may be warranted despite the appearance of severe cystic disease.

Juvenile polycystic disease/congenital hepatic fibrosis has been described in litters of Cairn Terriers, West Highland White Terriers, and cats.[42-44] This form is analogous to autosomal recessive polycystic kidney disease in humans, and the inheritance appears to be autosomal recessive in the few families of veterinary patients that have been described.[42,43] The liver cysts are thought to represent an intermediate defect in the development of the intrahepatic bile ducts.[1,40] The liver involvement is primarily microscopic including fibrotic portal areas containing abnormally structured, dilated small bile ducts. The result is a grossly enlarged and firm liver.[40] Renal cysts are present and are identical to those described for dogs with dilation of the large and segmental bile ducts.[40] Affected animals are usually presented at less than 8 weeks of age for abdominal distention because of renomegaly and hepatomegaly.[42,43] However, one 12-year-old cat with similar lesions has been reported.[44] Most affected animals are ill or have died at the time of presentation.[42,43] Liver enzyme activity has been increased in the few animals in which it was evaluated.[43] Abdominal ultrasound may identify cysts and definitive diagnosis is made based on histologic findings as described. Treatment has not been attempted, and the prognosis appears to be grave.

Adult polycystic disease is most commonly recognized as polycystic kidney disease in Persian cats.[44,45] It has also been reported in cats of other breeds and in dogs.[39,43] This is similar to autosomal dominant polycystic kidney disease in humans. Inheritance in Persian cats is autosomal dominant.[45] Liver cysts are thought to represent a late defect in the development of peripheral intrahepatic bile ducts. The liver may contain multiple cysts ranging from less than 1 mm to greater than 12 cm in diameter. These cysts typically contain clear, colorless fluid. Discrete fibrotic areas containing small, irregularly formed bile ducts, referred to as *Von Meyenburg complexes*, may be present.[1] In the kidneys, multiple cysts may form in any segment of the kidney but may involve only a small percentage of the nephron population. This is in contrast to the diffuse cysts seen with congenital dilation of the large and segmental bile ducts and juvenile polycystic disease.[1,40] Hepatic cysts are present in 10% to 40% of cats with polycystic kidney disease, while hepatic fibrosis is recognized in up to 48%.[44,45] The hepatic cysts are usually incidental findings and the animals are not clinically ill unless they develop renal failure secondary to cysts in the kidneys, which happens in adulthood.

Biliary Atresia

Biliary atresia is an extremely rare congenital disorder, having been reported in only one dog and one cat.[46,47] In both cases, the common bile duct was not patent because of atresia. In the dog, the occluded segment of bile duct was histologically comprised of fibrous tissue with minimal inflammation.[47] The etiology is unknown, but this lesion likely represents an embryologic nonfusion of the cranial (hepatic) and caudal (cystic) anlages of the bile ducts during development.[1] Other possible explanations include ischemic, toxic, traumatic, or infectious insults occurring pre- or postnatally.[47] Affected animals have presented at 4 to 6 months of age with clinical signs of depression, anorexia, vomiting, or lameness associated with rickets because of inadequate vitamin D absorption.[46,47] Affected animals show icterus, hepatomegaly and acholic feces. Serum biochemistry abnormalities are consistent with EHBDO. Definitive diagnosis is made at exploratory laparotomy. Surgical biliary diversion is a viable treatment option depending on the location of atresia, but it was unsuccessful in the one case reported.[47] A guarded prognosis should be given as for any animal undergoing a biliary diversion procedure (see discussion under "Neutrophilic Cholangitis").

Intrahepatic Cholestasis

Cholestasis is impaired bile flow resulting in the accumulation of bile components in the blood.[1] Intrahepatic cholestasis occurs secondary to a variety of primary or secondary hepatobiliary diseases.[1,48,49] Increased activity of serum liver enzymes, particularly ALP and GGT, is common with intrahepatic cholestasis but is not specific for the condition. Clinically patients may appear jaundiced, but the predominant clinical sign will be related to the underlying disease process. Cholestasis is marked by the presence of bile plugs in canaliculi, phagocytosed bile in Kupffer cells, and bile granules within hepatocytes. These changes are easily recognized in cytologic and frozen preparations, but are less apparent in paraffin-embedded specimens, particularly in cats.[1] When cholestasis is identified, EHBDO should be ruled out. This should be easily accomplished by abdominal ultrasonography as animals with intrahepatic cholestasis lack the dilation of intra- and extrahepatic bile ducts that is typical of EHBDO.[48] However, exploratory laparotomy should be considered in highly suspicious cases for confirmation.

Intrahepatic cholestasis is associated with extrahepatic bacterial infection in dogs.[49] This syndrome is well characterized in humans and may occur in other species such as the cat. It represents an important differential diagnosis for hyperbilirubinemia in animals without primary liver disease, as over 40-fold increases in total bilirubin have been reported. The physiology behind this mechanism is incompletely understood, but it is thought to result from reduction of bile salt–dependent and –independent bile flow caused by bacterial toxins and/or inflammatory mediators.[49]

