9 Liver

Liver biopsies are usually needle core biopsies. The most common reasons for a biopsy include monitoring disease progression in hepatitis, evaluating a transplanted patient for rejection or graft-versus-host disease (GVHD), and occasionally ruling out an additional disease process. Liver biopsies may also be done to diagnose a radiographic mass. At many institutions, all liver biopsy specimens are stained with trichrome (to evaluate fibrosis) or with an iron stain (to reveal abnormal iron in the tissue).

Anatomy

Blood comes in via the portal vein (from the gut) and the hepatic artery (from the aorta). These vessels ramify into, eventually, the arteries and veins within the portal tracts. Blood gets from the terminal portal vessels to the outgoing central veins via the sinusoids—the long channels lined by hepatocytes. Once in the central vein, blood exits the liver via the left and right hepatic veins, which join the inferior vena cava (joining blood from the lower extremities).

Bile is created by the hepatocytes and exits into the bile canaliculi, which eventually coalesce into ductules and ducts in the portal tracts. These exit the liver via the hepatic ducts, which join the cystic duct (from the gallbladder) to enter the duodenum as the distal common bile duct.

Normal Histology

The liver is composed of three main components – the *hepatocytes*, the *biliary system*, and the *vessels*. Hepatocytes are large pink polygonal cells with dense round nuclei. Nucleoli, and occasional binucleate cells, are okay. The hepatocytes are organized into plates that are one hepatocyte thick and lined by reticulin. Between these plates are the sinusoids for blood. Running perpendicular to the sinusoids, and essentially invisible to light microscopy, are the *bile canaliculi*: tiny intercellular channels between the hepatocytes.

Bile from the canaliculi makes its way to the bile ducts. The bile ducts are tubular structures with a low cuboidal epithelium (Figure 9.1). They are found in the *portal tracts*, which also contain branches of the hepatic artery and portal vein. These three components are also called the *portal triad*. Blood in both vessels is flowing *into* the liver; bile is flowing *out*. The portal tract also contains a small amount of connective tissue, which makes it stand out on a trichrome stain. The hepatocytes immediately surrounding the portal tract are called the *limiting plate*. The portal tract is usually the hotspot for inflammatory processes in the liver and so is important to identify on biopsy.

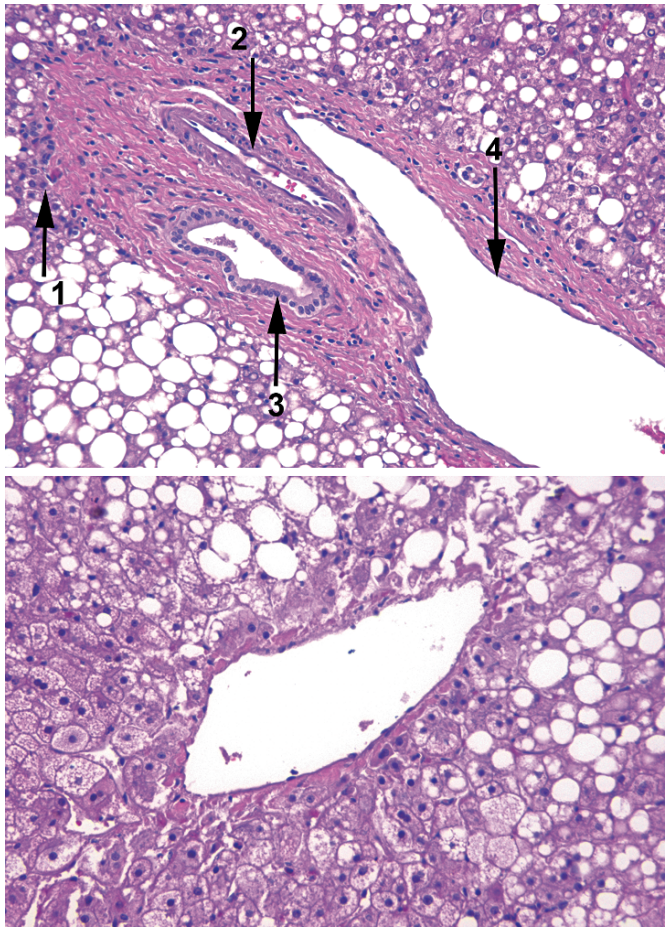

Figure 9.1. Portal tract and central vein. The upper panel shows a typical portal tract surrounded by the limiting plate of hepatocytes (1) and containing a branch of the hepatic artery (2), bile ductule (3), and portal vein (4). The lower panel shows a central vein from the same liver. Both panels show extensive steatosis.

The third vessel in the liver unit is the *central vein* or *terminal venule*. This is a thin-walled vessel surrounded by hepatocytes and nothing else (see Figure 9.1). It contains blood on its way out of the liver.

The lobule is an architectural unit with the central vein as its center and portal tracts at the periphery. *Centrilobular* refers to a process involving the central vein. This is the most easily visualized anatomic unit (Figure 9.2).

The acinus is an architectural unit with the portal tract at the base (as the source of blood flow) and the central vein at the tip. In this model, the area closest to the source of blood and oxygen is zone 1, and the most peripheral cells are zone 3. Ischemia and toxic insults affect the zones differentially. This is more of a physiologic unit and the one used when describing liver findings.

Nonneoplastic or Inflammatory Disease Categories

It is helpful to think of the different liver compartments separately, because histologic findings can often be grouped as well:

- Diseases of hepatocytes: the viral hepatitides, autoimmune hepatitis, steatohepatitis and alcoholic disease, and drug toxicity

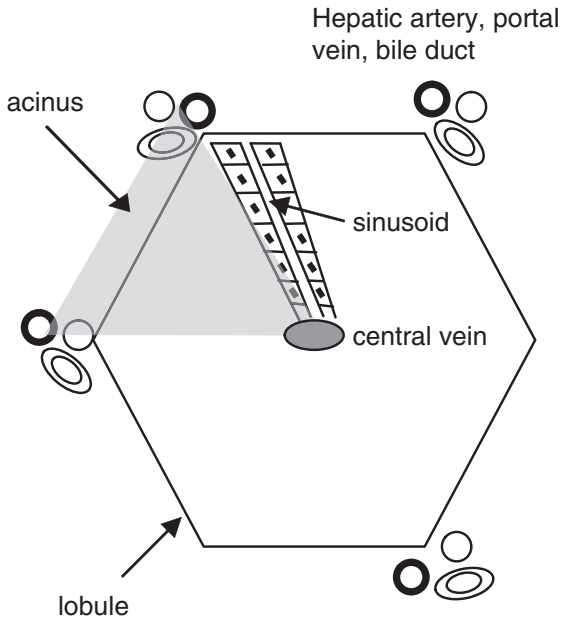

FIGURE 9.2. Liver organization. The acinus is a triangular, physiologic unit, while the lobule is a hexagonal anatomically based unit.

- Diseases of the biliary system: autoimmune biliary diseases (primary sclerosing cholangitis and primary biliary cirrhosis), obstruction, atresia, transplant rejection, GVHD, and drugs
- Diseases of the vasculature: transplant rejection, GVHD, and systemic vasculitides

The portal tract represents a collision of all three compartments. Therefore, inflammation of the portal tract is found in all of these diseases.

Pathologic Findings

The liver has only so many ways to respond to an insult or injury. An acute injury in the liver looks similar to that in any other organ: widespread edema, acute and chronic inflammation, and/or necrosis. Subacute or chronic injury generally has mainly mononuclear inflammatory cells as well as individual cell necrosis or degeneration. The final result of chronic injury from many causes is cirrhosis, or end-stage liver disease. Therefore, many diseases in the liver have histologic overlap, and, in the case of cirrhosis, often you cannot tell what the original disease process was. For this reason, the most important skill in interpreting the liver biopsy is recognizing injury to the different compartments. Attaching a diagnosis to this collection of findings requires clinical information. The most common patterns of injury are the following:

- Hepatocellular compartment
  - Portal inflammation: Inflammatory cells are present within the portal tract. In chronic hepatitis and autoimmune disorders, the infiltrate is predominantly mononuclear.
  - Interface activity (periportal hepatitis, piecemeal necrosis): Inflammation, usually lymphocytic, occurs in the limiting plate. This looks like portal inflammation spilling out into the hepatocytes (Figure 9.3). Note that the word *activity* when describing something in the liver does not mean *neutrophils*.
  - Lobular inflammation: Inflammation, usually chronic, and/or necrosis of the hepatocytes are at a distance from the portal tracts. Also called *spotty necrosis*, this appears as little clusters of lymphocytes destroying individual hepatocytes out in the lobules. Do not count lymphocytes in the sinuses, which are physiologic.

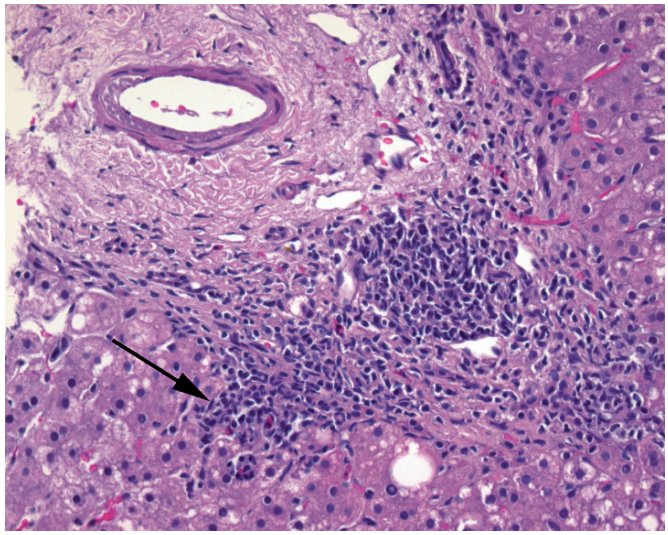

Figure 9.3. Portal inflammation. This is an example of chronic viral hepatitis. Lymphocytes in the portal tract spill out into the limiting plate of surrounding hepatocytes (arrow).

- Vacuolar degeneration (balloon cell change): This is one way in which hepatocytes can die. The cytoplasm swells and becomes feathery and pale.
- Acidophilic bodies: This is another way in which hepatocytes die. These cells are similar to dyskeratotic cells in the skin; they are bright pink, rounded up, with pyknotic nuclei.
- Fibrosis: *Fibrosis* is a general term indicating too much collagen. Fibrosis begins as an increase in collagen around the portal tract (portal fibrosis), and eventually spreads to connect adjacent portal tracts or central veins by thin webs of collagen (bridging fibrosis). The end stage of the process is cirrhosis, which is the division of the liver into individual nodules separated by thick bands of fibrosis (Figure 9.4).
- Steatosis: *Steatosis* literally means fat in the hepatocytes. Steatosis can be physiologic in small amounts (<5%), but 30%–60% involvement is considered moderate steatosis. Over 60% involvement is marked or severe disease. Macrovesicular steatosis means large single vacuoles in each hepatocyte and is typical of fatty liver, alcoholic disease, and non-alcoholic steatohepatitis. Pure microvesicular steatosis looks like foamy cytoplasm and is characteristic of mitochondrial injury such as in Reye's syndrome.
- Steatohepatitis: Steatohepatitis is steatosis plus inflammation or injury. Neutrophils are not necessary for the diagnosis, but some evidence of hepatocyte injury *is* (Figure 9.5). This includes lobular inflammation, hepatocyte necrosis, pericellular fibrosis, balloon cells, and Mallory's hyaline (see below).
- Mallory's hyaline (Mallory bodies): Mallory bodies are irregular worm-like pink blobs of condensed cytoskeleton in the cytoplasm, especially within balloon cells (Figure 9.6). They are associated with steatohepatitis, especially alcoholic disease.
- Megamitochondria: Megamitochondria are markedly enlarged mitochondria, which look like red blood cells entrapped in the hepatocyte cytoplasm.
- Iron accumulation: Abnormal levels of iron are detected with either hematoxylin and eosin or iron stain. If severe, iron accumulation may indicate hemochromatosis or be secondary to other hepatocellular processes.

- Biliary compartment
  - Cholestasis: Cholestasis is the backup of bile in the liver. This may be caused by extrahepatic obstruction to flow, intrahepatic biliary disease, or impaired excretion by the hepatocytes themselves.

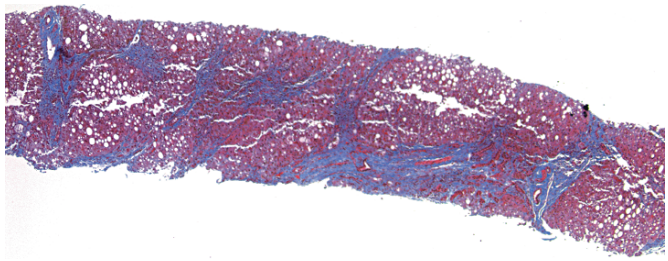

FIGURE 9.4. Cirrhosis in a biopsy specimen. In this trichrome stain, collagen is blue, while hepatic parenchyma is red. Collagen can be seen outlining the lobules of the liver, bridging the portal tracts and creating a nodular pattern.

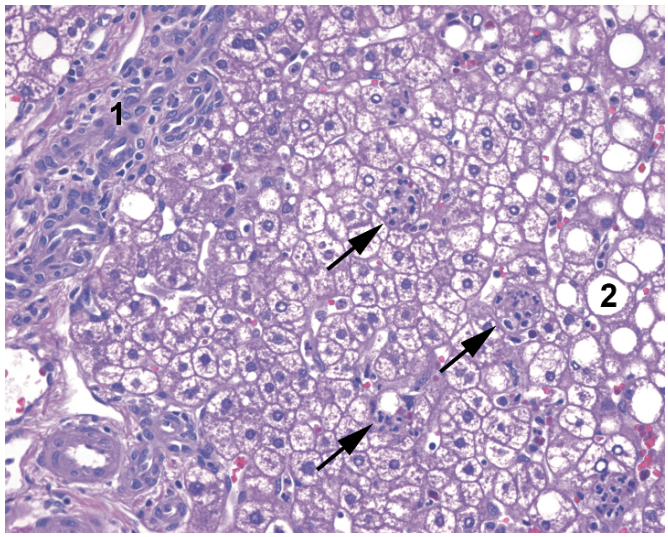

FIGURE 9.5. Steatohepatitis. An adjacent portal tract (1) shows minimal inflammation. In the lobule, there is macrovesicular steatosis (2) and collections of neutrophils attacking individual hepatocytes (arrows).

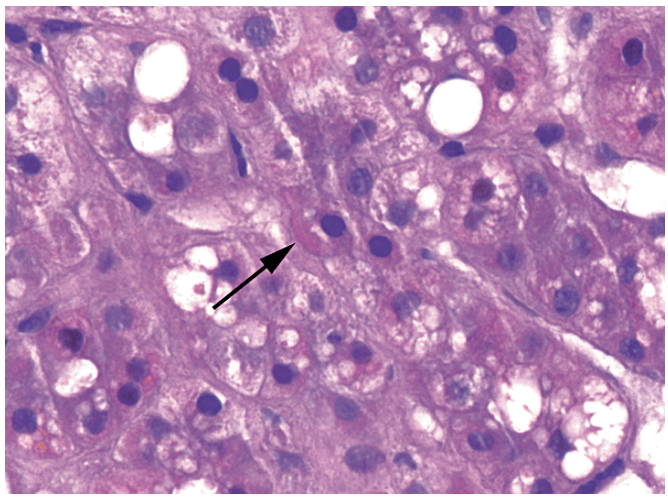

FIGURE 9.6. Mallory's hyaline. In the background of steatosis and inflammation, a pink refractile worm-like structure in the hepatocyte (arrow) is evidence of cytoskeletal collapse.

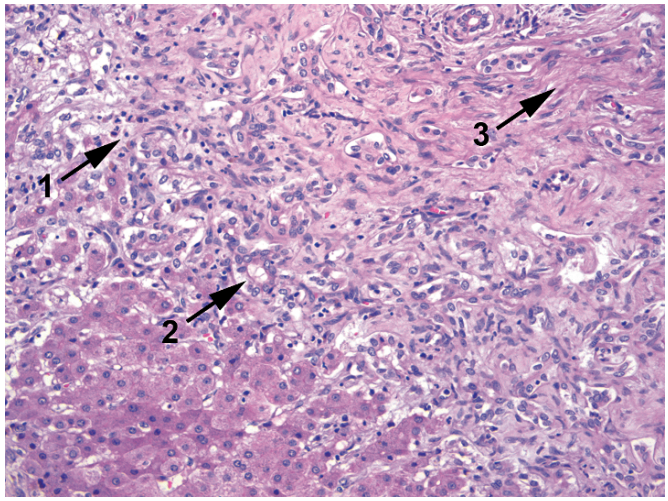

Figure 9.7. Bile stasis. In this example of congenital biliary atresia, the downstream obstruction to flow creates the triad of acute inflammation (1), a proliferation of poorly formed bile ductules (2), and the accumulation of golden globs of bile (not seen here). This will progress to fibrosis (3) and eventually loss of ductules.

- Bile duct proliferation: An increase in the number of bile duct profiles occurs in each portal tract; on average, there should be one to two per tract. Many of the new ductules are small, peripheral, and poorly formed. Bile duct proliferation occurs as a response to obstruction to flow. Other findings in obstruction include visible bile in hepatocytes or canaliculi, edema and inflammation (especially acute) in the portal tracts, eventually ductular atrophy, and finally widespread fibrosis (Figure 9.7).
- Bile duct injury: Bile duct injury is identified by lymphocytes in the bile duct epithelium and vacuolar degeneration or dropout of the epithelial cells. The end stage is ductopenia. Injury to the bile ducts can indicate a biliary disease, such as autoimmune (primary biliary cirrhosis) or rejection. Bile duct injury is usually patchy, so multiple portal tracts must be examined.
- Ductopenia: Ductopenia is loss of bile ducts, an indicator of chronic damage to the biliary system. Recognizing ductopenia, a diagnosis of absence, requires a conscious effort to look for bile ducts. Finding a bile duct in less than 80% of the portal tracts is abnormal.

- Vascular compartment
  - Venulitis (endothelitis): Venulitis is damage to the endothelium of the portal or central veins by inflammatory cells. It is usually an indication of rejection or GVHD.
  - Extramedullary hematopoiesis: Hematopoietic precursors (megakaryocytes are the most distinctive) are present in the liver sinuses. It is generally an indication of bone marrow disease (but is physiologic in fetuses and infants).

Chronic Hepatitis

Biopsies in chronic hepatitis, generally hepatitis C, are done to track disease progression, with the ultimate endpoint being cirrhosis. Sign out of a hepatitis biopsy specimen should include three key prognostic factors: *etiology* (if known), *grade* (degree of inflammation and necrosis), and *stage* (degree of fibrosis), plus any other disease process present (such as steatohepatitis).

There are many different scoring systems used to quantify grade and stage, as all clinicians love a number. However, most numeric scoring systems can be translated to or from adjectives, which convey the same information (for example, scores 0 to 4 corresponding to none,

