1 CORE PATHOLOGY PATTERNS IN MEDICAL LIVER SPECIMENS

One of the keys to strong diagnostic skills is becoming a master at pattern recognition. This begins by recognizing pure or isolated patterns. For example, a biopsy specimen may show a fatty liver disease pattern and nothing else. As part of this, one must master both the core elements of the pattern as well as the edges of the pattern, the limits at which any given histologic (or clinical) finding is no longer acceptable for a given core pattern. The next level of skill revolves around confidently recognizing when more than one pattern of injury is present. Mastery of this core skill takes time and effort, but is well rewarded.

Patterns are composed of a constellation of smaller findings or building blocks, findings evident at the cytologic, architectural, and special stain levels. A common pitfall is to confuse a single finding as an entire pattern of injury. For example, bile ductular proliferation is a key building block of the biliary obstruction pattern, but it does not always indicate biliary obstruction since it is also found in several other injury patterns. These smaller building blocks each contribute importantly to recognizing the overall pattern of injury, but in general do not have the same diagnostic strength as the pattern in its entirety.

Many of these building blocks have specific names and reasonably well-accepted definitions, which are included in the sections below. These patterns have their greatest clinical specificity when they are used together with laboratory, clinical, and imaging findings.

NORMAL LIVER HISTOLOGY

This brief review of normal liver histology focuses on those aspects that are most relevant to surgical pathology. It is not intended to be a comprehensive review of anatomy or physiology.

Portal Tracts

In the normal liver, most portal tracts (also termed portal triads) have a single bile duct, single hepatic artery, and single portal vein, all embedded in a collagen-rich matrix (fig. 1-1). The amount of collagen depends on the overall size of the portal tract, but in all cases, the edge of the portal tract has a smooth interface with the adjoining lobules (fig. 1-2). There is some

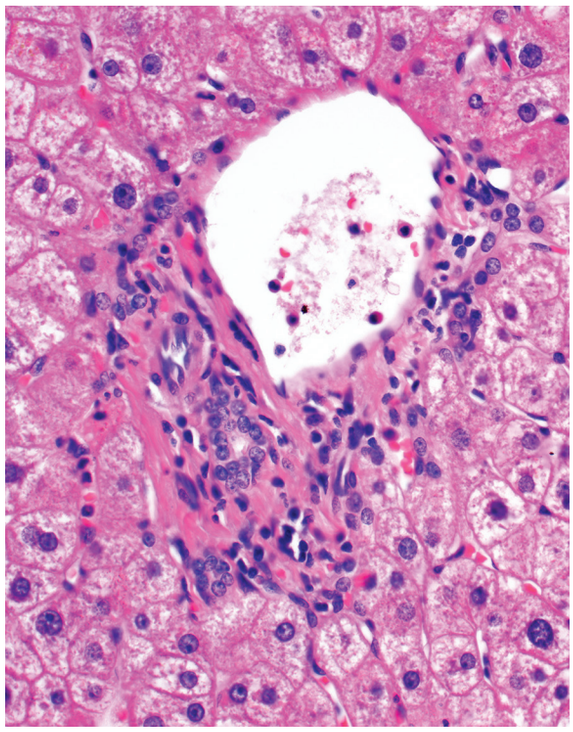

Figure 1-1

NORMAL PORTAL TRACT

The normal portal tract has a portal vein, hepatic artery, and bile duct, all ensconced in a collagenous matrix. The portal vein is typically at least five times larger in diameter than the hepatic artery.

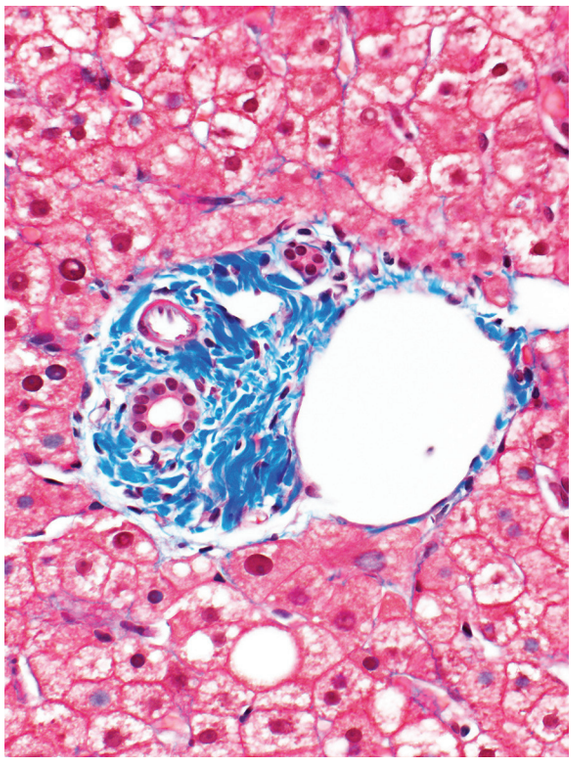

Figure 1-2

NORMAL PORTAL TRACT

The portal tract has relatively smooth edges (trichrome stain).

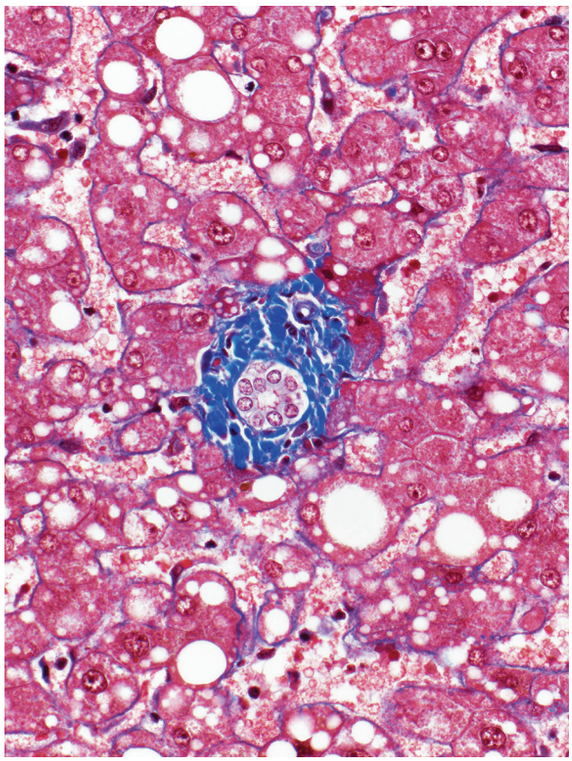

Figure 1-3

NORMAL PORTAL TRACT: PORTAL DYAD

No portal vein is seen in this small portal tract (trichrome stain).

variation in the structures within normal portal tracts, as many have several bile ducts or several hepatic artery profiles. Some of the smaller portal tracts, often near the periphery of the liver, have only two of the three structures present, and are called portal dyads (fig. 1-3): 7 percent of the smallest portal tracts lack a visible bile duct on hematoxylin and eosin (H&E)-stained slides (1).

The hilar region of the liver has very large portal tracts, which branch and arborize, diminishing in size as they extend out to the periphery of the liver. Nevertheless, there are smaller numbers of medium- to larger-sized portal tracts that extend out to nearly the liver capsule and can be sampled in peripheral liver biopsy specimens.

Regardless of the size of the portal tract, the diameter of the bile duct and the hepatic artery is similar (see fig. 1-1), while the portal vein is generally about five times larger in diameter than either the bile duct or the artery (see fig. 1-1). Portal tracts also contain lymphatics but these are inconspicuous in the normal liver. In some cases of cirrhosis, however, the lymphatic vessels are prominent (fig. 1-4). The larger portal tracts in the hilum can also contain nerves.

Hepatic Lobule

The lobules are composed of hepatocytes organized into thin plates (cords) that are one to two cells in thickness in the quiescent liver (fig. 1-5), but can be a bit thicker in the rapidly regenerating liver, focally up to three cells in thickness (fig. 1-6). The lobules are further divided into approximate thirds, zones 1, 2, and 3, with zone 1 surrounding the portal tracts, zone 3 surrounding the central veins, and zone 2 consisting of those hepatocytes between zones 1 and 3 (fig. 1-7). Zone 1 hepatocytes are the first to interact with nutrient- and oxygen-rich blood and have important roles in protein synthesis, while zone 3 hepatocytes play a greater role in detoxifying chemicals and in bile production (2).

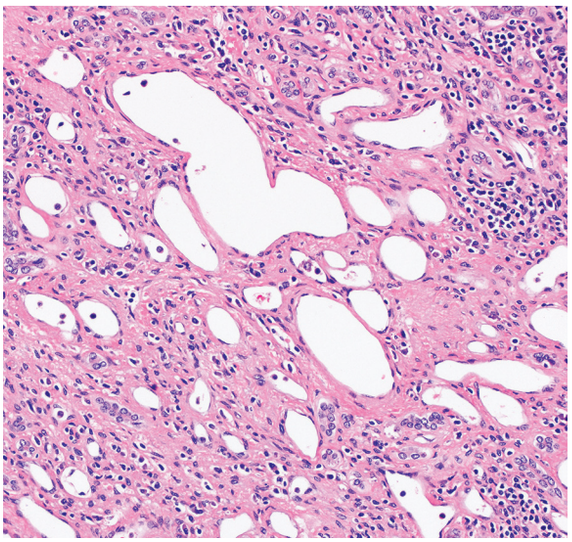

Figure 1-4

DILATED LYMPHATICS

The lymphatics are prominent in this cirrhotic liver, while normally they are inconspicuous.

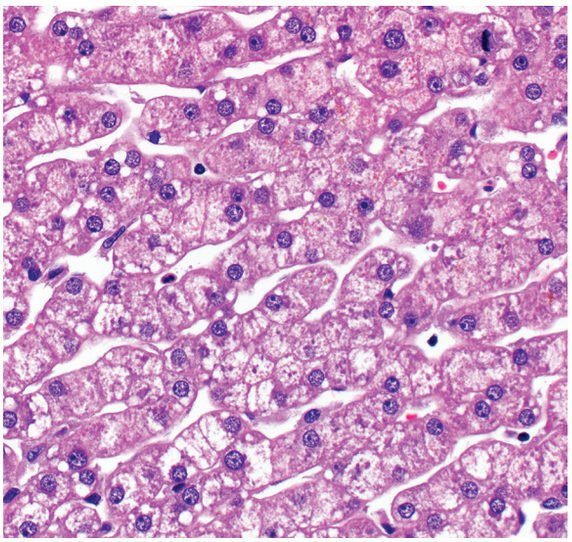

Figure 1-5

NORMAL LOBULE

The hepatic plates are 1 to 2 cells in thickness.

In some livers, lipofuscin is evident in the hepatocytes. Lipofuscin has a granular, red-brown appearance on H&E-stained specimens (fig. 1-8) and is more densely deposited in zone 3 hepatocytes, but can extend into other zones when deposition is heavy.

The hepatocytes form bile canaliculi where their cell membranes come together, each hepatocyte forming half of the bile canaliculi. The hepatocytes produce bile that is secreted into the bile canaliculi, moving from the lobules into the bile ducts of the portal tracts. The bile canaliculi are not evident on H&E unless there is cholestasis (fig. 1-9).

The hepatic plates are separated from each other by the hepatic sinusoids (fig. 1-10). The hepatic sinusoids in the normal liver are lined by fenestrated endothelium, allowing exchange between the blood and hepatocytes of nutrients, signaling molecules, and other substances. The sinusoids also contain Kupffer cells and stellate cells, but these cells are inconspicuous in the normal liver, becoming visible with special stains or in disease conditions when they undergo both hyperplasia and hypertrophy (figs. 1-11, 1-12). Occasional lymphocytes and rare eosinophils are also normally present in the sinusoids. Eosinophils can be more prominent

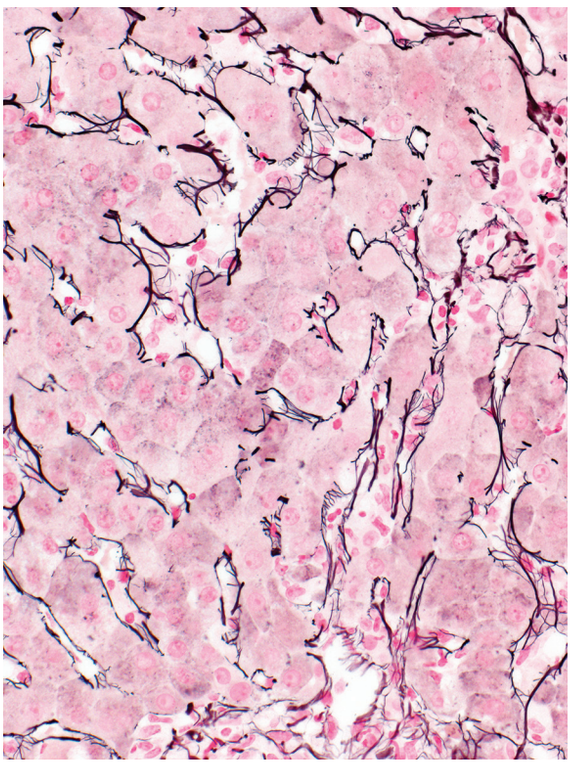

Figure 1-6

REGENERATING LIVER

In focal areas, the hepatic plates are 2 to 3 cells in thickness in this case of acute hepatitis.