Neoplastic Disorders

Tumors of biliary origin in dogs and cats include cholangiocellular adenoma, cholangiocellular carcinoma, and carcinoid.[50] They are uncommon, representing less than 1% of all canine and feline neoplasms.[51-54] The tumors of epithelial origin, cholangiocellular adenoma and carcinoma, are the most common, comprising 40% of all hepatic neoplasms in dogs[51-53] and 56% to 80% of all hepatic neoplasms in cats.[54-56] Tumors showing characteristics of both hepatocellular and cholangiocellular carcinoma have been reported rarely.[50,52] In dogs, 70% to 100% of biliary epithelial tumors are malignant,[51,52] while in cats 35% to 43% are malignant.[54-56] Cholangiocellular adenomas commonly contain cystic components, especially in cats, and have been referred to as biliary or hepatobiliary cystadenomas in this species.[57,58] Cholangiocellular tumors arise predominantly from intrahepatic bile ducts in both species. Extrahepatic location is more common in cats than in dogs and is always associated with malignancy.[51-54,56] In both species, cholangiocellular carcinomas are more likely to present as multiple or diffuse tumors than are adenomas.[51,52,54] Carcinomas are highly metastatic (70% to 90% rate) with local lymph nodes, peritoneum, and lung being the most common sites of metastasis.[51,52,54] The etiology of biliary neoplasia is unknown. Affected dogs and cats are typically middle-aged to older, although animals with malignancy may present at a younger age than those with benign disease.[52,56] Clinical signs are usually vague (such as anorexia and lethargy) and somewhat chronic. Malignant tumors are more likely to cause clinical signs, as many benign tumors are incidental findings not associated with illness.[51,56-58] Hepatomegaly or the presence of a cranial abdominal mass may be identified on physical examination. Increased activity of serum liver enzymes is more commonly associated with malignancy, often being absent with benign tumors.[51,56,58] Even though abdominal radiographs will often identify the presence of hepatomegaly or an hepatic mass, ultrasonography is the preferred imaging method for identification of biliary neoplasms. This modality allows for determination of the cystic nature of the tumors[58] and for the evaluation of metastatic potential. Fine-needle aspiration and cytology are of limited utility for diagnosis of biliary epithelial tumors. Carcinoma was correctly identified via liver mass aspiration in only 20% of cases in one study.[14] However, cytology is recommended as it likely exhibits high specificity, especially for metastatic lesions. Surgical excision and biopsy is the optimal diagnostic and therapeutic technique for tumors confined to one or two liver lobes. Surgical excision appears to be curative in cats with benign tumors,[56,57] but malignant tumors carry a poor prognosis in both dogs and cats with many cases not surviving to discharge and survival greater than 6 months not reported in any case.[56,59] Adjunctive chemotherapeutic protocols have not been reported.

Carcinoids, also referred to as neuroendocrine tumors, are far less prevalent in dogs and cats than the tumors of biliary epithelial origin.[52,54,55] These tumors are thought to develop from neuroendocrine cells in the epithelium of bile ducts or gallbladder or from hepatic progenitor cells.[50] They may be identified at intrahepatic or extrahepatic locations. They appear to have a more aggressive course than cholangiocellular carcinomas, with the majority being present in multiple lobes and greater than 90% having metastasized at the time of diagnosis.[52,54,55]

EXTRAHEPATIC BILIARY DISORDERS

Michael D. Willard and Theresa Fossum

Etiology

The major pathogenetic mechanisms of canine and feline extrahepatic biliary tract disease are obstruction, inflammation, and exudation. The major causes of extrahepatic biliary tract obstruction (EHBO) are pancreatitis, gallbladder mucoceles (in dogs), cholelithiasis, parasitic infections (in cats), and tumors. EHBO is more common in dogs than cats. Biliary tract inflammation (e.g., cholecystitis, cholangitis) is primarily caused by bacterial infection, but can be nonseptic or parasitic, especially in cats. Gallstones may be associated with biliary tract disease (e.g., infection, obstruction), but the majority of them appear to be clinically silent. Stones in the gallbladder are termed *choleliths* while stones in the biliary tract are termed *choledocholiths*. Biliary tract exudation or leakage can be caused by traumatic (primarily of the bile ducts) or spontaneous rupture (primarily of the gallbladder). The latter is caused by necrotizing cholecystitis, which can be caused by sepsis (e.g., infectious cholecystitis), pressure necrosis (e.g., mucocele), or infarction. Biliary tract *tumors* are rare and of uncertain cause.

Pathophysiology

Extrahepatic Biliary Tract Obstruction

The common bile duct passes through the lesser omentum and the pancreatic parenchyma before entering the mesenteric wall of the duodenum. In the dog, it empties near the opening of the minor pancreatic duct at the major duodenal papilla, whereas in the cat it joins with the major pancreatic duct before emptying into the duodenum. Inflammation and edema with pancreatitis may be sufficient to cause compression and obstruction of the bile duct. This is probably the most common cause of canine EHBO.[1] There is, however, no consistent relationship between clinical severity of the pancreatitis and likelihood of EHBO, possibly because pancreatitis can affect different regions of the pancreas. Pancreatitis is a rare cause of EHBO in the cat. Chapter 60 discusses the breeds at increased risk for pancreatitis and causes.

Gallbladder mucoceles occur primarily in dogs. In such cases, the gallbladder is filled with inspissated, semisolid mucus that may extend into the bile ducts causing obstruction. Mucoceles can exceed the storage capacity of the gallbladder thereby causing pressure necrosis on the wall of the gallbladder. They can also spontaneously rupture (often at the fundus)[2,3] causing bile peritonitis. The cause of gallbladder mucocele is unknown, but might include dysfunction/hyperplasia of mucus-secreting cells in the gallbladder mucosa. Mucoceles may become secondarily infected.[2]

Gallstones are often clinically silent and observed incidentally only at the time of abdominal imaging. They can be associated with cholecytitis,[4] but they rarely cause EHBO because they must be small enough to enter the cystic bile duct but large enough to lodge there.

Parasites occasionally cause obstruction. *Platynosomum fastosum* (i.e., *P. concinnum*) is a fluke that inhabits the gallbladder and/or bile ducts of cats that are infected by eating lizards or toads.[5] Natural infections are found primarily in Florida, Hawaii, and the Caribbean. It may be asymptomatic or may cause obstruction or fibrosis. They are rare causes of cholecystitis.

