EMBRYOLOGY

At the beginning of fetal development (toward the latter part of the third week of gestation), the hepatic diverticulum buds from the ventral foregut. The hepatic diverticulum then gives rise to the transverse septum (septum transversum), a structure situated between the pericardial and peritoneal cavities (1,2). Mesenchymal elements of the transverse septum, which already exist, are invested by liver parenchyma formed from hepatic endodermal cells (also known as hepatoblasts) of the hepatic diverticulum to give rise to the liver buds (3). During fetal development the liver buds function as a hematopoietic organ, which is composed of hepatocytic cords, venous-sinusoidal plexus, and hematopoietic precursor cells, including Kupffer cells (macrophages residing in liver). The umbilical vein supplies most of the blood during development of the liver. The remaining smaller amount of blood is supplied by the portal vein and hepatic artery.

During fetal development, the placenta executes the major functions performed by the adult liver, including the absorption of nutrients and the excretion of bile containing waste products; bile secretion by fetal liver is negligible before birth. Nonetheless, the fetal liver produces alpha-fetoprotein and other plasma proteins, synthesizes bile acids, and stores fat, glycogen, iron, and copper.

The anterior portion of the hepatic diverticulum forms the liver and intrahepatic bile ducts, while the posterior portion gives rise to the gallbladder and extrahepatic bile ducts (2,4–6). The ultimate formation of intrahepatic bile ducts occurs late during development, and is completed after birth, during the first year of life. The development of small intrahepatic bile ducts begins when the ductal plate is formed, which is a single-layered sheath of small flat epithelial cells assembled by the periportal hepatoblasts in intimate contact with the portal mesenchyme surrounding a portal vein branch (2). In the weeks that follow, certain parts of the ductal plates are duplicated by a second layer of cells, to become double-layered ductal plates, which then dilate to form cylindrical structures to be integrated into the mesenchyme of the newly formed portal region (fig. 1-1). When they are integrated into the portal space, the immature tubules evolve into bile ducts that are embraced by connective tissue and gradually situated in their usual location in the portal tracts as the portal tracts increase in size (2,7–9).

GROSS ANATOMY

The liver can be divided into the right and left lobes by the middle hepatic vein and a plane between the inferior vena cava and the gallbladder fossa. Anteriorly, this division is visible by the falciform ligament. Viewed from the under surface, there are also the quadrate lobe in the vicinity of the gallbladder fossa and the caudate lobe which in part encases the vena cava.

There are dual afferent blood supplies in the liver: hepatic artery and portal vein. The hepatic artery (branches from the celiac artery) transports 30 to 40 percent and the portal vein (drains the intestine) transports up to 70 percent of the oxygenated blood to the liver. The sinusoids then carry blood from hepatic arteries and portal veins to the terminal hepatic venules, and subsequently through the hepatic vein to the inferior vena cava.

Functionally, the liver is better divided into eight segments based on the blood supply, known as the Couinaud classification, or Couinaud scheme (fig. 1-2). The segments are numbered in Roman numerals I to VIII. Segment I essentially represents the caudate lobe, which drains directly into the inferior vena cava. Segments II to VIII are then numbered in a clockwise manner in a frontal plane beginning superiorly.
Tumors of the Liver

Figure 1-1
FETAL LIVER

Left: The embryonic ductal plate, composed of cytokeratin-rich cells, forms a layer that surrounds the portal area. This immunostain for cytokeratin (CK) 18 shows that the ductal plate is discontinuous and in a few places forms a double layer of cells with small lumina.

Right: Higher magnification of another portal area shows the bile duct forming from the ductal plate, which will eventually disappear as the liver grows.

Figure 1-2
SEGMENTAL ANATOMY OF THE LIVER

The segments, designated by Roman numerals, can be resected surgically because each is supplied by a major branch of the hepatic artery and portal vein and drained by a major tributary of the hepatic vein.

from the left lobe. These segments are bounded by the three main branches of the hepatic veins: the left, middle, and right hepatic veins. Each segment drains into branches of the hepatic veins and then into the inferior vena cava. Overall, segments I to IV represent the functional left lobe, whereas segments V to VIII are considered the functional right lobe. Division of the liver according to the Couinaud scheme allows the surgical removal of a single segment, or two or more adjacent segments en bloc, in such a fashion to avoid damaging the remaining segments. The Couinaud scheme also serves as a common terminology for communicating among physicians of different disciplines: surgeons, hepatologists, oncologists, radiologists, and pathologists (10).
HISTOLOGIC ORGANIZATION

The liver is structurally organized into parenchymal, vascular, bile ductal, and interstitial components (1). Traditionally, the smallest functional unit has been conceptualized as the hepatic lobule or three dimensionally as the hepatic acinus, as defined by Rappaport (12). Most oxygenated blood flows from the terminal branches of portal veins and hepatic arteries in the portal tracts, via the sinusoids to supply the hepatocytes, and finally draining into the terminal branches of the hepatic (central) veins at the peripheral part of the acinus (11). The acinus can also be conceptualized as a three-dimensional spherical structure with portal tracts at the center. The spherical area of those hepatocytes surrounding the portal tract is referred to as zone 1, which has the richest oxygenated blood. The area outside of zone 1 is referred to as zone 2. Zone 3 is further out, corresponding to the region surrounding the terminal hepatic vein, where the oxygen content in blood is the lowest (fig. 1-3).

The two-dimensional concept of the lobule, as seen on slides, serves the purpose of histologic visualization, but zonal subdivision of the acinus incorporates the physiologic concept into histologic organization and facilitates the recognition of certain liver injury patterns. These patterns include the gradual increasing vulnerability to ischemic and toxic/metabolic
Tumors of the Liver

Hepatocytes and Endothelial Cells

Left: Normal hepatocytes are polyhedral cells arranged in plates that are one cell thick. They have granular, eosinophilic cytoplasm and usually one nucleus. The sinusoids between the hepatocytes are lined by inconspicuous endothelial cells.

Right: The hepatocytic plates are normally one cell layer in thickness and are separated by the sinusoidal space (reticulin stain).

Bile ductules and canals of Hering are structures at the periphery of the portal tracts and are not conspicuous by hematoxylin and eosin (H&E) stain in normal liver. They are the conduits between the biliary tree and hepatocyte canaliculi. The canals of Hering drain bile from bile canaliculi into bile ductules, and then into the interlobular bile ducts. Canals of Hering are important for liver regeneration since the hepatic progenitor cells reside here.

HISTOLOGY

Cells of the Liver

Hepatocytes. Hepatocytes, which originate from endoderm, comprise the primary cell population in liver, accounting for 75 to 80 percent of the liver volume. They are hexagonal or polyhedral in cross section, and have an eosinophilic cytoplasm containing a centrally placed round nucleus; occasional hepatocytes may be binucleate. In the adult liver, hepatocytes are

injuries from zone 1 through zone 2 to zone 3, as well as the distinction between adult nonalcoholic steatohepatitis (zone 3 accentuated) and pediatric nonalcoholic steatohepatitis (zone 1 accentuated) (13–15).

Hepatocytes are formed in plates of one cell in thickness, which can be highlighted by a reticulin stain (fig. 1-4, right). The hepatocytic plates are organized in a radial fashion between the portal tracts and terminal hepatic venules (fig. 1-5), and are separated by sinusoids lined by endothelial cells, with oxygenated blood flowing from the portal tracts to the terminal hepatic venules. The radial alignment is most pronounced in the hepatocyte plates surrounding the terminal hepatic venules. In normal liver, the portal tracts are composed of at least one hepatic artery branch, one portal vein branch, and one interlobular bile duct (fig. 1-6). The boundary between the portal tracts and the first layer of hepatocytes in the parenchyma is termed the limiting plate.
Hepatocytes extend from the portal area (lower left) to the terminal hepatic venule or central vein (upper right). Blood that enters the liver through branches of the portal vein and hepatic artery perfuses the sinusoids and exits through tributaries of the hepatic vein.

Bile is produced exclusively by hepatocytes and secreted into the bile canaliculi, which are gutter-like structures between two or three adjacent hepatocytes in the hepatocytic plates (11). The canalicular surface is lined by glycoprotein I, which cross reacts with polyclonal carcinoembryonic antigen (CEA), an immunohistochemical marker specific for hepatocytic differentiation (fig. 1-7), although its sensitivity is not optimal and its interpretation requires experience. Canalicular staining is also observed using a CD10 immunostain (19). Immunohistochemical stains for cytokeratin (CK) 8 and 18 are positive in hepatocytes, as is CAM5.2 (20). Since the hepatocytes are rich in urea cycle proteins, antibodies against some urea cycle enzymes have been used widely as hepatocytic markers, such as hepatocyte paraffin-1 (HepPar1) (21) and arginase-1 (22). Various cytoplasmic contents, pigments, and materials are also present within the hepatocytes, including fat, glycogen, bile, lipofuscin, and hemosiderin. However, they are not always seen nor are they absolutely specific for hepatocytic differentiation.
**Tumors of the Liver**

**Biliary Epithelial Cells.** The lining epithelial cells of the septal or larger intrahepatic bile ducts are tall columnar to cuboidal, while those of the interlobular bile ducts are typically cuboidal (fig. 1-8). These cells are encased by a layer of periodic acid–Schiff (PAS)-positive basement membrane and are further surrounded by connective tissue of variable amount, depending on the size of the bile ducts. The interlobular bile duct and hepatic artery are typically adjacent to each other, with a similar diameter and a distance apart approximately the same as their own diameters. The bile ducts are connected to the bile canaliculi via the canals of Hering. On cross section, the configuration of the bile duct appears as a chain of pearls (fig. 1-8). Bile ductal cells express CK7 and CK19, reflected by immunohistochemical staining. Like the hepatocytes, they also express CK8 and CK18.

**Peribiliary Gland Cells.** Peribiliary glands are small accessory glands of the bile ducts in liver. They communicate with the lumens of the intrahepatic and extrahepatic large bile ducts via specialized conduits (7,23,24), densely populated in the hilar bile ducts, cystic duct, and periampullary areas (24–28). Peribiliary glands are composed of glandular and branched tubuloalveolar structures that form small acini that surround the intrahepatic and extrahepatic large bile ducts. The glandular cells are cuboidal, clear (fig. 1-9), and seromucinous in nature, secreting lactoferrin and lysozyme (26).

**Ductular Cells.** Bile ductules are also known as cholangioles. The terms bile ductules and canals of Hering are sometimes used interchangeably, however, they represent two different physiologic and histologic structures. The canals of Hering are the physiologic link between the biliary tree and hepatocyte canaliculi (29). They are lined partially by cholangiocytes and partially by hepatocytes. They continue into bile ductules, which represent the conduits between canals of Hering and the interlobular bile ducts. Bile ducts are lined entirely by cholangiocytes.

The bile ductules are situated at the edge of the portal tracts. They may also penetrate the limiting plate so they not only have an intra-portal segment, but also have an intralobular...
Figure 1-10

**BILE DUCTULES**

A: The ductules, or cholangioles, are normally inconspicuous, but they become apparent and increased in number in reaction to various pathologic conditions. In this case of primary sclerosing cholangitis, the ductules are apparent and increased in number in reaction to biliary obstruction.

B: Immunohistochemical staining for CK19 highlights the reacting ductules.

C: Ductular reaction in a case of submassive hepatocellular necrosis due to acetaminophen overdose. The ductules reflect a regenerative phenomenon in response to liver injury.

**segment (11,29).** Unlike the interlobular bile duct, the bile ductules are not accompanied by a hepatic artery branch (11). Their lumens are also smaller than those of interlobular bile ducts (29). The lining cells of the canals of Herring stain for CK7, CK19, and the hepatic stem cell marker NCAM/CD56, demonstrating the scattered presence of hepatic progenitor cells in these structures (16,17).

Ductular reaction is a phenomenon seen in diseased liver, either acute or chronic disease (fig. 1-10). It is a reaction of ductular phenotype, but not necessarily of ductular origin (29), as it may arise from proliferation of preexisting cholangiocytes, progenitor cells (either local and/or circulating cells such as bone marrow-derived), or biliary metaplasia of hepatocytes (rare) (29).

**Endothelial Cells.** The endothelial cells lining the portal veins, hepatic arteries, and terminal hepatic venules share the same properties and immunophenotype as the endothelial cells lining the arteries, veins, and capillaries in other organs. Their single layer of flattened endothelial cells is attached above a basement membrane and lines the vascular spaces of these vessels. They are immunoreactive to antibodies against CD31, CD34, and factor VIII-related antigen (von Willebrand factor).

In contrast, endothelial cells lining the sinusoidal spaces do not have a basement membrane
(fig. 1-11). In normal liver, the sinusoidal spaces are fenestrated and are lined by endothelial cells that are immunohistochemically nonreactive to CD31 or CD34. The fenestration allows plasma to be transported between the sinusoidal spaces and the hepatocytes underneath the subendothelial space of Disse. When there is neoplastic change of the hepatocytes, the fenestration is decreased, and the sinusoidal endothelial cells become reactive to CD31 or CD34, a phenomenon known as capillarization of the sinusoids. These changes are also observed in preneoplastic lesions, i.e., dysplastic nodules (30–32). Capillarization also occurs in livers with chronic injury or cirrhosis, beginning at the periportal region and extending to the hepatic lobules, although the changes are not as significant as in neoplasia.

**Kupffer Cells.** Kupffer cells are specialized macrophages of the mononuclear phagocyte system that line the walls of the sinusoidal spaces. Normally they are not prominent, but enlarge and become noticeable when they engulf and phagocytose debris resulting from liver injury (fig. 1-12). Hemosiderin pigments may be deposited in Kupffer cells, due to secondary iron overload, or more rarely, hereditary hemochromatosis due to mutation in the SLC40A1/ferroportin 1 gene (33). Lipofuscin pigment may also appear in Kupffer cells.

Kupffer cells are variably PAS positive and diastase resistant since they contain many lysosomes. They are also positive for macrophage markers such as CD68 (KP1).

**Hepatic Stellate Cells.** The compartment between the hepatocyte cytoplasmic membrane and the sinusoidal space, underneath the fenestrated sinusoidal endothelial cells, is the space of Disse, where hepatic stellate cells reside. A number of synonyms were previously used for these cells, such as Ito cells, fat-storing cells, perisinusoidal cell lipocytes, and parasinusoidal cells, but the preferred nomenclature by the
HEPATIC STELLATE CELLS

Within the subendothelial spaces of normal liver, these cells store vitamin A (retinoid) droplets in their cytoplasm (35). In their quiescent phase, they are not conspicuous in formalin-fixed normal liver, but may become discernible by immunohistochemistry using anti-smooth muscle actin (fig. 1-13, left) or anti-desmin antibodies. Hepatic stellate cells are also prominent during hepatotoxicity associated with hypervitaminosis A, becoming bubbly cells due to the deposition of fat in the cytoplasm, with hyperchromatic and scalloped nuclei (fig. 1-13, right).

Hepatic stellate cells are responsible for hepatic fibrogenesis. They can transform into myofibroblasts when they are activated in response to chronic liver disease with activated TGF-β and other signaling pathways. This phenotypic change triggers a series of accumulation as well as inhibition of the breakdown of matrix proteins, hence leading to liver fibrosis (36).

Pit Cells. Another cell residing in the space of Disse is the pit cell. Pit cells are large granule-containing lymphoid cells possessing natural killer cell properties that are liver specific (37). Pit cells are not readily identifiable under light microscopy, but have been characterized by electron microscopy as cells containing multivesicular body-related dense granules and rod-cored vesicles (38).

Inflammatory Cells. In normal liver, inflammatory cells are inconspicuous, except for occasional lymphocytes, Kupffer cells, and neutrophils in the sinusoids. The portal tracts have few lymphocytes and macrophages, and their number appears to correlate with age, likely reflecting a remnant response to previous injuries in the liver.

consensus of investigators studying this cell is hepatic stellate cell (34).
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