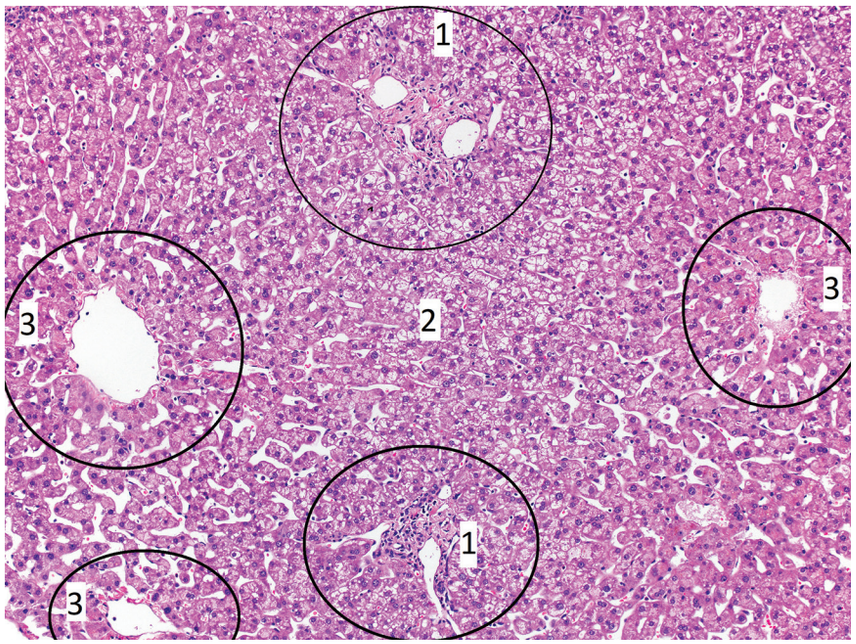

Figure 1-7

NORMAL LIVER

Zones 1, 2, and 3 are designated.

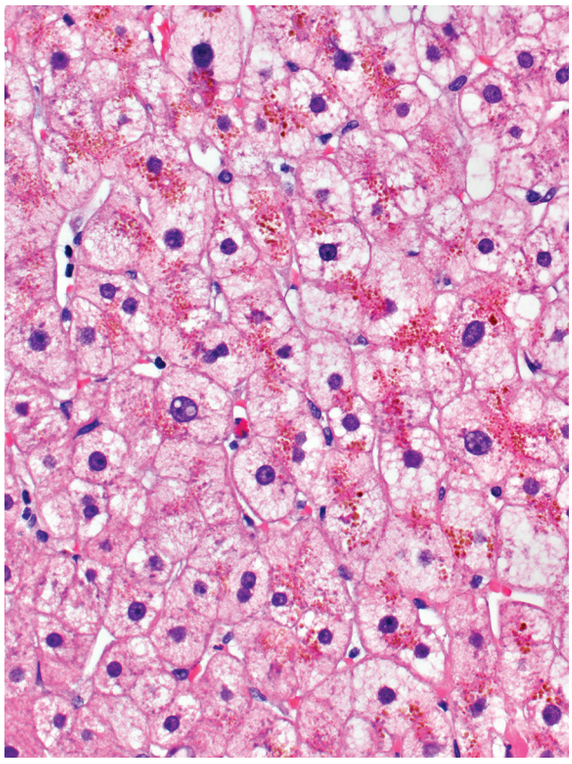

Figure 1-8

NORMAL LIVER: LIPOFUSCIN

The hepatocytes show heavy pigmentation.

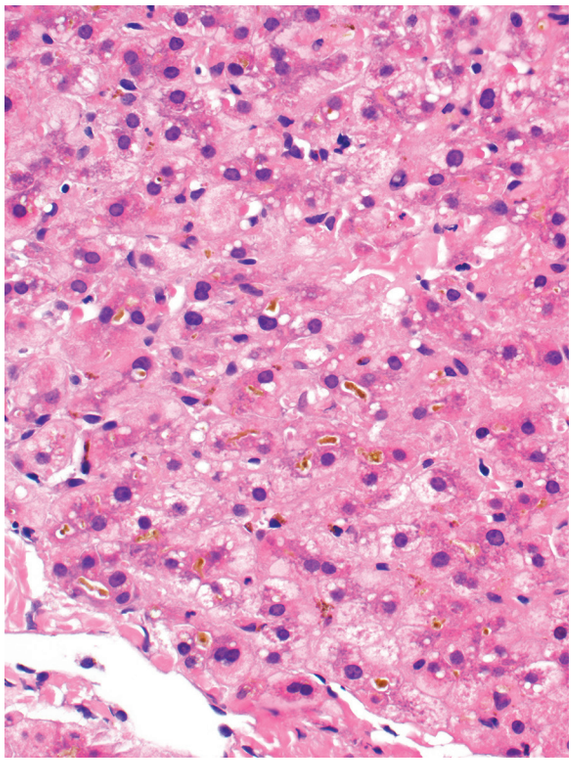

Figure 1-9

BILE CANALICULI

The bile canaliculi are evident due to cholestasis in this case of biliary obstruction.

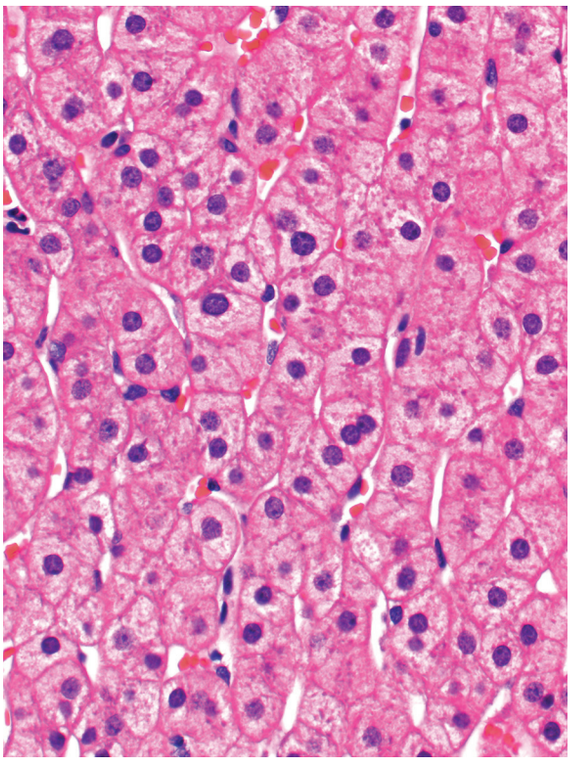

Figure 1-10

HEPATIC SINUSOIDS

The normal liver sinusoids are thin, linear, interconnecting structures lined by cells with flattened nuclei and inconspicuous cytoplasm, representing Kupffer cells, endothelial cells, and stellate cells.

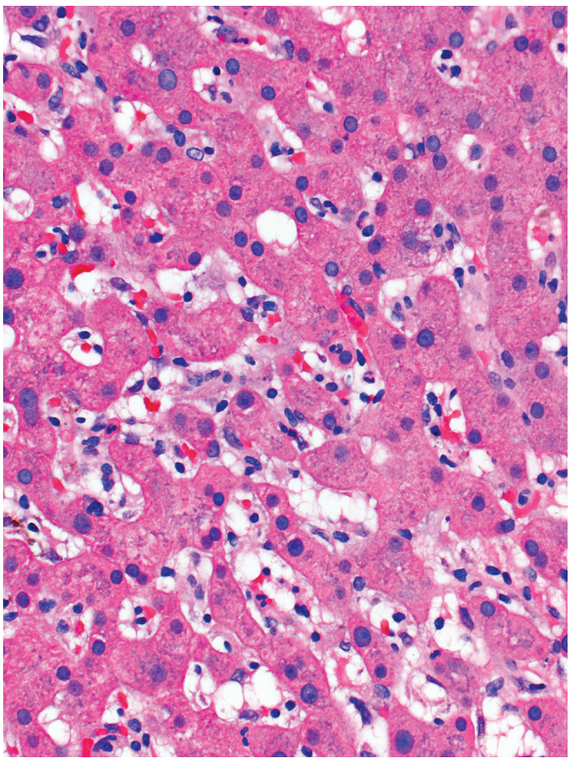

Figure 1-11

KUPFFER CELL HYPERPLASIA

The sinusoids show Kupffer cell hyperplasia in this liver biopsy from an older man with hemophagocytic syndrome, clinically thought to be viral associated.

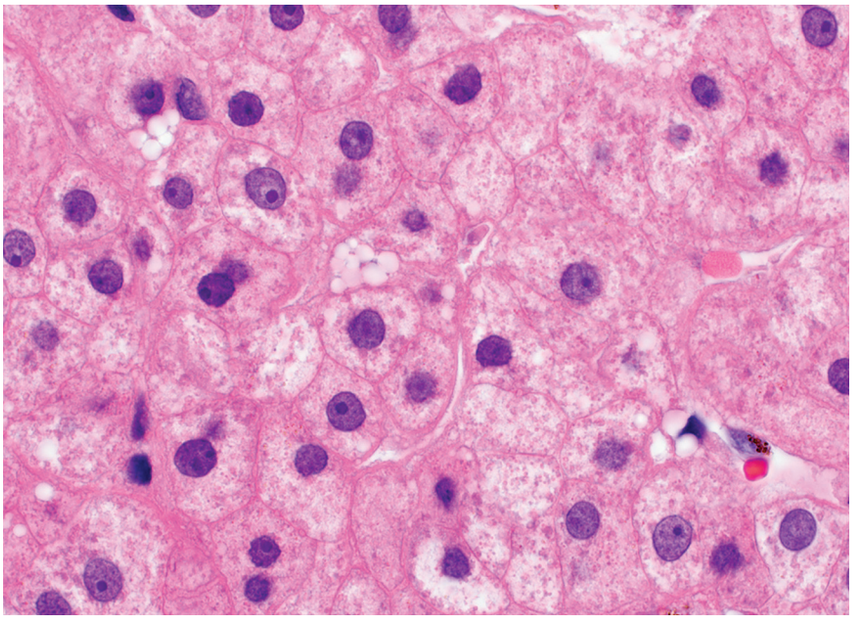

Figure 1-12

STELLATE CELL HYPERPLASIA

The sinusoids showed numerous enlarged stellate cells, their cytoplasm filled with many tiny vacuoles, often indenting their dense basophilic nuclei. Three stellate cells are evident in this image.

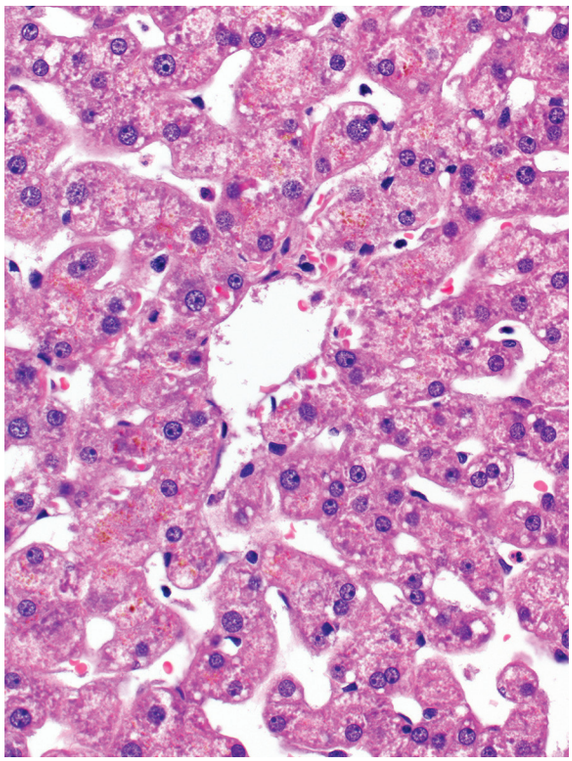

Figure 1-13

NORMAL CENTRAL VEIN: SMALL

There is minimal, inconspicuous collagen around this small central vein.

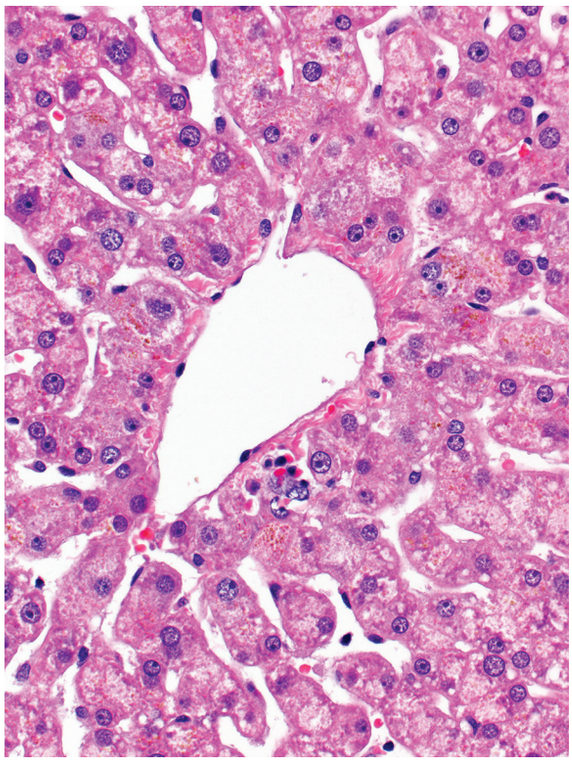

Figure 1-14

NORMAL CENTRAL VEIN: MEDIUM SIZED

A collar of collagen can be seen with a hematoxylin and eosin (H&E) stain.

if there is peripheral eosinophilia. Megakaryocytes are also rarely present, especially if there is bone marrow disease.

Both the portal veins and the hepatic arteries drain into the sinusoids, where the two streams of blood mix. After passing through the sinusoids, the blood drains out via the central veins. Medium- and larger-sized veins have a thin cuff of normal collagen visible with an H&E stain (figs. 1-13, 1-14). The hepatocytes surrounding the central veins, also called zone 3 hepatocytes, receive less oxygen-rich blood, and are the first to show ischemic injury and often the first to show injury from various liver toxins.

ACUTE LIVER FAILURE

The term *acute hepatitis* has a different meaning than *acute liver failure*. Both have commonly accepted definitions based on clinical parameters, with acute hepatitis defined as liver enzyme elevations of less than 6 months in duration, regardless of the degree of elevation, while acute liver failure is defined as severe liver disease that begins abruptly and quickly leads to encephalopathy and coagulopathy (Table 1-1). The clinical definitions for acute liver failure also commonly include subclassifications to indicate the severity of liver injury (3). Hyperacute liver failure occurs when the clinical presentation to encephalopathy or coagulopathy is less than 1 week; acute liver failure occurs when the clinical presentation to encephalopathy or coagulopathy is 1 to 4 weeks; and subacute liver failure occurs when the clinical presentation to encephalopathy or coagulopathy is 5 to 13 weeks.

The histologic findings of acute liver failure are determined by both the etiology and the time interval between the injury and when the sample for histology is taken. In most cases, the early patterns of acute liver failure fall into one of two large categories: severe inflammation (fig. 1-15), often with necrosis, or extensive bland

Table 1-1

CAUSES OF ACUTE LIVER FAILURE

Acetaminophen: 40 percent (in USA, Canada, and Europe; less frequent elsewhere)
Idiopathic: 30 percent; no definite cause identified after full clinical and histologic evaluation
Drug-induced liver injury: 20 percent; includes herbal medications; incidence varies widely throughout the world
Acute viral hepatitis: 10 percent; most cases are acute hepatitis A or B; incidence varies widely throughout the world
Rare causes: 1 percent; alcoholic hepatitis, autoimmune hepatitis, Budd-Chiari, nonhepatotropic viruses such as adenovirus and herpes simplex virus, Wilson disease

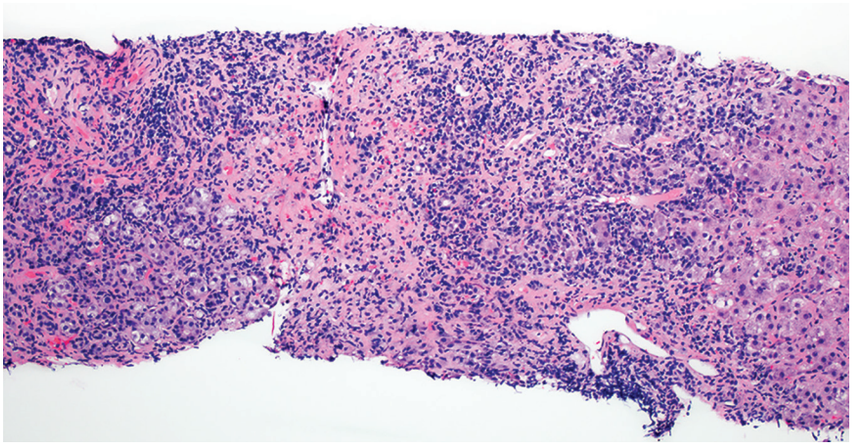

Figure 1-15

ACUTE LIVER FAILURE: MARKED HEPATITIS

There is marked lobular hepatitis and extensive necrosis in this case of acute autoimmune hepatitis.

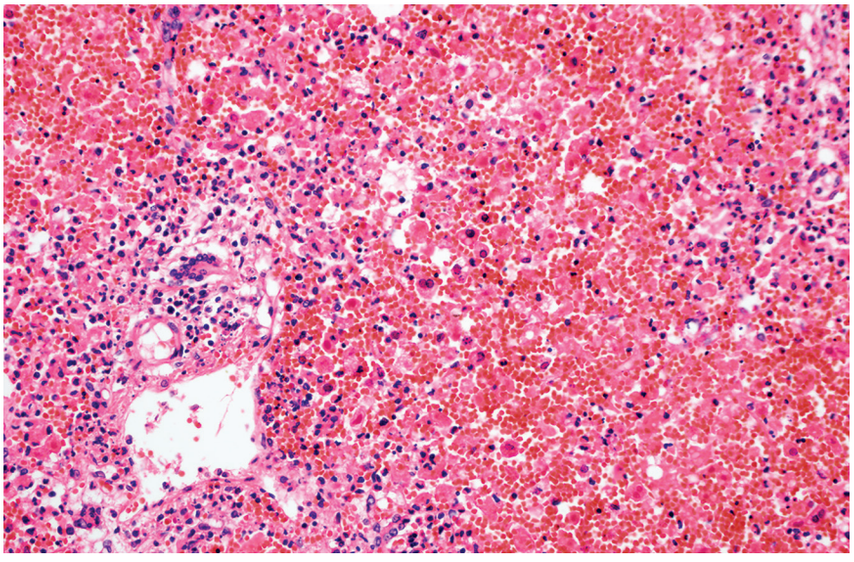

Figure 1-16

ACUTE LIVER FAILURE: BLAND NECROSIS

There is extensive hemorrhagic necrosis in this case of liver failure from a patient with acute herpes simplex virus (HSV) hepatitis.

necrosis with little or no inflammation (fig. 1-16). Over time, the histologic findings tend to converge for all etiologies and become nonspecific, dominated by collapsed parenchyma, mild chronic inflammation, bile ductular proliferation, and patchy parenchymal regeneration, a pattern called *subacute necrosis* or *submassive necrosis* (fig. 1-17). When the regenerating nodules are prominent, they can be mistaken for tumors or for underlying cirrhosis. Fibrosis or cirrhosis can be present when there is acute on chronic injury.

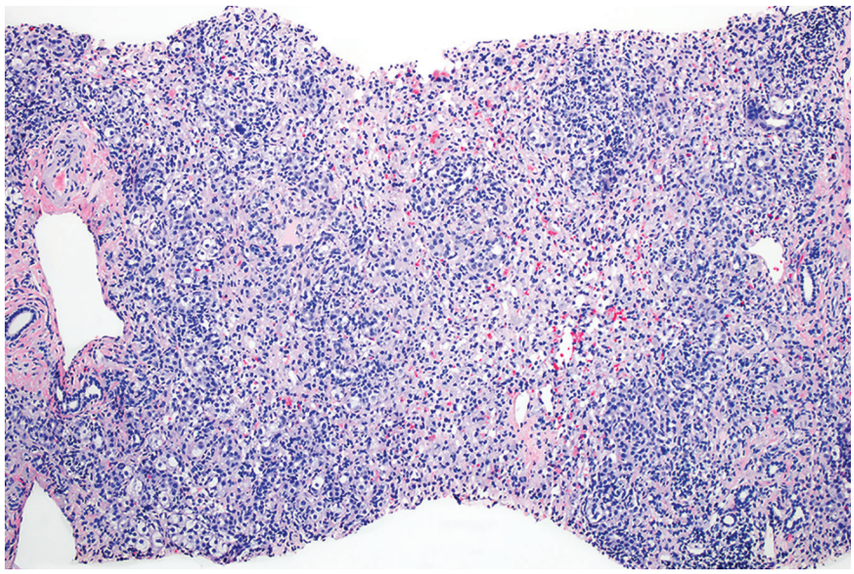

Figure 1-17

SUBMASSIVE LIVER NECROSIS

The hepatocytes have been destroyed, leaving behind inflammation, ductular proliferation, and collapsed stroma in the lobules. Areas of regeneration were also present (not shown).

Table 1-2

MOST COMMON LIVER INJURY PATTERNS

General necrosis patterns
Biliary predominant patterns of injury
Biliary obstruction
Ductopenia
Bland lobular cholestasis
Fatty liver disease
Alcohol
Metabolic syndrome
Other rare causes
Abnormal accumulation of substances in hepatocytes
Hepatitic patterns of injury
Bland lobular necrosis
Vascular disease
Granulomas
Amyloid

LIVER INJURY PATTERNS

The most common patterns of liver injury are shown in Table 1-2.

General Necrosis Patterns

Histologic necrosis patterns are generally subclassified as *spotty, confluent, zonal, panacinar, submassive,* or *massive*. In some cases, there are mixed patterns, for example, some areas of the specimen show a zonal pattern of necrosis and other areas a panacinar pattern of necrosis.

Spotty necrosis is defined as single or small clusters of dead hepatocytes. This pattern is common in many types of acute and chronic hepatitis and can be seen with or without accompanying inflammation. *Confluent necrosis* refers to somewhat larger clusters of dead hepatocytes, involving at least three hepatocytes, and in most cases is first evident in zone 3. Confluent necrosis indicates a more severe degree of acute lobular injury than spotty necrosis. Of course, it is neither feasible nor reliable to try to count the number of dead hepatocytes in order to distinguish spotty necrosis from confluent necrosis. Instead, the cut-off of three hepatocytes is used to compare and contrast the scattered, singly necrotic hepatocytes in spotty necrosis with the larger groups of dead hepatocytes, usually located in zone 3, that defines confluent necrosis. Confluent necrosis can range from mild, involving portions of a few zone 3 regions, to more extensive confluent bridging necrosis, connecting central veins to central veins or central veins to portal tracts.

Zonal necrosis most commonly has a zone 3 pattern (fig. 1-18), but rare etiologies can lead to zone 1 or zone 2 necrosis (fig. 1-19). *Panacinar necrosis* (also termed *multiacinar necrosis*) is defined as necrosis involving multiple adjacent acini (see figs. 1-15, 1-17). *Massive necrosis* is defined as necrosis involving a large proportion of the sampled tissue (e.g., greater than 75 percent). With massive necrosis, there can be foci of surviving

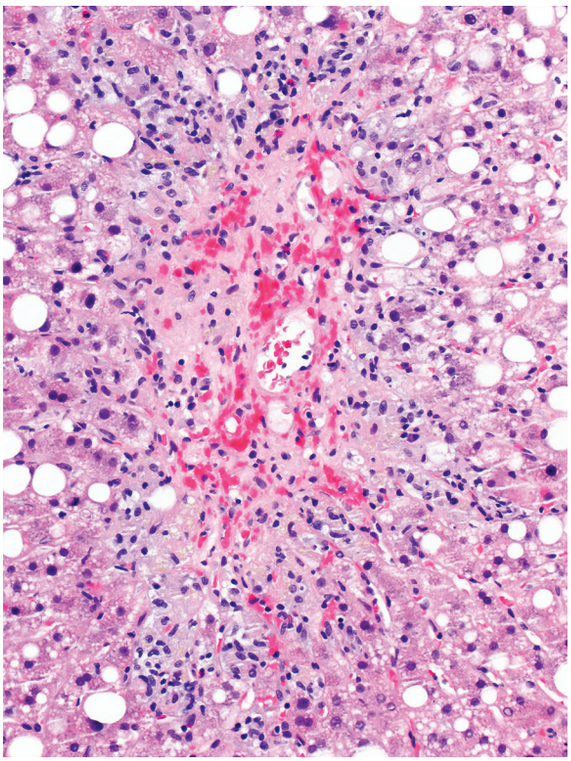

Figure 1-18

ZONE 3 NECROSIS

An elderly woman with fatty liver disease had severe zone 3 ischemic necrosis from severe, sudden hypotension.

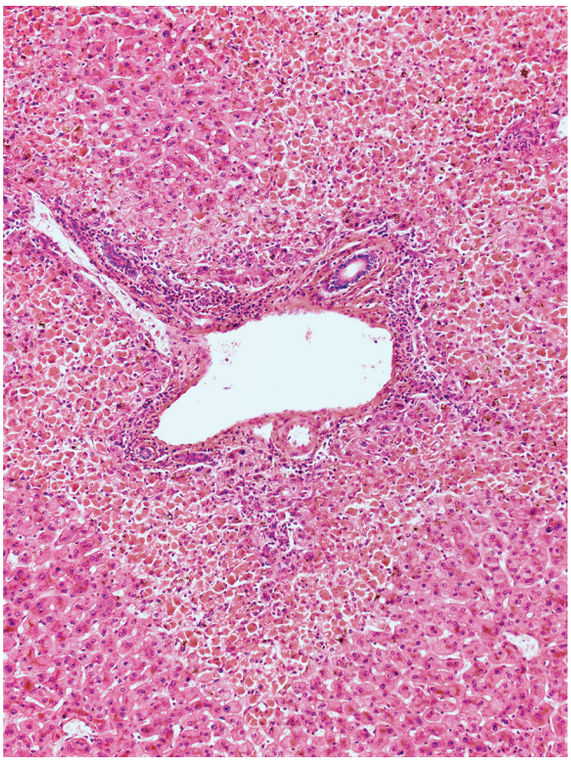

Figure 1-19

ZONE 1 NECROSIS

There is necrosis of the zone 1 hepatocytes, from a case of toxic exposure to phosphorus.

hepatocytes, often as small rims of viable hepatocytes in zone 1, hugging the portal tracts.

The term *submassive necrosis* is used in two settings, settings that are unfortunately different from each other, so the context of usage is important to understand the intent. In the first usage, the necrosis is acute and its extent is more than panacinar and less than massive. Formal definitions are not well established, but a reasonable approach is to define submassive necrosis as necrosis involving more than 25 percent but less than 75 percent of the tissue specimen. The second usage of the term submassive necrosis reflects the time since injury as well as the extent of the initial necrosis. In this setting, there has been sufficient time for the liver to undergo a least some regeneration after the initial episode of severe necrosis, which could have been either massive or submassive in terms of the percent of necrosis, with regeneration leading to nodules interspersed with large areas of parenchymal collapse. This latter pattern is most commonly seen in liver explants in patients who did not fully recover from acute liver failure, but there was sufficient time for partial liver regeneration, with a typical interval of weeks to months between the time of initial liver injury and subsequent liver transplantation. *Subacute necrosis* is also used to describe this pattern.

Biliary Obstructive Pattern

Bile ducts can be obstructed by several different mechanisms including biliary stones, biliary strictures, and mass lesions of the pancreas impinging on the common bile duct. The histologic findings vary depending on the severity of the obstruction and whether the obstruction is acute or chronic, but not so much on the cause of the obstruction. Most liver biopsy specimens with a *biliary obstruction pattern* tend to be chronic, as patients who present with acute

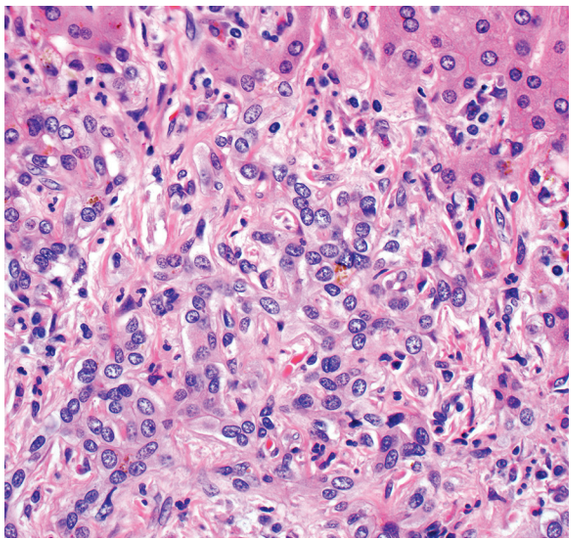

Figure 1-20

BILIARY OBSTRUCTION: BILE DUCTULAR PROLIFERATION

The common bile duct was obstructed.

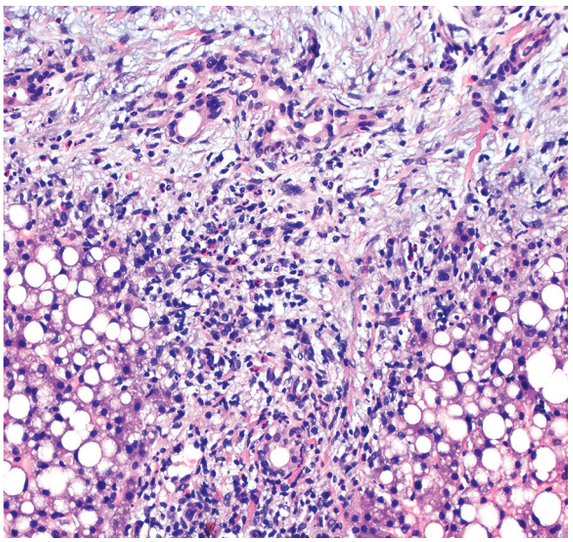

Figure 1-22

BILIARY OBSTRUCTION: PORTAL EDEMA

The portal tract stroma has a myxoid bluish tint, referred to as portal edema.

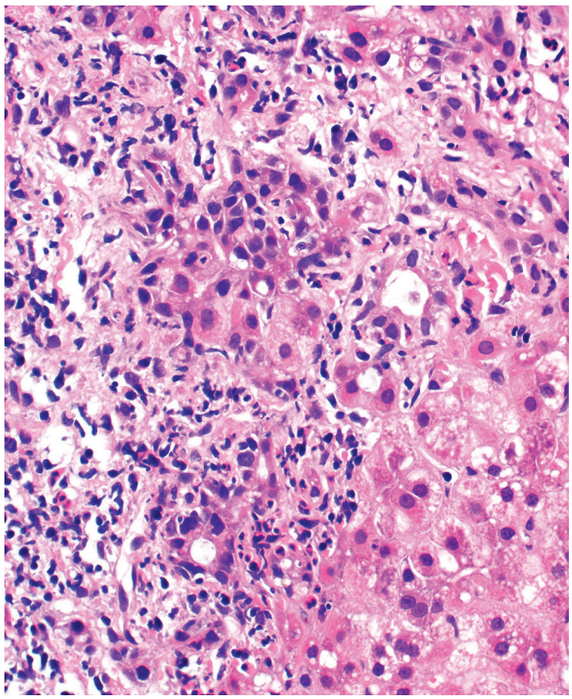

Figure 1-21

BILIARY OBSTRUCTION: MIXED INFLAMMATION

The portal tracts show mixed inflammation, with lymphocytes and neutrophils. Rare eosinophils and plasma cells are also present.

biliary obstruction tend to have a common set of clinical findings (acute right upper quadrant pain, often episodic), laboratory findings (disproportionate elevations in serum alkaline phosphatase and gamma-glutamyl transferase [GGT]), and imaging findings (dilated bile ducts) that are sufficient to indicate the diagnosis; liver biopsies are usually not needed for management.

The histologic findings in acute biliary obstruction consist of bile ductular proliferation (fig. 1-20), portal tract inflammation (fig. 1-21), and sometimes portal tract edema (fig. 1-22). The portal inflammation is mixed, with lymphocytes, neutrophils, and occasional eosinophils. The neutrophils are in the portal tract stroma. In contrast, large numbers of neutrophils in the bile duct lumen would suggest ascending cholangitis. There can also be mild patchy lymphocytosis of the bile duct epithelium and rare apoptotic bodies in the biliary epithelium, but these are never dominant findings. The lobules can also show cholestasis.

Cases of chronic biliary tract obstruction, such as primary sclerosing cholangitis, also typically have bile ductular proliferation and mixed inflammation, although the changes are often less prominent than those seen with acute obstruction. In other cases of chronic biliary obstruction,

Table 1-3

FEATURES OF CHOLATE STASIS

Results from chronic cholestasis of any etiology, but is seen most commonly with chronic biliary obstruction or ductopenia
Periportal (zone 1) hepatocytes show rarified cytoplasm with a foamy appearance, which can include fully ballooned hepatocytes and Mallory hyaline (also called feathery degeneration or pseudoxanthomatous change)
Hepatocytes with cholate stasis often contain copper, which can be highlighted with a rhodamine or similar copper stain
CK7 is positive in hepatocytes with cholate stasis; CK7-positive hepatocytes are also referred to as intermediate hepatocytes; not all intermediate hepatocytes show changes of cholate stasis, but virtually all hepatocytes with cholate stasis are CK7 positive

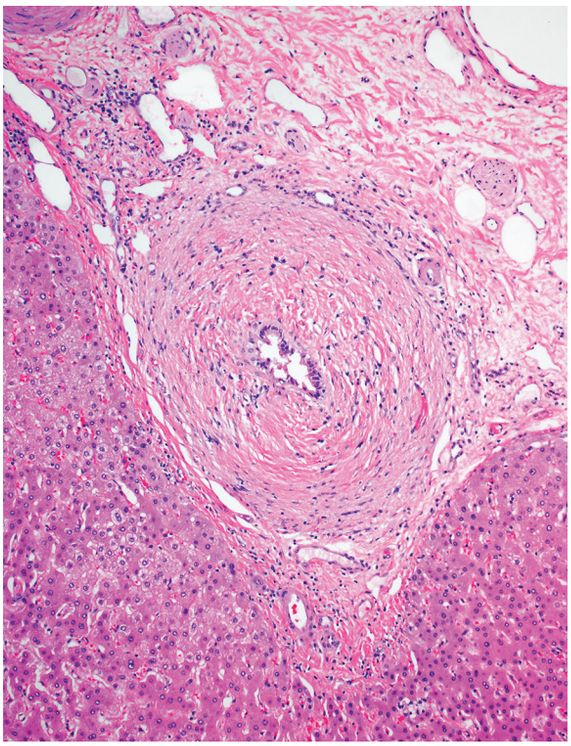

Figure 1-23

ONION SKIN FIBROSIS

The bile duct has a thick rind of lamellar fibrosis.

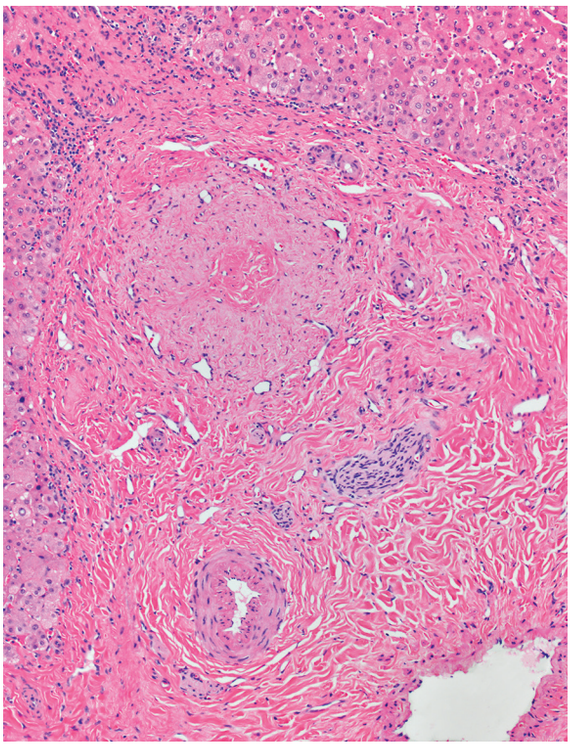

Figure 1-24

FIBRO-OBLITERATIVE DUCT LESION

The bile duct has been replaced by a fibrous scar.

usually low-grade obstruction, the histologic changes are limited to only patchy mild bile ductular proliferation, occasional neutrophils, and mild lymphocytic portal inflammation.

Longstanding cases of biliary obstruction can lead to additional findings of bile duct duplication, bile duct loss, and portal fibrosis. With longstanding disease, some of the medium-sized bile ducts develop a distinctive pattern of periductal fibrosis, called *onion-skin fibrosis* (fig. 1-23), or can be completely replaced by fibrous scars, called *fibro-obliterative duct lesions* (fig. 1-24).

The zone 1 hepatocytes can show cholate stasis (Table 1-3; fig. 1-25) and sometimes the lobules show cholestasis, the latter usually with advanced fibrosis or decompensated disease. The differential diagnosis of chronic biliary obstructive disease includes primary sclerosing cholangitis,

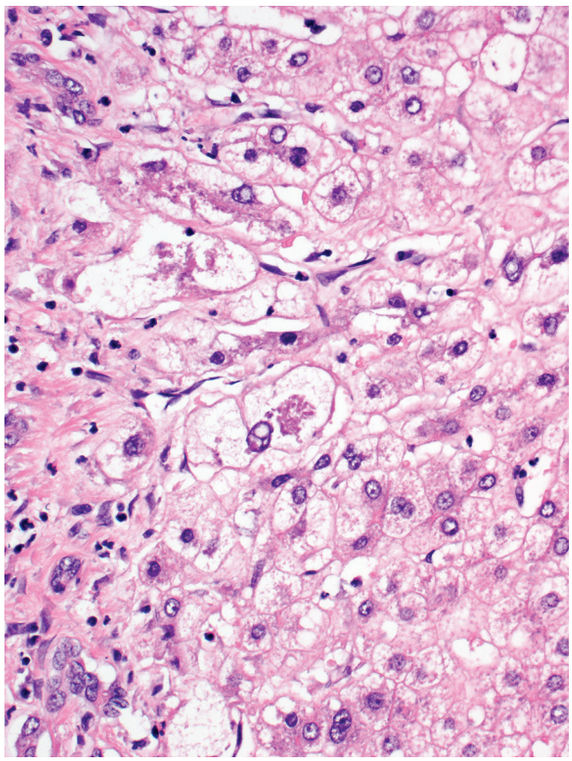

Figure 1-25

CHOLATE STASIS

The hepatocytes are ballooned and show Mallory hyaline.

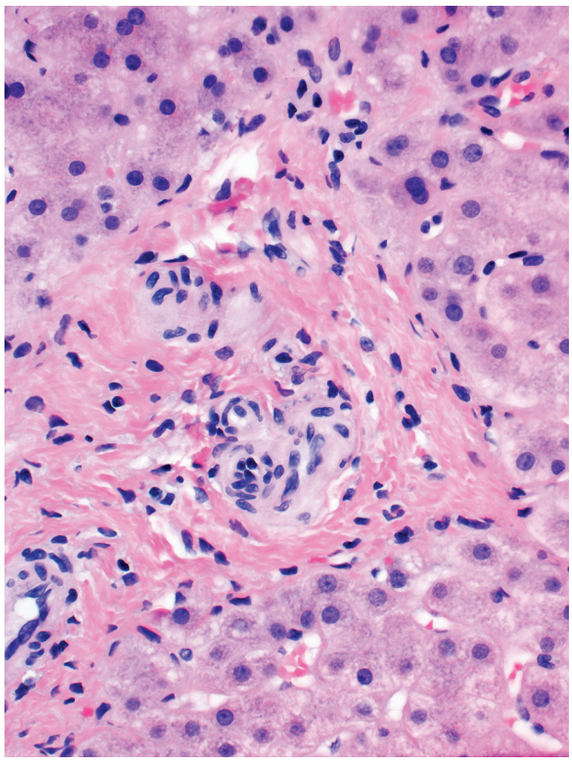

Figure 1-26

DUCTOPENIA

There is no bile duct in this portal tract.

ischemic bile duct strictures, chronic biliary stone disease, chronic pancreatic disease which can lead to strictures of the common bile duct, and a number of other rare causes of biliary strictures.

Ductopenia

Bile duct loss, also called *ductopenia*, most often results from chronic cholestatic liver diseases such as primary sclerosing cholangitis or primary biliary cholangitis, but has a wider differential that includes drug-induced liver injury (4) and paraneoplastic effects (Table 1-4). In liver transplant patients, chronic rejection is an important cause of bile duct loss. When no clear cause is identified for ductopenia, the pattern of injury is sometimes called the *vanishing bile duct syndrome* (5) or *idiopathic ductopenia*, the latter term being preferred. The lobules do not always show cholestasis, even with established ductopenia.

In the normal liver, about 10 percent of the smallest portal tracts do not contain bile ducts (1), so the diagnosis of ductopenia requires more substantial loss of bile ducts, with at least 50 percent of the portal tracts missing their bile ducts (fig. 1-26). The biopsy should be reasonably sized to assess bile duct loss, containing at least 10 portal tracts. The portal tracts do not have to be complete to be included in the count of 10, but should be sufficiently sampled so that the bile duct would be evident if it was there. Bile ducts and hepatic arteries are always paired, have similar diameters, and are typically located close together, within 1 to 2 hepatic artery diameters (6); thus, unpaired arteries are a useful clue to the diagnosis of ductopenia, although there are several potential pitfalls to avoid. When there is significant zone 3 fibrosis from either fatty liver disease or venous outflow obstruction, there can be arterialization of the fibrotic central vein region, leading to an artery next to a large vein, all embedded in fibrosis, a finding that can mimic ductopenia (7–9).

Table 1-4

DIFFERENTIAL DIAGNOSIS OF BILE DUCT LOSS (DUCTOPENIA)

Cause	Comment/Representative Reference
Chronic biliary tract disease	
Primary sclerosing cholangitis	
Primary biliary cholangitis	
ABCB4/MDR3 deficiency	Including heterozygous mutations
Secondary causes of chronic biliary tract disease	
Sarcoidosis	
Intra-arterial chemotherapy	
Mast cell cholangitis	
Portal biliopathy	Results from portal vein thrombosis and remodeling that impinges on the bile ducts, leading to obstruction
Extrahepatic bile duct disease	Any chronic extrahepatic biliary tract obstruction including stones, strictures, pancreatic disease, hilar lymph nodes with granulomas, or malignancy that enlarge lymph node and obstruct biliary tree
Infections	
Recurrent pyogenic cholangitis	
Acquired immunodeficiency syndrome (AIDS)	Thought to result from infection in most cases; potential etiologies include *Cryptosporidium*, *Microsporidium*, cytomegalovirus, and *Cyclospora* (89,90,92–96)
Pediatric liver disease	
Paucity of intrahepatic bile ducts	Can be syndromic or nonsyndromic
Neonatal hepatitis	Often subtle and does not reach full threshold of 50 percent of portal tracts with missing ducts
Biliary atresia	End-stage disease
Alpha-1-antitrypsin deficiency	
Drug/medication	Many different drugs, including total parenteral nutrition (99)
Paraneoplastic syndrome	
Lymphoma	Hodgkin disease (97), peripheral T-cell lymphoma (98)
Carcinoma	
Transplantation related	
Ischemic cholangiopathy secondary to hepatic artery thrombosis	
Chronic allograft rejection	
Biliary anastomotic strictures	
Bone marrow transplant related	Graft versus host disease, drug-induced liver injury
Idiopathic	

Another useful observation is that medium-sized and larger portal tracts should always have bile ducts. If they do not, then their absence strongly suggests ductopenia. CK7 is also helpful for identifying bile duct loss and adds additional value by highlighting intermediate hepatocytes in zone 1, a finding that is almost invariably seen with true bile duct loss (Table 1-5; fig. 1-27). As a diagnostic pitfall, especially if there is fibrosis, the zone 3 hepatocytes can also be CK7 positive in cases of vascular outflow disease (10).

Bland Lobular Cholestasis

Patients with the *bland lobular cholestasis pattern* of injury can present with itching or jaundice, but many patients present with nonspecific symptoms such as fatigue. Bilirubin levels are disproportionally elevated, with normal or mild elevations in aspartate aminotransferase (AST) and alanine aminotransferase (ALT), typically less than 5 times the upper limit of normal (ULN), and mild elevations in alkaline phosphatase.

Table 1-5

FEATURES OF INTERMEDIATE HEPATOCYTES

CK7-positive hepatocytes
Intermediate hepatocytes in zone 1 suggest chronic cholestatic liver disease (can be from biliary tract disease or other causes)
Intermediate hepatocytes in zone 3 suggest chronic vascular outflow disease
CK7-positive hepatocytes in zone 1 can range in appearance from essentially normal to showing changes of cholate stasis; in zone 3, they range in morphology from essentially normal to smaller and somewhat basophilic

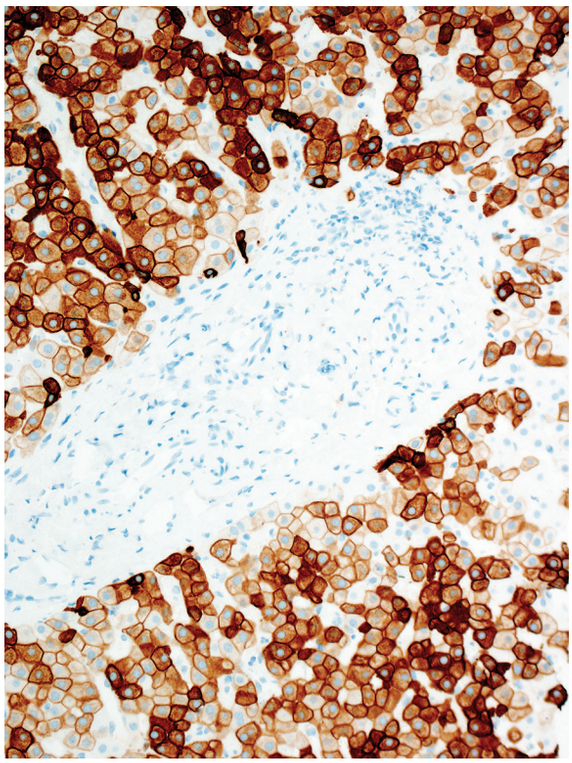

Figure 1-27

INTERMEDIATE HEPATOCYTES: CK7

No bile duct is seen. The zone 1 hepatocytes show CK7 positivity, a finding called intermediate hepatocyte. The amount of intermediate hepatocytes in this case is more dramatic than most.

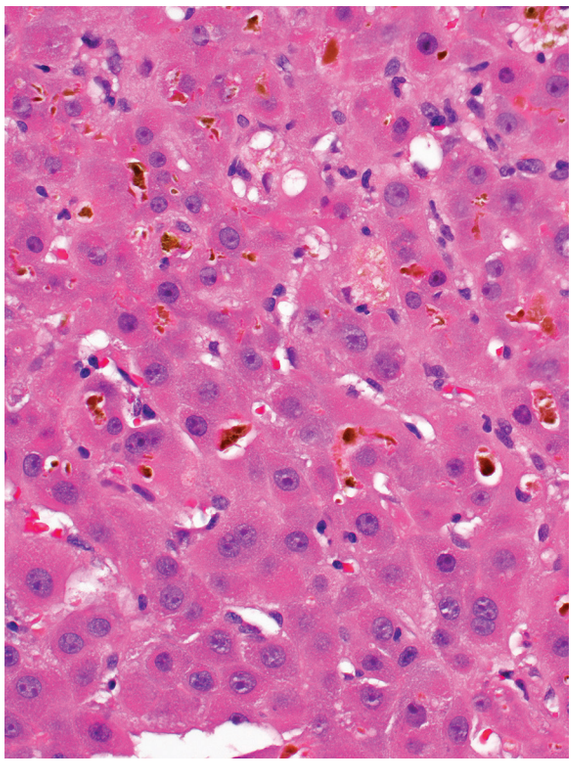

Figure 1-28

BLAND LOBULAR CHOLESTASIS

The lobules show cholestasis with no inflammation in this example of drug-induced cholestasis.

The bland lobular cholestasis pattern is well named because it perfectly captures the histologic pattern: there is lobular cholestasis with bile pigment in hepatocytes and or bile canaliculi (fig. 1-28) but not much else, with no significant inflammation in the portal tracts or lobules, no fatty change, and no other significant findings. The portal tracts are essentially normal with no evidence of the biliary obstruction pattern and no bile duct loss.

The most common cause of this pattern is drug-induced liver injury, but the differential includes sepsis as well as several other rare etiologies (Table 1-6). The location of the bile, whether it is in the hepatocytes, the bile canaliculi, or both, is not relevant to the etiology and does not need to be specified in the pathology report.

Table 1-6

POTENTIAL CAUSES OF BLAND LOBULAR CHOLESTASIS

Debilitating illnesses, including patient in the intensive care unit
Drug-induced liver injury
Intrahepatic cholestasis of pregnancy
Paraneoplastic syndromes Lymphomas are most common Others include renal cell carcinoma (Stauffer syndrome)
PFIC disorders *ATP8B1* mutations (PFIC1); mutated protein is called FIC1 *ABCB11* mutations (PFIC2); mutated protein is called BSEP *ABCB4* mutations (PFIC3); mutated protein is called DR3
Sepsis
Thyroid disease (mild, not usually biopsied) Hyperthyroid Hypothyroid

Ascending Cholangitis

Ascending cholangitis results from bacterial infections of the biliary tree. Common risk factors include anatomic abnormalities of the biliary tree and immunosuppression. In most cases, the diagnosis is made clinically and a biopsy is not necessary. The bile ducts in the portal tracts become dilated, the epithelium is thinned, and the lumens are filled with neutrophils (fig. 1-29). Additional findings of biliary obstruction are also commonly present.

Granulocytic Epithelial Lesions

Rarely, and almost always focally, liver specimens can show portal tracts that have neutrophils located primarily in the bile duct epithelium (not primarily in the stroma, not primarily in the lumen). This pattern of injury is called a *granulocytic epithelial lesion* (GEL) (fig. 1-30) and is generally not a pattern that indicates obstruction or ascending cholangitis, but instead has a differential that includes drug-induced liver injury, type 2 autoimmune pancreatitis involving the liver (11), syphilis, and idiopathic causes.

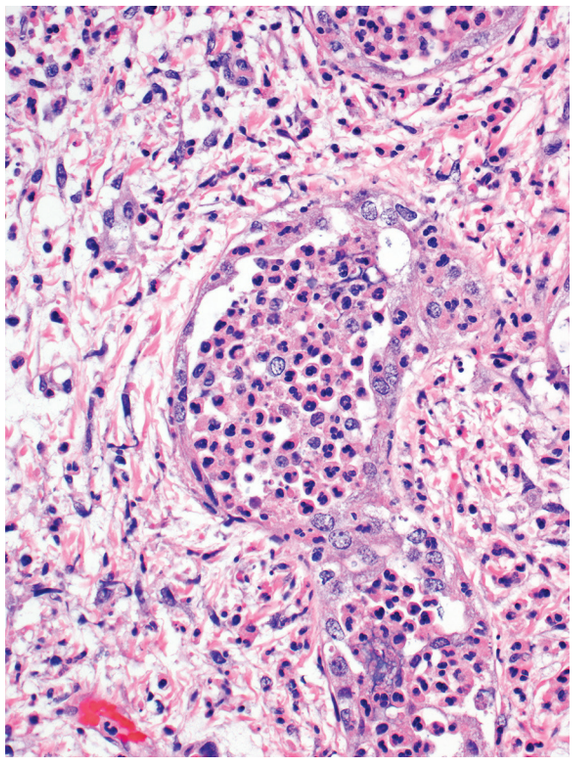

Figure 1-29

ASCENDING CHOLANGITIS

The bile duct is dilated, the ductal epithelium is attenuated, and the lumen is filled with neutrophils. Not all cases are this dramatic.

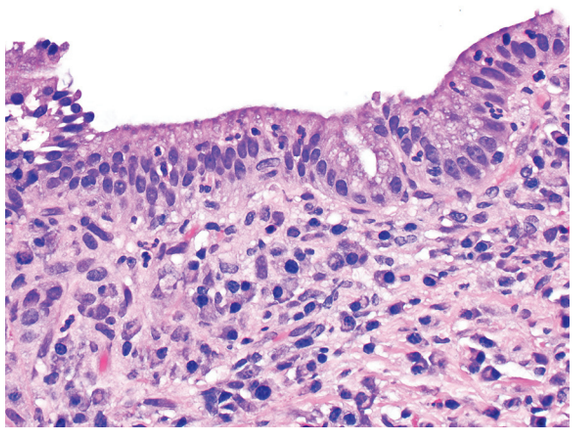

Figure 1-30

GRANULOCYTIC EPITHELIAL LESION: SYPHILIS

The bile duct epithelium shows neutrophilic inflammation. There also is a cuff of plasma cell-rich inflammation surrounding the bile epithelium.

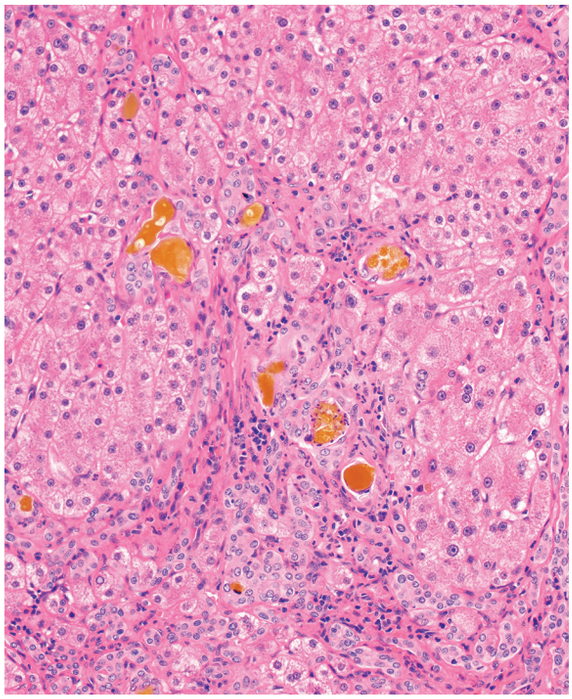

Figure 1-31

CHOLANGITIS LENTA

The bile ductules show inspissated bile concretions. The patient had longstanding biliary obstruction but was not septic.

Cholangitis Lenta

The *cholangitis lenta pattern* is defined as the presence of inspissated bile in proliferating bile ductules at the edges of the portal tract (fig. 1-31). This pattern of injury is most often seen in patients with chronic high-grade biliary obstruction (for example, biliary atresia), longstanding chronic cholestasis from any cause, or chronic debilitating illnesses, often coexisting with lobular cholestasis. In the latter setting, some patients go on to develop sepsis, but cholangitis lenta is not an indicator of concurrent sepsis per se. Instead, cholangitis lenta and sepsis are common in the same patient population (debilitated patients).

Fatty Liver

Fatty liver is classified into two patterns, each with its own differential diagnoses: *macrovesicular steatosis* (fig. 1-32) and *microvesicular steatosis* (fig. 1-33). The most common pattern of fat in the liver is by far macrovesicular steatosis. In fact, many pathologists will go their entire career and never see a bone fide case of microvesicular steatosis, while seeing hundreds of cases of macrovesicular steatosis.

Macrovesicular steatosis is defined as the presence of a single large droplet of fat in the hepatocyte cytoplasm, typically displacing the

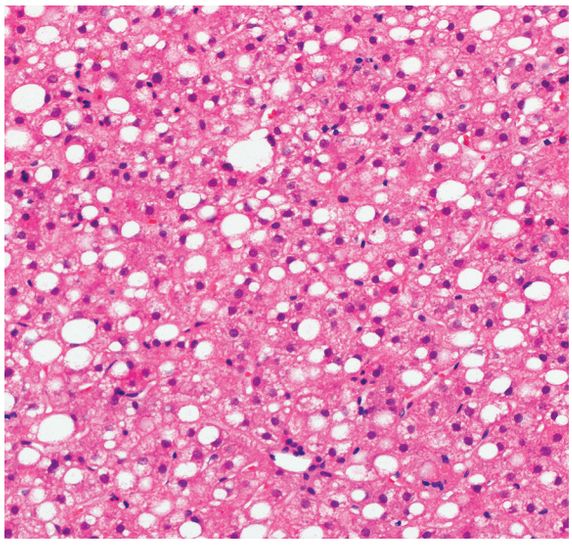

Figure 1-32

MACROVESICULAR STEATOSIS

The hepatocytes have large droplets of fat in their cytoplasm.

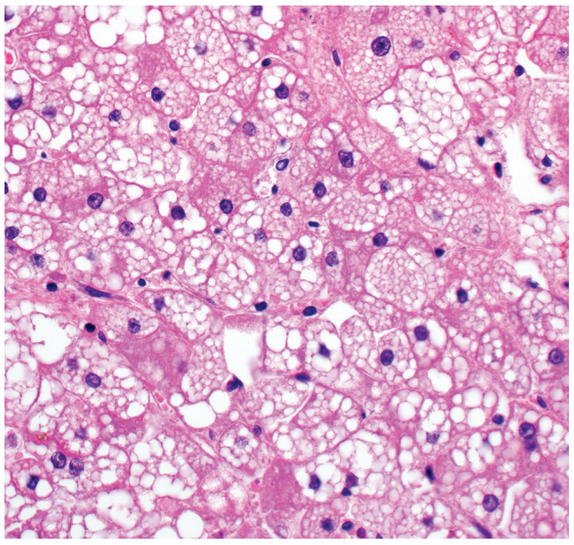

Figure 1-33

MICROVESICULAR STEATOSIS

The entire liver is diffusely involved by microvesicular steatosis (drug effect).

nucleus to the side. Hepatocytes with somewhat smaller droplets of fat, and sometimes several droplets of fat, are also commonly present (fig. 1-34) but are fully recognized as part of the macrovesicular steatosis pattern and are not particularly special. Because of this, there is no need to mention them and terms like *mixed microvesicular and macrovesicular steatosis* or *mixed small and large droplet fat* or *mixed intermediate and large droplet fat* are unnecessary.

In more than 90 percent of liver specimens, the macrovesicular steatosis pattern is associated with the metabolic syndrome or alcohol use. Other possible causes include drug-induced liver injury, genetic diseases, and malnutrition (see chapter 9, Table 9-5). In cases resulting from the metabolic syndrome, AST and ALT serum elevations are typically mild, in the 40 to 50 IU/L range (12), although they can reach the low 100 or 200s, with another group of about 20 percent of patients having normal serum ALT levels (13).

Clinically, patients with the metabolic syndrome have obesity, diabetes mellitus, hypertension, and dyslipidemia. Not all components of the metabolic syndrome need to be present to have fatty liver disease. Liver enzyme elevation patterns do not reliably determine the cause of fatty liver disease. The normal AST/ALT ratio (also known as the De Ritis ratio) is about 0.8, and ratios above 2 have historically been associated with alcoholic liver disease. For many years, however, it has been known that the AST/ALT ratio is not sufficiently sensitive or specific to be clinically useful in the diagnosis of alcoholic steatohepatitis (14).

With the microvesicular steatosis pattern of injury, the hepatocytes are filled with numerous tiny droplets of fat. The hepatocyte nucleus tends to be in the center of the cell. In addition to the cytologic findings, the definition of microvesicular steatosis also has a distribution component: the finding needs to be present throughout the biopsy. This does not mean that every cell has to be affected, for there often is some zonal accentuation of the microvesicular steatosis, usually in zone 3 hepatocytes, with sparing of zone 1 and sometimes zone 2 hepatocytes. Nevertheless, even in this scenario, the zone 3 hepatocytes are affected throughout the liver specimen. Scattered or small clusters of hepatocytes with macrovesicular steatosis are also common with the microvesicular pattern of injury.

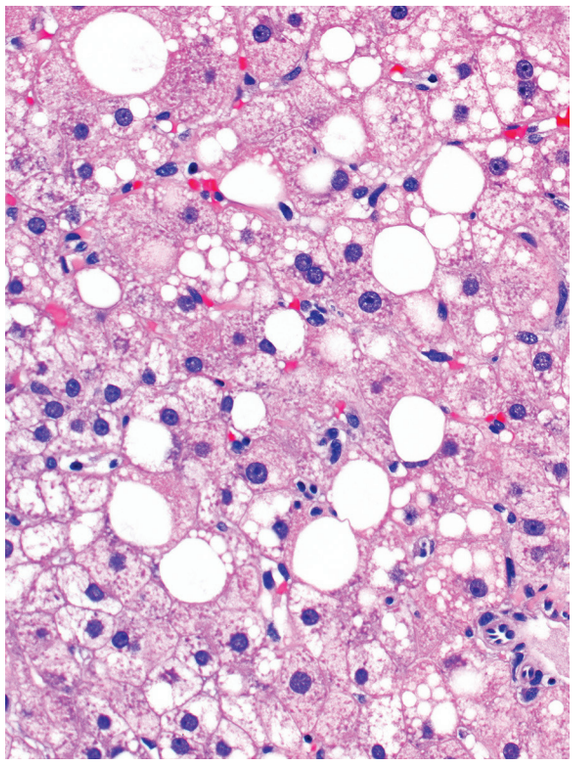

Figure 1-34

MACROVESICULAR STEATOSIS

There are a number of hepatocytes with intermediate-sized globules of fat, but this pattern is still macrovesicular steatosis.

The distribution component of the definition for microvesicular steatosis separates the true microvesicular pattern of injury from focal well-circumscribed areas of microvesicular steatosis that can be found in about 10 percent of cases of ordinary fatty liver disease (fig. 1-35) (15), but do not represent a true microvesicular pattern of injury. The true microvesicular steatosis pattern of injury results from various hepatocellular mitochondrial injuries. In adults, the most common etiologies are drug-induced liver injury, toxic injury, or fatty liver of pregnancy (see chapter 9, Table 9-6). In children, additional considerations include genetic disorders involving fatty acid oxidation or urea cycle defects.

The diagnosis of microvesicular steatosis should be based on the H&E findings. If no microvesicular steatosis is seen, then there is

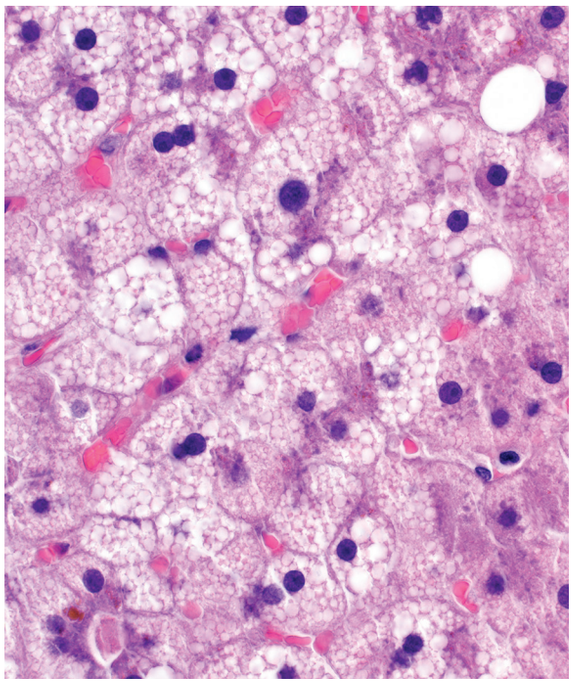

Figure 1-35

PATCH OF MICROVESICULAR STEATOSIS IN NONALCOHOLIC FATTY LIVER DISEASE

This biopsy shows a small distinct nodule of microvesicular steatosis, in a background of ordinary steatohepatitis. This pattern should not be equated with microvesicular steatosis.

no diagnosis of microvesicular steatosis. Some years ago, the literature emphasized an important role for oil red O stains on frozen tissue for diagnosing microvesicular steatosis, but subsequent experience has shown that such staining lacks specificity, since both the normal liver as well as many disease conditions, ones entirely unrelated to the microvesicular steatosis pattern of injury, can have numerous tiny cytoplasmic droplets of fat with the oil red O stain (fig. 1-36) (16). Finally, many causes of acute injury (17, 18) can result in hepatocytes having a few tiny droplets of fat in their cytoplasm (fig. 1-37). This finding is nonspecific and should not be classified as microvesicular steatosis.

Cytoplasmic Changes in Hepatocytes

In addition to fat, liver disease can lead to the accumulation of other material in the cytoplasm of hepatocytes. These changes are typically visible by H&E, but in several cases can be highlighted by special stains (Table 1-7).

Glycogen Accumulation. Glycogen accumulation can lead to diffusely swollen hepatocytes with clear, rarified cytoplasm. This pattern of injury in infants and young children can represent glycogen storage disease. In older children and adults, in contrast, this pattern almost always

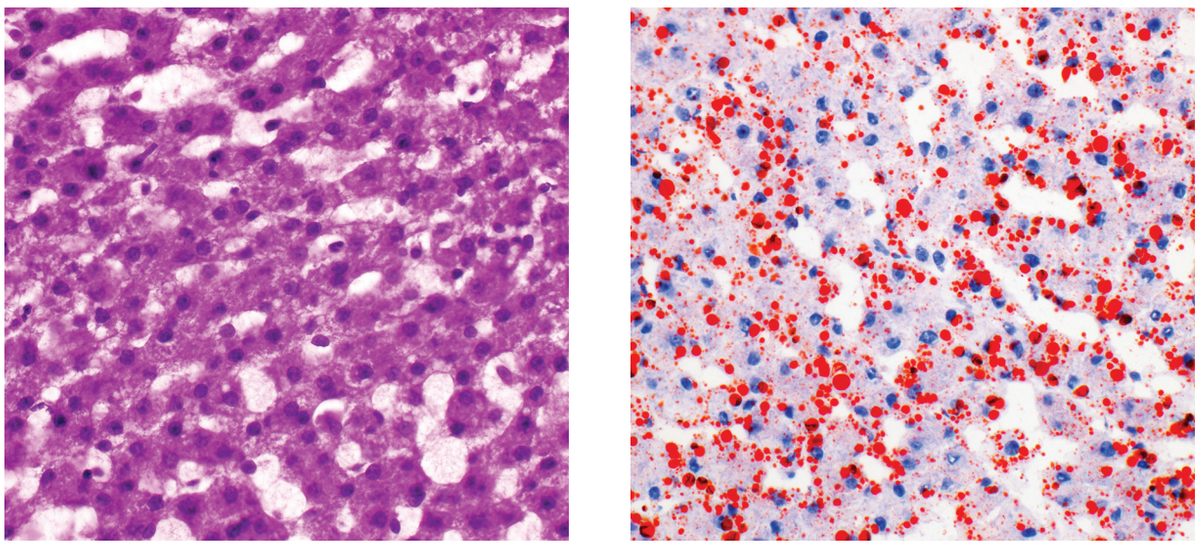

Figure 1-36

NORMAL LIVER: OIL RED O

This frozen section of a normal liver shows no microvesicular steatosis (left) but shows numerous small droplets of fat with an oil red O stain (right).

represents glycogenic hepatopathy (fig. 1-38). Glycogenic hepatopathy is strongly associated with poorly controlled type 1 diabetes mellitus (19). Periodic acid–Schiff (PAS) stains are brightly positive, but the diagnosis of glycogen accumulation requires an H&E stain, as even the normal liver can have abundant glycogen (fig. 1-39). Small discrete foci of hepatocytes with abundant glycogen can also be found as an incidental change (fig. 1-40), a finding called a glycogen storage focus, one with no strong clinical significance.

Table 1-7

ABNORMAL CYTOPLASMIC CHANGES IN HEPATOCYTES

Glycogen accumulation
Ballooned hepatocytes
Pigment accumulation: iron, copper, lipofuscin
Alpha-1-antitrypsin protein
Megamitochondria
Hepatitis B-associated ground-glass inclusions
Pseudoground-glass inclusions—most are drug affect associated
LECT2 amyloid

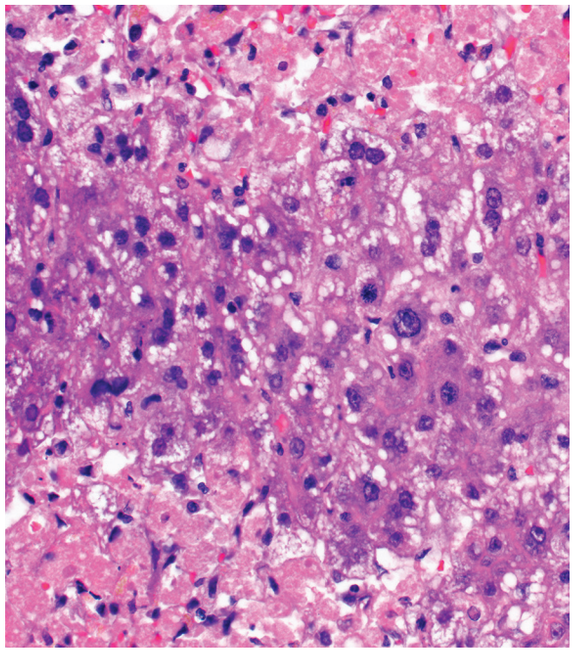

Figure 1-37

SMALL DROPLETS OF FAT IN ACUTE INJURY

The surviving hepatocytes have small droplets of fat in this case of severe panacinar necrosis secondary to acetaminophen. Necrotic hepatocytes are seen on both edges of this image, with viable hepatocytes in the middle.

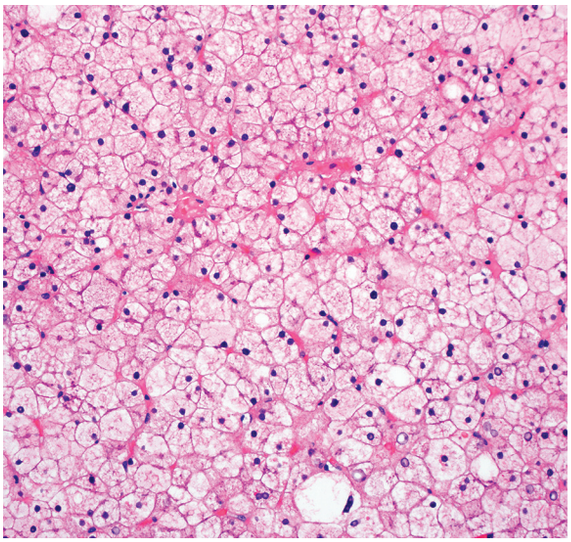

Figure 1-38

GLYCOGENIC HEPATOPATHY

The hepatocytes are enlarged and pale because they have abnormally large amounts of glycogen.

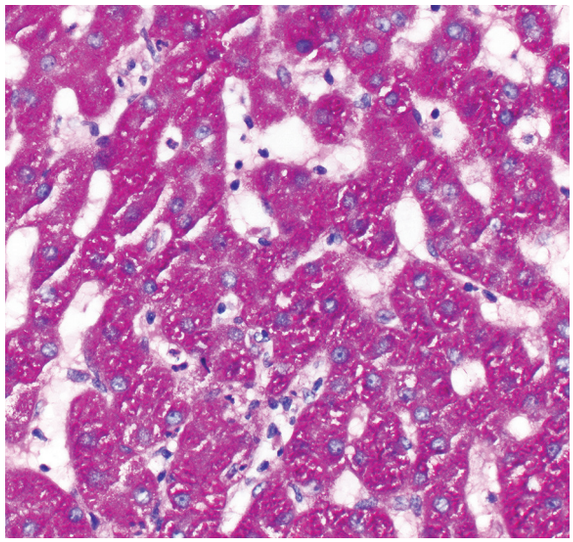

Figure 1-39

NORMAL LIVER

The hepatocytes are strongly positive for periodic acid–Schiff (PAS) stain, which does not indicate that there is abnormal accumulation of glycogen.

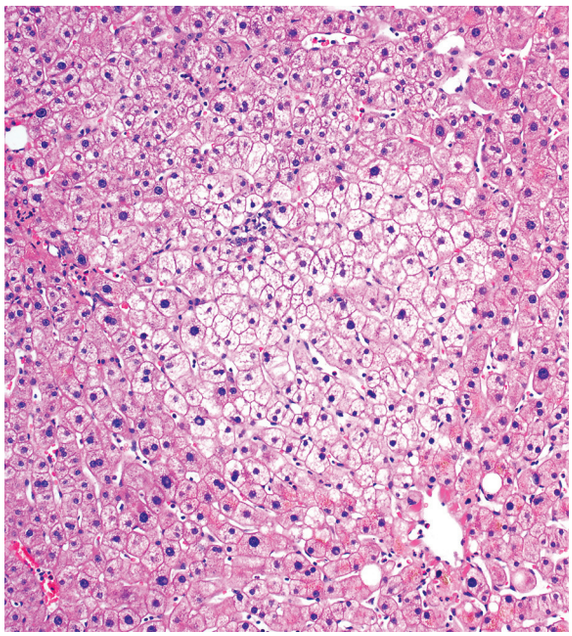

Figure 1-40

GLYCOGEN-STORING FOCI

At low power, a focus of hepatocytes with clear cytoplasm stands out, showing cytoplasmic clearing that results from glycogen accumulation, presumably in a small clone of hepatocytes.

Ballooned Hepatocytes. Ballooned hepatocytes represent a distinctive type of active hepatocyte injury. Ballooned hepatocytes are best identified at low to medium power (fig. 1-41), where they are seen as single or small clusters of hepatocytes that are typically bigger than their neighbors and have rarified, flocculent cytoplasm and sometimes Mallory hyaline. Mallory hyaline (fig. 1-42), also called Mallory-Denk bodies, is not necessary for the identification of a ballooned hepatocyte; it represents aggregates of damaged and ubiquinated cytoskeletal proteins. They can be highlighted by p62, ubiquitin, and keratins stains (20), but stains are not necessary for clinical care.

Ballooned hepatocytes are most commonly encountered in steatohepatitis but are also found with cholate stasis and can be seen as a nonspecific pattern of injury in severe acute hepatitis of various causes. The precise injury mechanism that leads to hepatocyte ballooning is unclear.

Hepatocyte Pigments. The most common pigments seen in hepatocytes are iron and lipofuscin. Lipofuscin typically has a strong zone 3 pattern of deposition and can be separated from iron, if needed, by special stains: iron deposits are positive with Perls iron and lipofuscin is positive with Fontana Masson stains (20).

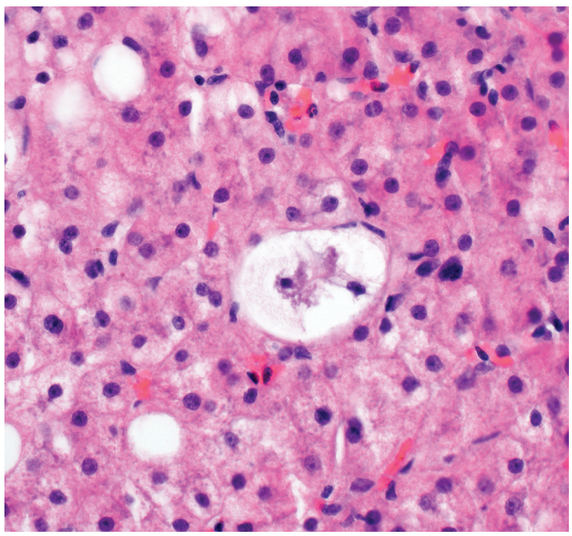

Figure 1-41

BALLOONED HEPATOCYTE

Located in the center of the image, the ballooned hepatocyte is larger than adjacent hepatocytes and has rarified cytoplasm with condensation of cytoplasmic proteins to create Mallory hyaline.

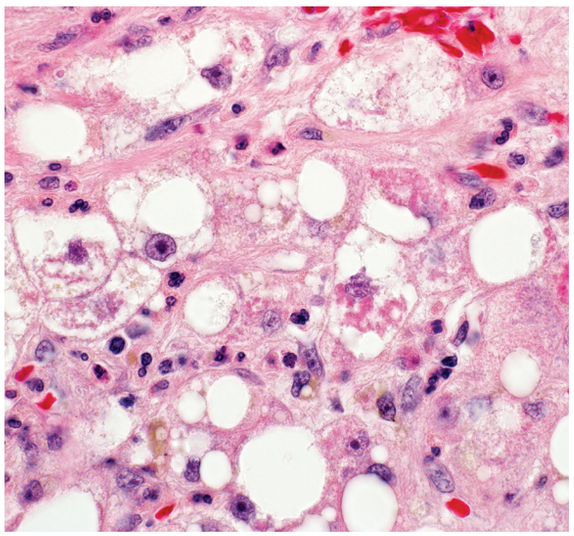

Figure 1-42

MALLORY HYALINE

Mallory hyaline (Mallory-Denk bodies) is abundant in this case of alcoholic hepatitis.

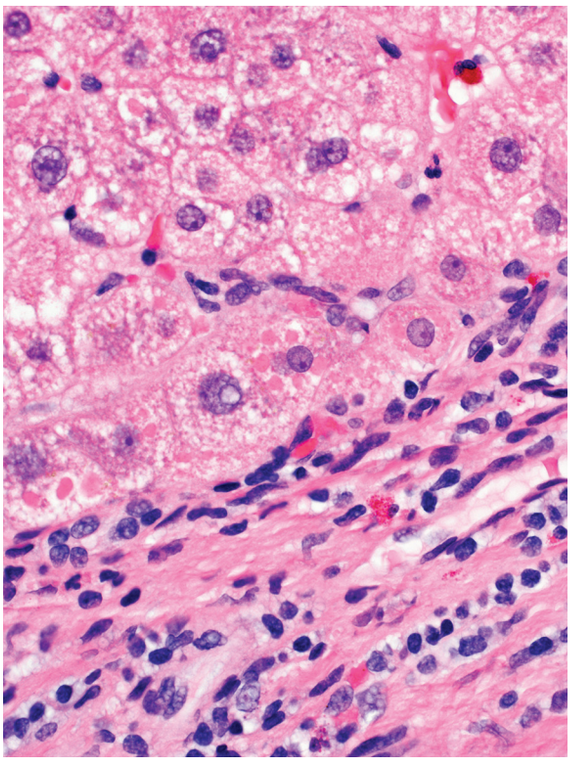

Figure 1-43

ALPHA-1-ANTITRYPSIN GLOBULES

Periportal hepatocytes have eosinophilic cytoplasmic globules. This biopsy is from a patient with cryptogenic cirrhosis; mutational status was unknown at the time of sign-out (figures 1-43 and 1-44 are from the same case).

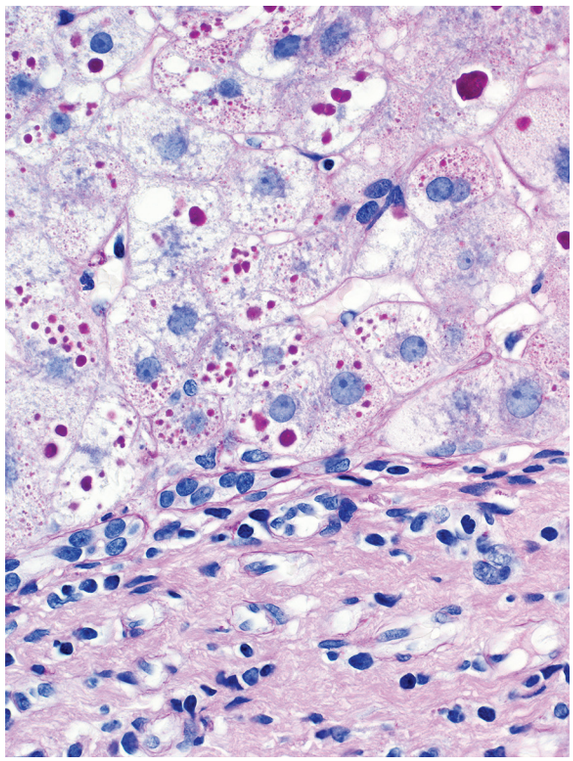

Figure 1-44

ALPHA-1-ANTITRYPSIN GLOBULES

The hepatocellular globules are easier to see with PASD.

Hepatocyte Globules and Inclusions. Eosinophilic globules in hepatocytes most commonly result from mutations in the *SERPINA1* gene, leading to misfolding of the alpha-1-antitrypsin protein (fig. 1-43). Most cases encountered in surgical pathology are incidental findings resulting from heterozygous mutations. The full clinical relevance is determined only after correlation with Pi testing, serum alpha-1-antitrypsin levels, and sometimes the genetic status. The globules of alpha-1-antitrypsin protein are highlighted as round, magenta, cytoplasmic inclusions on PASD (diastase digestion) stains (fig. 1-44). The globules of misfolded alpha-1-antitrypsin protein are deposited first, and have a heavier concentration, in zone 1 hepatocytes. Rarely, sparse but morphologically similar globules are seen in zone 3 hepatocytes in the setting of vascular outflow disease (21).

Megamitochondria are nonspecific signs of liver injury. Megamitochondria are commonly present in fatty liver disease, but require careful hunting to see them; their small round to elongated eosinophilic structures are of no particular diagnostic importance, although its still nice to know their name when you happen upon them (fig. 1-45). They are also common in various genetic metabolic disorders and mitochondrial disorders. Their importance lies primarily in that they are occasionally mistaken for globules of alpha-1-antitrypsin protein

Distinctive hepatocyte inclusions can develop as a result of chronic hepatitis B, where the finding is called *ground-glass inclusions* (22), and in association with medications (23), where the finding is called *pseudoground-glass inclusions* (figs. 1-46, 1-47). The inclusions are gray and homogeneous, often with a thin rim of more normal-appearing cytoplasm at the cell edge. The differential for the inclusions includes

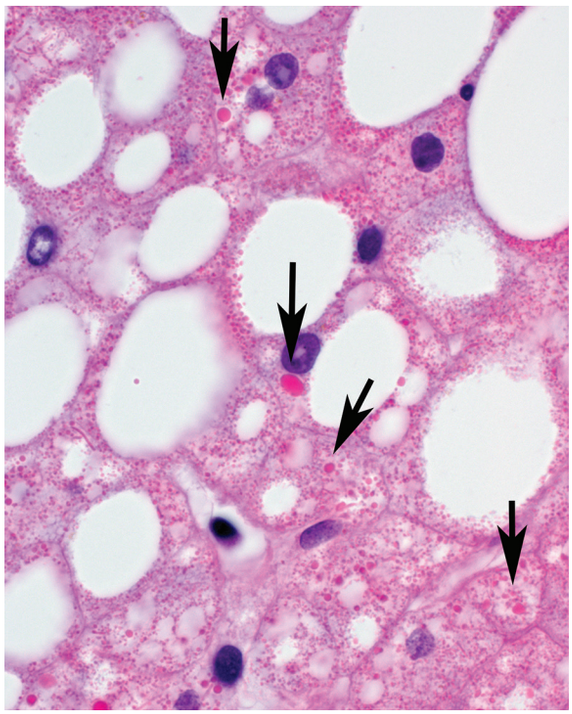

Figure 1-45

MEGAMITOCHONDRIA

The hepatocytes have small round to elongated eosinophilic megamitochondria, from a case of metabolic syndrome and fatty liver disease.

several rare conditions, conditions that are typically clinically evident and point to the correct diagnosis, such as LaFora disease, type IV glycogen storage disease, fibrinogen storage disease, or cyanamide, the latter used as an alcohol aversion agent and in agriculture to synchronize plant blooming. All of the different types of inclusions are PAS positive and all are PASD sensitive, except for the inclusions in fibrinogen storage disease, which are PAS negative. As an important caveat, the diastase digestion step can be incomplete in some cases, leading to partial retention of PAS staining.

In the setting of fibrinogen storage disease, the globules are fully resistant to digestion and can be highlighted by fibrinogen immunostaining. Fibrinogen-positive inclusions occur in two clinical settings. The first setting is patients, often young, with true dysfibrinogenemia, which can lead to either hypercoagulable or hypocoagulable states depending on the mutation. The second setting is that of older patients without coagulation abnormalities who appear to develop the inclusions when the liver is put under the stress of active injury (24). The underlying genetic predisposition in this setting is unclear.

LECT2 amyloidosis (previously known as globular amyloid) can also lead to round

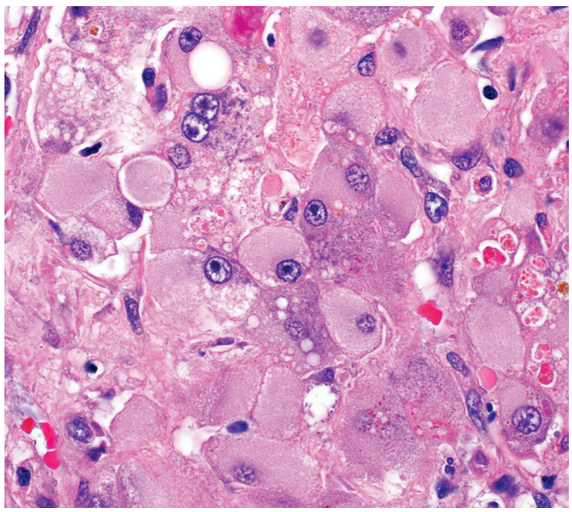

Figure 1-46

GROUND-GLASS CYTOPLASMIC CHANGE

In this patient with longstanding chronic hepatitis B infection, the hepatocytes have distinctive gray inclusions.

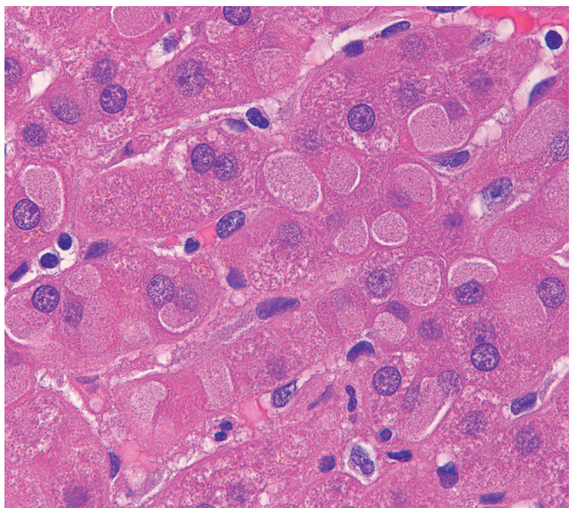

Figure 1-47

GLYCOGEN PSEUDOGROUND-GLASS CHANGE

This patient was immunosuppressed and on many medications. The patient was negative for hepatitis B infection.

inclusion-like deposits in hepatocytes (25,26). The inclusions often have a laminated appearance and can be intracellular or extracellular. They are Congo red positive and positive by LECT2 immunostain (26).

Giant Cell Transformation. Hepatocytes may undergo giant cell transformation, in which enlarged hepatocytes have three or more nuclei (fig. 1-48). This pattern is more common in children than adults and is not specific for etiology, but can be seen with a wide variety of injury patterns (Table 1-8), in particular, cholestatic liver disease. Giant cell hepatitis with autoimmune hemolytic anemia seems to cluster into a distinct syndrome in the pediatric population, one that often requires aggressive immunosuppression to manage (27,28). Ki-67 stains are typically negative in the multinucleated hepatocytes, suggesting that either the multinucleated giant hepatocytes have a very low proliferative rate or that they result from fusion of hepatocytes, without nuclear division.

Idiopathic post-infantile giant cell hepatitis is characterized by elevated liver enzymes, usually mild portal and lobular inflammation, but prominent transformation of hepatocytes. Mild elevations in autoimmune serologies are common (29), but this disease pattern is not typical of autoimmune hepatitis at the histologic or clinical level, so should not be classified as autoimmune hepatitis unless other typical histologic findings are present. The changes of giant cell hepatitis are typically persistent in subsequent biopsies (30), and some patients require transplantation for liver failure (31).

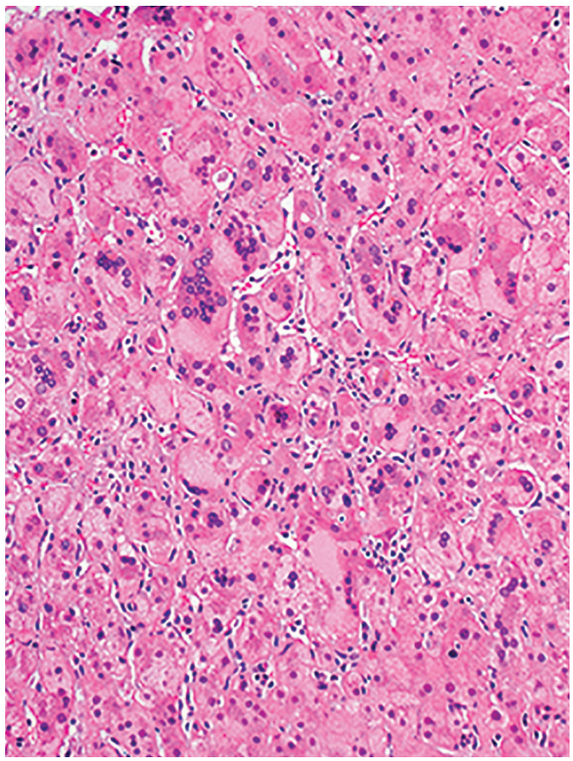

Figure 1-48

GIANT CELL TRANSFORMATION

There is extensive giant cell transformation of the hepatocytes in this case of idiopathic adult giant cell hepatitis.

Hepatitic Pattern

A *hepatitic pattern* of injury shows lymphocyte-predominant inflammation in the portal tracts and/or the hepatic lobules (fig. 1-49). The intensity of the inflammation varies from minimal to severe in both the portal tracts and lobules. In addition, the inflammation can be focal, patchy, or diffuse. The inflammation can also be more intense in the lobules than the portal tracts, or vice versa. Inflammation of the hepatocytes immediately adjacent to the portal tracts is called interface activity (fig. 1-50).

Acute Versus Chronic Hepatitis. The distinction of acute versus chronic hepatitis is formally defined by the duration of elevated liver enzymes (a common definition: chronic hepatitis has elevations persisting more than 6 months). The histologic findings also provide relevant information. Moderate diffuse or severe lobular hepatitis is essentially always an acute pattern of injury, since the liver cannot survive such degrees of inflammation for very long. On the other hand, definite fibrosis is evidence of chronic hepatitis. Other findings are not as useful. For example, while it is true that chronic hepatitis commonly has more portal inflammation than lobular inflammation, there are too many exceptions to make this clinically useful for their distinction. Thus, the presence of more portal inflammation than lobular inflammation should not be equated with chronic hepatitis, even though it was historically thought that this was true.

Acute on Chronic Hepatitis. Acute on chronic hepatitis occurs in two settings. The first is when there is a chronic hepatitis with

Table 1-8

DIFFERENTIAL DIAGNOSIS OF GIANT CELL CHANGE IN HEPATOCYTES

Cause	Comment/Representative Reference
Most common causes	
Idiopathic adult giant cell hepatitis	(29)
Chronic hepatitis C	Especially injection drug users, persistent in subsequent biopsies but not associated with grade or stage (100,101)
Autoimmune hepatitis	(29,102)
Cholestatic liver diseases	Various causes Can be striking in some cases of neonatal hepatitis, leading to pattern called postinfantile giant cell hepatitis (30,103)
Viral infections	CMV (104) Hepatitis E (105) EBV (106) HIV (107) HHV-6A (108), can also involved bile ducts (109) Possible novel paramyxovirus (110)
Other infections	Syphilis (111)
Autoimmune diseases	In all of these, the frequency is rare Autoimmune hemolytic anemia (112) Graves disease (113) Immune thrombocytopenic purpura (114) Primary biliary cirrhosis (115) Systemic lupus erythematosus (116) Ulcerative colitis (117,118)
Drug-induced liver injury/herbal reaction	Overall, very rare (119-121)
Hematologic disorders	Non-Hodgkin lymphoma (29) Chronic lymphocytic leukemia (122) Necrobiotic xanthogranuloma (123)
Genetic causes	*IGHMBP2* mutations (124) *CYP27A* mutations (125) *2MACR* mutations (126) Mitochondrial DNA depletion syndrome (127) Mitochondrial phosphoenolpyruvate carboxykinase deficiency (128) Wilson disease (104); rare, limited to case reports and strength of association is unclear

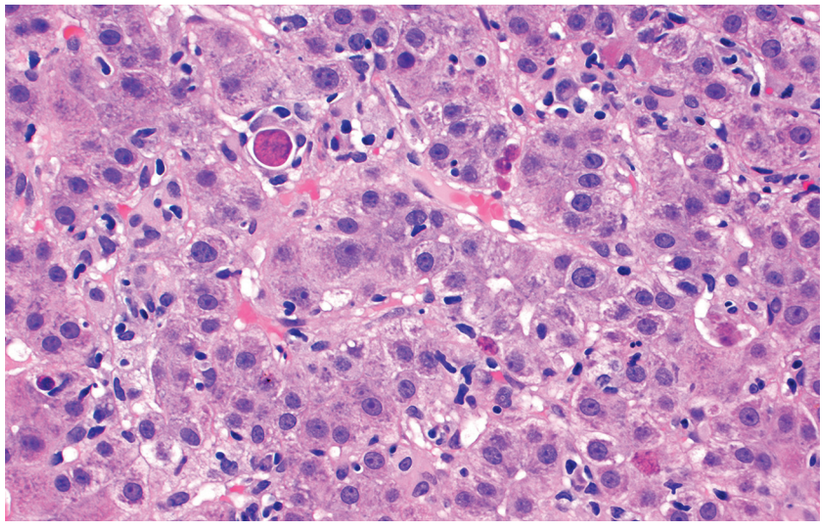

Figure 1-49

HEPATITIC PATTERN OF INJURY

The lobules show inflammation and apoptotic hepatocytes in this case of autoimmune hepatitis.

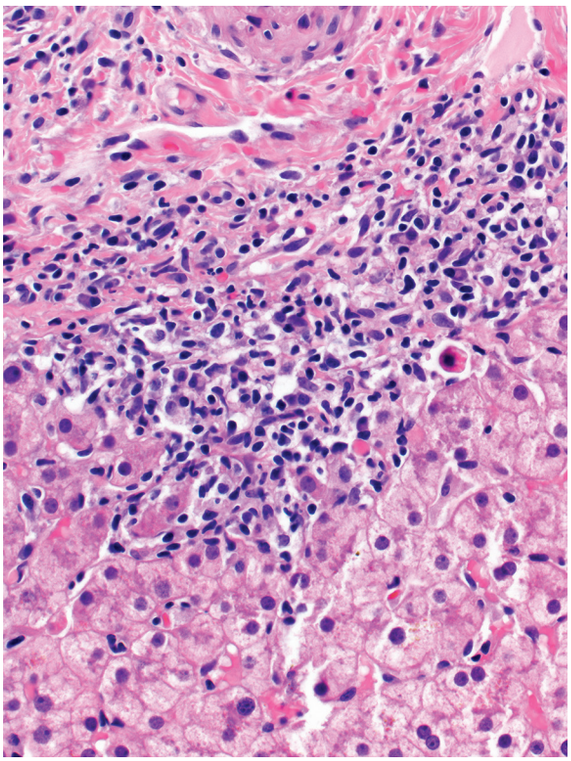

Figure 1-50

INTERFACE ACTIVITY

The portal tract shows a focus of interface activity, from a case of primary biliary cholangitis (PBC)-autoimmune hepatitis overlap syndrome.

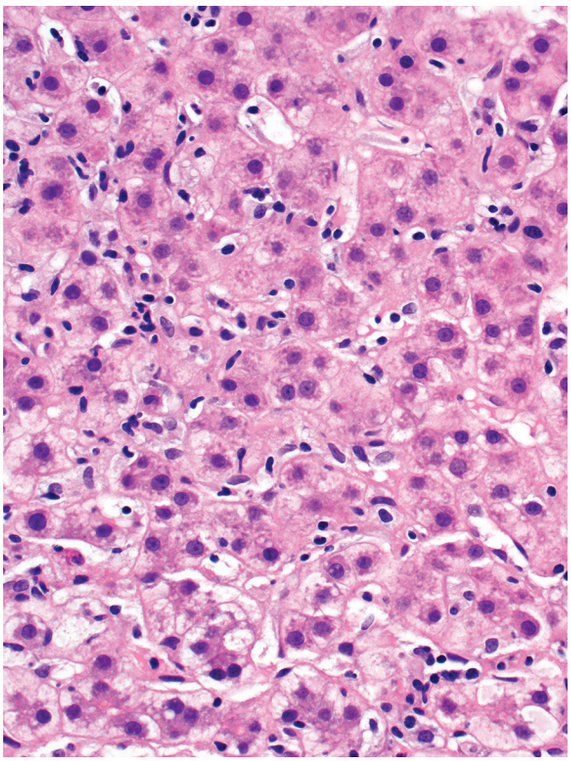

Figure 1-51

ACUTE ON CHRONIC HEPATITIS

This patient with longstanding chronic hepatitis C and cirrhosis had low levels of baseline liver enzyme elevations. An acute flare of liver enzymes led to this biopsy and was eventually diagnosed as acute hepatitis B, superimposed on the chronic hepatitis C virus infection.

superimposed acute injury (fig. 1-51). Fibrosis is helpful but not necessary to recognize the underlying chronic liver disease. One example is a person with underlying chronic steatohepatitis with no fibrosis who then develops ischemic necrosis. As a second example, a patient with underlying autoimmune hepatitis may develop an acute superimposed HSV hepatitis. Histologic findings that support acute on chronic hepatitis include two distinct patterns of injury: one that is low grade, such as fatty liver disease, and one that is high grade, such as acute necrosis. Other findings that can support a component of underlying chronic liver disease include fibrosis or bile duct changes such as bile duct loss, periductal fibrosis, or bile duct duplication.

The second setting in which acute on chronic hepatitis can occur is primarily seen with chronic hepatitis B and autoimmune hepatitis, where persons with relatively stable chronic liver disease have disease flares, leading to sudden liver enzyme elevations, often 10 times or greater than baseline. Histologically, there is moderate to marked lobular hepatitis, and often necrosis. In this setting, the flares of hepatitis are part of the natural history of a single disease and not a second disease process.

Lobular Hepatitis. Lobular hepatitis can range from minimal to severe (figs. 1-52, 1-53). Evaluation is commonly performed with a 10X or 20X lens. In the Ishak system (32), lobular inflammation is scored with a 10X lens as absent, minimal (up to 1 foci on average), mild (2 to 4 foci on average), moderate (5 to 10 foci on average), or severe (greater than 10 foci on average). With fatty liver disease and the nonalcoholic steatohepatitis (NASH) clinical research network (CRN) and the SAF grading