Tumors may cause obstruction, and may be one of the more common causes in cats.[6,7]

Inflammation and Necrosis

Cholecystitis is most commonly caused by bacterial infection, ostensibly from bacterial migration up the bile duct. Various Gram-positive, Gram-negative, aerobic, and anaerobic bacteria (e.g., *Clostridium*, *Staphylococcus* spp., *Enterococcus* spp., *Streptococcus* spp., *Klebsiella* spp., *E. coli*, *Helicobacter* spp.) have been reported.[8-11] Infections caused by gas-producing bacteria can produce emphysematous cholecystitis. Because of a shared biliary and pancreatic ductal system in the cat, hepatobiliary disease is a well-established risk factor for pancreatitis in the cat. Aseptic inflammation of the gallbladder (necrotizing cholecystitis) has been reported, and infarction is one such cause.[11,12]

Exudation

Leakage of bile into the abdomen can be a result of mechanical forces (e.g., automobile trauma) that cause a shearing effect resulting in transaction of the common bile duct or one of the other bile ducts. Mechanical rupture has also been reported following gunshot trauma. Necrosis of the gallbladder raises the risk for rupture of the gallbladder and occasionally the bile ducts.

Gallstones

Canine and feline gallstones are typically composed of cholesterol, bilirubin, or may be mixed (as opposed to human gallstones which are usually caused by cholesterol).[4] Feline gallstones are typically calcium carbonate or mixed stones.[13]

Clinical Examination

Biliary tract diseases can cause severe clinical signs (e.g., anorexia, depression, vomiting, icterus, abdominal pain) or they may be relatively asymptomatic. Most symptomatic patients have serum biochemical abnormalities (e.g., increased serum ALT, ALP, and serum bilirubin). Hypercholesterolemia is common in patients with EHBO. Hyperbilirubinemia by itself does little besides cause icterus; therefore, patients with extremely high serum bilirubin concentrations can be relatively asymptomatic. Renal failure has been attributed to excessively high serum bilirubin concentrations, but it is unclear that bilirubin is the cause or that this is common. Shetland Sheepdogs[14] appear to have an increased risk for biliary tract disease, and Cocker Spaniels might also (see Chapter 62).

Besides icterus, clinical signs in patients with EHBO are primarily a result of the cause of the obstruction, not the obstruction itself. Canine pancreatitis in particular may cause severe clinical signs (e.g., anorexia, vomiting, abdominal pain—see "Complications of Liver Disease" section). However, not all patients with pancreatitis-induced EHBO have severe pancreatitis. Causes of EHBO that are insidious (e.g., mucoceles, tumors, and stones) are often unsuspected until the patient becomes icteric.

Biliary tract inflammation such as septic cholecystitis is well-documented in dogs and cats. Shetland Sheepdogs appear to be at a greater risk of inflammatory biliary tract disease than most other breeds.[14] Clinical signs vary, but most animals are clinically ill with anorexia and vomiting as more prominent symptoms. Fever is uncommon, icterus inconsistent, and leukocytosis is often insignificant, even with marked bacterial infections of the biliary tract.

Gallstones are generally asymptomatic. They can be associated with cholecystitis or EHBO.

Bilious abdomen can be a clinically mild condition, or it can be associated with life-threatening signs. Septic bilious abdomen causes extremely severe peritonitis with systemic inflammatory response syndrome (e.g., anorexia, vomiting, abdominal pain, poor perfusion, fever, and death). These patients may be in the initial hyperdynamic state (e.g., red mucus membranes, bounding pulse, fever, or hypothermia) or, if initially undiagnosed, can be in the late hypodynamic state (e.g., pale mucus membranes, weak pulse, and hypothermia). Intraperitoneal bile seems to make septic peritonitis more severe. In contrast, some animals with sterile bilious abdomen (e.g., as a consequence of automobile trauma) are essentially normal except for ascites and icterus.

Diagnosis

Plain radiography is occasionally diagnostic of biliary tract disease. Some gallstones are radiopaque (Figure 61-30).[4] Finding air in the gallbladder or in the wall of the gallbladder (i.e., emphysematous cholecystitis; Figure 61-31) is diagnostic of infection with a gas-producing bacterium. "Porcelain" gallbladder (i.e., a radiopaque gallbladder because of intramural mineralization of the gallbladder) is associated with carcinoma.[15]

Ultrasound is generally accepted as the most important and sensitive method for diagnosing extrahepatic biliary tract diseases.[7] Many patients that are not suspicious for biliary tract disease are fortuitously diagnosed when ultrasound or radiographs are requested for various reasons.[14] In difficult cases (e.g., partial EHBO versus complete EHBO), nuclear scintigraphy techniques can be used[16]; however, this seems to be rarely required.

Diagnosis of EHBO generally relies upon the use of ultrasound. Dilation of the bile ducts (normal canine bile ducts are ≤3 mm; normal feline bile ducts are ≤2 to 2.5 mm) is primarily caused by EHBO, and is generally seen by 3 days postobstruction. If one is unsure whether the ducts are dilated, repeating the examination in 3 days should be helpful. Enlargement of the gallbladder is not diagnostic of EHBO because anorexia and starvation of any cause may do the same thing. However, failing to find a dilated gallbladder

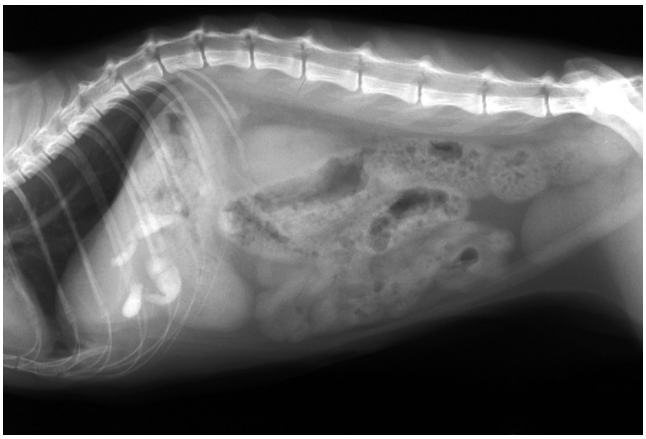

Figure 61-30 A lateral radiograph of a cat. There are several radiodense choleliths. These were serendipitous findings; the cat had no signs referable to abdominal disease. The cat was not treated for the stones and did well.

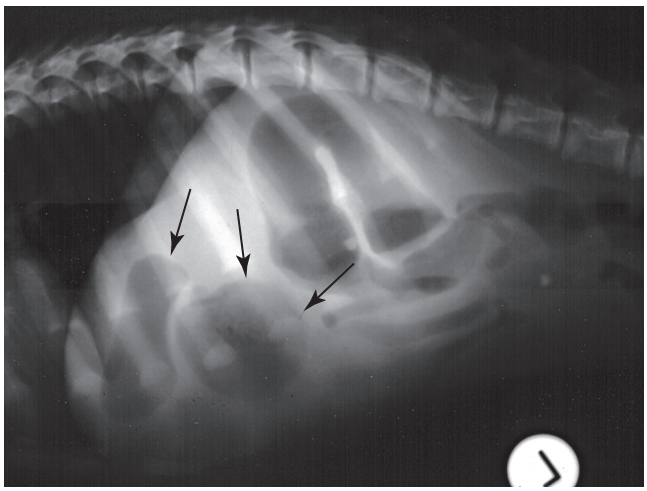

Figure 61-31 A lateral radiograph of a dog with emphysematous cholecystitis. The air-filled structure pointed out by *small arrows* is the pylorus; the air-filled structure pointed out by *larger arrow* is an air-filled gallbladder.

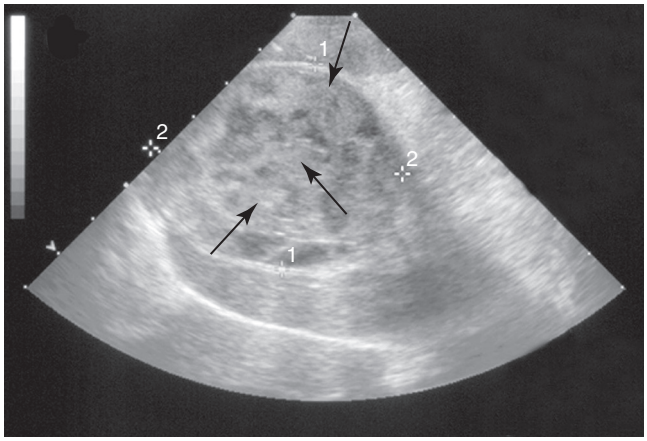

Figure 61-32 An ultrasonographic image of a Dachshund's gallbladder. The gallbladder is filled with hyperechoic material (*arrows*) that was not gravity dependent. The dog was asymptomatic for biliary tract disease and was still in good health without therapy for the gallbladder 10 months after this image was taken.

in a patient with clearly dilated bile ducts suggests biliary tract inflammation or prior EHBO. Any dog with EHBO should be suspected of having acute pancreatitis until proven otherwise. Chapter 60 details the diagnosis of pancreatitis. If acute pancreatitis is eliminated in a patient with EHBO, then one should look for gallstones and tumors, first by imaging and then by exploratory surgery or laparoscopy if imaging fails to provide a diagnosis.

Mucoceles are readily diagnosed by ultrasound, although there is some debate about what constitutes a mucocele. "Classic" mucoceles are described as producing a "kiwi fruit" appearance without gravity-dependent bile movement. "Sludge" in the gallbladder is a common finding (and it has gravity-dependent movement), but is not clinically significant.[17] The gallbladder of clinically normal dogs may appear abnormal on ultrasound examination (Figure 61-32), while patients with significant biliary tract disease may appear essentially normal. Whether or not patients with ultrasonographic abnormalities of the gallbladder will become symptomatic cannot be reliably predicted.

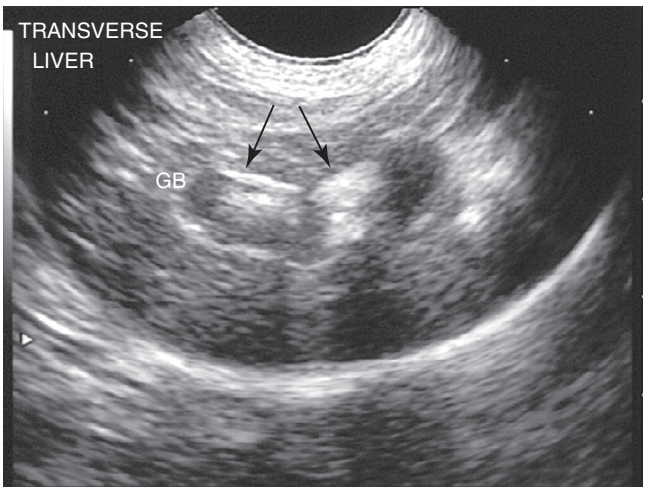

Figure 61-33 An ultrasonographic image of the gallbladder of a dog with gallstones (*arrows*). The stones were found fortuitously during an abdominal ultrasound; there was no evidence that they were causing any clinical signs.

Cholecystitis is often diagnosed by ultrasound-guided percutaneous aspiration of gallbladder bile for cytology and culture. This is a relatively sensitive and specific procedure for diagnosing biliary tract infection.[2,18] The finding of a dilated bile duct coincident with a normal-size gallbladder suggests cholecystitis, prior EHBO, or a rare congenital problem such as Caroli disease.[19] It is important to note that patients with bacterial cholecystitis may have no ultrasonographic or gross abnormalities. Ultrasound may be suggestive, but is relatively insensitive for cholecystitis.[8,20] Consequently, it is probably best to routinely aspirate bile for cytology and culture in patients with hepatobiliary disease.

Gallstones are relatively easy to diagnose, some can be found by radiographs, but almost all can be found by ultrasonography (Figure 61-33).

Treatment

With EHBO, the underlying cause is always the primary concern. EHBO in and of itself is not the primary consideration when deciding upon therapy. Pancreatitis, for example, is primarily a medical disease (see Chapter 60). Surgery is rarely appropriate in the management of pancreatitis even when it is causing EHBO. If deemed necessary, EHBO caused by pancreatitis can be relieved by percutaneous aspiration[21] or placement of a biliary tract stent.[22] If absolutely necessary, a cholecystoduodenostomy may be performed, but this surgery should be avoided if possible. These procedures are seldom necessary because almost all patients with EHBO caused by pancreatitis will experience resolution of the obstruction with medical therapy.

Gallstones should be removed only if they are causing obstruction or cholecystitis. It is usually better to perform a cholecystectomy[4,13] as opposed to a cholecystotomy; the former has a lower morbidity and mortality rate in people and presumably in dogs and cats as well. Biliary tract tumors can seldom be cured surgically.

If a patient has EHBO caused by something that cannot be treated medically (e.g., tumor, pancreatic stricture, traumatically torn bile duct), then a biliary bypass procedure can be performed. Cholecystoduodenostomy can relieve the obstruction, although the surgery requires special surgical skills. This surgery can predispose the patient to recurrent, ascending cholecystitis or other complications[10,23] and should only be performed in patients that

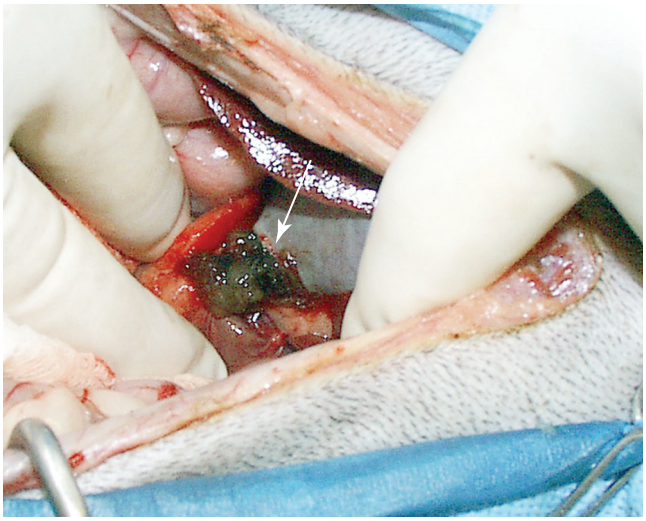

Figure 61-34 A photograph taken at surgery. The abdomen has just been opened, and bile has just begun to escape from a spontaneous rupture of the gallbladder wall (*arrow*) caused by necrotizing cholecystitis.

absolutely require it. Generally speaking, EHBO caused by pancreatitis that is not resolving as quickly as desired is not necessarily a good indication for this procedure; patience and medical therapy generally resolve the problem. In fact, pancreatitis can be a complication of surgery in this region.[24]

Many animals with hepatic disease are concurrently treated with antioxidants and other hepatoprotectants. Although most of these drugs will not hurt patients with biliary tract disease (in fact, they may be beneficial if the disease is extending into the hepatic parenchyma, such as cholangitis/cholangiohepatitis), care must be taken before administering UDCA. UDCA is a choleretic agent that stimulates bile flow. This could be disadvantageous in a patient with complete EHBO.

Mucoceles usually need to be removed surgically.[2,3,14] Medical management may be attempted (e.g., fat-restricted diet plus UDCA), but there is a substantially increased mortality rate for patients that experience gallbladder rupture (Figure 61-34); consequently, surgery is probably the safest course. Most patients are not at risk of immediate rupture. As long as ultrasonography does not suggest impending rupture, one should make sure the patient is an optimal anesthetic risk.

Bacterial cholecystitis, uncomplicated by EHBO or stone, should generally first be treated with antibiotics, usually for 4 to 6 weeks. However, some patients with bacterial cholecystitis cannot be cured with antibiotic therapy, ostensibly because the infection has localized in the gallbladder mucosa. These patients consistently relapse after discontinuation of antibiotics, and therefore a cure may be achieved only with antibiotics plus cholecystectomy. When performing this surgery, great care should be taken to avoid causing a stricture or obstruction of the common bile duct. Postcholecystectomy bile duct obstruction may prove fatal. It is also very important to be avoid traumatizing the pancreas to avoid severe pancreatitis.[24]

Biliary tract leakage is treated based upon the underlying cause. If the gallbladder has ruptured, cholecystectomy is usually most appropriate. If the bile duct has ruptured, it is very difficult to successfully anastomose the two ends. In that instance, one generally must perform a cholecystoduodenostomy and ligate both ends of the torn bile duct. It is important to avoid biopsying the gallbladder as that has a risk of dehiscence. In general, one should either remove the gallbladder, aspirate gallbladder bile, gently express the gallbladder, or leave it undisturbed. If the leakage is associated with septic peritonitis, then that is a true emergency and requires immediate, aggressive medical and surgical therapy.

Gallstones may be monitored if the patient is asymptomatic.

Tumors can rarely be resected; they are generally inoperable when found. Rare examples exist of patients with biliary tumors being cured surgically.

Biliary flukes can be treated with praziquantel (20 to 40 mg/kg SQ for 3 days).

Prognosis

Chapter 60 discusses pancreatitis in greater detail. Biliary mucoceles that have not ruptured often have a good prognosis; however, one report found a 32% mortality rate in patients without a rupture versus 68% mortality in patients with bile peritonitis.[14] The report authors recommended a more preemptive approach (i.e., versus waiting until the patent is symptomatic). Another group found no difference in mortality between rupture and nonruptured mucoceles (i.e., 21%).[3] Gallstones generally are innocuous, and even when they are causing signs have a good prognosis as long as rupture has not occurred. Bacterial cholecystitis has a good prognosis as long as the gallbladder is intact and not at risk for rupture. Septic bilious peritonitis has a very guarded to poor prognosis, depending upon the severity of the peritonitis. Gallstones are usually asymptomatic, but can occasionally cause a problem. They are usually easily removed and resolved. Biliary tumors are usually a poor prognostic finding[6,7] because of late diagnosis. Flukes can be treated, but without early recognition, extensive tissue injury may or may not resolve after therapy.

COMPLICATIONS OF LIVER DISEASE

Penny Watson

The liver serves many important functions including metabolism (carbohydrate, protein, lipid, nucleic acid, xenobiotics, porphyrins, vitamins, minerals, glutathione, endogenous hormones), coagulation factor synthesis, biliary secretion, and immune surveillance (see Chapter 1). It is not surprising, therefore, that animals with liver disease experience a broad range of complications reflecting perturbations in one or more of these functions.

Portal Hypertension and Its Consequences

The hepatic portal venous system accounts for up to 75% of the total hepatic circulation and serves to transport nutrients from the GI tract to hepatic sinusoidal capillaries (and hepatocytes) before coalescing once again into central veins, hepatic veins, and caudal vena cava. In health, the multiple branching of the main portal vein, venules, and capillaries reduces the overall resistance to blood flow (circuits in parallel reduce resistance), and therefore the pressure required to perfuse these capillaries is maintained at a low level of less than 5 to 6 mm Hg.[1,2] Disease processes that obstruct flow through the intrahepatic branches of the portal vein or sinusoids elevate this pressure and result in significant portal hypertension. Hepatocyte swelling impedes portal flow in acute pathophysiologic states, and fibrosis further impedes this flow in

chronic pathophysiologic states. Sustained portal hypertension is associated with many, but not all, of the complications of liver disease.

Portal hypertension is an important, potentially life-threatening complication of liver disease in the dog. It is rare or poorly documented in the cat. Portal hypertension develops most often in dogs with chronic liver disease and cirrhosis. It is occasionally recognized as a congenital lesion in young animals with arteriovenous fistulas,[3] or as a hypoplastic disorder of the intrahepatic portal vein branches resulting in a condition referred to as *noncirrhotic portal hypertension*.[4,5] Prehepatic portal hypertension can develop secondary to portal vein thrombosis or congenital hypoplasia of the extrahepatic portal vein, but these are less common. Sustained portal hypertension may progress to splanchnic congestion, GI ulceration, ascites, and encephalopathy.

Gastrointestinal Ulceration

Portal venous hypertension produces vascular stasis and venous congestion and increases the risk of GI ulceration, particularly in conjunction with other risk factors such as anorexia and steroidal and nonsteroidal antiinflammatory drug usage. Portal hypertension–related ulceration in the dog is typically duodenal although bleeding esophageal varices, similar to those reported in humans, are occasionally observed.[3] Glucocorticoids should be used only with great caution in dogs with portal hypertension.

Ascites

Reduced systemic blood pressure is another consequence of portal hypertension and splanchnic venous congestion. Changes in systemic blood pressure activate the renin–angiotensin–aldosterone system (as described in Chapter 8) and renal sodium retention, and thus increase total circulating fluid volume. The increase in circulating fluid volume (the "overfill" hypothesis) is believed to be the triggering event for the development of ascites in animals with portal hypertension.[6] This is why aldosterone antagonists are the initial treatment of choice in ascites caused by portal hypertension (for more details see Chapter 8). Ascites is a negative prognostic indicator in dogs with chronic hepatitis,[7] although individual animals with chronic hepatitis and ascites can be managed and maintained for many months.

Acquired Shunts

With sustained portal hypertension, multiple acquired PSSs develop and serve as a conduit for portal blood flow directly into the systemic circulation. Shunts serve to dissipate some of the increased portal pressure thus reducing the risk of adverse complications such as venous congestion, GI hemorrhage, and ulceration. They do, however, raise the risk of yet another complication of liver disease—hepatoencephalopathy.

Hepatoencephalopathy

HE is a syndrome of potentially reversible brain dysfunction resulting from impaired liver function. It results from either severe hepatocyte dysfunction or more commonly the presence of portosystemic collateral circulation, either congenital or acquired, where a variable combination of shunting of portal blood and hepatocyte dysfunction contributes to the clinical signs. It can be acute or chronic in presentation. Acute HE is most often a result of acute fulminating liver failure (see "Consequences of Hepatocyte and Biliary Tract Injury" section) and carries a poor prognosis. More chronic HE is usually a result of congenital or acquired PSSs.[2]

Table 61-10 Clinical Signs Attributed to Pathophysiology

Clinical Sign	Dysfunction
Anorexia, weight loss	Decreased metabolism; hepatic inflammation
Icterus	Biliary obstruction (intra- or extrahepatic) or dysfunction
Melena, hematuria	GI ulceration as a consequence of portal hypertension; coagulopathy
Ascites	Portal hypertension; hypoalbuminemia (reduced production in liver)
Polyuria/polydipsia	Multifactorial and poorly understood; may be contributions from hepatoencephalopathy; decreased urea cycling; increased antidiuretic hormone and cortisol and other factors
Hepatoencephalopathy	Hyperammonemia and other triggers (see text)
Depression, weakness	Hypoglycemia, anemia, hepatoencephalopathy
Vomiting, diarrhea	Portal hypertension (GI congestion); ascites; hepatic inflammation; hepatoencephalopathy; decreased xenometabolism

Clinical signs associated with HE in dogs and cats include depression, behavioral changes, circling/head-pressing, ataxia, apparent blindness, abnormal swallowing or salivation, stupor, seizures, and coma (Table 61-10). Salivation is much more common in cats than dogs with HE.

Brain dysfunction in HE was historically considered to be a neurotransmitter dysfunction but current evidence suggests low-grade cerebral edema caused by astrocyte swelling is the predominant pathologic change.[8] There is consensus that ammonia is the key toxin in HE but blood ammonia concentrations do not always correlate with severity of clinical signs. This is because a large number of other factors interact with the effects of ammonia to precipitate HE. Astrocytes play a central role because they express glutamine synthetase and detoxify the ammonia that reaches the CNS. Intraastrocyte accumulation of osmotically active glutamine in HE results in astrocyte swelling and thus low-grade cerebral edema.[8] This is largely reversible if the precipitating factors are treated, but edema can become severe and result in irreversible CNS changes in severe and acute HE. Precipitating factors include inflammatory cytokines, benzodiazepine-type sedatives, and disturbances in amino acid metabolism and dopaminergic neurotransmission.[8-10] The source of ammonia is primarily absorption from the gut, although other sources also exist as a result of interorgan metabolism. Gut-derived ammonia was traditionally assumed to be a by-product of intestinal bacterial metabolism in the colon. This remains an important source in some conditions such as melena. However, recent studies in other species suggest that small intestinal enterocyte metabolism of glutamine as their main energy source is the most important source of postprandial ammonia absorption in the portal vein.[9,10] This is also likely the case in most dogs on normal diets as it is very unusual for undigested protein to reach the colon, although the source of gut-derived ammonia has never been investigated in dogs. In normal dogs, ammonia is transported to the liver via the main portal vein, and further metabolized

to urea by hepatocytes in the Krebs-Henseleit cycle. With portosystemic shunting or severe hepatocyte dysfunction, ammonia accumulates in the brain (and other tissues) where it is taken up by astrocytes and results in edema as described previously. Dogs with HE also show disturbances in CNS aromatic amino acid metabolism.[11,12] The aromatic amino acids (tyrosine, tryptophan, and phenylalanine) accumulate in, the CNS in portasystemic shunting. In the brain, β-phenylalanine and tyrosine are metabolized to phenylethanolamine and octopamine, both of which can act as false neurotransmitters. However, dietary supplementation with branched-chain amino acids (e.g., leucine, isoleucine, valine) does not convincingly improve HE in either dogs or humans.[9,12] However, some dietary protein sources appear to be better than others in dogs with HE. Dogs on soya protein diets show a lower plasma ammonia concentration than those fed meat protein.[13] Dogs with HE have also traditionally been fed a protein-restricted diet. However, protein restriction is no longer advocated in humans with HE[9] and it may be the digestibility and type of protein rather than a reduced amount that are most important in dogs. More studies are needed to investigate this.

Several other metabolic alterations exacerbate clinical signs associated with HE, including acid–base disorders, electrolyte abnormalities, particularly hypokalemia, hypoglycemia, hypoxemia, and arginine deficiency (cats). An important trigger in humans and rodents is inflammation: Recent studies confirm that inflammatory cytokines are synergistic with ammonia in precipitating HE and that controlling inflammation in other organs is an important part of managing the patient with HE.[14,15] There is anecdotal evidence that this is also true in dogs.

Consequences of Hepatocyte and Biliary Tract Injury

Functional Reserve

The liver has significant structural and functional reserve capacity to support ongoing metabolic needs during mild to moderate forms of liver injury. Moreover, the liver has the ability to regenerate liver volume and cell mass during the recovery phase of most forms of liver injury. Signs of liver failure develop earlier with acute forms of liver injury than with chronic, progressive liver disease.

Zones 1, 2, and 3 hepatocytes of the hepatic acinus have differing functions. Zone 1 (periportal) hepatocytes, for example, have a high capacity to cycle ammonia through the urea cycle thereby reducing the toxicity of ammonia. Zone 3 hepatocytes (nearer the hepatic vein) have a lesser capacity for ammonia and instead convert it to glutamine.[16,17] In health, zonation permits flexibility in hepatic function such that in metabolic acidosis, for example, the liver can rapidly divert ammonia toward glutamine production, which is necessary for H+ ion excretion in the kidney. In severe acute liver injury, this becomes an important "tradeoff" because acute selective destruction of periportal (zone 1) hepatocytes more readily results in signs of encephalopathy because of the reduced ability of zone 3 hepatocytes to detoxify ammonia. In chronic liver disease, if hepatocytes undergo piecemeal necrosis at different rates in different zones, the remaining hepatocytes can assume some of those functions, so that clinical signs of deficiency are not seen until later in chronic disease processes.

Coagulopathy

Coagulopathy is a complication of both acute and chronic liver disease in dogs and cats. A recent study reported one or more coagulation abnormalities (prolongation of coagulation times, changes in platelet counts, D-dimers, fibrinogen, or protein C) in 24 (57%) of 42 dogs affected with liver disease.[18] Coagulation abnormalities are also common in cats with liver disease and one study found abnormalities in 18 (82%) of 22 cats with liver disease.[19]

Multiple mechanisms of coagulopathy are possible in liver disease patients. In ALF cases such as xylitol toxicity in dogs,[20] HL in cats,[21] and cirrhosis in dogs,[18] loss of normal hepatocyte function results in severe coagulation factor deficiency. Vitamin K deficiency has also been implicated in coagulopathy particularly in cats in which cholestasis impedes bile salt secretion, emulsification, and micellarization of fat and fat-soluble vitamins and fat-soluble vitamin absorption.[21] Concurrent inflammatory bowel disease and pancreatitis exacerbate this condition in many cats with cholangitis.[21] Finally, platelet abnormalities (cytopenia and cytopathy) may contribute to coagulopathy in dogs with liver disease.[18,22]

Regeneration

The liver has the unique ability to regulate its growth and mass. Hepatocyte loss caused by viral, bacterial, or chemical injury, or partial hepatectomy triggers hepatocyte replication.[23,24] Liver injury not only stimulates hepatocyte turnover but may also stimulate biliary proliferation and activation and proliferation of HSCs. These changes usually occur together in an orchestrated wound-healing response. In the case of hepatocyte loss, normally quiescent hepatocytes replicate to restore the liver functional capacity and mass. These are the main cells that regenerate liver mass. However, in severe injury or where hepatocyte turnover is inhibited by senescence, a progenitor cell reserve may also replicate and regenerate liver mass.[24] Although hepatocytes are capable of replication, they have very slow turnover in a normal liver and there are negative consequences of long-term increased stimulation and turnover in chronic liver disease. It has been shown that cycling hepatocytes suffer irreversible erosion of telomeres, which leads to senescence.[25] Functional capacity is a relative rather than absolute parameter. The set point for growth regulation is the ratio between liver mass and body mass rather than liver mass per se. The optimization of the ratio indicates that the liver reaches a state in which it performs the amount of metabolic work needed to meet the functional requirements of the body.[24]

Gene expression in the regenerating liver is a multistep process with at least two critical steps: the transition of quiescent hepatocytes into the cell cycle ("priming"), and the progression beyond the restriction point in the G_1 phase of the cell cycle. Hepatocytes must first be primed before they can fully respond to growth factors. As many as 70 different genes participate in the early response to hepatectomy, but TNF and IL-6 appear to be the major cytokines involved in the priming of hepatocytes.[20] The proliferative effect of TNF on hepatocytes is further influenced by reactive oxygen species, nitric oxide, and glutathione content, and at least four transcription factors (nuclear factor kappa B, STAT3, AP-1, and C/EBPβ) play major roles in the initiation of early liver regeneration.[23,24] Progression through the cell cycle beyond the initiation phase requires growth factors, primarily hepatocyte growth factor and TGF-α. The subsequent expression of cell-cycle genes, particularly cyclin D_1, establishes the stage at which replication becomes growth factor–independent and autonomous. At this point, the hepatocyte is irreversibly committed to replicate and the cell-cycle replication machinery takes over. The regenerative capacity of the residual hepatocytes may restore liver mass and function after as much as 65% to 70% hepatectomy.[23,24] Progenitor cells are only activated in severe liver injury. They appear to reside in a "niche" that is a particular regulatory environment.[24] A recent immunohistochemical

study suggests that canine and human liver progenitor cells are functionally very similar.[26] Further characterization of the molecular events regulating hepatocyte replication and liver regeneration should improve outcome in animals affected with severe liver disease.

Fibrosis and the Wound-Healing Response

The normal ECM of the liver provides cells with positional information and a mechanical scaffold for adhesion and migration. The ECM consists of collagens, glycoproteins, proteoglycans, glycosaminoglycans and molecules that are bound specifically by the ECM, such as certain growth factors, cytokines, matrix metalloproteinases, and processing enzymes such as tissue transglutaminase and procollagen propeptidases. A normal liver contains a very small amount of fibrous tissue as a percentage of its total mass.[27,28] Acute or chronic liver injury causes a dynamic wound-healing response with both production and removal of fibrosis.[29] HSCs are the major source of the collagens that comprise fibrosis and cirrhosis, as well as of the tissue inhibitors of metalloproteinases (TIMPs) that inhibit collagen degradation. It is the balance between collagen production by HSCs and its degradation by matrix metalloproteinases that determines the severity and reversibility of the fibrotic response. Following acute or chronic liver damage, HSCs are stimulated to multiply and to undergo a complete phenotypic transformation from quiescent vitamin A–storing cells to contractile myofibroblasts which synthesize large amounts of ECM.[25,29] Important stimuli for HSC transformation and multiplication in liver injury include oxidative stress; chemokines including platelet-derived growth factor; VEGF and TGF-β; adipokines; and parts of the innate immune system including Toll-like receptor ligands.[29] The role of adipokines in stimulating fibrosis is increasingly being recognized in humans, where nonalcoholic fatty liver disease can lead to fibrosis and cirrhosis. They are produced by HSCs themselves, as well as fat cells, and increased leptin and reduced adiponectin drive fibrosis.[29] Their importance in dogs is unknown and it is also unknown whether vacuolar or fatty liver diseases progress to fibrosis in dogs, but these are important questions to answer in the future given the widespread occurrence of obesity in dogs.

The contractile function of activated HSCs contributes significantly to the development of portal hypertension,[28] and increases in angiogenic chemokines such as VEGF and platelet-derived growth factor not only contribute to fibrogenesis by HSCs, but also to the development of portal hypertension, so that the two pathologic processes are inextricably linked.[29]

Activated HSCs have greatly increased production of TIMPs, particularly TIMP1 and TIMP2, which prevent the action of matrix metalloproteinases in the ECM. The degree of fibrosis and reversibility then depend on the balance between perpetuation of HSC proliferation and secretion and resolution of HSC by either apoptosis or senescence of HSCs or, indeed, their reversion to an inactive state. Many factors contribute to HSC apoptosis, senescence, or reversion, including reduction in TIMPs and nuclear factor kappa B and increased Fas and p53.[29] However, in spite of all this understanding of the molecular mechanisms of fibrosis, a truly effective treatment for hepatic fibrosis in either humans or dogs has yet to be found.[29]

It is important to remember that a normal fibrotic response (scar) is important in walling off pathogens and tissue injury and inhibiting this response without removing the inciting cause (e.g., a viral cause) could lead to spread of the pathology.[28] Future treatment strategies for fibrosis should therefore incorporate treatment of the underlying cause of disease. This is clearly a problem in dogs where the cause of chronic hepatitis is usually unknown.[30] There is increasing evidence that fibrosis and even some forms of cirrhosis in humans and rodent models are reversible if the underlying cause is removed.[28,31] The challenges are removing the cause and also defining the point at which cirrhosis moves from a reversible to irreversible state. Increased fibrous septal thickness, smaller nodule size, and reduced cellularity together with increased collagen cross-bridging have all been associated with an irreversible cirrhotic state in rodents and humans.[28]

It is unknown whether liver fibrosis or cirrhosis in dogs is reversible clinically. Cases of chronic hepatitis in dogs very rarely have sequential liver biopsies over a long period of time to assess progression of disease and noninvasive markers of fibrosis remain to be validated. Serum hyaluronic acid is increased in dogs with cirrhosis[32] as is TGF-β[33] but the usefulness of these markers in following progression in clinical cases has not been assessed. Identifying a reliable noninvasive marker of fibrosis for sequential studies in humans and dogs remains a challenge.[34]

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STRUCTURE AND FUNCTION

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