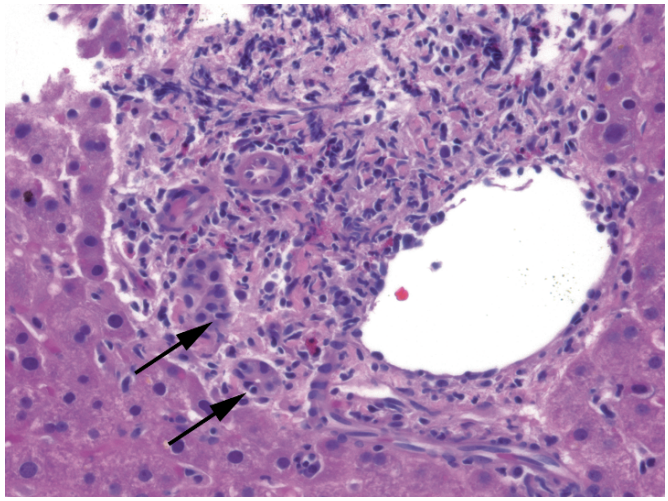

Figure 9.8. Acute rejection. Acute rejection refers to the attack on the bile ducts and venules by lymphocytes, which are seen invading the duct epithelium (arrows).

minimal, mild, moderate, or severe inflammation; and none, portal, periportal, bridging, or cirrhotic fibrosis). Calibrating these levels takes some experience, and thresholds may vary by institution.

The changes in viral hepatitis are nonspecific. The differential includes most other hepatocellular processes.

Transplant Biopsy for Rejection or Graft-Versus-Host Disease

The changes seen in cellular rejection and GVHD are histologically similar; one occurs in the setting of a liver transplant and the other in a bone marrow transplant. Both are divided into acute and chronic. (Hyperacute rejection implies an antibody response and is rare and immediate, not usually diagnosed by biopsy.)

- Acute cellular rejection: Acute rejection usually occurs 5–30 days after transplant, but can be longer. Changes include the following:
  - Mixed portal tract inflammation, including lymphocytes, neutrophils, and eosinophils
  - Venulitis
  - Bile duct inflammation and damage (Figure 9.8)
- Chronic rejection: Chronic rejection usually occurs after more than 1 year. Changes are primarily those of ductopenia and fibrosis.

Note that the changes of rejection must be differentiated from recurrent hepatitis C, an inevitable occurrence in patients who lost their first liver to hepatitis C (occurs from 3 to 9 months after transplantation).

Primary Biliary Cirrhosis and Primary Sclerosing Cholangitis

Primary biliary cirrhosis and primary sclerosing cholangitis are hard to keep straight. The bullet version is as follows:

- Primary biliary cirrhosis (occurs much more often in women than in men):
  - Primary biliary cirrhosis is a chronic destructive *intrahepatic* cholangitis (inflammation of the intrahepatic bile ducts).
  - Cirrhosis is an end-stage feature.
  - It is associated with antimitochondrial antibody.

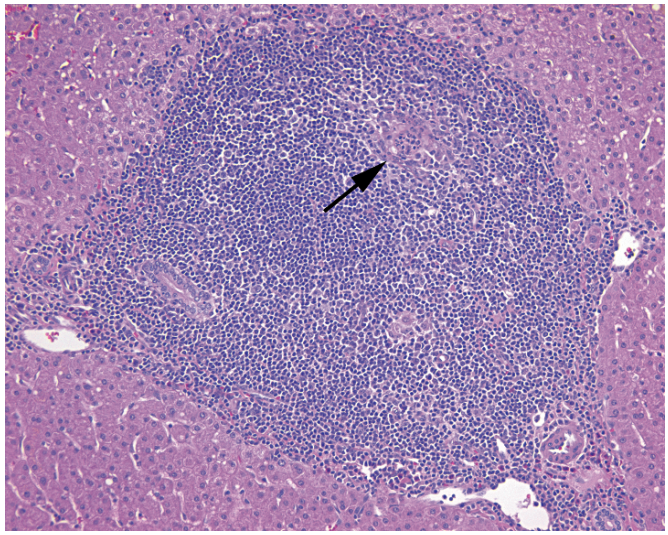

Figure 9.9. Primary biliary cirrhosis. There is a granulomatous inflammation of the portal tract, with destruction of a bile ductule (arrow).

- Findings are nonspecific and patchy but include inflammation and injury to the bile ducts, especially granulomatous, followed by proliferation and cholestasis, then eventually ductopenia and cirrhosis (Figure 9.9).
- The etiology is direct damage to bile duct epithelium.

- Primary sclerosing cholangitis (occurs more often in men than in women):
  - Primary sclerosing cholangitis is an inflammatory disease of the *extrahepatic* (and large intrahepatic) ducts.
  - It leads to patchy stricturing lesions, visible on cholangiogram.
  - It is associated with inflammatory bowel disease and p-ANCA.
  - The histologic picture is also nonspecific but dominated by ductular proliferation and cholestasis.
  - The etiology is unknown but may be a fibrotic process of the connective tissue surrounding the bile ducts, causing secondary stricture and damage.

Mass Lesions (Neoplasms)

The most common cause of mass lesions in the liver is metastatic disease. However, there are primary lesions of all three components of the liver: hepatocytes, biliary epithelium, and vessels. Within each category, it can be difficult to differentiate neoplastic from nonneoplastic, and benign from malignant, on resection, let alone on biopsy. However, here is a brief list of features that favor one over the other.

- Hepatocellular
  - Focal nodular hyperplasia: Focal nodular hyperplasia is essentially an island of cirrhosis occurring in the background of a noncirrhotic liver. This is not a clonal process, so there is more than one cell type; in addition to hepatocytes are bile ducts and fibrous septae. There is no capsule but sometimes a central scar. The lesion is composed of nodules divided by bands of fibrosis and thick vessels.
  - Adenoma: Adenomas are benign clonal neoplasms. They occur mainly in noncirrhotic livers of adult women taking oral contraceptive pills. Adenomas are circumscribed, partially encapsulated masses of uniform, bland-looking hepatocytes with no central veins or

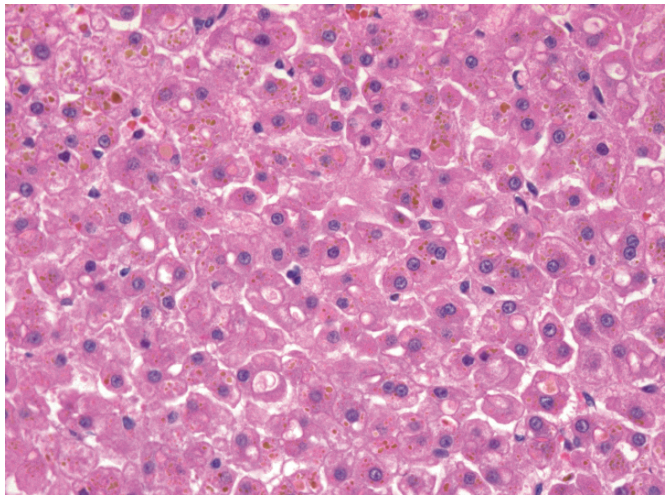

Figure 9.10. Well-differentiated hepatocellular carcinoma. Golden bile can be seen in the tumor cells, which are hard to differentiate from normal liver. Portal tracts are absent.

bile ducts (although there are diffuse prominent arterioles). The cells may be pale due to steatosis or glycogen or discolored with bile (which has no place to go). When visualized with reticulin stain, the hepatocyte plates are still only one to two cells thick (every cell touches reticulin).

- Well-differentiated hepatocellular carcinoma: Well-differentiated hepatocellular carcinoma (HCC) can be very difficult to distinguish from an adenoma histologically. However, HCC generally occurs in the setting of cirrhosis, unlike the adenoma. As with an adenoma, there are no bile ducts or central veins, and you may see intracellular bile (Figure 9.10). Nuclei may be large, hyperchromatic, and irregular. A reticulin stain shows a breakdown in architecture, and plates may be three or more cells in thickness.
- Poorly differentiated hepatocellular carcinoma: Poorly differentiated HCC can be very pleomorphic and hard to identify as hepatic. The presence of bile, if any, is still a give away.
- Fibrolamellar hepatocellular carcinoma: Fibrolamellar HCC is a variant of well-differentiated HCC occurring in children and young adults. It is typified by oncocytic cells with prominent nucleoli in a dense fibrotic stroma.

- Biliary
  - Bile duct adenoma: A bile duct adenoma is usually <1 cm and subcapsular (often sampled on frozen section), with a tangle of small simple tubules, with or without inflammation and fibrosis. It may produce mucin but not bile. Think of this as a benign biliary epithelial neoplasm (Figure 9.11).
  - Bile duct hamartoma: Also called von Meyenburg complex, a bile duct hamartoma is also usually < 1 cm and subcapsular (often sampled on frozen section). However, it generally shows more dilated and angular tubules in a loose connective tissue stroma and often produces bile. Think of this as a disordered reduplication of the portal tract. The consequences of confusing the hamartoma with the adenoma are minimal.
  - Cholangiocarcinoma: Cholangiocarcinoma is a primary malignancy of the bile ducts that appears as a nondescript adenocarcinoma infiltrating the liver. There is no definitive way to distinguish it from a metastatic lesion except by history. Although bile is *not* present in a cholangiocarcinoma, mucin is common, as is an intense desmoplastic response (Figure 9.12).

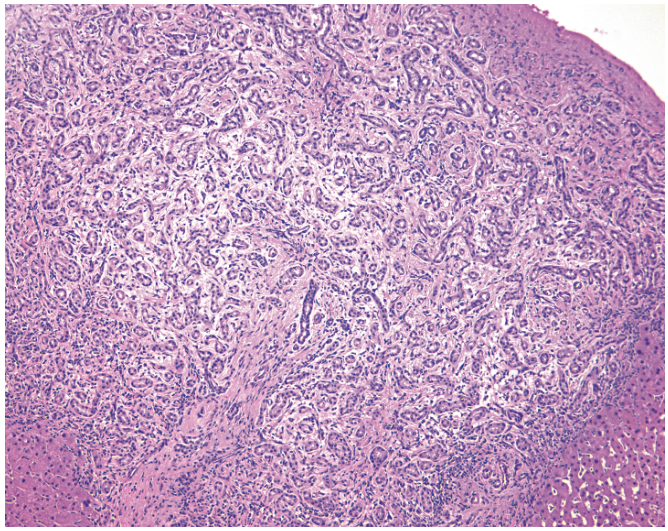

Figure 9.11. Bile duct adenoma. This is a benign tangle of proliferating bile ducts, surrounded by edema, which may mimic desmoplasia. Bile is absent, as is any cytologic atypia.

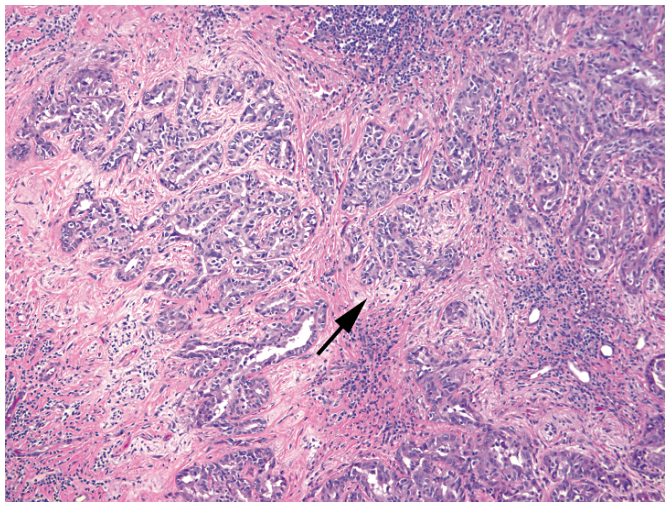

Figure 9.12. Cholangiocarcinoma. A nondescript adenocarcinoma, cholangiocarcinoma produces an intense desmoplastic response in the stroma (arrow; the pale swirling fibrosis surrounding the malignant glands).

- Vascular lesions
  - Cavernous hemangiomas are benign vascular lesions.
  - Epithelioid hemangioendotheliomas have a low malignant potential.
  - Angiosarcomas are malignant.