1 Structure Function, and Repair

1 Structure Function and Repair of the Liver

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The liver is an organ that has fascinated mankind ever since medicine existed. Ancient medicine was aware of the liver's central role in nutrition, and for Galen it was a "principal instrument" of the body. In Greek mythology, Prometheus—the friend of mankind who was chained to a rock by the god Zeus as punishment for giving humans the use of fire—suffered daily as an eagle devoured his liver, only for it to restore itself overnight. This association with Prometheus and the capacity of the liver to regenerate has been quoted many times in textbooks, editorials, and reviews.

Most lay people understand the principal roles of the heart, brain, and kidney, but are unfamiliar with the liver. Patients and families find it difficult to understand the functions of the liver and the implications of liver failure, and this has to be taken into consideration when counseling children and their families. In order to gain an understanding of liver disease, it is necessary to study the basics of the development, anatomy, and function of the liver and its responses to injury.

Structure

Development

Overview

The development of the liver has been extensively studied.[1,2] Human liver development begins during the third week of gestation from the ventral foregut endoderm (the future duodenum), which gives rise to the liver bud or hepatic diverticulum. The liver bud grows into the septum transversum and the cardiac mesoderm. These structures provide connective tissues to the developing liver and appropriate gene expression, which is regulated in a time-specific manner by liver-enriched transcription factors such as hepatocyte nuclear factor 6 (HNF6),[3] required for normal development in the endoderm and is mesoderm. This process is termed "mesoderm inductive signaling."[4,5] In this environment, cells from the liver bud form thick plates of hepatoblasts surrounding sinusoids fed from vitelline vessels derived from the wall of the yolk sac. Sheets of liver cells are initially many layers thick, but by 5 months after birth, the plates are two cells thick. The adult pattern of plates one cell thick (Figure 1.1) is not seen until at least 5 years of age.[6] The liver reaches a peak of relative size at the ninth gestational week, accounting for 10% of fetal weight. In the healthy neonate, it represents up to about 5% of the body's weight; during adolescence, this decreases to the final adult proportion of 2% of body weight, or a weight of 1400 g in the female and 1800 g in the male.

Vascular development

The liver grows under the influence of its blood supply. Initially, blood is provided by the symmetrical vitelline veins, which ultimately join to form the portal vein. Later, blood is supplied by the left and right umbilical veins, rich in oxygen and nutrients, from the placenta. The right umbilical vein then disappears, leaving the left umbilical vein as the principal supplier. Blood in the left umbilical vein takes one of three routes—supplying sinusoids on the left side of liver; supplying sinusoids in the right half of the liver via retrograde flow through a connection with the left branch of the portal vein; or to the inferior vena cava via the ductus venosus. Ultrasound studies in fetuses near term have shown that the left lobe receives almost exclusively nutrient-rich umbilical vein blood, whilst the right lobe only receives 50% of its supply from the umbilical vein, with the remaining 50% coming from the nutrient-poor portal vein.[7] The left lobe is therefore significantly better perfused *in utero*, and as such is relatively larger than in adults and better able to withstand hypoxic insults. At birth, the left umbilical vein becomes the ligamentum teres, and the ductus venosus becomes the ligamentum venosum. Hepatic artery branches appear later in development, emerging in portal tracts (Figure 1.2) first near the hilum and then toward the periphery. This spatial and temporal sequence mirrors that seen in the developing bile ducts. The artery appears before the definitive bile duct and may be formed at least in part from portal constituents, specifically myofibroblasts, rather than growing into the portal tracts from the hilum.[8]

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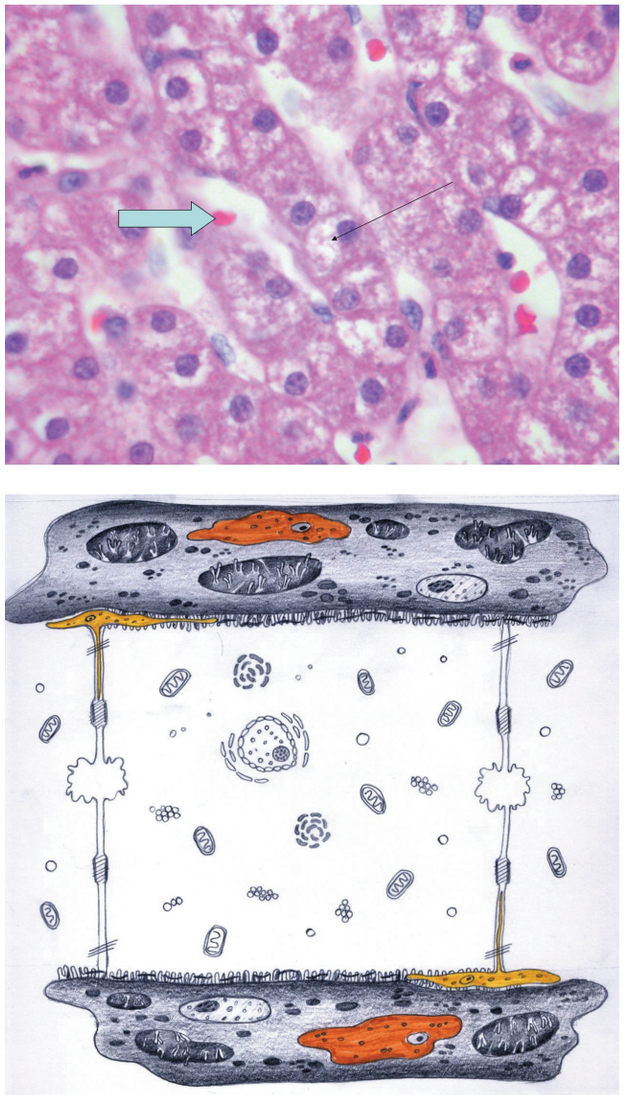

Figure 1.1 Mature hepatic plates and sinusoids. **A** Mature hepatic plates and sinusoids are easily identified on light microscopy. The small black arrow shows a hepatocyte in a plate one cell thick, while the large blue arrow shows an erythrocyte in a sinusoid. (Hematoxylin–eosin, original magnification × 400.) **B** Schematic view of the cellular arrangement of the hepatocyte in the hepatic plate, with stellate cells (yellow) located in the space of Disse and Kupffer cells (brown) in the sinusoidal lumen.

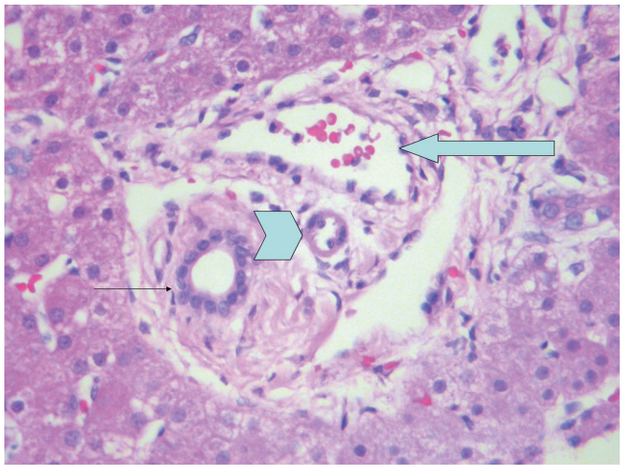

Figure 1.2 Normal portal tract. The normal portal tract consists of the hepatic artery (blue arrowhead), the portal vein branch (blue arrow), and bile duct (small black arrow). (Hematoxylin–eosin, original magnification × 200.)

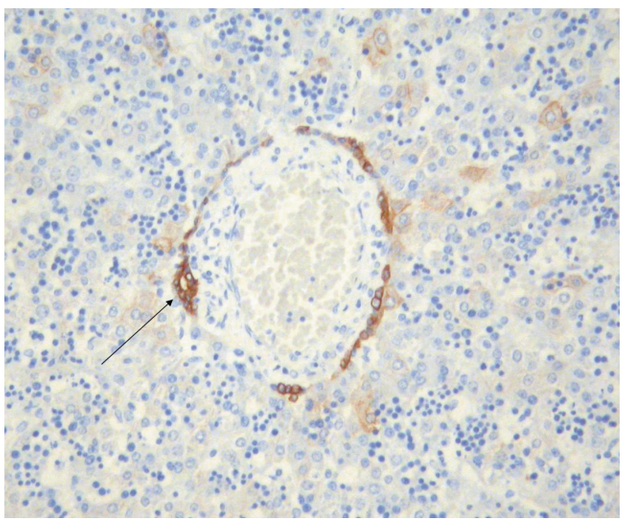

Figure 1.3 The ductal plate. The oval-shaped ductal plate highlighted in this 17-week fetus on cytokeratin immunohistochemistry (AE1/AE3) is undergoing the process of remodeling. A tubular structure has formed within the ductal plate (arrow), which will subsequently become incorporated into the developing portal tract to occupy a position as seen in Figure 1.2. (Original magnification × 200.)

Biliary development

The extrahepatic and intrahepatic biliary systems develop from the endoderm as two independent subunits, which merge at the end of the developmental process. The extrahepatic bile ducts and gallbladder develop from the elongated stalk of the hepatic diverticulum as the duodenum withdraws from the septum transversum. Formation of the intrahepatic bile duct system begins around the eighth week of gestation. The hepatoblasts around the margins of the mesenchyme of the portal tracts become smaller and strongly express cytokeratins (intermediate cytoskeletal components, of which there are many types). This sleeve of cells surrounding the portal vein branch, with its associated mesenchyme, is the *ductal plate* (Figure 1.3).

A discontinuous second layer of cells now forms around the first, resulting in a double layer around variable stretches of the portal perimeter. Within this double layer, slit-like lumens appear. The cells destined to form ducts express biliary-

type cytokeratins, identifiable by immunohistochemistry; hepatoblasts not involved in the evolution of the ductal plate differentiate toward mature hepatocytes and express different cytokeratins. The early liver cells are bipotential, capable of differentiating into biliary epithelial cells or mature hepatocytes. Contact with the portal mesenchyme orchestrates the differentiation toward biliary epithelium; the portal myofibroblasts have been implicated specifically in this process.[8] Signals include bone morphogenic protein and transforming growth factor-β.[3] The unique nature of the portal mesenchyme in inducing this differentiation is evidenced by the fact that ductal plates do not form around the central veins. From 12 weeks' gestation onward, the ductal plate is *remodeled*.

Both ductal plate development and its subsequent remodeling begin in the largest portal areas near the hilum and proceed outward toward the smaller portal tracts.[9] The tubular structures that have formed in the double-layered ductal plate become surrounded by portal mesenchyme and separated from the parenchyma. Connections are retained between the newly forming duct in the portal tract and the ductal plate and hence to the canaliculi (canals of Hering). As only a single duct persists, remodeling requires the disappearance of unwanted elements of the ductal plate by apoptosis. Failure of the precise scheme of spatial and temporal remodeling leads to persistence of the ductal plate, known as "ductal plate malformation," which can affect any caliber of portal tract.[10] Periportal cells may retain the ability to differentiate toward bile duct epithelium, seen as the ductules that appear at the portal tract margins in biliary diseases. Speculation surrounds the origin of the ductules—either from metaplasia of mature hepatocytes or biliary epithelial cells, or from progenitor cells located in the canals of Hering, possibly of bone-marrow origin.[5,11] This topic is further considered in the section on regeneration below.

At term, the remodeling process has only just reached the smallest peripheral portal tracts, where a ductal plate may therefore persist. Canaliculi appear as intercellular spaces between hepatocytes before bile secretion begins,[12] from about 12 weeks, and the intrahepatic biliary system is in luminal continuity with the extrahepatic bile duct. However, the proliferation and development of the intrahepatic biliary system is not complete by 40 weeks of gestation, and bile duct genesis continues postpartum. The number of bile ducts per portal tract continues to increase and only reaches the adult 1 : 1 pairing of hepatic arteries and bile ducts per portal tract at about 15 years of age.[13–15]

Mature macroanatomy

The liver occupies most of the right upper quadrant of the abdomen. Physical examination demarcates the borders of a normal liver in the midclavicular line, from the fifth intercostal space to just below the costal margin. In infants, a liver palpable below the right costal margin is normal. A normal liver span on percussion and palpation can be estimated as:

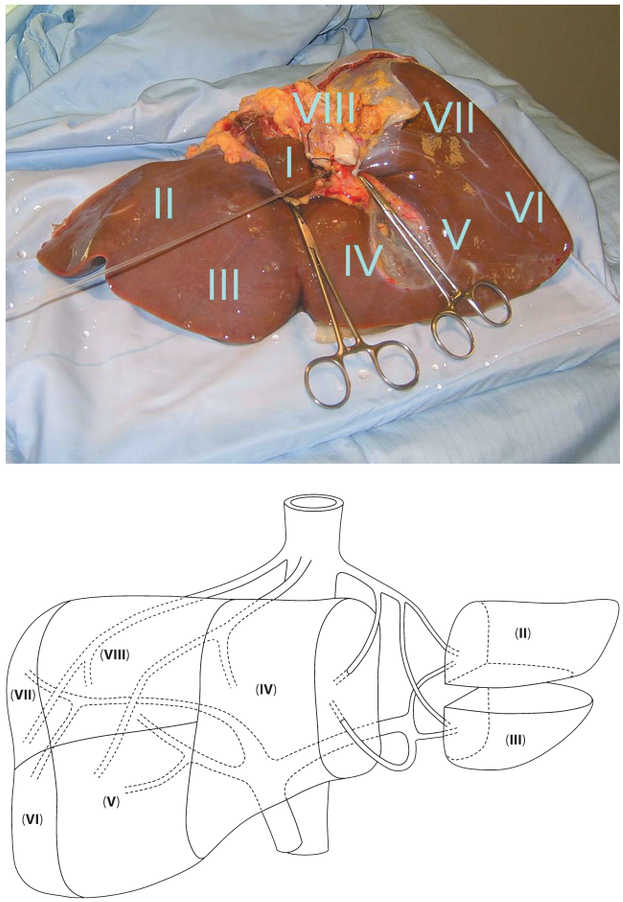

Figure 1.4 Segmental anatomy of the liver. **A** Dorsoposterior view of a normal adult liver. All segments can be seen only from this perspective. **B** Schematic view of the anterior aspect of a normal liver. The retrograde blood supply of segment IV is shown, which is of relevance in split-liver techniques in liver transplantation. Segments II and III are also used for reduction hepatectomies and living related donor transplantation.

- < 1 year: 4–5 cm
- 1–5 years: 6–7 cm
- 5–12 years: 8–9 cm

A prominent left lobe that is palpable in the epigastrium may be normal in infants, but in older children is suggestive of pathology.

The macroscopic division of the liver into the right, left, quadrate, and caudate lobes does not correspond to the segmental organization into eight segments (Figure 1.4). The right and left lobes of the liver are defined by the principal plane, or "Cantlie's line," from the gallbladder bed anteriorly to the left side of the inferior vena cava posteriorly and between the right and left branch of the portal vein, with the quadrate lobe and most of the caudate lobe functionally belonging to the left hemiliver.[16] The right and left halves of the liver are further subdivided into two sectors by the right and left fissures, which roughly correspond to the positions

of the right and left hepatic veins. The shape of the left lateral segment (segments III and II) varies greatly between a thin, "flatfish" lobe and a short, thick lobe—particularly segment III—or "blowfish" shape. This has particular relevance in monosegmental liver transplantation.[17]

More important than the topographic description of macroscopically visible lobes is the segmental organization of the liver, which provides the basis for all major liver surgery, including liver transplantation.[18,19] The caudate lobe is segment I, and the remainder of the segments are labeled according to their clockwise position. Each segment has its own independent vascular and biliary supply, which is surrounded by a fibrous sheath, the extension of Glisson's capsule.[20] Partial hepatectomies for tumor surgery or liver transplantation follow these segmental borders and are different from the traditional lobar macroanatomy.[21,22]

Portal venous anatomy

The portal vein, a valveless vein, drains blood from the splanchnic area and commences behind the neck of the pancreas as a cranial continuation of confluence of the superior mesenteric vein and the splenic vein. It is interesting that in several animal species, the portal vein takes a helical or spiral shape. This has been less well documented in humans.[23] The significance of this helical structure and the implications for its effect on blood flow have yet to be defined, but the structure has been documented using color duplex Doppler ultrasonography.[24] The portal vein is also unique in that, instead of the normal pattern of solely circular smooth-muscle fibers, there are two distinct muscle layers: a relatively thin inner layer consisting of circular smooth-muscle cells, resembling the normal media of a vein, and an outer layer of longitudinal muscle with abundant vasa vasorum—architecture that closely resembles that of the gastrointestinal tract.[25] The portal vein branches in an extrahepatic position at the hilum into a right and left portal vein; the latter supplies the caudate and quadrate lobe before it enters the parenchyma. The venous return from the gallbladder drains into the right branch of the portal vein. Each segment of the liver is supplied by its own branch of the portal vein. Anomalies of the portal vein are rare, but those most frequently seen are an abnormal position anterior to the head of the pancreas, typically associated with the biliary atresia and splenic malformation syndrome (absent intrahepatic inferior vena cava, polysplenia, situs inversus, and malrotation) and an abnormal communication with the inferior vena cava, resulting in a congenital portocaval shunt (Abernethy syndrome).[26]

Hepatic artery anatomy

The arterial supply to the liver and biliary tree is notorious for the variation in its origin and course relative to the surrounding anatomy, due to the complex embryological development of the celiac and superior mesenteric arteries.[27] The usual arrangement of the hepatic artery, originating from the celiac axis and dividing into a right and left branch above the level of the gastroduodenal artery, is only present in about 60% of cases. In about 25% of individuals, the right hepatic artery arises from the superior mesenteric artery and may act as a fully replaced right hepatic artery or as an accessory artery. This artery runs through the head of the pancreas and lies posterior to the common bile duct and has particular relevance in its supply to the right liver bile ducts and gallbladder.

In a similar proportion of individuals, the left lobe of the liver may be partially or completely supplied by an artery arising from the left gastric artery, which runs in the gastrohepatic omentum and enters the hilar plate at the level of the umbilical fissure. Other less common anomalies are a very short common hepatic artery with long right and left arteries, with the gastroduodenal artery arising from the right hepatic artery or even arising separately from the celiac trunk.

The blood supply to the bile ducts is entirely arterial and may be divided anatomically into hilar, supraduodenal, and pancreatic sections. The blood supply to the mid-portion of the common duct is axial, with a 3-o'clock and a 9-o'clock artery running alongside the duct, receiving an average of eight contributions from all of the surrounding named vessels. There is a 60% contribution from the gastroduodenal artery and 40% from the right hepatic artery. An additional supply to the supraduodenal duct is a consistent retroportal artery, arising from the celiac axis or superior mesenteric artery close to their origin from the aorta.[28] These all form a plexus of vessels surrounding the bile ducts, which extend into the liver. The ducts at the hilum receive blood from the right and left hepatic arteries and multiple small vessels that enter the caudate lobe. These vessels may be arranged in an arcade pattern, suggesting good collateral supply, or in a tree-like fashion from either the left or right hepatic arteries. It is also important to note the frequency of segment IV arterial supply either from the right, proper, or left hepatic artery, which has important implications for split-liver transplantation. From corrosion-cast studies, it is obvious that a very important role for the hepatic arteries is the nourishment of the biliary system, and impairment of this blood supply will lead to ischemic consequences, with necrosis or stricture.[28,29]

Hepatic vein anatomy

The hepatic venous anatomy is relatively simple, as there are three main hepatic veins, which lie above the portal structures within the liver. They divide the liver into sectors along an oblique plane; thus, the right hepatic vein divides the right lobe of the liver into posterolateral and anteromedial sectors; the middle hepatic vein separates the liver into right and left, and the left hepatic vein also divides the liver into a posterolateral sector (segment II) and an anteromedial sector (segments IV and III). The caudate lobe also has bilateral drainage with a relatively clear median plane, with direct venous channels into the inferior vena cava—more on the left, as this part of the caudate lobe is the larger and more consistent.

The right hepatic vein may not be dominant, and much of the right posterior sector may drain into the inferior vena cava (IVC) as a large accessory, caudally placed vein. There is a short extrahepatic course, and in 60% of cases there are no branches just before joining the IVC, which lends itself to separate dissection and ligation.

There are multiple other "dorsal" hepatic veins that drain directly into the IVC, which are thin-walled and fragile and require delicate ligation during right hepatectomy. The middle hepatic vein drains into the left hepatic vein within the liver substance, resulting in a common confluence in most cases, and receives branches from the right and left liver to a variable extent—mainly segments V, IVb, and VIII. This venous drainage area becomes crucially important in living-donor right liver transplants, as adequate drainage must be ensured for the donor (segment IV) as well as the graft (segments V and VIII) (Figure 1.4).

Biliary anatomy

The interlobular or terminal bile ducts belong to the portal triad and have a diameter of < 100 μm. They are accompanied by arterial vessels, which supply oxygenated blood to the bile ducts and also play a role in the immediate reabsorption of organic compounds from primary bile into the general circulation. Bile is then drained into the septal, segmental, and right or left hepatic ducts. The left hepatic duct drains segments II, III, and IV, and the right hepatic duct drains segments V, VI, VII, and VIII. Segment I, the caudate lobe, has its own biliary drainage. Variations of this are common, and in 78% of individuals the caudate lobe drains into both the left and right hepatic duct.[30] The right and left hepatic ducts join to form the common hepatic duct. The left hepatic duct lies predominantly outside the liver parenchyma, and this can be used to advantage in dealing with more distal bile duct strictures.[31]

An important and common anomaly is for the right sectional (sectoral) duct to cross to the left and drain into the left hepatic duct. There is considerable variation in ductal anomalies, which are recorded in textbooks of anatomy and surgery. In about 70% of cases, there is a clear right–left confluence, and in 12% there is a trifurcation of the ducts at the porta hepatis,[32] but many patterns of drainage are discernible. The right hepatic posterior and anterior sectoral ducts may drain separately at different levels or may join the left duct, as mentioned. A right posterior sectoral duct may join the hepatic duct as low as the insertion of the cystic duct or may even drain into the gallbladder.

The cystic duct joins the hepatic duct in most cases at an acute angle on the right side. However, the level of insertion is variable and may be anterior or on the left, with a spiral or parallel configuration around the duct. The term "hepatocystic triangle" describes the inferolateral base, with the cystic duct and hepatic duct medially and the inferior surface of the liver superiorly. Calot's triangle is the inferior part of this, with the cystic artery as the base of the inverted triangle.[33] The cystic duct drains the gallbladder, which lies in the median plane between the two functioning halves of the liver on its anterior undersurface. The length and diameter of the cystic duct also vary greatly—from 4 mm to 65 mm in length and from 3 mm to 9 mm in diameter.

The gallbladder lies wrapped in the extension of Glisson's capsule and may be embedded within the liver substance to a variable degree, or may even have a mesentery of its own suspended from the undersurface of the liver.

The common bile duct, with a mean diameter of 6 mm in adults, passes distally behind the duodenum and sometimes through the pancreas to reach its destination in the mid-second part of the duodenum, surrounded by sphincter muscle. At its terminal portion, it is joined by the pancreatic duct, with a short common channel in most cases. However, not infrequently there may be pancreaticobiliary malunion with a long common channel, which is associated with choledochal dilation and cystic change due to pancreatic juice reflux (see the section on choledochal cysts in Chapter 19, pp. 441–444).

Lymphatics

Hepatic lymph is generated in the space of Disse, which is continuous with the lymph vessels. Lymphatic vessels originate in the connective-tissue spaces within the portal tracts and flow toward the hepatic hilum. Lymphatics in the hepatic capsule drain to vessels either at the hilum or around the hepatic veins and inferior vena cava and eventually into the thoracic duct.[1]

Microanatomy

Microanatomy is intimately related to function and is best considered by linking individual cellular constituents and their local relationships with function. Blood from the hepatic artery and portal vein needs to come into intimate contact with hepatocytes to allow the metabolism of dietary molecules and detoxification of compounds, and to distribute the diverse proteins synthesized by the liver. In order for the liver to fulfill its exocrine function, bile secreted into intercellular canaliculi has to find its way to the biliary duct system and ultimately to the intestine. These functions require a complex interaction between individual cells, as well as regulation of blood supply and innervation. The way in which groups of cells are organized into "functional units" has been the subject of much debate and is discussed further below.

Cellular constituents of the liver

The liver parenchyma consists of a number of different cell types. About 80% are hepatocytes; biliary epithelial cells account for 1%, sinusoidal endothelium 10%, Kupffer cells (hepatic macrophages) 4%, and lymphocytes 5%.

Hepatocytes, arranged in branched and anastomosing cords, are between 30 and 40 μm in size. In keeping with

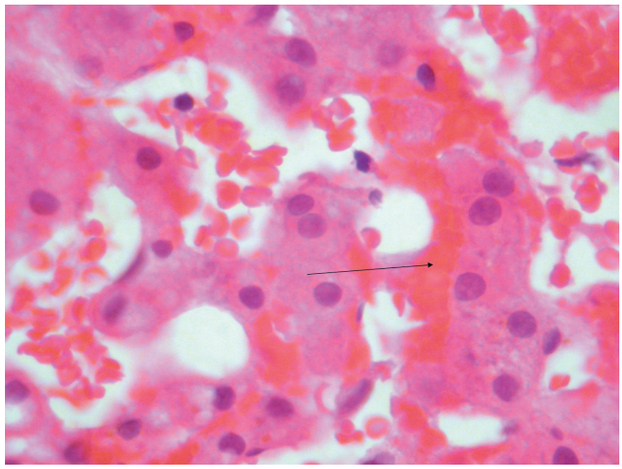

Figure 1.5 The space of Disse. Liver histology in a child with Budd–Chiari syndrome. The space of Disse is not normally visible, but in this image from a patient with Budd–Chiari syndrome, blood has been forced into the space of Disse and renders it visible. (Hematoxylin–eosin, original magnification × 400.)

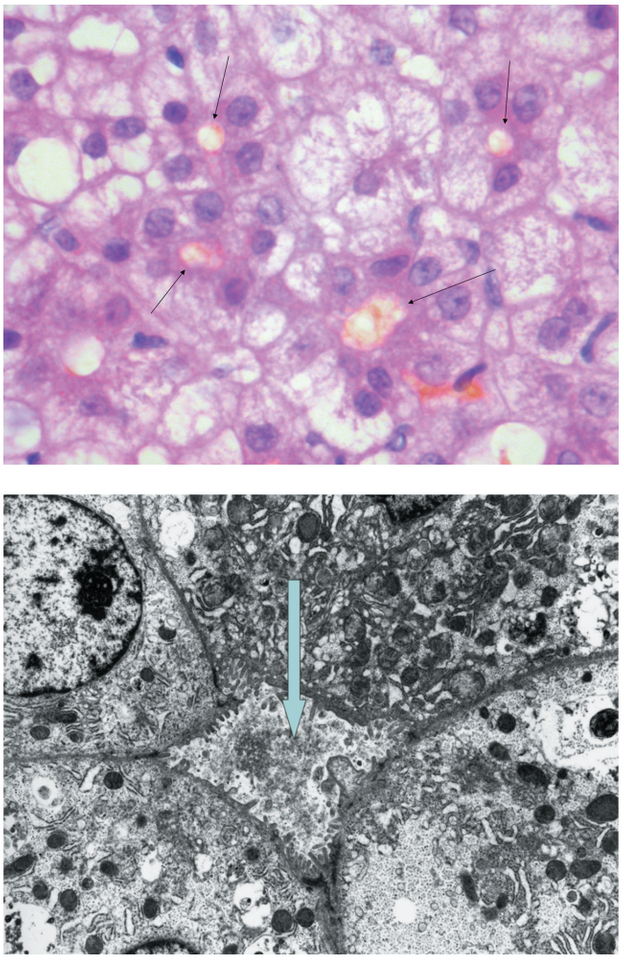

Figure 1.6 Bile canaliculi in cholestatic liver disease. **A** Canaliculi in a child with neonatal cholestasis. The canaliculi are not visible in the normal liver. In this child with neonatal hepatitis, they are distended by bile plugs, making them prominent (arrows). (Hematoxylin–eosin, original magnification × 400.) **B** Electron microscopy of a canaliculus. The arrow shows granular bile in a canaliculus in a child on parenteral nutrition. There are microvilli lining the edge of the canaliculus.

their diverse functions, they are rich in organelles, up to 1000 mitochondria can be seen in a single cell, and apparatus for protein production is also abundant (e.g., endoplasmic reticulum and Golgi complex).[6,32] Particulate glycogen forms much of the "background" of the cell. The hepatocytes have different surfaces or "domains," where they abut other hepatocytes, with which they communicate via gap junctions (lateral domain). The basal domain is where the hepatocyte contacts blood in the sinusoid, and the apical domain forms the canaliculus. The latter two domains are covered with microvilli, providing an enlarged surface area. The sinusoids are lined by a specialized endothelium, which has fenestrae (apertures) to facilitate the transfer of molecules and particles. The sinusoidal endothelium lacks a basement membrane, further facilitating exchange between blood and hepatocyte.

Between the endothelial cells and the basal aspect of the hepatocytes lies the space of Disse (Figure 1.5). This is not normally visible with light microscopy, but can be seen if there is hepatic venous obstruction. The space of Disse contains extracellular matrix components, including type IV collagen, laminin, and proteoglycans. This matrix is not merely an extracellular scaffold, but also interacts via adhesion molecules with the hepatocytes. The extracellular matrix can modulate the cell phenotype and serves as a reservoir for cell growth factors, cytokines, and albumin, which can be released by matrix degradation.

The apical domain constitutes 15% of the hepatocyte cell membrane and forms the bile canaliculus. Again, canaliculi are not normally visible on light microscopy, but become so in cholestatic disease (Figure 1.6). The canaliculus is delineated from the third (lateral) domain by tight junctions. The bile canaliculi constitute the outermost reaches of the biliary tree. They are spaces 1–2 μm wide, which are interconnected and form a network of intercellular channels, which receive the bile secreted from hepatocytes. Actin and myosin filaments of the hepatocyte propel the bile into the canals of Hering (ductules or cholangioles), which are lined with a mixture of biliary epithelium and hepatocytes.[34] They have a diameter of less than 15 μm and are located at the periphery of a portal triad. They are not visible with routine light microscopy.

Kupffer cells are located on the luminal side of the endothelial wall. They have a phagocytic function and are also an

important source of cytokine secretion. Hepatic stellate cells (previously known as Ito cells) produce extracellular matrix, store vitamin A and lipid, and have fine extensions surrounding the sinusoids, possibly related to control of vascular tone. When activated, they transform into myofibroblasts and have an important role in fibrosis.

Functional anatomy/regulation of blood supply

The dual blood supply to the liver, by the hepatic artery and portal vein, is almost unique in the body. In resting conditions, the liver receives about a quarter of the cardiac output. About 30% of this hepatic inflow is oxygen-rich blood via the hepatic artery; the remaining 70% is nutrient-loaded blood from the intestine and spleen, supplied by the portal vein. Arterial and portal blood mixes freely at the level of the sinusoids. Total blood flow into the liver varies considerably and is reduced during sympathetic stimulation or sleep. In contrast, portal blood flow increases following a meal. It is most stimulated by a protein-rich feed and only moderately by carbohydrates, with little effect following lipids. The arterial blood supply is not determined by oxygen demand. In normal livers, only half of the oxygen supplied is extracted, and in situations in which metabolic rates increase, oxygen extraction rises without an increase in arterial flow. Portal and arterial flow are closely related, and an experimental reduction of portal flow in dogs resulted in arterial hyperemia. Clinically, this phenomenon becomes apparent in liver transplantation, when thrombosis of either the hepatic artery or the portal vein leads to compensatory flow rates in the other vessel.

About 20–25% of the normal liver consists of blood, which is situated in the large vessels. This is about 10–15% of the body's total blood volume, and the liver thus serves as a reservoir with capacitance function. Liver blood volume can increase by 4% for each 1-mmHg increase in hepatic venous pressure and may be tripled to about 60% in states of severe outflow obstruction. In hemorrhagic shock, in sympathetic stimulation, and in vascular dehydration, the liver can replace systemic volume rapidly. In animal studies, it has been observed that 7% of the total blood volume can be replaced from the liver, while in dogs a 60% decrease in liver blood content can be achieved within seconds by sympathetic stimulation.

Portal vein perfusion pressure is approximately 6–10 mmHg. Arterial perfusion pressures depend on systemic perfusion pressures. The sinusoidal perfusion pressure is determined by a number of factors in the afferent and efferent vessels, including muscular sphincter, autonomic nervous innervation, and paracrine function.

In the normal liver, the sinusoids consist of a fenestrated endothelial capillary that receives blood from arterioles and venules, with a perfusion pressure of 2–4 mmHg. The distribution of blood flow in the sinusoids is determined by variation in the size of the Kupffer and endothelial cells, which swell and shrink to control the patency of the sinusoidal lumen. The role of stellate cells, which are thought to be myofibroblasts, remains unclear, although their ability to induce fibrosis appears to be the dominant effect on sinusoidal perfusion in states of liver disease.

Functional versus anatomical units

In the absence of connective-tissue septa delineating structural units, different models have been used to define the smallest functional unit in the liver (Figure 1.7):

- The *classic lobule*, hexagonal in shape, was described in 1833.[35] It corresponds to the unit that is outlined by connective tissue in other species, such as the pig. It has a hepatic vein branch ("central vein") at its center. Blood arriving in the portal tracts at the periphery of the hexagon will feed sinusoids around the whole of their circumference, rather than all draining into the interior of the hexagon. It therefore has limited application as a true primary unit. The *primary lobule*, described by Matsumoto *et al.*,[36] uses the portal vein branches to act as the center of the functional unit, giving rise to tortuous and branching three-dimensional units surrounding portal vein branches, but it does include the *classic lobule* as a secondary structure.[6,32] This model is based on actual vascular reconstruction (rather than the gelatin infusions used in the acinar concept, below) and is gaining widespread acceptance.[37] Descriptive histology in the lobular models hence includes such terms as "centrilobular" hepatocytes (those around the central vein).
- The work of Rappaport *et al.* in 1954 defined the functional unit as an acinus.[38] The axis of the acinus is formed by the terminal branch of the portal vein, not visible in routine microscopy. The three zones of the acinar concept are illustrated in Figure 1.7. Descriptive histology in the acinar concept refers to these three acinar zones, and it should be noted that these do not equate to the regions described in the lobular concept. "Acinar zone 3" is not exclusively "perivenular," but rather extends in an arc-like fashion from one portal tract to another. The acinar concept proved popular for pathologists from an observational point of view. In severe liver damage, necrosis is presumed to occur in the least well oxygenated, most vulnerable hepatocytes first. In the lobular concept, the least well oxygenated hepatocytes would be centrilobular, and necrosis would therefore be seen in the perivenular region exclusively. In practice, however, necrosis occurs in a portal–central distribution, and this corresponds to the most peripheral acinar regions (zone 3; Figure 1.7). Many studies of functional heterogeneity within the liver do not support the acinar concept, however, and as mentioned above, the *primary lobule*, based on the actual branching of the portal vein, is gaining in popularity.

However the functional unit is defined, the function of the hepatocytes, sinusoidal endothelium, Kupffer cells, and

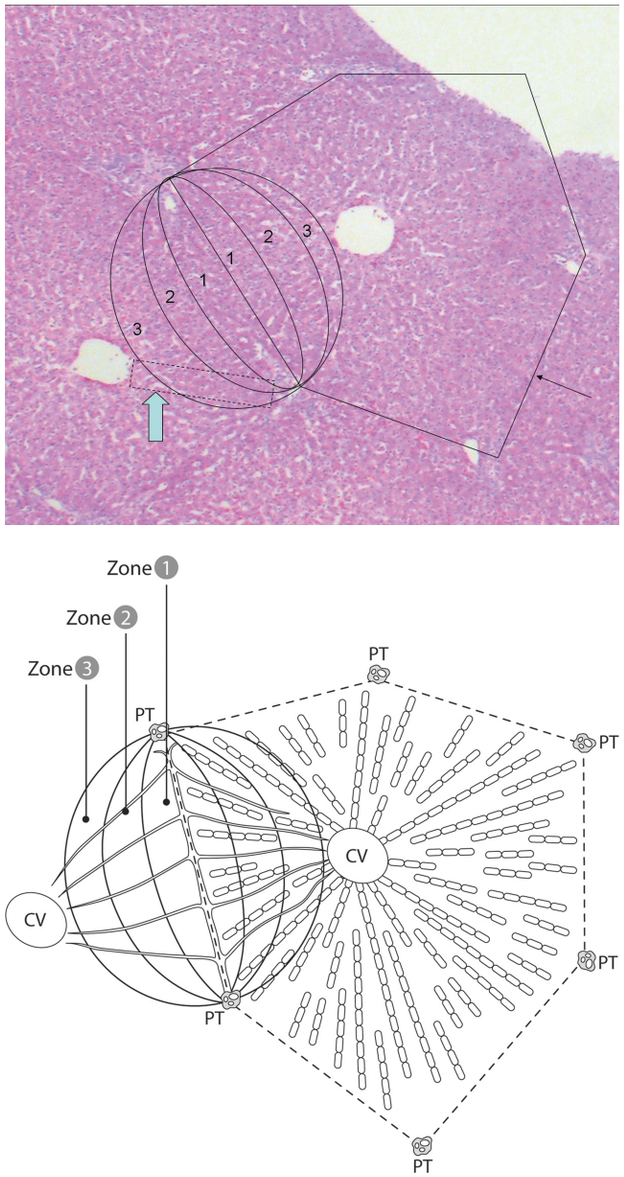

Figure 1.7 **A** Light microscopy of normal liver tissue. The small arrow points to the approximate outline of a classic hepatic lobule, centered around a central vein. In schematic diagrams, this is often illustrated as a regular hexagon, with portal tracts at four points and "nodal points of mall" at the other two. This is rarely reproducible in practice, leading to the slightly irregular hexagon shown. The elliptical structure denotes postulated acinar zones 1, 2, and 3, centered around a terminal portal venule (not visible). This occupies portions of two adjacent classic lobules. The dotted rectangle shows the location of portal central bridging necrosis, which is observed in the clinical situation and which made the acinar concept popular from a pathological point of view. (Hematoxylin–eosin, original magnification × 40). **B** Schematic view of the same anatomical and functional units of the liver.
CV, central vein; PT, portal tract.

extracellular matrix composition, varies between regions. "Periportal," "perivenular," and—although it does not correspond to a true acinar zone—"midzonal" serve as useful descriptors for considering functional differences or gradients. Gene expression also shows a functional gradient.[32] The phenotypic variation may be determined by the declining gradient in oxygen concentration, the decreasing glucagon–insulin ratio, or other autocrine signals. Periportal hepatocytes are responsible for oxidative energy metabolism, such as beta-oxidation and amino acid catabolism, bile formation, and cholesterol synthesis. Perivenous hepatocytes are involved in glucose uptake for glycogen synthesis, glycolysis, liponeogenesis, and ketogenesis.

Innervation

The liver is innervated by the autonomic nervous system, through sympathetic nerve fibers from the celiac ganglia and some parasympathetic input from the vagus nerve. Sympathetic nerves supply a dense perivascular plexus around the hilar blood vessels into the sinusoids, where nerves course in the space of Disse and surround isolated hepatocytes and stellate cells. Parasympathetic nerve fibers accompany the hepatic inflow system, forming a plexus around hepatic artery and portal vein, but there is little cholinergic innervation beyond the portal tract.[39]

It has been suggested that gap junctions may also provide direct electrical coupling between cells, bypassing the need for nervous innervation. Cholinergic stimuli increase metabolic activity, whereas adrenergic stimuli increase glucose mobilization into the blood. The realization that hepatic function is effective even in the denervated graft following liver transplantation has challenged long-standing views about the role of the autonomic nervous system in regulating metabolic activity in the liver. More recent studies have suggested that α-adrenergic innervation is involved in hepatocyte replication.

Function

The liver is the central organ for metabolic homeostasis. Its main functions are:

- Regulation of uptake and processing of nutrients from the intestinal tract
- Synthesis and biotransformation of proteins, carbohydrates, and lipids
- Excretion of bile and elimination of hydrophobic compounds
- Regulation of energy metabolism
- Endocrine functions and mediation of normal growth and development
- Immunological function
- Drug metabolism
- Regulation of fluid balance

Uptake and processing (synthesis, storage and degradation) of proteins, carbohydrates, and lipids

Proteins

The liver accounts for 15% of total body protein production, and the majority of these proteins are secreted as plasma proteins. Proteins are synthesized following the activation of genetic promoter sequences by transcription factors. Following translation and modification, proteins are secreted from the sinusoidal aspect of the hepatocytes into the circulation. Nutritional status and hormone secretion regulate the level of protein production. There is a surge of protein production in acute illnesses—the acute-phase response, in which C-reactive protein is the most commonly measured sign. The liver is responsible for synthesizing many proteins, such as albumin, transport proteins such as ceruloplasmin, coagulation and fibrinolytic proteins, complement, and protease inhibitors. Proteins are not stored in the liver, but amino acids are recycled to synthesize new molecules. The liver also plays a role in protein and glycoprotein degradation. Amino acid degradation takes place in the liver, generating the highly toxic metabolite ammonia, which is associated with hepatic encephalopathy (see Chapters 7 and 15). The urea cycle, which is active almost exclusively in the liver, is largely responsible for its removal, and urea cycle defects present with severe encephalopathy (see Chapters 5 and 13).

Carbohydrates

Glucose, fructose, and galactose are taken up by the hepatocytes from portal blood. Glucose is converted to glucose-6-phosphate and used to replenish glycogen stores, or else used in triglyceride production. The liver, under the influence of hormones—principally insulin (which reduces glucose output) and glucagon (which increases glucose output)—has a major role in maintaining blood glucose. Glucose is either released from glycogen (glycogenolysis) or synthesized from substrates such as lactate (gluconeogenesis). In conditions of stress or fasting, glucose uptake is reduced and glucose production is increased from glycogenolysis. Hypoglycemia is a sensitive test of liver function and is a sign of severe hepatic necrosis, indicating loss of liver function (see Chapter 7). For the same reason, many infants with severe liver disease are unable to maintain their blood sugar levels during prolonged fasts.

Lipids

The liver is essential for cholesterol and lipoprotein metabolism. Cholesterol is a component of all cell membranes and is essential for the production of steroid hormones and bile acids. Cholesterol homeostasis is controlled by uptake from lipoproteins and chylomicrons, which increase hepatic cholesterol, and by the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA), which synthesizes cholesterol *de novo*. The amount synthesized in the liver is twice that absorbed from the diet. In the liver, cholesterol is either "free" or stored as cholesterol ester. The degradation of cholesterol takes place through the synthesis of bile acids and biliary excretion of cholesterol (see below). A number of cholestatic liver diseases (e.g., biliary atresia or Alagille's syndrome) lead to elevated plasma cholesterol due to deficient biliary excretion and catabolism.

Chylomicrons, which transport water-insoluble lipids, carry dietary fat from the intestine to the circulation. They deliver triglycerides to peripheral tissues, and the resulting cholesterol-rich chylomicron remnant is taken up by the liver. The liver also synthesizes fatty acids from glucose in times of dietary excess, and these are subsequently stored as triglycerides, which are the principal source of energy. Fatty acids that are not converted to triglycerides or used in the synthesis of other molecules are oxidized, following modification, to ketone bodies in the mitochondria, or in the case of very-long-chain fatty acids in the peroxisomes. Microvesicular steatosis in hepatocytes is a sign of mitochondrial or peroxisomal disease or drug toxicity (see Chapters 5, 9, and 13).

Very-low-density lipoproteins (VLDLs) are the main lipoproteins secreted by the liver and carry triglyceride and cholesterol to other tissues, where they are converted to low-density lipoproteins (LDLs). High-density lipoproteins (HDLs) carry cholesterol from the peripheral tissues back to the liver. Fatty liver occurs when the synthesis of triglycerides exceeds the liver's capacity for export or internal metabolism.

Bile and bile acids

Bile is produced in hepatocytes and is modified in the bile ducts. In adults, about 600 mL of isotonic watery bile with a pH of 7.8 is produced daily in order to facilitate the excretion of many compounds, including drugs, toxins, and waste products, and to provide bile salts to the intestine for the emulsification and absorption of dietary lipids. Bile formation is an osmotic process and is traditionally classified as "bile salt–dependent" (the relationship of canalicular bile flow to bile salt excretion) and "bile salt–independent" (the active secretion of electrolytes and other solutes).

The main components of bile are bile acids (12%), phospholipids (4%), cholesterol (0.7%), and conjugated bilirubin (0.1%). Lecithin increases the solubility of cholesterol in bile by micelle formation exponentially to allow a 10-fold concentration of bile acids and cholesterol in the gallbladder. Of the electrolytes in bile, only sodium is concentrated to about 280 mmol/L; other electrolytes and bicarbonate are less concentrated, or unchanged. The primary bile acids—cholic acid and chenodeoxycholic acid—are synthesized from cholesterol by 7α-hydroxylase and subsequently conjugated with taurine and glycine to enhance affinity to both acids and bases ("amphophilia").

Primary bile salts are transformed by intestinal bacteria into secondary bile salts—cholic acid into deoxycholic acid

and chenodeoxycholic acid into lithocholic acid and subsequently to ursodeoxycholic acid (UDCA). They are reabsorbed in the ileum and returned to the liver via the portal vein. In normal conditions, UDCA represents only 3% of the bile salt pool. It is more hydrophilic than the other bile salts and is used therapeutically to stimulate bile secretion; it may prevent the hepatocytes from damage caused by hydrophobic bile salts. Only lithocholic acid is poorly reabsorbed and excreted, so that the gallbladder bile consists of the four bile salts at a ratio of 10 : 10 : 5 : 1. In chronic liver disease, this balance is shifted to a predominant production of chenodeoxycholic acid, which lowers the bile pH.

Hepatic bile formation and the biliary excretory function are closely related. The rate of bile flow is determined by the enterohepatic circulation of bile salts and by the rate of secretion of bile salts, cholesterol, phospholipids, and glutathione, which means that if bile excretion is impaired in liver disease, there is reduced excretion of both endogenous and exogenous compounds. Bile salts are the main organic solutes in bile, and their active transport against a 1000-fold concentration gradient into the bile canaliculus is the driving force for hepatic bile formation. In adults, the enterohepatic circulation of bile salts occurs more than six to eight times in 24 h, enabling the body to retain most of the 5–6 g in the body bile salt pool. Neonates have about half the bile salt pool of an adult, and ileal bile salt reabsorption is lower. Their intestinal bile acid concentration may be low, leading to poor micelle formation and reduced uptake of fat-soluble vitamins and dietary lipid in comparison with older children and adults. Although this is rarely a cause of malnutrition and/or steatorrhea, it needs to be considered in cholestatic conditions when early supplementation of fat-soluble vitamins is indicated. Bile acid uptake from portal blood is physiologically lower in neonates in comparison with older children, and elevated levels of bile acids may be mistaken for cholestatic liver disease.

Intrahepatic and extrahepatic bile salt transport

The transport processes for bile salts are complex, and—despite the recent discovery of different membrane-bound bile salt transporters—they are still not fully understood. Hepatocytes are polarized cells that absorb substrates from the blood in the sinusoids, such as bile salts, phospholipids, and metabolites of toxic substances, and transport them across the cell to the canalicular membrane to secrete into bile. The sinusoidal uptake of conjugated bile salts (e.g., taurocholate) by hepatocytes at the basolateral plasma membrane is mediated by an active transport process driven by a sodium gradient via the sodium-dependent transporter for the uptake of bile salts, sodium taurocholate cotransporting polypeptide. The uptake of unconjugated bile salts at the sinusoidal membrane is sodium-independent and mediated by the organic anion transporting polypeptide. This transporter also transports steroids such as progesterone and cyclosporine. After uptake into hepatocytes, the intracellular transport across the cell is thought to be mediated by binding to cytosolic proteins, ligandins, and Y9 proteins or fatty acid–binding proteins. Some free intracellular bile salts reach the canalicular plasma membrane by diffusion.

Excretion of bile salts across the canalicular plasma membrane is the rate-limiting step in the transport of bile salts from blood into bile. Canalicular secretion of monovalent bile salts is facilitated by the adenosine triphosphate–dependent "bile salt excreting pump" (BSEP).[40] A defect in this transporter is responsible for the genetic condition known as progressive familial intrahepatic cholestasis (PFIC). In contrast to monoanionic bile salts, divalent sulfated and glucuronidated bile salts are excreted into bile by the multidrug resistance–associated protein 2 (Mrp2), also known as canalicular multispecific organic anion transporter (cMOAT). Failure to express Mrp2 at the canalicular membrane results in conjugated hyperbilirubinemia and forms the basis of the hereditary Dubin–Johnson syndrome. Canalicular phospholipid secretion is mediated by a different transporter protein, multidrug resistance protein type 3 (Mdr3), which is important in preventing bile salt–induced toxic damage to the biliary epithelium. Failure to express this transporter results in progressive familial intrahepatic cholestasis type 3 and biliary cirrhosis[41] (see Chapter 4). FIC-1 is an aminophospholipid translocator in the canalicular membrane of hepatocytes that is also found on the apical membrane of enterocytes. It is responsible for the transport of phosphatidylserine and phosphatidylethanolamine. A genetic defect in the expression or function of this transporter causes progressive familial intrahepatic cholestasis type 1 (PFIC-1) and benign recurrent intrahepatic cholestasis type 1 (BRIC-1; see Chapters 3 and 4).

Excretion of bilirubin

As well as its role in facilitating bile salt homeostasis, the biliary system also serves as the primary pathway for eliminating bilirubin, excess cholesterol, and hydrophobic xenobiotics. About 80% of bilirubin is derived from the breakdown of erythrocytes; the remainder stems from heme-containing myoglobin, cytochromes, and other enzymes. Mononuclear phagocytic cells oxidize heme to form biliverdin, which is then reduced to bilirubin. This unconjugated bilirubin is albumin-bound, transported to the hepatic sinusoids, and actively transported into the hepatocytes via the basolateral membrane. If the unconjugated bilirubin is displaced from albumin, it may diffuse across the blood–brain barrier and cause kernicterus in neonates.

Bilirubin uridine diphosphate (UDP) glucuronyltransferase (UGT1A1) conjugation with one or two molecules of glucuronic acid in the endoplasmic reticulum converts bilirubin (conjugated bilirubin), which is excreted as hydrophilic bilirubin glucuronides via the canalicular membrane. Following intestinal excretion, bacterial beta-glucuronidases

degrade most of these bilirubin glucuronides to colorless urobilinogen. About 20% of urobilinogen is reabsorbed in the ileum and colon and returned to the liver via the portal vein. Some of this urobilinogen is excreted into the urinary tract.

UGT1A1 belongs to the UGT family of conjugating enzymes, which catalyze glucuronidation of various substrates, including steroid hormones, carcinogens, and drugs, and which are expressed in a wide range of tissues. Splicing variation of the original transcripts of the *UGT1A1* gene on chromosome 2q37 leads to different mRNAs of the enzyme. Of several isoforms, only UGT1A1 is physiologically active. Decreased activity of the enzyme in the newborn period contributes to the physiological jaundice common in the neonate. Mutations in the *UGT1A1* gene either reduce the affinity of UGT1A1 toward bilirubin or reduce enzyme activity. Complete absence of UGT1A1 activity causes Crigler–Najjar syndrome type 1, and a significant reduction of activity causes Crigler–Najjar syndrome type 2. Only a very mild reduction of UGT1A1 activity by missense mutation or reduced expression of the enzyme is present in 6% of the general population, causing Gilbert's syndrome, in which there is a mild elevation of unconjugated bilirubin (see Chapter 4).

Regulation of energy metabolism

The energy metabolism of the body is integrated by the liver through glucose metabolism and fatty acid oxidation. The liver has a central role in maintaining blood glucose homeostasis at constant levels between 3.3 and 6.1 mmol/L in order to supply glucose as an energy substrate for the brain, renal medulla, or blood cells. This glucostat function of the liver is primarily achieved by controlling the storage and release of glucose from glycogen, followed by glycolysis and gluconeogenesis. The glycogen content of a liver of a 10-kg child is around 20–25 g, increasing to about 70–80 g in an adult. As the normal resting glucose requirement is between 4 and 6 mg/kg/min, the glycogen stores last for less than a day of fasting, after which gluconeogenesis is activated. In prolonged fasting, total body glucose requirements decrease from 160 g/glucose/day to 40 g/glucose/day after 5–6 weeks of starvation in the adult. The healthy body can tolerate this, because fatty acid oxidation becomes the main source of fuel for respiration. Soskin postulated as early as 1940 that the blood glucose concentration is the primary stimulus to control glucose uptake or output.[42] Glucose uptake into the hepatocyte is insulin-independent and has a direct regulatory effect on glycogen synthesis. Conversion of excess glucose to fatty acids only takes place when hepatic glycogen stores are complete. Such fatty acids are esterified to triglycerides and exported from the liver as very-low-density lipoproteins (VLDLs). Triglycerides in VLDLs from the liver and from the intestinal absorption of lipids are hydrolyzed by lipoprotein lipase and taken up in the peripheral tissues, where fatty acids are metabolized for energy or stored.

Endocrine function

The liver plays an active role in endocrine regulation. In response to growth hormone activation, the liver produces the majority of the circulating mitosis-inducing (mitogenic) polypeptides insulin-like growth factor 1 and 2 (IGF-1 and IGF-2), which have anabolic and metabolic effects and regulate the proliferation of various cells. The specific endocrine effect of the IGFs and other hormones, such as steroid hormones, is modulated by different binding proteins (IGF-binding proteins 1–6, sex hormone–binding globulin, or thyroid-binding globulin) that are synthesized in the liver. These binding proteins transport the hormones, regulate their metabolic clearance, and directly modulate hormone interactions with specific receptors.[43,44] Thyroxine (T_4) is converted into the metabolically active form of T_3 in the liver, which accounts for the low T_3 syndrome in patients with decompensated cirrhosis. Hormonal dysfunction in liver disease may develop from reduced clearance of hormones (e.g., gynecomastia in men), from portosystemic shunting, dysregulated synthesis of binding proteins, or impaired end-organ sensitivity to the hormone—i.e., insulin resistance in cirrhosis.

Immunological function

The liver contains many lymphocytes, both of the adaptive immune system, which require previous exposure to antigen for efficacy, and also cells of the innate immune system—natural killer (NK) cells (Pit cells).[45] The liver also contains a population of cells that express both T-cell and NK-cell markers,[46] which play a role in the clearance function of the liver in filtering gut-derived endotoxins and microorganisms. Kupffer cells are macrophages that are important in the phagocytosis of particulate material and cellular debris (they are conspicuous in acute hepatitis), but also play a role in cytokine release and antigen presentation. Following liver transplantation, donor Kupffer cells are rapidly (within days) replaced by recipient Kupffer cells infiltrating the liver.

Drug metabolism

The liver is the prime site for drug metabolism in the body, which occurs in two phases. The first is an oxidation reaction, mediated by the cytochrome P450 enzymes. Reactive oxygen species that are toxic to the cell are generated during this process and require a range of antioxidant mechanisms (molecules—e.g., glutathione and vitamin E; and enzymes—e.g., superoxide dismutase) to render them inert. The metabolized drug, which may itself be toxic, enters the second phase of metabolism, which involves conjugation with hydrophilic compounds—e.g., glucuronic acid or glutathione. Once rendered hydrophilic, the drug metabolite is excreted via the kidneys or the bile. The enzymes responsible for drug metabolism may be either induced or inhibited by other drugs or chemicals, and there can also be idiosyncratic differences between individuals in drug metabolism. Severe liver

failure reduces the ability to metabolize drugs, so that drug effects are prolonged (e.g., sedatives or anesthetic agents), or there may be an accumulation of toxic metabolites, which complicates hepatic encephalopathy.

Liver function and fluid balance

As described above (functional anatomy/regulation of blood supply), the liver can retain and release a significant volume of whole blood and/or plasma and hence influence the circulating blood volume. Although the direct interaction between the liver and kidney is not fully understood, impaired liver function leads to a reduced ability to excrete sodium and water. A number of factors are involved, which include: hyperaldosteronism and/or increased tubular sensitivity to aldosteronism; increased renal sympathetic nerve activity; and reduced renal perfusion. Splanchnic vasodilation is probably an initial adverse event that leads to renal vasoconstriction, followed by a reduction of renal blood flow and of the glomerular filtration rate. Sodium retention is the first sign of renal dysfunction, followed by water retention, leading to dilutional hyponatremia in plasma. Plasma volume expansion due to sodium and water retention, together with sinusoidal hypertension (portal pressure gradient of > 12%), is a key factor in the pathogenesis of cirrhotic ascites, which indicates the progression from compensated to decompensated cirrhosis.

Growth and repair

Functional development of the liver and physiological adaptations at birth

At birth, the change from placental to enteral nutrition stimulates bile acid secretion and the enterohepatic circulation. The switch from umbilical venous to portal blood supply means that new molecules and bacteria are carried to the immature neonatal liver by the portal vein. This is best demonstrated by the immaturity of bile formation and the development of physiological jaundice in neonates (see above). The liver is vulnerable in the presence of prematurity, hypoxia, sepsis, drug administration, or total parenteral nutrition.[47–49] α-Fetoprotein, one of the main fetal serum proteins, is synthesized by fetal hepatocytes 25–30 days after conception, and by the yolk sac and intestinal epithelium. Levels peak by the end of the first trimester and exponentially fall until normal adult levels are reached approximately at the end of the first year of life. Albumin levels are close to adult levels at birth, but coagulation proteins are low, increasing the risk of bleeding and hence the need for vitamin K administration at birth. Bile acids are synthesized from 5 to 9 weeks' gestation and bile secretion begins at 12 weeks, but canalicular transport mechanisms and the distal bile ducts, where the bile is extensively modified, are still under development for 4 weeks after birth.[6,49] γ-Glutamyltransferase, located at the canalicular surface of the hepatocytes, is slightly elevated in the serum in the first few months of life.

In utero, the placenta carries out most of the metabolic and detoxifying functions that normally take place in the liver. To cope with this change, hepatic enzymes are rapidly induced at birth. Many conjugation reactions are mature by 2 weeks, but some UDP-glucuronyltransferase genes are not fully expressed for 2 years.[50] The cytochrome P450 group and peroxisomal enzymes also show early functionality. The first feed stimulates insulin production and storage of glycogen. Term newborns have hepatic glycogen stores, but these are quickly depleted, making the infants prone to hypoglycemia unless fed frequently. If the infant is unwell, acute-phase proteins may have a long half-life, because the immature liver is unable to clear them.[48]

At 12 weeks' gestation, the liver is the main site of hemopoiesis, but the bone marrow becomes active from 5 months of gestation. It is normal to see evidence of residual hemopoiesis in the neonatal liver for up to 6 weeks after birth,[48] but it is particularly prominent in neonatal hepatitis. Hemosiderin (as hemopoiesis decreases) and copper-associated protein accumulate in the liver and are deposited in periportal hepatocytes. Both are normal constituents of the neonatal liver and are not indicative of disease.

Liver growth and regeneration

The expected life span of a hepatocyte is about 200–500 days. In normal children and adults, hepatic regeneration occurs by replication of mature cells. This process can be up-regulated—for instance, following trauma or partial hepatectomy, the liver can be reconstituted by proliferation of mature hepatocytes within days and weeks. The liver cell mass is highly flexible and varies throughout life, depending on metabolic demands such as disease or pregnancy. It is likely that liver is also regenerated from progenitor cells in the liver, bone marrow–derived stem cells, and mechanisms of cell fusion, but it is still not clear how this is controlled. Initial studies were based on the search for a growth factor that would stimulate hepatocyte regeneration and control of human hepatocyte replication. