Summary of the World Small Animal Veterinary Association Standardization Committee Guide to Classification of Liver Disease in Dogs and Cats

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KEYWORDS

• Pathology • Histopathology • Liver • Classification • Dog
• Cat

Liver disease is a frequently encountered problem in small animal practice. The diagnostic terminology to describe liver diseases used by pathologists from various countries and even different training programs is often inconsistent, leading to confusion for clinicians interpreting pathology reports and those who would like to compare the pathology results from separate studies. Because of these issues, the World Small Animal Veterinary Association (WSAVA) has formed a group of experienced clinicians and pathologists to develop a standardized format for diagnostic terminology that, it is hoped, leads to greater uniformity in diagnoses and better communication between clinicians and pathologists alike. Standardized criteria and nomenclature are also mandatory for setting up multicenter clinical trials. Based on the developed system, several international studies are now getting started. The aim is to find a sound scientific basis of diagnostic and treatment protocols for hepatobiliary diseases. An overview of that monograph[1] follows.

MORPHOLOGIC CLASSIFICATION OF CIRCULATORY DISORDERS OF THE LIVER

There are several congenital disorders of the vascular supply to the liver recognized in dogs and cats. The liver responds to insufficient portal blood flow in a relatively

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stereotypic fashion regardless of the impediment to normal perfusion. Consequently, there is considerable overlap in the histologic appearance of these disorders. Typical features include absent or diminished portal vein profiles in the portal tracts; an increase in arteriolar profiles in the portal tracts and periportal sinusoidal dilation may also occur, presumably attributable to the local increased blood pressure that accompanies the arteriolar blood flow into the sinusoids (**Fig. 1**). Bile duct proliferation may be evident as well. Hepatocytic atrophy is also common. Nonuniform distribution of portal flow most likely leads to irregular residual lobular architecture and the presence of abundant lipogranulomas formed from areas of hepatocyte loss, although other explanations are possible.

Abnormal venous anastomoses are often difficult to identify post mortem without benefit of antemortem imaging studies. Clinical data, such as the presence or absence of shunt vessels and the determination of portal vein pressure, are essential to achieve a final diagnosis.

Congenital Portosystemic Shunts

Congenital portosystemic shunts occur in the dog and cat. Congenital portosystemic shunts are single (almost always) large-caliber connections between the portal vein and systemic veins. A congenital shunt can be intrahepatic or extrahepatic in location. Intrahepatic congenital shunts, often attributable to a patent ductus venosus, occur most often in large-breed dogs. Extrahepatic congenital shunts, which occur in small-breed dogs more often than the intrahepatic shunts, may connect the portal vein to any of several veins, including the splenic, azygous, or renal vein, or to the caudal vena cava.

The typical histologic appearance is characterized by loss or diminution of portal veins; arteriolar proliferation; hepatocytic atrophy, often with abnormal lobule formation; and the presence of lipogranulomas. Dogs with congenital portosystemic shunts do not develop portal hypertension, because any increased portal pressure would lead to increased flow into the systemic venous system, restoring normal blood pressure.
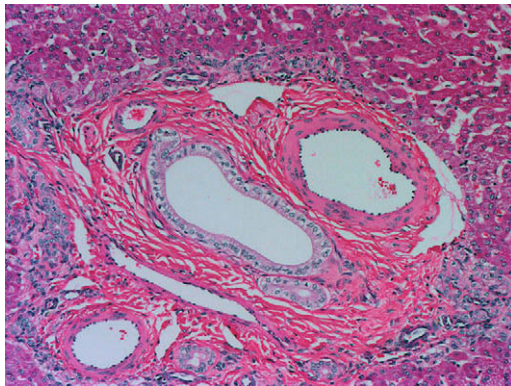

Fig. 1. Portal tract with a portosystemic shunt in a dog. The alterations are similar in a variety of disorders leading to portal vein hypoperfusion of the liver. These changes include an absent or dramatically reduced portal vein profile, prominent hepatic artery branches, arteriolar proliferation, and atrophy of hepatocytes.

Disorders Associated with Portal Hypertension

Portal vein obstruction

Flow through the portal vein can be obstructed through thrombosis or invasive neoplasms or through compression from local inflammatory processes, such as pancreatitis or peritonitis. Infection with schistosomal parasites can also produce disturbed portal vein blood flow. Because the disorder is typically acquired rather than congenital, the liver is characterized histologically by diminution of the portal vein profiles depending on the proportion of the flow that is interrupted, but without prominent arteriolar changes.

Primary hypoplasia of the portal vein (microvascular dysplasia and noncirrhotic portal hypertension)

There has been considerable confusion surrounding this disorder because of the various terms used to describe it, including *microvascular dysplasia* and *noncirrhotic portal hypertension*. The preferred term proposed by the WSAVA Liver Study Group is *portal vein hypoplasia*. Portal vein hypoplasia is a congenital vascular anomaly that occurs in dogs, and occasionally in cats, in which the intrahepatic vein and, in some circumstances, the extrahepatic portal vein are abnormally small or absent. The abnormally small or absent extrahepatic vein or intrahepatic portal vein results in diminished hepatic perfusion. There is potential for portal hypertension, along with ascites and the formation of acquired shunt vessels because of the restricted intrahepatic portal blood flow. These alterations can be present early in the course of the disease or may develop over time or not at all. Affected animals have small livers and the typical histologic pattern of portal vein hypoperfusion. It is important to remember that this condition differs from portosystemic shunts, because there is no abnormal connection between the portal vein and systemic venous system.

There are histologic variants of this disorder that are characterized by increased, possibly abnormal, connective tissue within the portal tracts that may bridge between portal tracts in some cases. This variant has previously been called noncirrhotic portal hypertension or, when the portal tract connective tissue is particularly abundant, hepatoportal sclerosis.

Intrahepatic arteriovenous fistulas

Intrahepatic arteriovenous fistulas, acquired or congenital, occur in the dog and cat. These shunts arise from a direct communication between a branch of the hepatic artery and branches of the portal vein. They may occur anywhere within the liver and can be single or multiple. A distended throbbing fistula can often be appreciated at surgery. Affected areas of the liver contain a convoluted hepatic artery branch and an aneurysmal portal vein branch with abnormal thickened walls. The liver adjacent to the fistula has the typical appearance of portal hypoperfusion. Shunting of blood may lead to portal hypertension or reversal of the direction of portal blood flow, subsequent development of acquired portocaval shunts, and ascites. Most of these lesions are diagnosed before biopsy.

Incidental Vascular Disorders

Peliosis hepatis

Peliosis hepatis is defined as a random distribution of dilated vascular spaces in the hepatic parenchyma. Grossly, these areas appear as variably sized dark blue foci within the liver that vary from pinpoint to several centimeters in size. It occurs in old cats, and occasionally in dogs, in which it can be mistaken for a vascular tumor, such as hemangioma or hemangiosarcoma.

MORPHOLOGIC CLASSIFICATION OF BILIARY DISORDERS

Acquired and congenital biliary diseases can be divided into four categories:

1. Biliary cystic diseases
2. Cholestasis and cholate stasis
3. Inflammation (cholangitis and cholangiohepatitis)
4. Diseases of the gallbladder

Solitary Biliary Cysts

These lesions are uncommon in cats and dogs. They are single round cysts lined with a flattened single layer of biliary epithelium. They may be acquired or a congenital disorder.

Congenital Biliary Cystic Disease

Congenital biliary cystic diseases are a complex and often confusing collection of conditions that affect dogs and especially cats. They can all be attributed to an abnormality of the development of the primordial biliary ductular system arising from the ductal plate. Congenital cystic disease is characterized by dilation of portions of the biliary tree and associated fibrosis. Lesions may affect only the large extrahepatic bile ducts in a pattern resembling Caroli's disease in human beings or may affect only the small-caliber ducts, with formation of fibrotic portal tracts and abnormal, often dilated, irregular profiles of biliary epithelial-lined ducts that can lead to extensive lesions with bridging fibrosis and associated abnormal bile ducts, diagnosed as congenital hepatic fibrosis (**Fig. 2**). A third pattern is characterized by multiple unilocular or multilocular biliary cysts that range from a few millimeters to several centimeters in diameter and contain clear fluid (**Fig. 3**). In animals affected with this form of so-called "Von Myenburg complexes," discrete fibrous areas with small, often irregular, bile duct profiles are also frequently found. These lesions are often confused with benign tumors of the bile duct epithelium. Dilated renal tubules can also occur in some circumstances as well. The pattern of lesions and inheritance in domestic animals is not as clearly separated in domestic animals as it is in human beings. Persian cats are more frequently affected than other feline breeds.
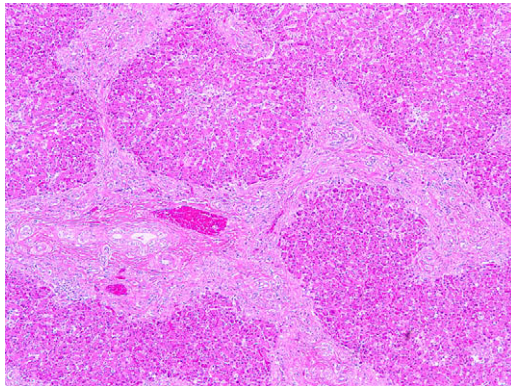

Fig. 2. Congenital hepatic fibrosis in a cat. The affected liver is characterized by thick bands of connective tissue that bridge between portal tracts and contain proliferated small bile ducts.
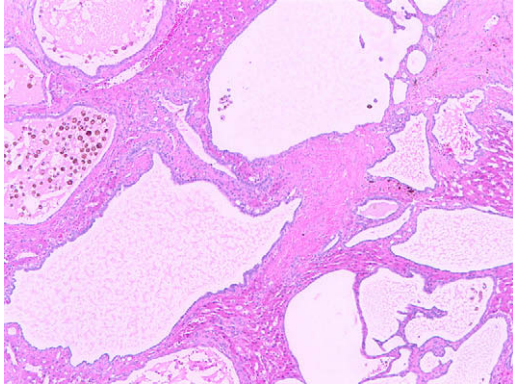
Fig. 3. Biliary cystic disease in a cat. Multiple biliary cysts, lined by a single layer of cuboidal to flattened biliary epithelium, are present within the hepatic parenchyma. Occasional hepatocytes are entrapped between the cysts.

Cholestasis

Cholestasis is an accumulation of substances normally secreted in the bile (eg, bilirubin, bile acids) in the blood. Cholestasis can be categorized as follows:

Intrahepatic cholestasis (attributable to decreased or blocked bile flow in the canaliculi) occurs in association with a wide spectrum of conditions affecting hepatocytes (drug toxicity, lipidosis, necrosis, hepatitis, and others) or, in some circumstances, dramatically increased bile production associated with hemolysis.

Extrahepatic cholestasis (attributable to blockage of extrahepatic bile ducts) results from luminal obstruction by bile calculi (gall stones), inspissated bile (often associated with gallbladder mucoceles in dogs), or extraluminal compression attributable to neoplasia or inflammation (particularly of the pancreas or duodenum).

Histologically, the most prominent feature of intrahepatic cholestasis is the presence of bile, seen as fine linear deposits between hepatocytes. These deposits are called canalicular bile plugs, and they are most abundant in the centrilobular region. Bile may also be found within Kupffer cells. Retention of bile salts is damaging to cell membranes, and swollen and pigmented hepatocytes (termed *feathery degeneration*) may also be found in the periportal region. Extrahepatic cholestasis is typically characterized by edema and neutrophils in portal areas and ductal bile plugs. Over time, concentric rings of fibroblasts or myofibroblasts expand, forming an "onion skin" appearance around interlobular bile ducts, proliferation of bile ducts, and, eventually, bridging portal-portal fibrosis (**Fig. 4**).

INFLAMMATION
Cholangitis

Cholangitis, inflammation of the bile ducts, can be differentiated in (1) neutrophilic cholangitis, (2) lymphocytic cholangitis, (3) destructive cholangitis, and (4) chronic cholangitis associated with liver fluke infestation.
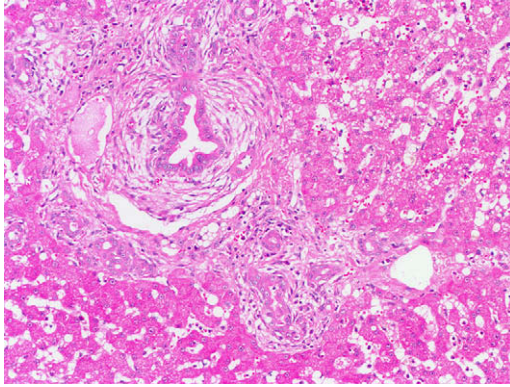
Fig. 4. Chronic biliary obstruction in a dog. Affected bile ducts typically have concentric spindle-shaped cells that may be myofibroblasts. Biliary hyperplasia of small ducts is also present. Moderate numbers of mononuclear inflammatory cells and pigmented macrophages are usually present.

Neutrophilic cholangitis

Neutrophilic cholangitis is more common in cats than in dogs. The pathogenesis is thought to involve ascending bacterial infections, but this is not always demonstrated. Affected bile ducts have neutrophils in their lumen, between the biliary epithelial cells, or in close association with the bile ducts (**Fig. 5**). When the inflammation extends beyond the limiting plate and spills into the hepatic parenchyma, the diagnosis becomes cholangiohepatitis. This can be the result of bile duct rupture and release of the contents of the bile duct, leading to necrosis and abscesses. This term should no longer be used for mild accumulations of inflammatory cells within the portal tracts.

Lymphocytic cholangitis

This condition occurs in cats. It is characterized by dense aggregates of lymphocytes that form a cuff around bile ducts but usually do not penetrate the lumen of affected ducts or invade the biliary epithelium (**Fig. 6**). Biliary hyperplasia is usually also present but can be difficult to appreciate when the inflammation is intense. The primary
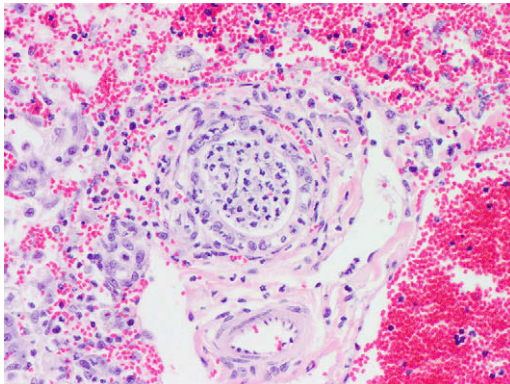

Fig. 5. Neutrophilic cholangitis in a cat. Neutrophils are found within the lumen and invade the biliary epithelium.
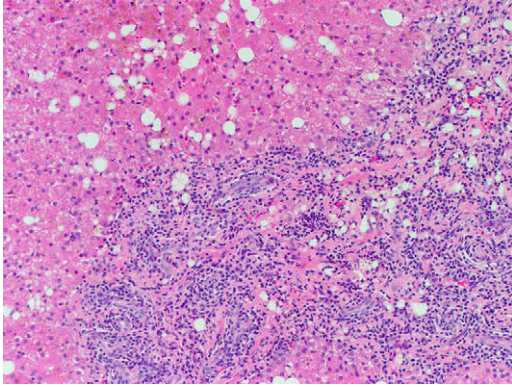
Fig. 6. Lymphocytic cholangitis in a cat. Moderate to intense infiltrates of mature lymphocytes centered on bile ducts and associated biliary hyperplasia are characteristic of lymphocytic cholangitis in cats.

differential diagnosis is hepatic lymphoma. The presence of biliary hyperplasia supports a diagnosis of lymphocytic cholangitis and lymphoid aggregates in such areas as the periphery of the central vein, suggesting lymphoma. Use of special techniques, such as the polymerase chain reaction for antigen receptor rearrangements assay, to assess the presence or absence of monoclonal lymphocyte populations (characteristic of lymphoma) may be needed in difficult cases.

Destructive cholangitis

Destructive cholangitis is uncommon and is the result of biliary epithelial necrosis in smaller branches of the biliary tree. Histologically, portal tracts from affected animals lack a normal-caliber bile duct and contain aggregates of pigmented macrophages and small numbers of inflammatory cells, predominantly neutrophils or eosinophils (**Fig. 7**). Portal fibrosis can also occur. Destructive cholangitis is most likely attributable to an idiosyncratic reaction to some drugs (ie, trimethoprim sulfa). It is not known if ducts can completely recover.

Chronic cholangitis

In chronic cholangitis, the inflammatory cells include lymphocytes and plasma cells, often in association with neutrophils. There are varying amounts of bile duct hyperplasia and fibrosis. Portal-portal bridging fibrosis is present in severe cases. This condition results from persistent inflammation, such as unresolved bacterial infections or, particularly in cats, liver fluke infestation, in which dramatic ductular distention and fibrosis can occur.

Chronic cholangitis associated with liver fluke infestation

Chronic cholangitis associated with liver fluke infestation is regularly observed in cats, and less frequently in dogs, in endemic areas. Infections are caused by members of the family Opisthorchiidae. The lesion is microscopically characterized by dilated larger bile ducts with papillary projections and marked periductal and portal fibrosis (**Fig. 8**). A slight to moderate inflammation may be seen within the ducts (neutrophils and macrophages) and in the portal areas (neutrophils, lymphocytes, and plasma cells). Eosinophils may be present. The number of liver flukes and eggs within the dilated bile ducts varies markedly, and it is often difficult to find liver flukes or eggs.
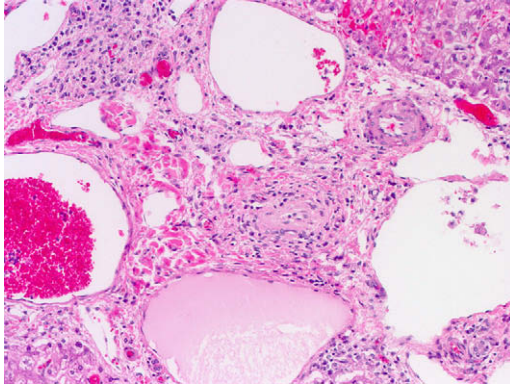
Fig. 7. Destructive cholangitis in a dog. The affected liver is characterized by loss of the normal bile duct profile in the portal tract. In the center of the field, there is a remnant outline of the bile duct with peripheral accumulation of mononuclear inflammatory cells.

GALLBLADDER LESIONS
Cholelithiasis

Cholelithiasis is not common in any domestic species, although bile calculi can occur in dogs and cats. Inspissated bile may cause bile duct obstructions. A histologic pattern typical of extrahepatic obstruction can result.

Cystic Mucosal Hyperplasia

This condition is relatively common in older dogs. The mucosa is thickened, sometimes dramatically, and contains variably sized mucus-containing cysts lined by hyperplastic columnar epithelium.

Gallbladder Mucocele

This is a condition characterized by the presence of a distended gallbladder filled with firm tenacious mucoid material, which may lead to gallbladder rupture (**Fig. 9**). The
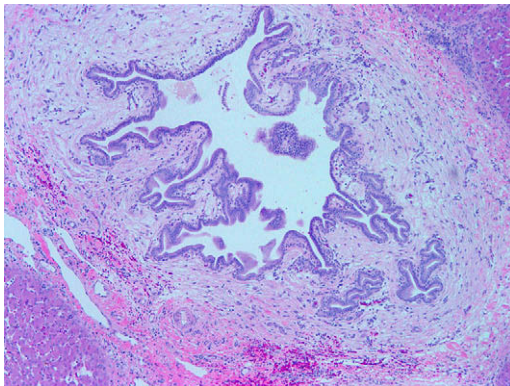

Fig. 8. Chronic cholangitis in fluke infestation. Chronic infection with intrabiliary flukes typically produces prominent ductular distention and an associated dramatic peripheral deposition of connective tissue and mononuclear inflammatory infiltrate. It is uncommon to find flukes in tissue sections, however.
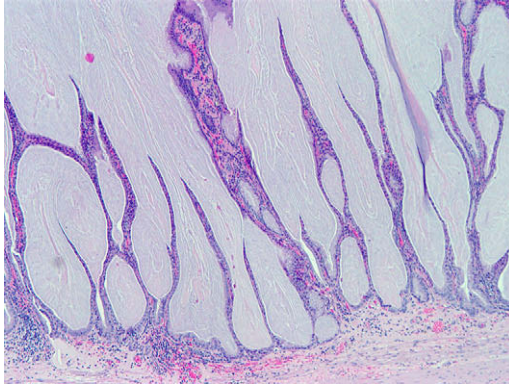
Fig. 9. Gallbladder mucocele in a dog. The affected gallbladder is characterized by distention attributable to an excess of tenacious mucus that fills the lumen of the organ. The gallbladder mucosa is often mildly proliferative with fine fronds of mucosa projecting into the lumen.

mucosa is variably hyperplastic. The viscous mucus may extend into the cystic or common bile duct, causing obstruction.

Gallbladder Infarcts

Gallbladder infarction is characterized histologically by transmural coagulation necrosis of the wall of the gallbladder, with minimal inflammation. The primary lesion seems to be thrombosis of arteries in the wall of the gallbladder without evidence of concurrent cholecystitis. Affected animals may have severe bile peritonitis as a result of rupture of the infarcted gallbladder.

Cholecystitis

Cholecystitis is an inflammation of the gallbladder. It can be acute or chronic and may be associated with inflammation in other areas of the biliary tree. Neutrophilic cholecystitis is frequently seen in cats, and rarely in dogs, and is, in general, associated with bacterial infection. The lesion is characterized by the presence of neutrophils in the lumen, epithelium, or wall of the gallbladder. Neutrophilic cholecystitis can be present as a solitary lesion or in combination with neutrophilic cholangitis.

Lymphoplasmacytic and follicular cholecystitis are characterized by the presence of a lymphoplasmacytic infiltrate or the presence of lymphoid follicles in the mucosa of the gallbladder. The gallbladder lining, like other mucosal surfaces, can normally contain a small number of lymphocytes and occasional lymphoid follicles.

MORPHOLOGIC CLASSIFICATION OF PARENCHYMAL DISORDERS OF THE CANINE AND FELINE LIVER
Introduction to Parenchymal Diseases

Parenchymal disorders of the liver in dogs and cats can be grouped into seven categories: (1) reversible injury (cell swelling, excess glycogen accumulation, and lipidosis); (2) amyloidosis; (3) hepatocellular death, apoptosis, and necrosis; (4) acute and chronic hepatitis; (5) hepatic abscesses and granulomas; (6) hepatic metabolic storage disorders; and (7) miscellaneous conditions.

Lesions involving the hepatocytes are the hallmarks of most of these disorders; however, inflammation, necrosis, fibrosis, and bile duct proliferation are not restricted

to parenchymal disorders and may be prominent in primary biliary or circulatory disorders.

Reversible Injury

Hepatocellular swelling

The earliest manifestation of injury is termed *cloudy swelling* and is attributed to loss of cell membrane functionality, leading to an influx of water into the cytoplasm. This change is difficult to appreciate histologically because of its subtlety in most circumstances.

Vacuolar change

Cytoplasmic accumulation of various substances leading to vacuole formation in hepatocytes can occur for a variety of reasons. The finding of vacuolar hepatopathy is consequently a vague and often uninformative diagnosis. In young animals, particularly those with abnormal growth, storage disorders should be considered. In older animals, the vacuoles are almost always lipid or glycogen containing. Identification of the contents can aid in sorting out the pathogenesis; however, in many circumstances, the diagnosis of hepatocellular vacuoles in an animal with altered liver-related biochemistry does not provide much assistance in determining the pathogenesis of the problem. Some disorders associated with vacuole formation are discussed here.

Steroid-induced hepatopathy

This condition occurs in dogs and is characterized by excessive accumulation of cytoplasmic glycogen. Typically, hepatocytes are swollen with clear cytoplasm attributable to glycogen accumulation and contain fine irregular strands of eosinophilic cytoplasm and a central nucleus (**Fig. 10**). The distribution can be diffuse or zonal or may involve individual cells. Periodic acid–Schiff staining with or without diastase may help to identify glycogen accumulation in mild cases, although frozen sections are usually needed to preserve glycogen content. Other hepatic changes associated with glycogen accumulation are marginated neutrophils in sinusoids and occasional foci of extramedullary hematopoiesis. This condition is caused by an excess of endogenous or exogenous corticosteroids. Possible additional causes include an excess of other types of steroid hormones and, occasionally, drugs, such as D-penicillamine.
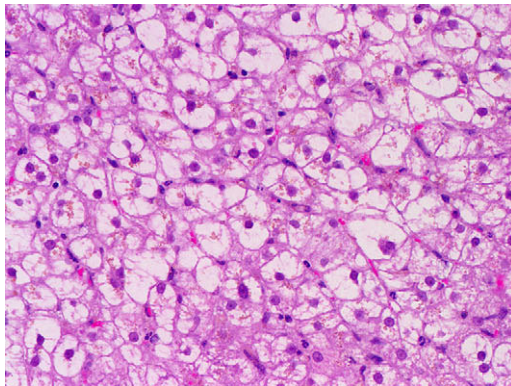

Fig. 10. Corticosteroid hepatopathy in a dog. Excessive endogenous or exogenous corticosteroids can produce distended hepatocytes with irregular clear vacuoles and diaphanous strands of cytoplasm. The vacuoles contain glycogen that is removed during fixation and processing.

Hepatocellular steatosis (lipidosis and fatty change)

Hepatocellular steatosis is a nonspecific accumulation of lipid-filled vacuoles in the cytoplasm of hepatocytes, is a reversible form of cellular change, and can be a physiologic change in some circumstances.

Hepatic steatosis has two phenotypic forms. In macrovesicular hepatocellular steatosis, the accumulation of lipids forms vacuoles that are larger than the size of the nucleus and tend to displace the hepatocellular nucleus (**Fig. 11**). These lipid vacuoles most commonly form as a result of dietary excess or starvation or in some forms of feline hepatic lipidosis. A particular form of lipidosis, identified as microvesicular lipidosis (vacuoles smaller than the size of the nucleus), is associated with injury to mitochondria and more severe liver dysfunction than macrovesicular lipidosis (**Fig. 12**). This form occurs in juvenile hypoglycemia of small-breed puppies and in uncontrolled diabetes mellitus, and it may occur after exposure to some toxins. Mixed microvesicular and microvesicular lipidosis is common in the syndrome of feline hepatic lipidosis.

Hepatocellular steatosis should be described by distribution (zonal or diffuse), severity, and size of the cytoplasmic vacuoles (microvesicular, macrovesicular, or mixed).

In routine formalin-fixed paraffin-embedded tissues, lipid vacuoles are empty vacuoles because of the loss of fat in processing. Frozen sections of unfixed or fixed tissue can be stained for fat using special stains, such as oil red O, Sudan IV, or osmium tetroxide, and are helpful in recognizing microvesicular lipidosis and in differentiating fat from other substances that may accumulate in vacuoles.

Amyloidosis

Amyloid in the liver is almost always secondary or reactive (serum amyloid associated) and develops over the course of a chronic inflammatory disorder. Hepatic amyloidosis is commonly associated with inflammatory conditions in other organ systems; however, in breeds with a predisposition to amyloid deposition (Chinese shar-pei dogs and Abyssinian, Siamese, and other oriental cats), inflammation in other organs may be slight or negligible. Amyloid appears as eosinophilic material in the space of Disse and sometimes in the walls of vessels and in portal areas (**Fig. 13**). Deposition may be diffuse, zonal, or multifocal, and there is frequently atrophy of the adjacent hepatocytes and dilation of the sinusoids because of disruption of the blood hepatocyte interface. Special stains (Congo red or Stokes) may be needed to identify or
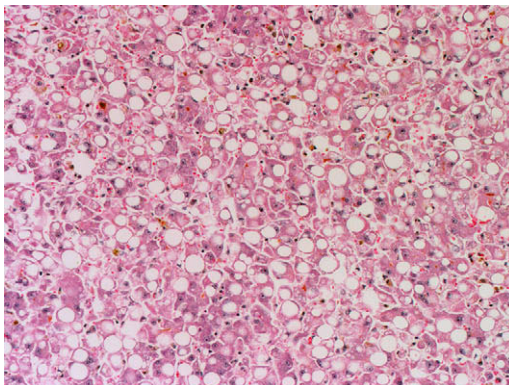

Fig. 11. Macrovesicular steatosis in a dog. Lipid vacuoles are distinct, round, and clear. Typically, these vacuoles displace the nucleus to the edge of the cell.
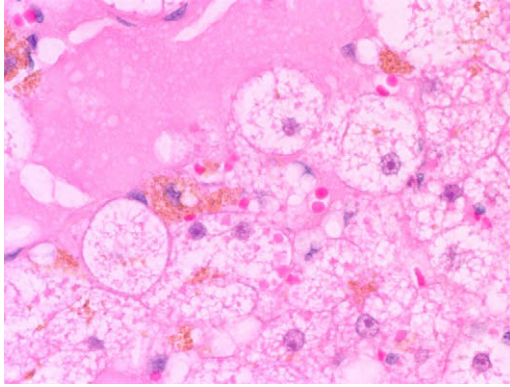
Fig. 12. Microvesicular steatosis in a cat. Microvesicular lipid vacuoles are smaller than the nucleus but retain a clear round outline, and the nucleus remains central in affected cells.

confirm the presence of amyloid. Spontaneous or biopsy-induced liver rupture with hemorrhage and hemoabdomen may occur in amyloid-infiltrated livers.

HEPATOCYTE DEATH (APOPTOSIS AND NECROSIS)

Hepatocytes may be killed by a broad variety of insults, including hypoxia, toxins, infectious agents, immunologic events, and severe metabolic disturbances. Cell death has been considered to occur through apoptosis or necrosis; however, recent evidence suggests overlap between the processes, because moderate exposure to some toxins causes apoptosis, whereas greater exposure may result in necrosis. Apoptosis is an active process of programmed cell death that results in shrinkage of the cell without loss of integrity of the cell membrane and subsequent fragmentation. Necrosis involves cytoplasmic swelling and abnormal cell membrane permeability and may result in coagulative necrosis or liquefactive necrosis. Coagulative necrosis is the result of sudden and catastrophic denaturation of the cytosolic protein and appears as swollen hepatocytes with acidophilic cytoplasm; preservation of the basic outline of the coagulated cell; and pyknosis, karyorrhexis, or karyolysis.
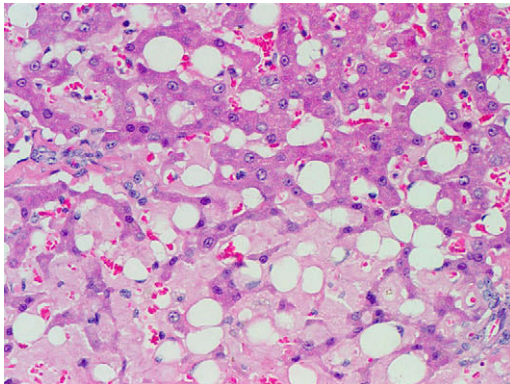

Fig. 13. Hepatic amyloidosis in a cat. Uniformly hyaline eosinophilic material within the sinusoids distends the sinusoids and leads to atrophy of the hepatocytes that remain.

Liquefactive necrosis appears as loss of hepatocytes and is the result of osmotic swelling and disintegration of hepatocytes, with subsequent collapse of the residual reticulin network or replacement by erythrocytes and, eventually, the presence of ceroid-laden macrophages. Necrosis may be followed by proliferation of Kupffer cells and infiltration of phagocytic cells, with subsequent resorption and lysis of the necrotic cells. Necrosis should be characterized by the pattern of injury, including focal, multifocal, confluent, bridging, massive, or piecemeal, because the pattern of necrosis can provide insight into the pathogenesis of the lesion.

Response of the Liver to Hepatocellular Injury

After destruction of hepatic parenchyma, regeneration of parenchyma, fibrosis, and ductular proliferation (bile duct hyperplasia) may occur. When hepatocyte destruction is limited and the reticulin network remains intact, regeneration with almost complete restitution of the liver structure can occur. Severe parenchymal destruction with extensive loss of hepatocytes is often followed by ductular proliferation, termed the *ductular reaction*, which may involve hepatic progenitor cells. With persistent parenchymal damage, fibrosis and postnecrotic scarring may occur, which may result in regenerative parenchymal nodules. In areas of collapse or fibrosis, intrahepatic portovenous vascular shunts may form.

Controversy still exists about the nomenclature of hepatic necrosis and acute inflammation. A morphologic diagnosis should emphasize the primary or most important process (necrosis versus inflammation), with appropriate modifiers indicating chronicity, severity, distribution, presence of inflammatory cells, and evidence of the cause, if known.

Acute Hepatitis

Acute hepatitis is characterized morphologically by a combination of inflammation, hepatocellular apoptosis, necrosis, and, in some instances, regeneration (**Fig. 14**). The proportion and detailed nature of these components vary widely according to the cause, the host response, and the passage of time, and it is necessary to include in the diagnosis the type, pattern, and extent of the necrosis and inflammation, in addition to the possible cause. The lesions are usually sufficiently diffuse within the liver to be diagnosed with confidence on small biopsy samples; however, although there may be histologic clues for a specific cause, it may be difficult to distinguish a cause for
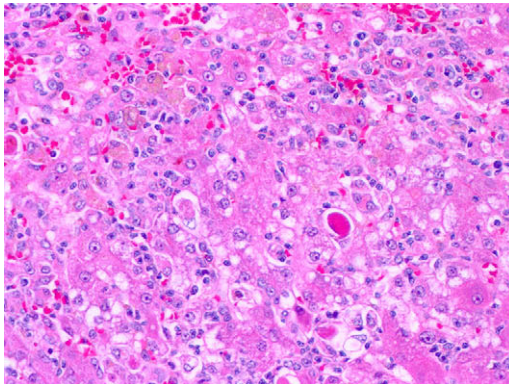

Fig. 14. Acute hepatitis in a dog. There is a diffuse distribution of neutrophils and mononuclear inflammatory cells, with scattered hepatocellular apoptosis evident.

hepatitis by morphologic means alone. The distinction between acute and chronic hepatitis can sometimes be difficult to distinguish because of overlapping features.

SPECIFIC INFECTIOUS CAUSES OF ACUTE HEPATOCELLULAR NECROSIS AND INFLAMMATION
Viral Diseases

Herpes virus
Canine and feline herpes virus infections in neonates often involve the liver in addition to other organs. Injury is characterized by multifocal randomly dispersed areas of acute hepatocellular and biliary necrosis, typically without inflammation. Inclusion bodies are intranuclear and eosinophilic but are difficult to find. The main differential diagnosis is acute bacterial septicemia.

Adenovirus
Infectious canine hepatitis attributable to canine adenovirus 1 causes a multisystemic disease involving the liver, kidney, brain, and other organs because of the ability of the virus to kill endothelial cells. Unlike most random multifocal patterns seen with viral infection, severe centrilobular to bridging necrosis with or without inflammation is typical. The centrilobular accentuation is attributed to the combined injury to hepatocytes and sinusoidal endothelial cells. Amphophilic to basophilic intranuclear inclusion bodies are usually easily found in hepatocytes and may also occur in endothelial cells and bile duct epithelium.

Corona virus
Feline infectious peritonitis involvement in the liver is characterized by multifocal random areas of necrosis, often extending in the portal and perivenular connective tissue, with a moderate to marked infiltration of macrophages and, at the periphery, plasma cells. Fibrosis may be present. A layer of fibrin with neutrophils and macrophages may be found on the liver capsule, with infiltration of the subcapsular parenchyma by plasma cells and some lymphocytes.

Bacterial Diseases

Septicemic bacterial diseases may lead to multifocal random and confluent necrosis with macrophage proliferation or infiltration with neutrophils and, in later stages, lymphocytes and plasma cells or may cause nonspecific reactive hepatitis. Enteric bacteria, such as *Escherichia coli* and *Salmonella* spp, are relatively common, as are *Streptococcus* spp, *Pasteurella* spp, and *Brucella* spp, and many other organisms are possible.

Clostridium piliformis (Tyzzer's disease) occurs in dogs and cats and is characterized by randomly dispersed areas of confluent necrosis restricted to the parenchyma with or without an inflammatory reaction. There are long slender bacilli within viable hepatocytes at the margin of the necrotic foci. These can sometimes be seen in hematoxylineosin preparations but are best seen with Giemsa or silver (Warthin-Starry) stains.

Leptospirosis occurs in dogs, and various serovars produce an acute multisystemic disease. Hepatocellular necrosis is usually unremarkable or minimal. The main and characteristic lesion is dissociation and separation of liver cell plates (particularly evident in postmortem material), with the presence of many hepatocytes with mitotic figures or binucleation.

Protozoal Diseases

Toxoplasma gondii causes multisystemic disease involving the liver, lung, brain, and other organs in the cat and dog. In the liver, there is confluent to panlobular necrosis,

often with neutrophils, macrophages, and other inflammatory cells. Areas of necrosis and the adjacent parenchyma often contain free tachyzoites or cysts containing bradyzoites.

Toxic liver injury
Many hepatotoxins affect dogs and cats, and the list of individual toxins is too extensive to cover in this article. Manifestations of liver toxicity, one or more of which may occur with each toxin, include no morphologic abnormalities, hepatocellular swelling, lipidosis, necrosis (usually in a specific pattern), inflammation, and eventual fibrosis if exposure is long term. Most acute drug toxicity produces lesions in the centrilobular region. This is attributable to the increased abundance of the cytochrome p450 enzymes in this area. Metabolism of parent drugs by the cytochrome p450 system can produce injurious metabolites in certain circumstances and can lead to regional or massive hepatocytic necrosis depending on the dose of the toxicant (**Fig. 15**).

Some hepatotoxins are found in the environment, such as aflatoxin B1; others are therapeutic agents that are given in toxic doses or have rare and usually unpredictable toxicity. Some toxins are termed *predictable*. This group affects most animals in a dose-related fashion, such as acetaminophen. Another class of toxins, idiosyncratic toxins, affects a tiny percentage of patients, however, and the effect can be unrelated to dose, as can be seen with various therapeutic drugs. Thus, drug toxicity should be considered in most cases of acute hepatic injury. Chronic intoxication can lead to fibrosis or cirrhosis, particularly in dogs.

Chronic hepatitis
Chronic inflammation of the liver is rare in cats but is a common problem in dogs. In most cases, the pathogenesis of this process remains unclear. It is likely that chronic hepatitis has many possible causes that may include infectious, autoimmune, drug-induced, and copper-associated causes. Copper-associated hepatitis, the most well-characterized form of chronic hepatitis, is discussed in more detail elsewhere in this article. Chronic hepatitis is characterized by hepatocellular apoptosis or necrosis, a variable mononuclear or mixed inflammatory infiltrate, regeneration, and fibrosis (**Fig. 16**). The proportion and distribution of these components vary widely. It is desirable for the pathologist to provide a subjective evaluation of the activity and stage of the disease to facilitate comparison among cases. The activity of the
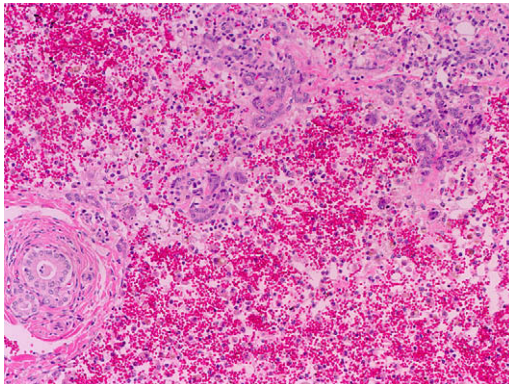

Fig. 15. Acute toxic injury in a cat. Acute hepatocytic necrosis and hemorrhage with a centrilobular and midzonal lobular distribution from a cat with diazepam-related toxicity.
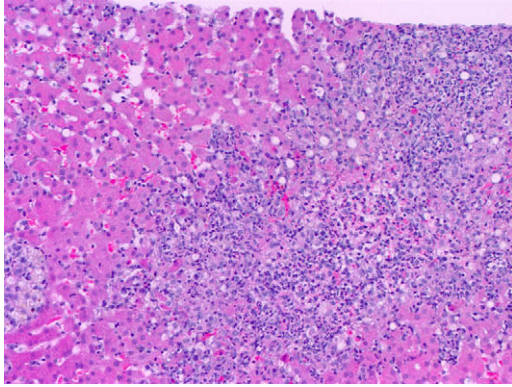
Fig. 16. Chronic hepatitis in a dog. There is pyogranulomatous inflammation, characterized by aggregates of macrophages and a diffuse infiltration of neutrophils within the lesion, in this section of tissue.

disease is determined by the amount of inflammation and extent of hepatocellular apoptosis and necrosis. The stage of the disease, and the prognosis, may be determined by the extent and pattern of fibrosis and the possible presence of architectural distortion. Fibrosis may be portoportal, portocentral, or centrocentral bridging, or it may dissect the lobule. Fibrosis may occur associated with interface hepatitis (inflammation extending from the portal tract and through the limiting plate) after parenchymal collapse with condensation of the residual reticulin network or by means of activation of hepatic stellate cells or related myofibroblasts, which synthesize collagen in the perisinusoidal space or in the portal tracts and around the central veins, respectively. Hepatocellular regeneration and regenerative nodules of hepatic parenchyma are often seen, in addition to proliferation of ductular structures at the periphery of the parenchyma and within fibrous septa. The amount and pattern of fibrosis, particularly in early and mild disease, can be best appreciated with appropriate stains, such as Sirius red or Masson's trichrome.

Copper-associated chronic hepatitis

In the Bedlington terrier, a genetic mutation in the COMM-D (formerly Murr 1) gene produces a defective protein involved in copper transport and leads to excessive accumulation of copper in hepatocytes, resulting in injury or necrosis. Copper accumulation leading to inflammation and necrosis seems to be familial in the West Highland white terrier, Skye terrier, Doberman pinscher, Labrador retriever, and Dalmatian. Typically, copper first accumulates in the centrolobular regions and, with progressive accumulation, results in hepatocyte necrosis, inflammation with copper-laden macrophages, and eventual chronic hepatitis and cirrhosis (**Fig. 17**).

Healthy dogs with normal livers may have copper levels up to 500 μg/g dry weight, and diseased dogs may have copper levels more than 2000 ppm dry weight. Hepatic copper levels in breeds with primary copper storage disease vary among individual animals and among breeds. In many circumstances, it is not clear if copper elevations are primary or secondary. Copper-induced chronic hepatitis and cirrhosis can occur in cats.

CIRRHOSIS

Cirrhosis is the end stage of chronic hepatitis and is a diffuse process of the liver characterized by fibrous septa, shunting of afferent and efferent vessels, and conversion of
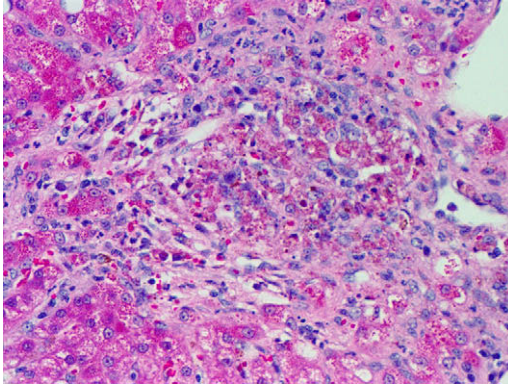
Fig. 17. Copper-related chronic hepatitis in a dog. In this liver, the aggregates of macrophages contain granular pigment, typical of copper, with an abundance of mononuclear inflammatory cells within the lesion. Surrounding hepatocytes also contain fine granular pigment as a result of copper retention.

normal liver architecture into abnormally structured parenchymal nodules (**Fig. 18**). As in chronic hepatitis, it is essential to include in the diagnosis the extent of the fibrosis, the activity of the disease, and the possible cause.

Cirrhosis is a relatively common condition in dogs and is often associated with the presence of multiple portosystemic collaterals. Some animals may have compensated cirrhotic disease and show no or minor clinical signs, whereas other animals may show decompensation of liver function and liver failure. Cirrhosis is only rarely seen in cats.

Lobular Dissecting Hepatitis

Lobular dissecting hepatitis is a particular form of cirrhosis with a rapid clinical course that is seen in young or young adult dogs as isolated cases or in groups of dogs from the same litter or kennel. The liver usually has a normal size with a smooth capsular
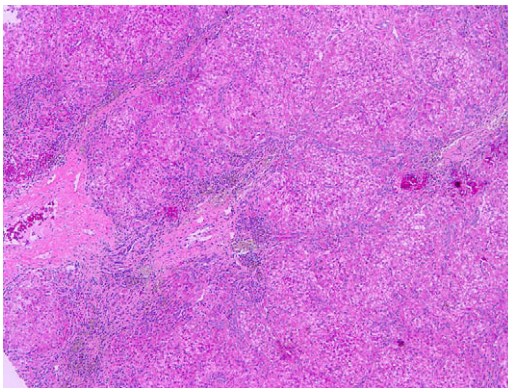

Fig. 18. Cirrhotic liver in a dog. Areas of bridging fibrosis and nodules of regenerative hepatocytes, characteristics of cirrhosis, are evident, along with aggregates of pigmented macrophages and a moderate mononuclear inflammatory infiltrate along the fibrous septa.

surface or some small nodules of regeneration. Microscopically, bands of fibroblasts and thin strands of extracellular matrix are seen between individual and small groups of hepatocytes, which cause dissection of the original lobular architecture (**Fig. 19**). Connective tissue stains (especially for reticulin) are helpful in demonstrating the pattern of connective tissue alterations. Inflammation and hepatocellular apoptosis or necrosis are usually slight to moderate.

Nonspecific Reactive Hepatitis

Nonspecific reactive hepatitis is an inflammatory response of the liver to a variety of extrahepatic disease processes, especially febrile illnesses and inflammation somewhere in the splanchnic bed, or it may be the residuum of previous inflammatory intrahepatic disease. The lesion is characterized by an inflammatory infiltrate in portal areas and in the parenchyma, without evident hepatocellular necrosis. In acute extrahepatic diseases, there is a slight to moderate infiltrate in the parenchyma or the portal areas composed mainly of neutrophils. The infiltrate varies in intensity among portal areas, and some normal portal areas may be found (**Fig. 20**). There is slight to marked leukocytosis, Kupffer cell proliferation in the sinusoids, and some neutrophils in the connective tissue around the hepatic veins. In chronic extrahepatic diseases or in the case of residual intrahepatic disease, the inflammation is usually mononuclear,
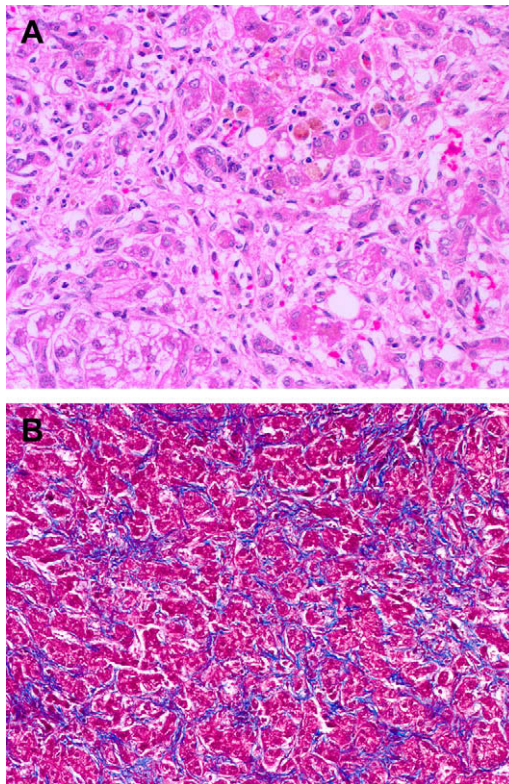

Fig. 19. Lobular dissecting hepatitis in a dog. (*A*) Normal hepatic parenchyma is disrupted by fine strands of collagen that separate hepatocytes and disrupt the normal architecture. (*B*) Trichrome stain aids the detection of the red collagen bundles that divide the hepatocytes.
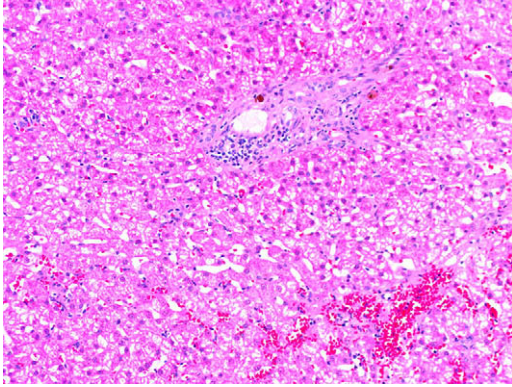
Fig. 20. Nonspecific reactive hepatitis in a dog. There is a light infiltration of lymphocytes and plasma cells in the portal tract connective tissue without evidence of hepatocellular necrosis.

with plasma cells, lymphocytes, and pigmented macrophages in the portal areas and around the hepatic veins, in addition to some plasma cells and lymphocytes with or without single or small aggregates of pigment-laden macrophages in the parenchyma.

Eosinophilic Hepatitis

Eosinophilic hepatitis is most likely a form of nonspecific reactive hepatitis. Eosinophils appear mostly as scattered elements in portal and perivenous infiltrates and, less frequently, within the sinusoids. Marked eosinophilic inflammation in the liver is a rare condition in dogs and cats and may be associated with parasitic infections (eg, migrating nematode larvae, schistosomiasis, liver fluke infestation) usually at and near the site of the parasitic lesion; a more diffuse lesion is sometimes seen in drug-induced liver lesions.

Hepatic Abscesses and Granulomas

Hepatic abscesses usually are the result of bacterial infections with subsequent neutrophil accumulation and lysis. Hepatic abscesses in dogs and cats are particularly seen in newborn animals as a result of umbilical infection by bacteria. In adult animals, hepatic abscesses may be the result of infections with *Yersinia* spp, *Nocardia asteroides*, and *Actinomyces* spp (**Fig. 21**). Hepatic abscesses may occur in association with central necrosis in hepatocellular neoplasms.

Hepatic granulomas may occur in a wide variety of diseases, but most are part of a generalized disease process. They consist of aggregates of epithelioid macrophages or multinucleated giant cells with or without lymphocytes and plasma cells. Infectious causes for hepatic granulomas in dogs include mycobacteria (eg, *Mycobacterium avium intracellulare*, *Mycobacterium tuberculosis*), *Bartonella* spp, systemic mycoses (eg, *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Coccidioides immitis*), *Leishmania* spp, and other parasitic infections.

Hepatic Storage Disorders

Hepatic metabolic storage disorders are usually inherited but can be acquired in certain circumstances. The histologic appearance of hepatic storage disorders can be quite varied but is most often characterized by pigment or vacuole accumulation within the hepatic cytoplasm. The types of contents range from clear to granular or
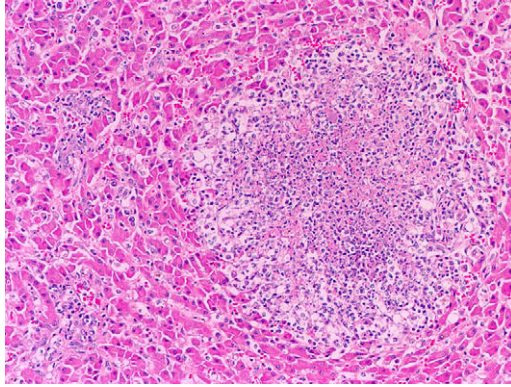
Fig. 21. Hepatic abscesses in a cat infected with *Yersinia*.

hyaline, and pigment may be evident in other circumstances. In addition to hepatocytes, Kupffer cells and macrophages may be affected. Some storage disorders may be limited to the liver, but others affect other organs as well. Metabolic storage disorders are usually evident early in life, and the index of suspicion should be higher in young animals that do not have normal growth patterns than in adult dogs. Identification of the abnormal stored material can be facilitated by ultrastructural examination and the use of special stains and frozen liver samples. A metabolic screening test is most likely to clarify the metabolic disorder, however.

HEPATIC NEOPLASIA AND HYPERPLASIA
Nodular Hyperplasia

Nodular hyperplasia of hepatocytes is a common proliferative lesion in dogs older than 8 years of age. Nearly all dogs are affected by 14 years of age. Nodular hyperplasia is of no clinical significance but should be distinguished from metastatic masses. Cats have a low incidence of nodular hyperplasia.

Nodular hyperplasia of the liver is characterized by multiple distinct spherical to oval masses that are randomly distributed throughout the liver. The liver is usually normal otherwise. They can be difficult to distinguish from hepatocellular adenomas by gross pathologic morphology alone.

Histologically, nodular hyperplasia is characterized by an expansile nodule of hepatocytes that retains normal lobular architecture and may compress adjacent normal tissue. Hepatocytes within the nodules are often vacuolated.

Regenerative Nodules

Nodules of regenerative hyperplasia are distinct from nodular hyperplasia for several reasons. They are much more common in dogs than cats. They are believed to originate from the outgrowth of surviving hepatocytes in a chronically injured liver. As a result of the outgrowth, regenerative nodules lack normal lobular architecture and there is typically only a single portal tract within the regenerative nodules. Regenerative nodules can be difficult to distinguish from hepatocellular adenomas on the basis of histology alone, although there are a few distinguishing features. Regenerative nodules are composed of hepatic plates that are no more than two cells thick, and adenomas may have thicker hepatic plates. Hepatocellular adenomas are more likely

to be solitary lesions and usually do not typically arise in a background of hepatic injury and fibrosis.

Hepatocellular Adenoma

Hepatocellular adenomas are benign neoplasms of hepatocytes. They have most likely been under-diagnosed for many years by veterinary pathologists, because older diagnostic terminology in dogs and cats tended to include only nodular hyperplasia, regenerative nodules, and hepatocellular carcinomas. The neoplasms usually are single, unencapsulated, variably sized, red or brown masses that compress adjacent parenchyma. They lack normal nodular architecture and are composed of well-differentiated hepatocytes, which form uniform plates that may be two to three cells thick. The adenomatous hepatocytes tend to abut normal adjacent hepatocytes at right angles. Cystic areas containing hemorrhage or serum and foci of extramedullary hematopoiesis can be present.

Hepatocellular Carcinoma

Hepatocellular carcinomas are malignant neoplasms composed of hepatocytes. They are uncommon in dogs and cats. These neoplasms are often solitary, frequently involve an entire lobe, and are well demarcated, although diffuse involvement of large portions of the liver may occur. They usually arise in a liver that is otherwise normal. Metastasis occurs within the liver (intrahepatic metastasis) and to the hepatic lymph nodes most often, but distant metastasis can occur.

Histologically, hepatocellular carcinomas can be composed of trabecular, pseudoglandular, and solid patterns. Mixtures of these patterns can be found within individual tumors. In the trabecular pattern, hepatocytes form irregular trabeculae three or more cells thick and with distended vascular spaces between the trabeculae. Pseudoglandular patterns are formed by malignant hepatocytes that are arranged into complete or incomplete acinar structures. The solid pattern lacks acini or trabeculae and is characterized by sheets of neoplastic hepatocytes; this pattern often contains the least well-differentiated hepatocytes. Cells forming the neoplasm range from well-differentiated hepatocytes to atypical or bizarre forms (**Fig. 22**). Mitoses are common, and multinucleate cells can occur. In the absence of metastasis, which is obviously indicative of malignancy, the separation of a well-differentiated carcinoma from an adenoma can be difficult, although invasion by malignant hepatocytes at the margin of the adjacent compressed normal hepatocytes and hepatocellular pleomorphism and atypia are useful indicators of malignancy. Metastasis is uncommon in the author's experience.

Cholangiocellular Adenoma (Biliary Adenoma)

Biliary adenomas are nonencapsulated, irregular, pale white to pale gray, multilocular masses. Adenomas of the biliary ducts are rare in dogs and cats. They are usually small and asymptomatic, discovered as an incidental finding at necropsy.

Histologically, they are characterized by circular acini lined with well-differentiated biliary epithelial cells. Cysts may be mildly dilated. Biliary adenomas should be differentiated from solitary biliary cysts, multilocular cysts, or von Meyenberg complexes.

Cholangiocellular Carcinoma (Biliary Carcinoma)

Cholangiocellular carcinomas are malignant neoplasms of biliary epithelium that usually arise from the intrahepatic ducts, but extrahepatic bile ducts can be affected. They are found in dogs and cats.
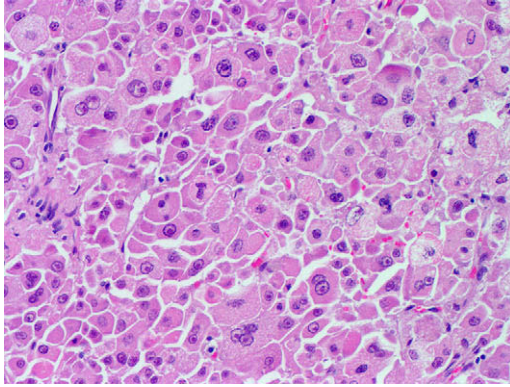
Fig. 22. Hepatocellular carcinoma in a dog. Neoplastic hepatocytes form a broad sheet of pleomorphic and multinucleate hepatocytes.

Tumors are typically white and firm, often with an umbilicated and lobulated appearance. The borders of the lesions are generally well delineated from the adjacent hepatic parenchyma, although the border is frequently irregular because of local invasion. Areas of necrosis can be found in the central regions. The tumors are composed of cells that can be quite pleomorphic but usually retain a resemblance to biliary epithelium. Characteristically, well-differentiated carcinomas are organized into a tubular or acinar arrangement (**Fig. 23**). In less well-differentiated neoplasms, some acinar arrangements can be detected among solid masses of neoplastic cells. Mucin is frequently evident in the lumen of well-differentiated areas of the tumors. The epithelial components of the neoplasms are usually separated by abundant fibrous connective tissue, termed a *scirrhous response*. Metastasis to extrahepatic sites is common.

Mixed Hepatocellular and Cholangiocellular Carcinomas

Occasionally, carcinomas with a mixture of hepatocellular and biliary characteristics can arise.
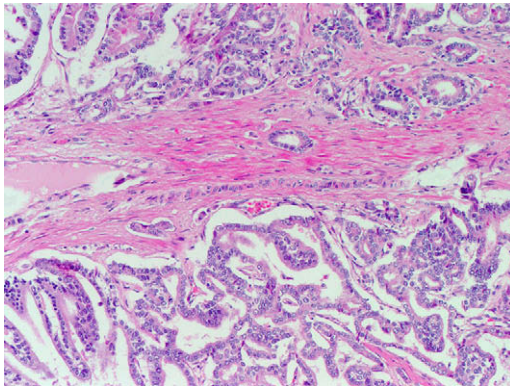

Fig. 23. Cholangiocellular carcinoma in a cat. The neoplasm forms multiple papillary projections lined by moderately pleomorphic biliary epithelial cells. Bands of fibrous tissue separate the neoplastic cells.

Carcinoids

Carcinoids are uncommon tumors of the liver. They are believed to arise from neuro-endocrine cells that lie within the intra- or extrahepatic biliary epithelium ductular tree, gallbladder, or, possibly, hepatic progenitor cells. Often, they form a single mass, but multiple nodules can occur, probably secondary to intrahepatic metastasis. They tend to have an aggressive course, and extrahepatic metastasis can occur. Cells tend to be small, elongated, or spindle shaped and form ribbons or rosettes, and the tumors are highly vascular (**Fig. 24**). Immunohistochemical detection of neuroendocrine markers, such as chromogranin A or neuron-specific enolase, may be used to confirm the diagnosis in some cases, but there are no definitive markers.

Miscellaneous primary mesenchymal neoplasms of the liver

Primary neoplasms can arise from any of the normal cellular constituents of the liver. Primary hepatic hemangiosarcoma is well recognized in dogs, although it is a relatively uncommon site of origin for this neoplasm compared with the skin and spleen. Other primary tumor types can involve mesenchymal neoplasms derived from the liver's connective tissue, including fibrosarcoma, leiomyosarcoma, and osteosarcoma (probably secondary to another mesenchymal tumor initially). Myelolipomas are well-demarcated benign tumors of cats and wild felids characterized by a mix of adipose tissue and myeloid elements.

Metastatic neoplasms

The liver is one of the two more common sites for metastatic spread of malignant neoplasms, a distinction shared with the lung. Given the higher frequency of metastasis compared with primary neoplasia, a complete necropsy and medical or surgical history are necessary to distinguish metastatic neoplasms from primary neoplasia of the hepatobiliary tissue.

Hematopoietic neoplasms, particularly lymphoma, frequently involve the liver as part of the generalized disease process. Neoplastic lymphocytes are characteristically distributed around the vessels of the portal tract and, to a lesser extent, the central and sublobular veins. The gamut of other myeloid neoplasms can also involve the liver.
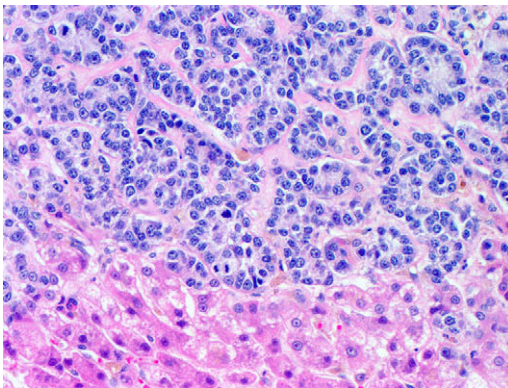

Fig. 24. Hepatic carcinoid in a dog. The tumor is composed of oval pale basophilic cells with oval to carrot-shaped nuclei that form ribbon-like arrays or rosettes, as in this case. Mitotic figures are common.

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