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Normal Liver

The liver is a unique organ that has numerous structural and physiological functions. It is most important when discussing liver pathology that one understands first the normal liver histology before one can best understand the basic pathophysiologic concepts of the numerous liver diseases. The pathologist plays a fundamental role in assessing the various morphologic features seen in liver tissue, whether by fine needle aspirates, needle or wedge biopsies, partial hepatectomies, liver explants, or autopsy material. The pathologist also has not only routine but also numerous special histochemical and immunohistologic stains as well. Yet correlating the histologic findings with the most pertinent clinical and laboratory data enables the pathologist to better arrive at a diagnosis and the most pertinent differential possibilities.

This introductory chapter addresses all aspects of the normal liver, reviewing the embryologic development, gross and microscopic features, the pertinent intracytoplasmic components and how their function varies with their location within the hepatic lobule, and the importance of stem cell function within the liver. Additionally the various useful stains and laboratory values will also be presented, as well as a brief outline of how best to organize pathologic readings and signouts of liver biopsy specimens.

Embryology

The hepatic primordium anlage initially appears at the end of the third week of gestation and is first seen as a hollow midline outgrowth stalk (*hepatic diverticulum*) of the endodermal epithelium at the distal aspect of the foregut. By the fourth week, the diverticulum enlarges from proliferation of the endodermal cell strands (*hepatoblasts*) and projects cranially into the mesoderm of the septum transversum, eventually giving rise to the liver hepatic parenchyma and intrahepatic ducts. The cephalic end ultimately develops into the right and left hepatic lobes, while the stalk between the diverticulum and foregut narrows and forms the extrahepatic biliary system and gallbladder.

Solid cords are initially formed by proliferating endodermal cells. These eventually anastomose to form vesicles and cribriform tubules with centrally located lumenal structures (*biliary canaliculi*). The cords eventually merge and develop small channels and capillaries that subdivide the cords to eventually form the hepatic sinusoids. The individual hepatoblasts are progenitor cells that develop into mature hepatocytes, with those immediately adjacent to the portal mesenchyme becoming the ductal plates. The rapid growth rate of the hepatic cords enables the development of sheets of cells (*muralium multiplex*) that persist until birth, after which the cell sheets narrow to two cells (*muralium duplex*) and eventually evolve within the first year of life into a one cell thick trabecular cord (*muralium simplex*). The perisinusoidal cells and Kupffer cells appear by three months gestation.

The mesoderm from the septum transversum initially surrounds the liver and is directly in

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contact with the lesser curvature of the stomach, duodenum, and ventral body wall. The mesoderm eventually forms the lesser omentum, the falciform, coronary, and triangular ligaments, with a portion developing into the liver (Glisson) capsule. The mesoderm on the liver surface is also in continuity with the peritoneum, and the portion that makes contact with the future diaphragm remains uncovered (*bare area*). The developing hepatic artery and vagus nerve branches follow the mesoderm along and adjacent to the portal vein.

The mesoderm is the main focus in the development of *hematopoiesis*, which begins at about 6 weeks and becomes most active during the fifth month of gestation. This process regresses with increase in bone marrow activity. The erythroid precursors are most prominent during fetal development within the hepatic sinusoids while the myeloid and megakaryocytic precursors reside mostly within the portal structures (Figure 1.1). This hematopoiesis is responsible for the enlarged size of the liver (up to 10% body weight by the tenth week of gestation, with the right and left lobes taking up an equal volume), but this size significantly regresses at birth (5% of body weight) at which time only rare small clusters of normoblasts can be seen. By 4 weeks of age hematopoietic activity has usually ceased.

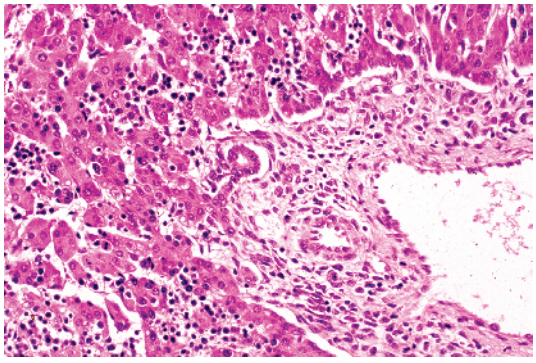

Figure 1.1 Embryonic development**.** A developing bile ductule is seen at the border of the portal tract and parenchyma. The portal tract and sinusoids contain hematopoietic precursors (extramedullary hematopoiesis).

Additionally with time the left lobe diminishes in size, and the caudate and quadrate lobes develop as subdivisions of the right lobe.

The *vascular network*, originally derived from the development of the vitelline and umbilical veins, occurs at the same time as proliferation of the hepatoblasts, with the sinusoids forming from anastomosis of the hepatic cords and vessels. By the fifth week of gestation most of the major vessels are present and include the right and left umbilical veins, the transverse portal sinus, and the ductus venosus, which shunts blood from the umbilical vein into the inferior vena cava. The portal vein initially develops from the vitelline vein and then subdivides into the right and left branches. The hepatic and portal vein branches divide the parenchyma into the individual lobules and acini. At birth, a sphincter mechanism closes the ductus venosus, resulting in cessation of blood flow through the umbilical vein, with the liver now receiving blood from the left branch of the portal vein.

The *biliary apparatus* develops from membranous infoldings between the junctional complexes located between individual hepatoblasts and initially appears as intercellular spaces with no distinct wall. The biliary canaliculi are first seen at 6 weeks of gestation, with synthesis of bile occurring by the ninth week and secretion of bile by the twelfth week. The ductal plate, which is initially two layers thick, is formed from the periportal hepatoblasts. A lumen develops by the third month (see Figure 1.1) with eventual formation of double-layered tubular (ductular) structures. The true interlobular bile ducts occur immediately after birth from remodeling of these ductular elements. This biliary network receives its blood supply from a complex of arterioles and capillaries formed from the peribiliary plexus. The extrahepatic biliary tree develops from the stalk of the original hepatic outgrowth.

Individual *cell functions* become apparent at different stages of the embryologic development. α-Fetoprotein, found in high amounts at birth, initially is seen within the hepatocytes by one month gestation and continues throughout fetal development, with high serum levels at

birth. Fatty change (steatosis), glycogen and glycogen synthesis become most apparent by two to three months gestation, with the glycogen eventually diminishing due to rapid glycogenolysis. Hemosiderin is usually seen early on but gradually decreases, with some often occurring in the periportal hepatocytes at birth.

Gross Anatomy

The adult liver weighs from 1200 to 1800 g, dependent on the overall body size, takes up the majority of the right upper abdominal cavity beneath the rib cage, and extends from the right lateral aspect of the abdomen 15–20 cm transversely toward the xiphoid process. Although the weight of the adult liver constitutes about 1.8–3.1% of the total body weight, at birth the liver is larger compared with adjacent thoracic and abdominal viscera and constitutes about 5–6% of the body weight.

Anatomically, the liver has four lobes: right, left, caudate, and quadrate. The right lobe accounts for one-half to two-thirds of the total liver volume and is divided from the left lobe by the falciform ligament on gross inspection; however, functionally the right and left lobes are of about equal size and are divided by a line extending from the inferior vena cava superiorly to the middle of the gallbladder fossa inferiorly.

A total of *eight functional segments* are present, each having its own vascular supply and biliary drainage: the right posterolateral (VI and VII), right anterolateral (V and VIII), left anterior (IV), left posterior (II and III), and the caudate lobe (I), the latter being a watershed area of both the right and left lobes blood supply (Figure 1.2).

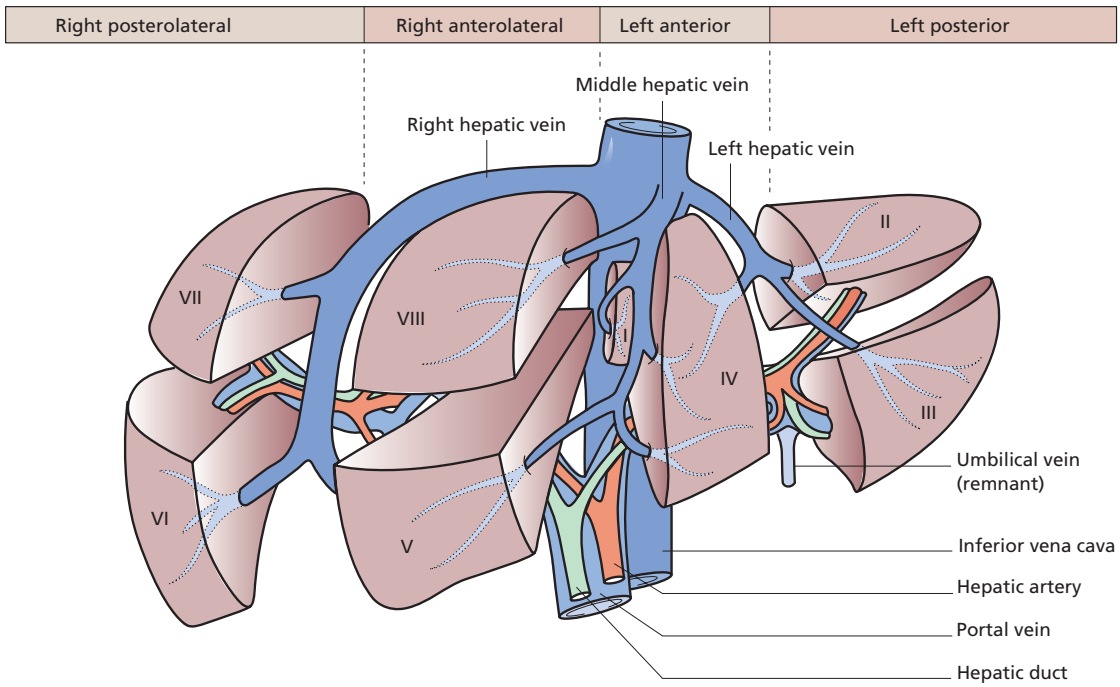

Figure 1.2 Schematic anatomical vascular arrangements of the liver. The liver is divided into eight functional anatomical segments, each having its own vascular supply and biliary drainage: the right posterolateral (VI and VII), right anterolateral (V and VIII), left anterior (IV), left posterior (II and III), and the caudate lobe (I). Source: Wanless IR. Physioanatomic considerations. In: *Schiff's Diseases of the Liver*, 11th edn. Oxford: Wiley Blackwell, 2012. Reproduced with permission of John Wiley & Sons.

The *portal vein*, which is the main route of vascular drainage of the gastrointestinal tract, is formed by merger of the superior mesenteric and splenic veins, with additional blood supply from the coronary and cystic veins. The portal vein divides at the porta hepatis into the right and left main branches. The right branch divides early into anterior and posterior segments, while the left branch divides into the pars transversus, which extends to the left in the porta hepatis, and the pars umbilicus, which descends into the umbilical fossa. The caudate lobe veins arise from both the right and left main portal vein branches.

The *hepatic vein* is composed of three major tributaries: right, middle, and left. The middle and left hepatic veins often converge to form a single outflow vessel before draining into the inferior vena cava, while the right hepatic vein opens through a separate ostium. The caudate lobe drains directly into the inferior vena cava.

The *hepatic artery* is a branch of the celiac artery and ascends along the hepatoduodenal ligament, eventually dividing into the right and left main branches. The right hepatic artery, usually located behind the common hepatic duct after giving rise to the cystic artery, eventually divides into the anterior and posterior branches. The left hepatic artery passes obliquely upward and to the left in the porta hepatis, eventually dividing into the medial and lateral branches. The quadrate lobe is fed by a branch of the middle hepatic artery, while the caudate lobe is fed by both right and left hepatic artery branches.

The *biliary system* originally arises from the bile canaliculi within the hepatic lobule and is first seen on gross inspection in the larger interlobular branches. The biliary drainage of the right lobe is derived from anterior and posterior segmental branches that merge to form the right hepatic duct, while the lateral and medial segmental branches merge to form the left hepatic duct that drains the left lobe. The caudate lobe is drained from three duct branches directly into the right and left hepatic ducts. The smaller interlobular bile ducts do not have a wall, but the larger septal branches have a thin wall of collagen fibers. The intra- and extrahepatic bile ducts are directly fed by the hepatic artery and its anastomosing branches, which parallel the ducts as they progress through the various hepatic divisions.

The *lymphatic channels* are divided into *deep* and *superficial* branches. The deep branches parallel the portal and hepatic vein branches, while the superficial branches arise from Glisson capsule and drain through the adjacent falciform ligament, diaphragm, esophagus, and hilar lymph nodes.

The *nerve supply* parallels the main hepatic artery and portal vein and is divided into parasympathetic and sympathetic fibers. The nerve supply enters the hepatic hilum through both anterior and posterior routes, feeds the arteries and bile ducts through sympathetic innervation, and branches through the main portal tracts, with smaller unmyelinated branches feeding the periportal hepatocytes. Many of the nerve fibers terminate on endothelial cells lining the smallest arterioles and along Kupffer cells, stellate (fat-storing) cells, and hepatocytes.

Histology

Basic Architecture Arrangement

The basic *microanatomical structure* of the liver can best be appreciated on a three-dimensional drawing of the portal tracts, parenchyma, and vascular blood flow (Figure 1.3). The portal tract – terminal hepatic (central) venule – portal tract arrangement of the hepatic lobule is evenly distributed throughout all of the hepatic lobules. Although the architecture may at times be difficult to assess when acute hepatic injury occurs, on recovery the arrangements return to normal; however, architectural distortion seen in advanced chronic liver disease and cirrhosis is for the most part irreversible, although regression of fibrosis has been documented in patients with chronic viral hepatitis with severe fibrosis who respond to anti-viral therapy.

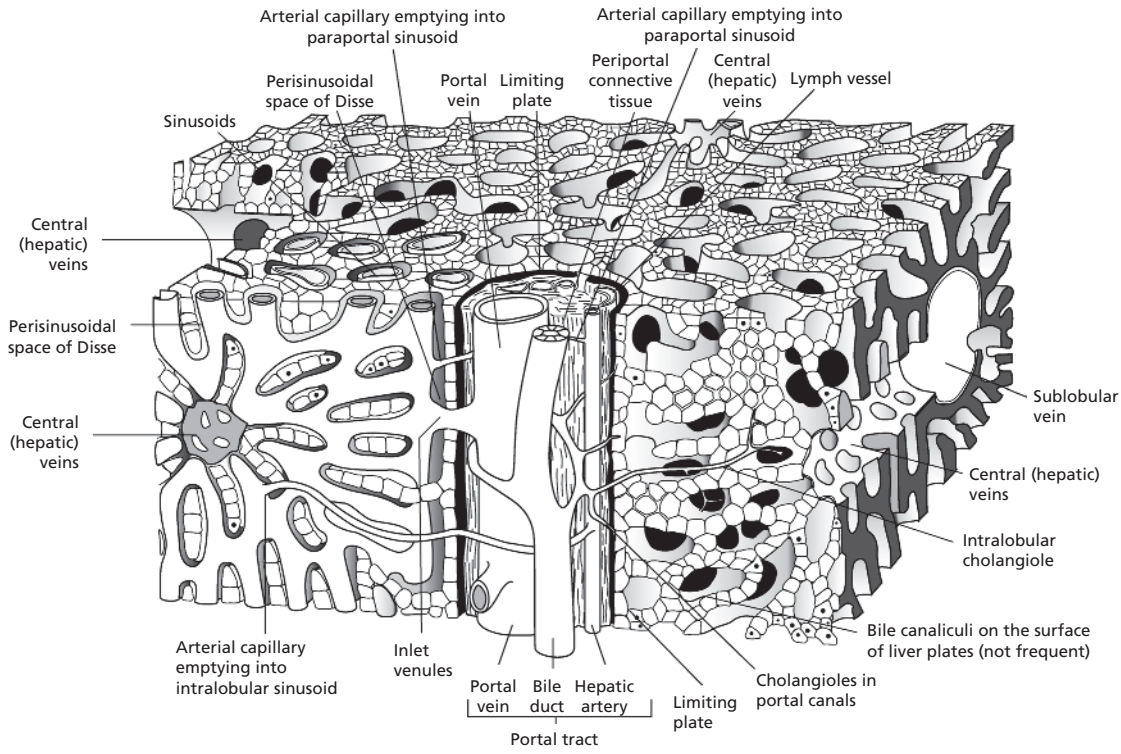

Figure 1.3 Structure of the normal liver. Source: Sherlock S and Dooley J. *Diseases of the Liver and Biliary System*, 11th edn, Blackwell Science, 2002. Reproduced with permission of John Wiley & Sons.

Portal Tracts

The *portal tracts* are composed of five individual components (Figure 1.4). The *interlobular bile ducts* number from one to two per portal tract, although in infants the ducts appear early on to be slightly less frequent. The ducts are usually seen immediately adjacent to the *hepatic arterioles*, which are responsible for their blood supply. The hepatic arterioles are usually present singly. The *portal venules* are a single vascular structure. It is important to note that transverse cuts of the portal tracts in biopsies can errantly appear as if two or more ducts and vessels are present; however, a true increase in portal venules and lymphatics are characteristic of portal hypertension and are usually seen in conjunction with severe portal fibrosis or cirrhosis. The *fibroconnective tissue* is composed of mature collagen and supports the major portal tract components. It often varies in degree, depending on the distance of the portal tracts from the hepatic hilum, and can mistakenly be

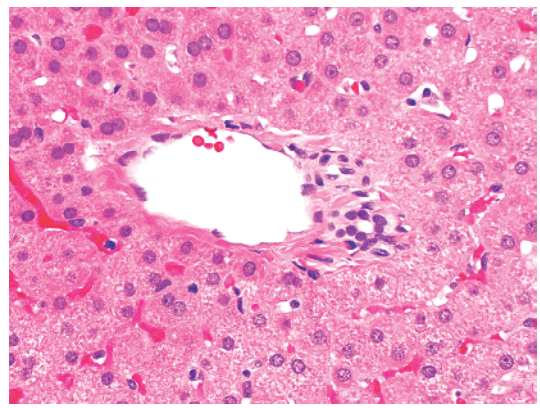

Figure 1.4 Portal tract. This normal portal structure exhibits a single interlobular bile duct, portal venule, and small hepatic arteriole.

described as fibrotic when the biopsy specimen is taken toward the hepatic hilum. The *cellular inflammatory components* within the portal tracts consist usually of scattered lymphocytes. Although in the normal liver these portal inflammatory cells are usually absent, it is not infrequent that a few scattered lymphocytes may appear within these portal structures in an otherwise normal liver.

Parenchyma

The *hepatic lobules* comprise about 80% of the total hepatic volume and are mainly composed of liver cell cords one cell thick made up of polyhedral hepatocytes. The adjacent sinusoids are lined by both endothelial and Kupffer cells, with the perisinusoidal space located between the endothelial cells and hepatocytes. Stellate cells and collagen fibers also occur along the perisinusoidal space, but on routine H&E staining of normal liver tissue these structures are usually inconspicuous. The sinusoids drain from the portal venule and hepatic arterioles into the terminal hepatic (central) venules (Figure 1.5).

Hepatocyte

The *hepatocyte* comprises about two-thirds of the total number of cells within the liver and about four-fifths of the total liver volume. Hepatocytes measure 25–40 μm in diameter, the size dependent on the age of the patient and the zonal location. The cells are polyhedral, are arranged in cords one cell thick, and have three dimensional boundaries (1) the *sinusoidal (basolateral)* surface area lined by both microvilli that extend into the perisinusoidal spaces and plasma membranes that exhibit various membrane pits and infoldings for both secretory and absorptive functions, (2) the *lateral (intercellular)* membranes lying between adjacent hepatocytes that form *gap junctions*, *intermediate* and *tight junctions,* all related in various degrees to transport of metabolites, cell membrane resilience, and membrane permeability to macromolecules, and (3) the *canalicular* membranes lined by microvilli and containing various contractile microfilaments allowing transport of bile secretions.

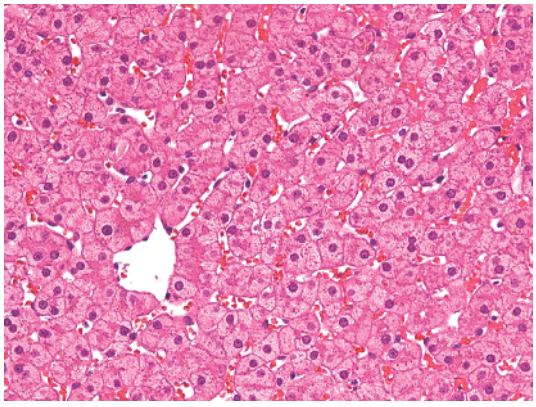

Figure 1.5 Parenchyma. The hepatic cords are one cell thick lined by flattened Kupffer and endothelial cells. The sinusoids are open with the circulating blood draining into a terminal hepatic (central) venule.

Nucleus

The *liver cell nucleus* is round to oval, contains clumped chromatin and small nucleoli, and measures approximately 10 μm in diameter. The nuclear membrane is composed of two envelopes separated by a narrow zone, these membranes having numerous apertures or pores that provide communication between the cytoplasm and the nucleus. The outer membrane is often lined by ribosomes, which are in direct communication with adjacent rough endoplasmic reticulum (ER) fibers. The intranuclear chromatin fibers are composed of heterochromatin (round to irregular dense metabolically inactive granules) and euchromatin which actively transcribes RNA. From one to six nucleoli are often present. The hepatocyte usually contains one nucleus, although bilobed forms may also be seen predominantly in the perivenular zone (zone 3 of Rappaport) and are more frequently present in the elderly patient population. Various nuclear inclusions may be present and include glycogen (most frequent in diabetic patients), lipid, and cytoplasmic invaginations (pseudo-inclusions, nuclear membrane irregularities).

Cytoplasm

The *cytoplasm* constitutes about 90% of the volume of the hepatocyte and contains numerous functionally important organelles (Figure 1.6). The superstructure of the cell is maintained by the cytoskeleton of the hepatocyte and includes three major subdivisions: *microfilaments*, *microtubules*, and *intermediate filaments* that are responsible for the overall three-dimensional framework of the hepatocytes as well as organization of the various intracellular functions. Additionally the structure and function of the cells vary depending on their zonal location (Tables 1.1 and 1.2). The cytoplasm contains the following important components:

- *Mitochondria* are one of the most prominent intracellular organelles that average up to 2200 per hepatocyte, are oval to oblong and measure 0.4–3.5 μm in diameter. They are divided into outer and inner membranes separated by a gap, with folds (cristae) projecting into the body of the mitochondria, increasing the total mitochondrial membrane area. They have numerous critical functions that include oxidative phosphorylation and fatty acid oxidation and contain components essential for the urea and citric acid cycles.
- *ER* is composed of a convoluted network of cisternae, saccules, tubules, and vesicles that are distributed throughout the liver cell cytoplasm. The ER is divided into two components: the *rough ER* (seen predominantly surrounding the nucleus and biliary channels) and *smooth ER* (forming a meshwork of small tubules that are devoid of ribosomes and often communicate with the Golgi apparatus). The ER is where various important functions such as protein synthesis and fatty acid metabolism occur. The cytochrome p450 oxidative system is also located in the ER and plays a large part in drug metabolism and toxin degradation.
- *Golgi apparatus* complexes are composed of highly polarized, parallel, flattened, dilated saccules or vesicles, are about 1 μm in diameter, and may number from 40 to 60 per liver cell. Vesicles arranged at the periphery of the sacs detach and transfer secretory material such as lipoproteins into the sinusoids or biliary canaliculi. Golgi function also includes bile secretion, incorporation of carbohydrates into proteins, and membrane synthesis and repair.
- *Lysosomes* appear as electron-dense, pleomorphic, single membrane-bound vesicles

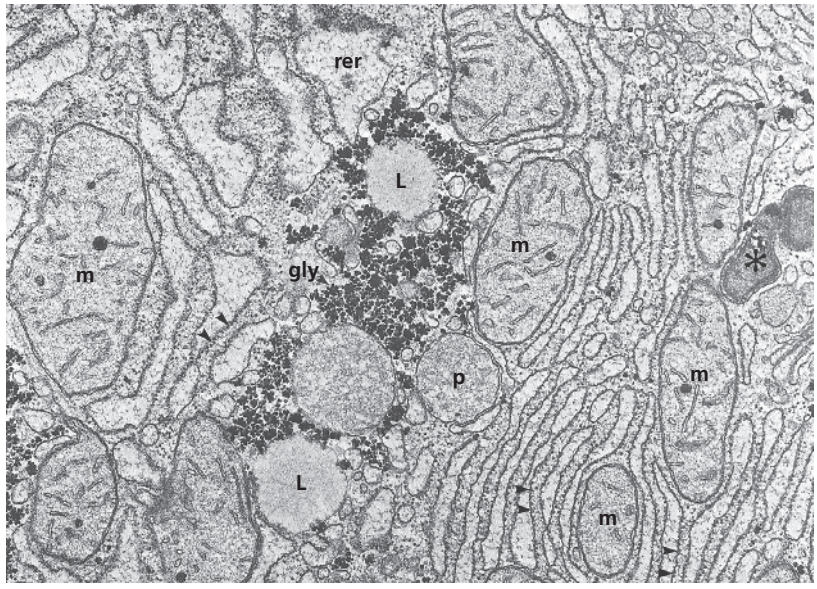

Figure 1.6 Liver cell cytoplasm. Using electron microscopy, the cytoplasm is composed of rough endoplasmic reticulum (rer), mitochondria (m), glycogen (gly), lipid droplets (L), peroxisomes (p), and secondary lysosomes (*). Source: Phillips. *The Liver: An Atlas and Text of Ultrastructural Pathology.* Raven Press, 1987. Reproduced with permission from Wolters Kluwer Health.

Table 1.1 Liver cell structural zonal variations

Zone 3 (perivenular)	Zone 1 (periportal)
Mitochondria round, less numerous and smaller, fewer inner membranes	Mitochondria oval and oblong, larger diameter, larger volume and number, greater cristae area
Peroxisomes prominent	Rough endoplasmic reticulum more abundant
Lysosomes numerous	Bile canaliculi with numerous microvilli, larger in diameter
Bile canaliculi with fewer microvilli, smaller in diameter	Kupffer cells more numerous
Surface area of smooth endoplasmic reticulum larger	Sinusoids form interconnecting polygonal network, are smaller (6 μm), more tortuous, more numerous in number
Sinusoids form parallel vessels that open into terminal hepatic venules, are wider (30 μm), fewer in number	Abundant Golgi-rich volume
Endothelial cells more numerous but smaller, increase in number of fenestrations and porosity	Endothelial cells larger, endothelial fenestrations larger but less numerous
Larger nuclear volumes	Numerous large granular lymphocytes (pit cells)
Increase in number of microbodies	Predominant collagen types IV, V
Slight increase in stellate (Ito) cells[a]	
Predominant collagen types I, III, VI	

[a] Zone distribution varies considerably depending on the nutritional state.
Source: Adapted from Kanel GC. Anatomy, microscopic structure, and cell types of the liver. In: Yamada T (ed.) *Textbook of Gastroenterology*, 4th edn. London: Wolters Kluwer–Lippincott Williams & Wilkins, 2003.

Table 1.2 Liver cell functional zonal variations

Zone 3 (perivenular)	Zone 1 (periportal)
Glycolysis	Gluconeogenesis
Glycogen synthesis from glucose	Glycogen synthesis from lactate
Lipogenesis	β-Oxidation of fatty acids
Removal of ammonia from blood by glutamine	Amino acid catabolism
Detoxification, biotransformation of the majority of drugs and toxins (p450 enzymes)[a]	Urea synthesis
	Cholesterol synthesis
Ketogenesis	Bile acid secretion
Bile acid synthesis	Bile salt-dependent fraction of bile formation; bile acid uptake (sodium dependent)
Bile salt-independent fraction of bile formation, bile acid uptake (sodium independent)	
Glucuronidation	
Mixed function oxidase	
Increase in Kupffer cell phagocytic activity	

[a] Certain drugs and toxins (e.g., allyl formate, phosphorus) are metabolized and may cause liver cell injury in zone 1 due to different pathophysiological mechanisms.
Source: Adapted from Kanel GC. Anatomy, microscopic structure, and cell types of the liver. In: Yamada T (ed.) *Textbook of Gastroenterology*, 4th edn. London: Wolters Kluwer–Lippincott Williams & Wilkins, 2003.

containing and storing enzymes such as acid phosphatase, esterases, proteases, and lipases. They are most frequently identified adjacent to the canalicular membrane and are divided into *primary* and *secondary* lysosomes. The primary lysosomes digest intracellular degradation products ("auto-phagocytosis"), forming secondary lysosomes that secrete these vacuoles into the biliary system. Various pigments such as lipochrome, hemosiderin, and copper also may accumulate within lysosomes with time and form *residual bodies.*

- *Peroxisomes (microbodies)* are single membrane intracellular organelles that vary from 0.2 to 1.3 μm, are round to oval, exhibit a finely granular homogeneous matrix, and contain various oxidases and catalases. Their primarily function involves the oxidation and degradation of numerous substrates with formation of hydrogen peroxides. They are also involved in oxidation of long-chain fatty acids.

There are various other *intracellular components* seen by light microscopy that contribute to the function and appearance of the hepatocyte.

- *Lipochrome* is a finely to coarsely granular brown pigment that is derived from an increase in lysosomal activity and intracellular condensation of various cellular remnants.
- *Bile* is a clumped green to green-yellow globular pigment that is positive on the Hall stain for bilirubin. Usually the presence of intracellular bile is also accompanied by intracanalicular bile.
- *Hemosiderin* is a coarsely granular and golden brown pigment that is highlighted by the Perl iron stain and represents red blood cell degradation remnants.
- *Intracellular lipids* appear as clear distinct rounded vacuoles and are usually composed of neutral triglycerides. The size of the fat droplets varies, with the extremely small variants (*microvesicular* or *foamy*) often difficult to see on routine light microscopy and may require thin 1-micron sections on routine H&E stain or fat stains (Oil Red O) on frozen section material. The small lipid droplets can also be demonstrated by immunoperoxidase staining using *perilipin* and *adipophilin*, two proteins that play a role in lipid metabolism and appear at the rim of the lipid droplets.
- *Intracellular glycogen* is distributed throughout the cytoplasm but is more easily seen by periodic acid–Schiff (PAS) stain on frozen section material.
- *Nuclear glycogen*, when abundant, gives the nucleus a clear appearance and is more often present in a number of liver diseases including non-alcoholic steatohepatitis and Wilson disease.

Sinusoidal Lining Cells

Kupffer cells

Kupffer cells are sinusoidal lining cells that function as tissue macrophages. Although originally derived from the circulation, they eventually rest along the sinusoidal borders but maintain the ability to divide and migrate along the sinusoidal spaces, especially into regions of liver cell damage where it is not uncommon to find hyperplastic and hypertrophic Kupffer cell clusters and aggregates. Kupffer cells have oval to elongated nuclei, abundant pyramidal stellate cytoplasm, and measure up to 9 μm in length. They overlie but do not form junctional complexes with the smaller endothelial cells but may be seen in gaps between adjacent endothelial cells, with cytoplasmic processes extending through endothelial fenestrations. Kupffer cells contain various lysosomes, with their primary functions related to (1) phagocytosis and eventual clearance of particulate material, (2) clearance of bacteria, endotoxins, and degenerating cellular components, (3) synthesis and catabolism of lipids, (4) clearance of senescent erythrocytes, (5) sequestration of antigens, and (6) clearance of immune complexes.

Endothelial cells

Endothelial cells are flattened elongated sinusoidal cells that range from 50 to 80 nm and

contain numerous cytoplasmic projections and clustered fenestrae or gaps varying from 0.1 to 0.2 μm. These fenestrae function as a filtration barrier. These cells have slightly different functions from more typical endothelial cells seen in other organ systems, as they do not bind lectin or factor VIII-related antigen, and they normally express little CD31 or CD34, although activation and expression of these latter markers is common in endothelial cells lining trabecular cords in chronic liver diseases and in many benign and most malignant hepatocellular neoplasms. Although endothelial cells synthesize various substances such as prostaglandins and cytokines, their main function involves filtering of various macromolecules from the sinusoidal blood by receptor-mediated endocytosis, enabling substances such as glycoproteins and polysaccharides direct contact with the hepatocyte but excluding and protecting the liver cell from numerous larger cellular components.

Stellate (perisinusoidal, fat-storing, Ito) cells

Stellate cells are located within the perisinusoidal liver cell recesses along the space of Disse, range from 2 to 10 μm, and contain small star-shaped nuclei without prominent nucleoli. The cytoplasm often contains variably sized lipid droplets having a high concentration of vitamin A (retinoyl palmitate) that can easily be demonstrated on frozen sections by intensely green rapidly fading fluorescence when excited at a wavelength of 328 nm. Besides being the major source of vitamin A storage, the cells synthesize extracellular matrix by way of cytokine activation and resultant transformation to myofibroblasts in response to liver injury, with enhancement of protein and collagen synthesis. These cells also produce hepatocyte growth factor and play a role in the expression of the vascular contour of sinusoids.

Pit cells (liver-associated lymphocytes)

The *pit cells* are non-parenchymal T cells distributed within the sinusoidal lumen in loose contact with the endothelial and Kupffer cells, although they can occasionally be seen within portal tracts. These cells function as natural lymphocyte-activated killer (NK) cells and contain multivesicular body-related dense granules and rod-cored vesicles. These cells are often seen in direct contact with the endothelium in response to various immunologic mechanisms, produce cytokines, and can be targeted in viral hepatitis, acute post-transplant cellular rejection, and various primary and metastatic neoplastic processes where they are felt to play a role in the host immune reaction.

Stroma (Extracellular Matrix)

The *stroma* overall supports the basic hepatic architectural arrangement, produces intercellular cohesion and communication, and effects cellular differentiation. The capsule of Glisson, composed of dense hypocellular collagen, surrounds the liver and extends at the hilum into the hepatic parenchyma, forming the tensile structure of the portal tracts. Extension within the sinusoids into the space of Disse as reticulin fibers maintains the intralobular framework.

Five basic types of collagen are seen, with types I and III representing more than 95% of the total collagen. *Type I* represents mature collagen fibers and is present predominantly within the portal tracts but also around the terminal hepatic venules, sublobular veins and hepatic veins, while *type III* represents new collagen fibers which, along with the type IV collagen, comprise the sinusoidal reticulin framework. *Type IV* collagen is also present in the basal lamina (membrane) around small vascular structures and ducts, and represents about 1% of the total hepatic collagen. The *non-collagenous proteins* are numerous matrix glycoproteins and include (1) *laminin*, the major glycoprotein component within the basement membranes responsible in part for cell adhesion and formation of capillaries within the sinusoids, (2) *fibronectin,* synthesized by perisinusoidal cells responsible for collagen adhesion, and (3) *elastin,* which stabilizes blood vessel walls. Collagen deposition is often triggered by activation of stellate cells, the best example being alcoholic hepatitis where perivenular sinusoidal fibrosis is most prominent.

Biliary Network

The main function of the biliary tract is to transport bile synthesized in the hepatocyte into the gastrointestinal tract by way of the intra- and extrahepatic biliary network. The transport proteins synthesized by biliary epithelium aid in both the secretion of bicarbonate-rich fluid and the reabsorption of various fluids and solutes that generally enhance bile flow.

The biliary tract can be divided into its various structural components:

- *Biliary canaliculi* are located along the intercellular spaces between hepatocytes, range in diameter from 0.5 to 1 μm, and are lined by microvilli (Figures 1.7 and 1.8). The canaliculi have numerous anastomotic connections and may undergo contractions secondary to actin, myosin, and tropomyosin, enabling and enhancing forward bile flow.
- *Terminal bile ductules, periportal cholangioles, and canals of Hering* are formed from canaliculi that enter into the portal structures, are derived from hepatocytes located at the periportal limiting plate, and provide communication with the interlobular bile ducts. These ductules develop a basement membrane and have both liver cell and duct ultrastructural and histochemical features. Although in the normal liver these ductules are usually inconspicuous on routine light microscopy, their proliferation (*duct transformation, duct metaplasia*) is most apparent in instances of (1) liver cell damage, and (2) biliary tract obstruction and other cholestatic processes due to the accumulation of bile acids which trigger bile duct reduplication.
- *Interlobular bile ducts* are larger ducts that range from 15 to 20 μm, are located within the smaller portal structures, and are lined by a single layer of cuboidal cells with discrete round nuclei, usually inconspicuous nucleoli, and scanty eosinophilic cytoplasm. The luminal surface contains numerous pinocytotic vacuoles, with complex interdigitations present in adjoining duct epithelium. A basement membrane is apparent and easily demonstrated on PAS stain. Although the smaller ducts have no apparent wall, the larger interlobular ducts which measure up to 100 μm in diameter develop a small periductal fibrous sheath. The main blood supply is the smaller branches of the hepatic artery and the peribiliary plexus, which run in parallel with the duct structures. These ducts express class I major histocompatibility antigens, with cytokine-mediated class II expression in instances of liver allograft rejection and certain chronic biliary tract diseases that attack ducts, such as primary biliary cirrhosis.
- *Interlobar and septal ducts* measure more than 100 μm in diameter, have a fibrous wall, and are lined by a single layer of cuboidal to columnar epithelium, with nuclei located towards the basement membrane. Some degree of periductal fibrous tissue is common but should not be confused with the distinct periductal concentric fibrosis seen in long-term bile duct obstruction and primary sclerosing cholangitis.

Figure 1.7 Scanning electron micrograph of the canalicular biliary system. Source: Sherlock S and Dooley J. *Diseases of the Liver and Biliary System*, 11th edn. Blackwell Science, 2002. Reproduced with permission of John Wiley & Sons.

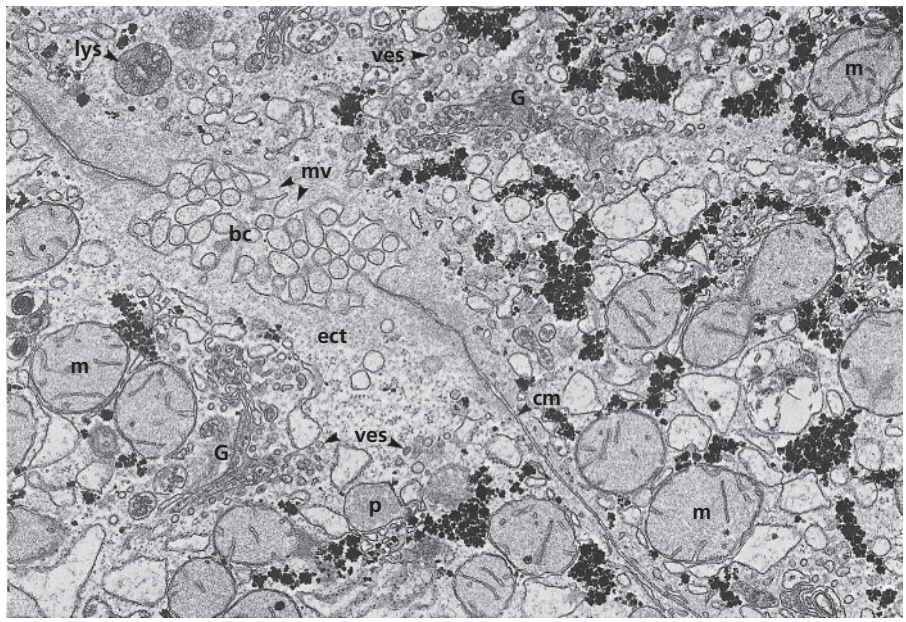

Figure 1.8 Biliary pole. Using electron microscopy, the bile canaliculi (bc) can be seen to the left of the field, surrounded by microvilli (mv) and pericanalicular ectoplasm (ect). Golgi complexes (G), vesicles (ves), mitochondria (m), peroxisomes (p), and lysosomes (lys) can also be seen in the adjacent liver cell cytoplasm. cm, liver cell membrane. Source: Phillips. *The Liver: An Atlas and Text of Ultrastructural Pathology*. Raven Press, 1987. Reproduced with permission from Wolters Kluwer Health.

- *Segmental ducts* are formed from the interlobar and septal ducts and measure up to 800 μm in diameter. These ducts eventually form the major *hilar ducts* that measure up to 1.5 mm in diameter. The hilar ducts ultimately branch into the *main right* and *left hepatic ducts*. The hilar ducts are lined by columnar mucus-secreting epithelium, have a distinct fibromuscular wall, and are associated with both intramural and extramural seromucinous peribiliary glands that communicate with the bile duct lumen.

Vascular and Lymphatic Networks

The major blood vessels that supply the liver are the *portal vein* and *hepatic artery*, the former supplying approximately two-thirds of the total blood flow. The portal vein develops into interlobar, segmental, interlobular veins and pre-terminal branches, with the terminal portal venules measuring about 20–30 μm in diameter that are present in the smaller portal tracts. The hepatic artery branches accompany the portal vein and divide within the smaller portal tracts into two segments: the *periportal plexus*, which branches around the portal vein and drains into the sinusoids, and the *peribiliary plexus*, which provides blood supply to the accompanying interlobular bile ducts by way of small capillaries that are layered around the ducts. Various connections are seen between the small arterioles and the sinusoids that are most prominent in the periportal zone (zone 1 of Rappaport).

There are a number of organizational approaches in assessing the structure as well as function of the liver lobule. On the basis of vascular injection studies, Rappaport *et al.* described the concept of the *liver acinus*, with a stratified order of *simple acini*, *complex acini*, and *acinar agglomerates*. The *simple acinus* (Figure 1.9) is the smallest functional parenchymal unit and

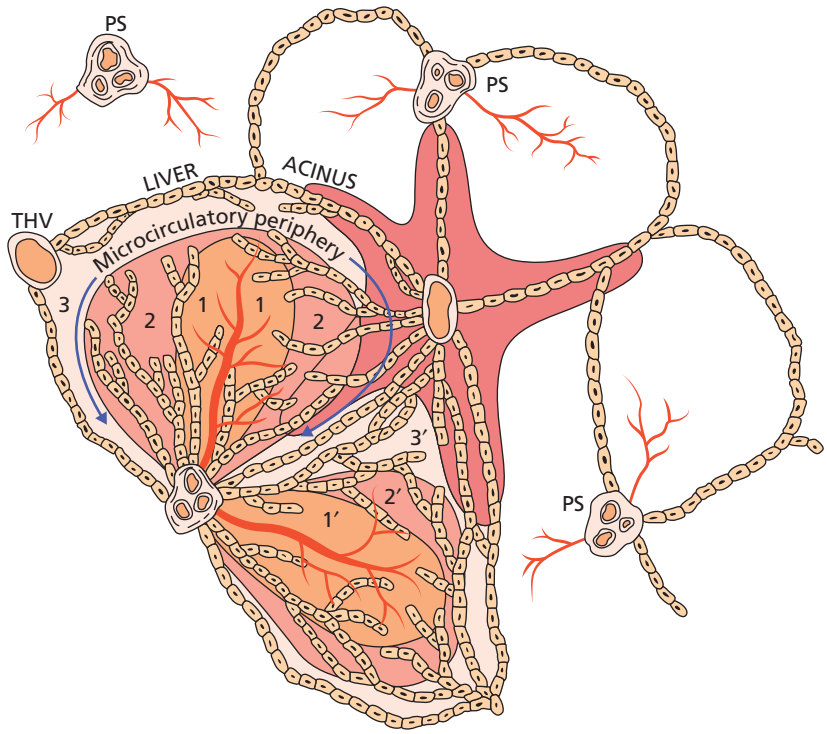

Figure 1.9 Hepatic acinus. The simple liver acinus demonstrates the three hepatic zones and their relationship to the microcirculatory blood supply. PS, portal structures; THV, terminal hepatic venule. Source: Sherlock S and Dooley J. *Diseases of the Liver and Biliary System*, 11th edn. Blackwell Science, 2002. Reproduced with permission of John Wiley & Sons.

centers around the pre-terminal portal venule, hepatic arteriole, and terminal bile ductule. The acinus is divided into three zones (zones of Rappaport): periportal (zone 1) which includes the limiting plate, midzone (zone 2), and perivenular (zone 3) with the terminal hepatic venule at its outer lateral margin. Biliary drainage runs parallel to the vascular sinusoidal circulation. The *complex acinus* is derived from three adjacent simple acini and is fed by a pre-terminal portal venule and arterial branches. The *acinar agglomerate* is composed of about four complex acini and is fed by a portal venous branch measuring 300–1200 μm in diameter.

The *space of Disse* lies between the hepatocyte and the endothelial cells, measures 0.2–1.0 μm in width, and forms a space that is not appreciated on routine light microscopy. Numerous microvilli can be seen projecting from the liver cell membranes into the space of Disse. The discontinuity of the adjacent endothelial cells allows plasma to have access to the liver cell membranes. The space of Disse contains reticulin fibers, and stellate or perisinusoidal cells (Ito cells) also protrude into this space. The sinusoids eventually drain into the terminal hepatic venules, which have no fibrous wall. These vessels then drain into the terminal hepatic and sublobular intercalated veins, and then exit the liver from the three main hepatic vein branches into the inferior vena cava.

Hepatic lymph is mostly derived from the space of Disse, whereas a minority comes from capillary leakage from the peribiliary plexus. Its main function is to drain excess proteinaceous fluid from the interstitial hepatic spaces. The hepatic lymph drainage within the space of Disse travels into the smallest lymphatic vessels within the portal tracts by way of *endothelial massaging* by circulating erythrocytes and leukocytes within the sinusoids. Small lymphatic branches can be seen along the hepatic venous outflow vessels. A lymphatic plexus is also present within Glisson capsule and communicates

with the intrahepatic lymphatics through anastomotic channels. Most lymphatics leave the liver at the porta hepatis, although lymphatic drainage is prominent through the capsule of Glisson in instances when the hepatic venous drainage is impaired (e.g., acute and chronic hepatic venous outflow obstruction, cirrhosis).

Neural Network

The nerve fibers are composed of both parasympathetic and sympathetic branches and release neurotransmitters from intrasinusoidal fibers that contribute to modulation of liver cell function including regulation of carbohydrate metabolism and sinusoidal blood flow. Small nerve segments can be seen within the larger portal tracts, but smaller unmyelinated fibers can be discerned by way of electron microscopy and immunohistochemical studies within the space of Disse.

Structural and Functional Components

The hepatocytes within the various parenchymal zones have many different specialized structural and physiological functions (see Tables 1.1 and 1.2). This functional heterogeneity applies not only to liver cells but also to the sinusoidal and perisinusoidal spaces, Kupffer and endothelial cells, the extracellular matrix, and bile duct epithelial cells. These functions are manifestations of (1) nutrient and hormonal gradients delivered to the various zones, (2) sinusoidal vascular perfusion and oxygen concentration gradients, (3) availability of innumerable substrates and co-factors, and (4) expression of various enzyme activities through gene expression and local (zonal) genetic variations.

Progenitor and Stem Cells

Progenitor and *stem cells* play a significant role in liver cell development and regeneration. The pluripotential *embryonic stem cells* are derived from blastocysts and first give rise to somatic stem cells followed by multipotent tissue-specific stem cells. *Oval cells* are a heterogeneous population of cells that under certain circumstances are activated and proliferate. These cells express various histologic markers of both hepatocytes (α-fetoprotein, albumin) and bile duct epithelium (cytokeratins 7 and 19) and are also known as *facultative stem cells.* Oval cells also express various isozymes of aldolase, pyruvate kinase, lactic dehydrogenase, and glucose-6-phosphatase, the latter a typical hepatocyte marker. Oval cells also express various markers of hematopoietic stem cells (e.g., Thy-1, CD45, Sca-1), although it is not felt that oval cells have a bone marrow origin. These cells are seen along the canals of Hering, behave like progenitor and stem cells, and have the ability to replicate and differentiate into hepatocytes under certain conditions. In fact, these cells appear as prominent bile ductular proliferation that is seen after significant confluent hepatic necrosis, with these ductular cells having the capacity to develop into mature bile ducts and hepatocytes (Figure 1.10). This is seen also in liver cell regeneration after partial hepatectomy and in partial livers after living-related transplantation; however, in chronic hepatitis with cirrhosis, the replicative ability of these cells is quite diminished.

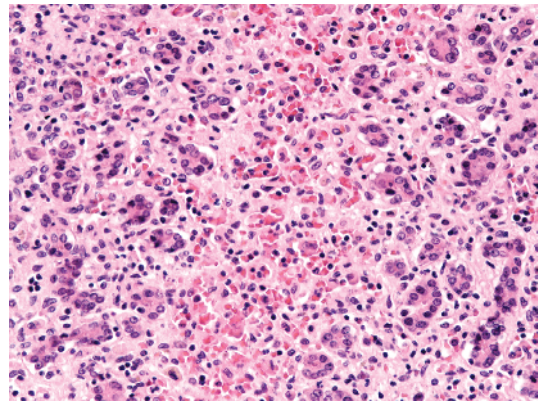

Figure 1.10 Stem cells. Prominent bile ductular proliferation is seen in this example of severe hepatitis with confluent necrosis, the ductules derived from facultative stem cells.

A very small number of *hematopoietic stem cells* are also present in fetal livers and may remain in adult livers as well. They are induced to proliferate by similar conditions that cause oval cell proliferation, and have been shown to have the ability to mobilize and migrate into the liver with differentiation into hepatocytes and duct epithelial cells. These bone marrow cells can generate into hepatocytes in transplanted livers as well, although the frequency is quite low with such cells not always detectable. Although experimentally it has been shown by some that oval cells in the liver can be generated from hematopoietic stem cells, the numbers are extremely small, with some studies also showing no evidence of oval cells generated from hematopoietic stem cells.

Evaluating a Liver Biopsy Specimen

Indications for Liver Biopsy

The liver biopsy is a useful tool in evaluating patients with known or suspected liver diseases. Often the clinical history and liver tests, and when appropriate special studies, provide the clinician with enough information to make a diagnosis and treatment choice without necessitating a liver biopsy; however, certain scenarios can occur that make a biopsy necessary (Table 1.3).

Table 1.3 Indications for liver biopsy

Clinical signs and symptoms of acute or chronic liver disease associated with normal liver tests
Clinical signs and symptoms of acute or chronic liver diseases associated with abnormal liver tests inconsistent with the suspected clinical diagnosis
Staging and grading of known chronic liver diseases
Evaluation of space-occupying lesions
Evaluation of liver transplant specimens
Acute liver failure of no known cause
Abnormal liver tests of no known cause

Clinical Signs and Symptoms of Acute or Chronic Liver Disease Associated with Normal Liver Tests

If a patient has signs of chronic liver disease such as esophageal varices, ascites, and/or splenomegaly, but the liver tests including albumin are normal, a biopsy may be necessary to see if chronic liver disease with cirrhosis is indeed present, as at times patients with advanced liver disease may in fact have normal liver tests; however, if the liver biopsy is normal or shows non-specific changes, then other non-hepatic causes of ascites and portal hypertension (e.g., portal vein thrombosis, cardiac failure with "cardiac" ascites, peritonitis) may be present and necessitate appropriate workup. Additionally, in certain conditions such as non-cirrhotic portal fibrosis and nodular regenerative hyperplasia, signs of portal hypertension may be clinically present with associated normal liver tests, with a biopsy again aiding in the correct diagnoses of these lesions as well.

Clinical Signs and Symptoms of Acute or Chronic Liver Diseases Associated with Abnormal Liver Tests Inconsistent with the Suspected Clinical Diagnoses

Another indicator for biopsy are patients with a known liver disease where the liver test results do not fit, hence bringing up the possibility of either an incorrect clinical diagnosis or two coexisting liver diseases. For example, patients with known alcoholic liver disease after binge drinking who a week later present with jaundice, hepatomegaly, and an abdominal bruit, all features most suggestive of alcoholic hepatitis, but have only minimally abnormal aspartate transaminase (AST) and alanine aminotransferase (ALT) values but a markedly elevated alkaline phosphatase activity, would be biopsied to rule out various causes of those liver test abnormalities including bile duct obstruction.

Staging and Grading of Known Chronic Liver Disease

Patients with chronic viral hepatitis periodically have liver biopsies for staging and grading of the disease even when the liver tests are only mildly abnormal, as even in those cases the

degree of transaminase elevations may not parallel the degree of necroinflammatory change and fibrosis seen on biopsy. The histologic features would then aid the clinician as to whether therapy is indicated. Additionally, even if the liver tests border on normal, a biopsy showing a bridging fibrosis alone may warrant therapy to prevent a severe fibrosis and eventual cirrhosis developing. Staging and grading systems are also used in patients with non-alcoholic fatty liver diseases and autoimmune hepatitis.

Evaluation of Space-Occupying Lesions

In patients with a hepatic lesion seen on imaging, a biopsy is frequently warranted for diagnosis. Even when the clinical indicators are most suggestive of a benign process such as a hepatocellular adenoma (e.g., in a young woman on oral contraceptive therapy with normal liver tests and a single solid liver mass on imaging), a biopsy is often necessary to rule other causes of a benign process such as focal nodular hyperplasia or cavernous hemangioma that require different treatment and follow-up. In addition a tissue diagnosis of hepatocellular carcinoma or other primary or metastatic malignant lesions is also oftentimes indicated before initiation of therapy.

Evaluation of Liver Transplant Specimens

It is not uncommon for various patients post-liver transplant to have identical abnormal liver tests yet have completely different histologic findings on biopsy, leading to different therapies. For example, acute cellular rejection, bile duct obstruction, bile duct ischemia, and harvesting injury can all be associated with hyperbilirubinemia with high alkaline phosphatase values and modest elevations of the aminotransferases, all indicators of the value of post-transplant liver biopsies.

Acute Liver Failure of No Known Cause

About 10–20% of patients with severe liver cell necrosis and liver failure have no known clinical cause. Transjugular liver biopsies are often then performed to assess for possible histologic changes (e.g., abundant plasma cells in unsuspected autoimmune disease, necrosis without inflammation in ischemic injury or due to certain medications such as acetaminophen) that would lead to a correct diagnosis.

Abnormal Liver Tests of No Known Cause

Although liver test values can often correlate with the clinical aspects of certain liver diseases, such as a high alkaline phosphatase with hyperbilirubinemia in known bile duct obstruction, at other times patients may present with abnormal liver tests for no known cause, especially when associated with fever or other systemic findings such as rash or lymphadenopathy. The biopsy can often then point to a specific cause or group of possibilities, such as multiple granulomas on biopsy (suggestive of an infectious process and other causes) or numerous portal eosinophils (suggestive of a possible drug-induced hepatitis).

Organizational Approach in Liver Biopsy Evaluation

Individual morphologic features are often not diagnostic of specific liver diseases; however, assessing the whole complex of portal tract and parenchymal changes oftentimes may lead to likely diagnoses with differential possibilities. Even in instances where the clinical diagnosis is known, all aspects of the morphology should be assessed to prevent missing an unexpected or additional diagnosis.

The *basic architectural arrangement* should be first evaluated as to whether it is intact, with regularly arranged portal tracts and terminal hepatic (central) venules, or whether there is portal fibrosis with bridging, incomplete, or complete fibrous septa (cirrhosis with regenerative nodule formation).

Each *portal tract* is then evaluated for a number of different parameters. The degree of *portal fibrosis* (if any) along with the degree of portal inflammation and the type of inflammatory

cells are assessed. Additionally the presence or absence and the degree of periportal interface inflammatory activity are evaluated. The bile ducts are reviewed as to whether they are normal in number and appearance or whether there is duct proliferation, duct dilatation (ectasia), periductal fibrosis, and inflammatory cells targeted towards the ducts (cholangitis). Duct cytologic atypia and the absence of ducts (ductopenia) are also important indicators of a number of different disorders (e.g., chronic allograft rejection). The portal vessels (arterioles, portal venules) should also be assessed as to whether there is thrombosis or occlusion, inflammatory cells targeted to the arteries (arteritis) or venules (pylephlebitis) and whether the venules are increased in number (a manifestation of portal hypertension).

The *parenchyma* is assessed as to the degree and type of inflammatory infiltrates and the degree of lobular necrosis, with any zonal accentuation noted. The individual hepatocytes are evaluated for the presence or absence of Mallory–Denk bodies, steatosis (degree and type [macrovesicular, microvesicular]), cholestasis, granuloma formation, pigments (bile, lipochrome, hemosiderin), and inclusions (nuclear [e.g., cytomegalovirus], cytoplasmic [e.g., "ground glass cells" in chronic hepatitis B virus infection]). The sinusoids are evaluated as to whether there is dilatation and congestion, acute hemorrhage, sinusoidal fibrosis (zonal, patchy, diffuse), and red blood cell extravasation into hepatic trabeculae. The Kupffer cells are noted as to whether they are hyperplasic or hypertrophic and whether cytoplasmic material (e.g., red blood cells) or microorganisms (e.g., *Histoplasma*) are present. Certainly the present of tumor cells (benign and malignant, primary and metastatic) and other space-occupying lesions (e.g., abscesses, cysts) are crucial features as well.

Routine and Special Histologic Stains in Liver Biopsy Specimens

Table 1.4 lists the routine and special histochemical stains used in liver biopsy evaluation. Each laboratory sets up a basic "panel" that includes routine H&E, trichrome and iron stains, but the addition of PAS after diastase and reticulin stains are also used in some centers as well. Examples of these special stains are shown in Figures 1.11, 1.12, 1.13, 1.14, 1.15, and 1.16. It is best to include at least two or three H&E stained slides for each biopsy, with these slides *not* consecutive in the block. This can avoid missing an important feature that is eventually seen on deeper special stains that may preclude accurate evaluation, especially in instances of a granuloma or neoplasm. The table also lists additional stains that are available to best evaluate other possible diagnoses. Of note is that if a clinical diagnosis is suspected that could require special stains for diagnosis (e.g., Grocott's methenamine silver, acid–fast stains for non-viral infection, immunohistochemical markers for neoplasms), then it may be appropriate to order unstained slides ahead of time for possible later use, as ordering them afterwards often means loss of tissue from the initial shavings when the paraffin block is re-set in the microtome.

Routine Laboratory Tests Aiding in Liver Biopsy Evaluation

The routine and special laboratory tests performed in evaluating patients with known or suspected hepatic disease are listed in Table 1.5. The most common tests include the transaminases (AST, ALT), total bilirubin and direct, alkaline phosphatase, albumin, and globulin values ("liver panel").

Aminotransferases

The *AST* or *SGOT* (glutamic oxaloacetic transaminase) is a pyridoxal phosphate-dependent transaminase enzyme that catalyzes the reversible transfer of an α-amino group between aspartate and glutamate, and is found in multiple organ systems besides the liver that include heart, skeletal muscle, kidneys, brain, and red blood cells. Two isoenzymes are present: a cytosolic isoenzyme derived mainly from red blood cells and myocardial fibers, and

Table 1.4 Routine and special stains for liver biopsy interpretation

Specific stain	Features	Clinical correlation
Routine histology panel for liver biopsies		
Hematoxylin and eosin	Routine assessment of liver histology	
Masson trichrome	Collagen (dark blue)	Chronic liver diseases
	Lobular confluent necrosis (light blue)	Severe (confluent, submassive, massive) hepatic necrosis (fulminant hepatitis)
Periodic acid–Schiff after diastase digestion (DiPAS)	Lysosomal activity in portal macrophages, Kupffer cells	Areas of liver cell necrosis, dropout, and phagocytosis (both mild non-specific changes and acute/chronic hepatitis)
	α_1-Antitrypsin inclusions in periportal hepatocytes	α_1-Antitrypsin deficiency (heterozygous and homozygous)
	Lipochrome pigment	Older patient population (perivenular hepatocytes)
	Lipochrome-like pigment	Dubin–Johnson syndrome, Gilbert syndrome
Perl's iron, Prussian blue	Hemosiderin	Hepatocellular iron: hemosiderosis, idiopathic hemochromatosis Kupffer cell iron: hemolytic anemias, multiple blood transfusions
Additional useful special stains		
Periodic acid–Schiff (PAS)	Glycogen Neutral polysaccarides Bile duct basement membranes	Steatosis of hepatocytes with microvesicular fat droplets highlighted (fatty liver diseases) Storage cells (Gaucher, Niemann–Pick diseases)
von Gieson	Elastic tissue fibers (medium and large vessels)	Fibrointimal thickening, vascular occlusion (non-cirrhotic portal fibrosis, allograft vascular rejection)
Reticulin	Assessment of the basic hepatic cord-sinusoid reticulin (type 3 collagen) framework	Evaluation of tumors: • Normal cord thickness: 1–2 cells • Thickened cords >2–3 cells thick, decrease to absent staining (hepatocellular carcinoma)
	Fibrosis	Chronic liver diseases
	Confluent necrosis (condensed fibers)	Severe (confluent, submassive, massive) hepatic necrosis (fulminant hepatitis)
Fouchet, Hall	Bile	Cholestatic liver diseases
Sirius red	Direct staining of mature (type 1) collage fibers	Fibrosis in chronic liver diseases
Congo red	Amyloid	Amyloidosis confirmed with positive apple-green birefringence under polarized light
Gram	Gram-positive and Gram-negative cocci and bacilli	Bacterial sepsis, abscesses
Grocott's methenamine silver (GMS)	Fungal micro-organisms, parasites	Infection by *Aspergillus*, *Candida*, *Cryptococcus*, *Pneumocystis*, and others

(continued)

Table 1.4 (*continued*)

Specific stain	Features	Clinical correlation
Ziehl–Neelson acid–fast (AFB)	Mycobacterium	Tuberculosis (*M. tuberculosis*) MAI infection (*M. avium-intracellulare* complex)
Warthin–Starry reaction	Spirochetes	Syphilis (*Treponema*)
Phosphotungstic acid hematoxylin (PTAH)	Fibrin (sinusoids, vessels)	Toxemia of pregnancy HELLP syndrome Q-fever Humoral allograft rejection
Rubeanic acid, rhodanine	Copper	Wilson disease
Orcein (Shikata), Victoria blue	Copper-binding protein (granular staining)	Indian childhood cirrhosis Certain chronic biliary tracts disorders (e.g., primary biliary cirrhosis)
Orcein (Shikata), Victoria blue	Hepatitis B surface antigen (diffuse cytoplasmic staining)	Chronic viral hepatitis type B
Oil Red O, Sudan Black	Neutral fats (frozen sections)	Fatty liver diseases
Useful immunohistochemical stains[a]		
HBsAg	Cytoplasmic staining of hepatocytes	Chronic viral hepatitis B
HBcAg	Nuclear, sometimes cytoplasmic staining of hepatocytes	Chronic viral hepatitis B
HCV Ag	Cytoplasmic staining of hepatocytes	Chronic viral hepatitis C
Delta antigen	Nuclear staining of hepatocytes	Acute and chronic delta hepatitis
Ubiquitin	Mallory–Denk bodies	Active alcoholic and non-alcoholic fatty liver diseases Chronic biliary tract disorders
α_1-Antitrypsin	Periportal intracytoplasmic inclusions	α_1-Antitrypsin deficiency
Cytokeratins 7, 19	Bile duct and ductular epithelium	Liver diseases associated with interlobular bile duct loss (e.g., primary biliary cirrhosis, chronic allograft rejection) with *absent* cytokeratin staining
Cytomegalovirus	Nuclear staining of hepatocytes	Cytomegalovirus infection, active and latent
Adenovirus	Nuclear "smudge" staining of hepatocytes	Adenovirus infection
Herpesvirus	Nuclear, sometimes cytoplasmic staining of hepatocytes	Herpesvirus infection
Molecular techniques		
Epstein–Barr encoded RNA (EBER) probe	Intrahepatic latent Epstein–Barr virus	Lymphoma Post-transplant lymphoproliferative disorder (PTLD)
Polymerase chain reactions (PCR)	Viruses, infectious agents, gene mutations	Chronic HBV, HCV hepatitis Hemochromatosis (HFE genes)
Gene micro-array analysis	Gene mutations	Hemochromatosis (HFE genes)

[a] Excluding tumor markers (see Chapters 13 and 14).

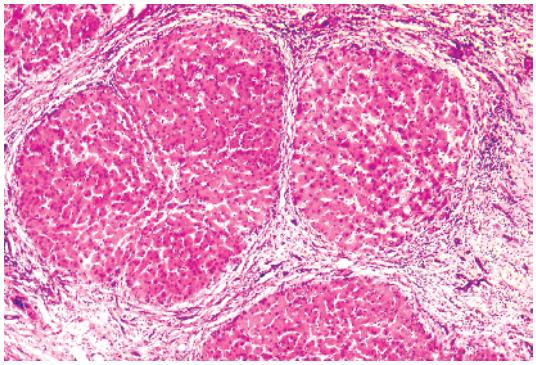

Figure 1.11 Trichrome stain. Mature type I collagen representing mature fibrous bands in this cirrhotic liver is present.

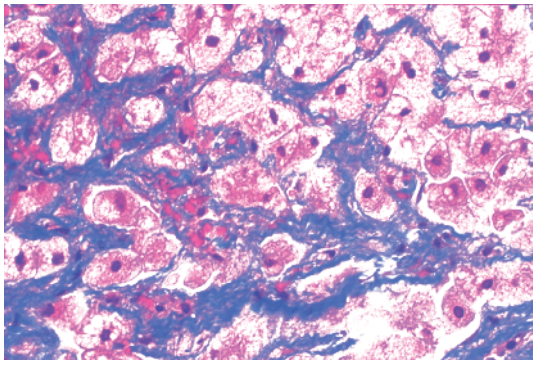

Figure 1.12 Trichrome stain. Type III sinusoidal collagen fibers in an alcoholic fibrotic liver are demonstrated.

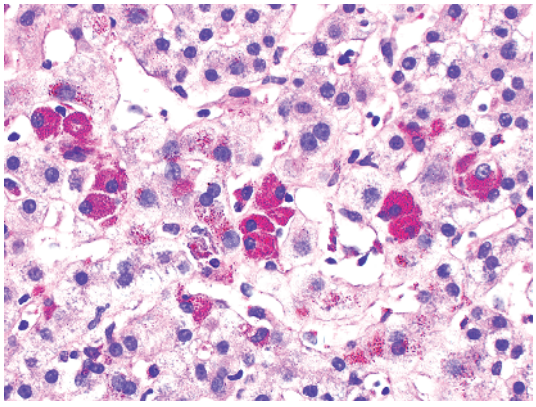

Figure 1.13 PAS stain after diastase digestion (DiPAS). Increase in lysosomal activity in areas of liver cell necrosis is seen.

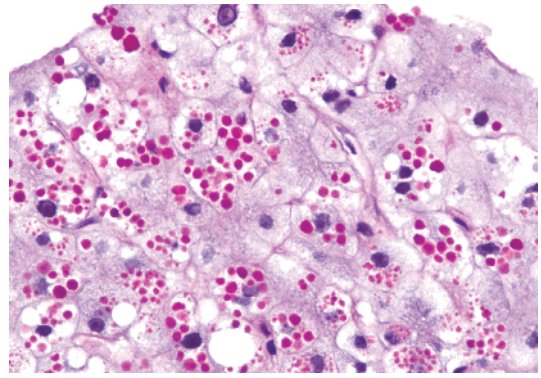

Figure 1.14 PAS stain after diastase digestion (DiPAS). α1-Antitrypsin inclusions in periportal hepatocytes are highlighted with this stain.

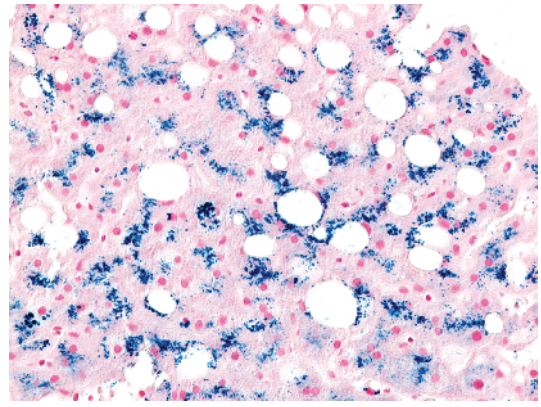

Figure 1.15 Perl's iron stain. Hemosiderin pigment stains intensely blue in the hepatocytes in this example of hereditary hemochromatosis.

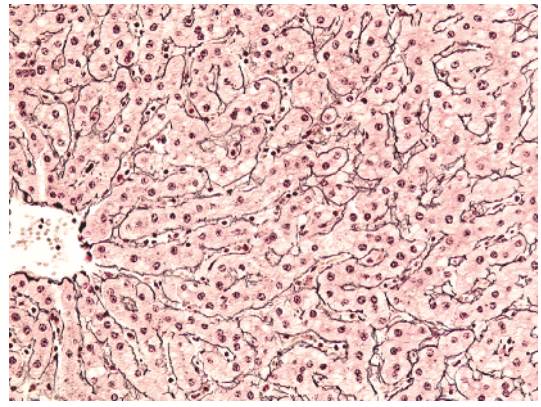

Figure 1.16 Reticulin stain. The reticulin fibers line the hepatocyte trabecular cords that are one cell thick.

Table 1.5 Routine and special laboratory tests for liver biopsy evaluation

Laboratory test	Cause of abnormal values	Examples
Routine liver tests		
AST (aspartate transaminase)	⇑ In hepatocellular damage	Various acute and chronic hepatitic reactions
ALT (alanine aminotransferase)	⇑ In hepatocellular damage	Various acute and chronic hepatitic reactions
Alkaline phosphatase	⇑ In cholestatic injury, bile duct damage, infiltrative processes	Biliary tract diseases including duct damage in transplant rejection Granulomatous hepatitis Primary and metastatic tumors
Total bilirubin, direct	⇑ In cholestatic injury, bile duct damage, functional hepatocellular damage	Biliary tract obstruction Hepatitis, acute and chronic with increased activity Advanced liver disease with liver failure Dubin–Johnson syndrome, Gilbert syndrome
Albumin	Values parallel liver cell synthetic function	Cirrhosis (decreased values)
Globulin	Values manifestations of liver injury	Cirrhosis, autoimmune hepatitis (increased values)
Additional useful tests		
INR (prothrombin activity)	⇑ In liver dysfunction	Severe hepatitis, cirrhosis
γ-Glutamyl transferases (GGTP)	⇑ In bile duct damage	Biliary tract disorders
5′-Nucleotidase (5′NT)	⇑In bile duct damage	Biliary tract disorders
Lactic dehydrogenase (LDH)	⇑ In hepatocellular damage	Drug-induced injury Liver cell ischemia
α-Fetoprotein (AFP)	⇑ In liver cell neoplasms ⇑ In liver cell regeneration	Hepatocellular carcinoma Fulminant hepatitis with hepatocellular regeneration
Iron/iron-binding capacity	⇑ With increased hepatic absorption ⇑ In hemolysis	Hemochromatosis Hematologic disorders (hemolysis, ineffective erythropoiesis)
α_1-Antitrypsin	⇑ In inflammatory diseases ⇓In inherited disease	Infections (increased values) α_1-Antitrypsin deficiency (decreased values)
Ceruloplasmin	⇓In copper storage disorders, copper deficiency	Wilson disease Aceruloplasminemia Menke disease
Hepatic tissue iron quantitation	⇑ In primary and secondary iron deposition	Hemochromatosis
Hepatic tissue copper quantitation	⇑ In hepatic copper	Wilson disease Indian childhood cirrhosis
Viral and autoimmune hepatitis serologies See Table 2.2 Viral hepatitis – serologic markers See Table 7.2 Autoimmune-associated liver diseases – serologies		

a mitochondrial isoenzyme present mainly in the liver.

The *ALT* or *SGPT* (glutamic pyruvic transaminase) catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate and requires the coenzyme pyridoxal phosphate. The enzyme is found in the cytosol of the hepatocytes. It is found in low concentrations in many other sources but is most frequently present and more specifically associated with liver damage.

The AST and ALT values are elevated to various degrees in almost all liver diseases at one point or another. Although the degree of enzyme elevations can give hints suggestive of certain liver diseases while making other less likely (e.g., marked elevations >1000 U/L in acute viral hepatitis, values in 100–200 range in chronic viral hepatitis), there is considerable overlap, as in chronic viral hepatis with reactivation associated with high aminotransferases. Of importance, the aminotransferases are not manifestations of true liver *function* but liver *status*. For instance, although the aminotransferases can be in the thousands in fulminant hepatitis, these values in the same disease can then decrease and even approach near normal associated with a rise in bilirubin values and INR due to the fact that there are few viable liver cells remaining to release the enzymes.

The *AST : ALT ratio* can often be very useful. In alcoholic liver disease, especially alcoholic hepatitis, the AST : ALT ratio is characteristically 2–3/1, with the AST ranging from 100 to 300 while the ALT is only slightly elevated or can even be normal. Conversely, in non-alcoholic fatty liver disease this ratio does not occur. In acute or chronic viral and autoimmune hepatitis, the AST and ALT are approximately the same, with the ALT often slightly higher, although in the cirrhotic stage the AST is usually higher than the ALT. In direct and indirect hepatotoxic (non-hypersensitivity-induced) injury from certain drugs (e.g., acetaminophen) and toxins and in ischemic liver injury the AST can be markedly elevated with the ALT only modestly increased.

Bilirubin

Bilirubin, a heme degradation molecule excreted from the liver via the biliary system, is water insoluble and requires glucuronidation by the enzyme bilirubin uridine diphosphate (UDP)-glucuronyltransferase (bilirubin-UGT) into the water-soluble bilirubin mono- and di-glucuronide forms for secretion into the biliary canaliculi. It is divided into both the unconjugated (indirect) and the conjugated (direct, a combination of the mono- and di-glucuronide) forms in laboratory testing.

The bilirubin values are elevated in a wide variety of liver diseases that hamper bile secretion. In various causes of severe hepatitis with or without liver failure (e.g., acute and fulminant viral or drug-induced hepatitis), elevated bilirubin values are the rule. Extrahepatic or large intrahepatic bile duct obstruction or stricture are also common causes. Elevated bilirubin values can also occur as a response to intracytoplasmic pathologic processes such as hereditary hyperbilirubinemias (e.g., Dubin–Johnson syndrome) and developmental disorders (e.g., paucity of duct syndrome).

Alkaline Phosphatase

The *alkaline phosphatase* enzymes are zinc metalloenzymes present in most tissues and are localized in the liver within the microvilli of the bile canaliculi. It is a hydrolase enzyme responsible for removing the phosphate groups from many molecules including nucleotides, proteins, and alkaloids (dephosphorylation). It is also present within bone, with elevated levels occurring during pregnancy due to its placental origin as well.

The alkaline phosphatase, like the serum bilirubin, is often elevated in acute and chronic biliary tract obstruction. It also is elevated in immune-mediated processes that attack interlobular ducts themselves (e.g., primary biliary cirrhosis, autoimmune cholangitis, post-transplant acute cellular rejection) while the bilirubin may be normal. The alkaline phosphatase is also increased in infiltrative processes such as granulomatous hepatitis, amyloidosis, or rapidly growing primary or metastatic tumors.

Serum Proteins Albumin and Globulin

Albumin is one of the most abundant proteins in the circulation and has many functions including transport of fatty acids, various metabolites, and drugs. It is synthesized in the hepatocyte as a pre-proalbumin, with the N-terminal peptide removed before the nascent protein is released from the ER as proalbumin. It is then glycosylated and released as the albumin protein. Although there are a number of non-hepatic causes for low values (hypothyroidism, nephrotic syndrome, malnutrition), its value also parallels the number of viable hepatocytes in the liver, hence low values often correlate with advanced liver disease and cirrhosis.

The serum *globulin* includes the α_1 and α_2, β, and γ (IgG, IgM, IgA) globulins. The values are elevated in numerous non-hepatic conditions (arthritis, infections, multiple myeloma) but are also useful in correlating with various liver diseases, as the value is usually elevated in advanced liver disease and cirrhosis and may be markedly elevated in autoimmune hepatitis.

Special Laboratory Tests

A wide range of additional testing is also listed in Table 1.5 that includes laboratory values targeted to specific liver diseases (e.g., low serum ceruloplasmin values in Wilson disease). The critical viral and autoimmune hepatitis serologic markers are discussed in Chapters 2 (Viral hepatitis) and 7 (Autoimmune hepatitis) and listed in the appropriate tables in each chapter.

Selected Reading

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Additional material for this chapter can be found online at:
www.wiley.com/go/kanel/liverpathology

This includes a full list of References, Case Examples, and Library Images to supplement this chapter.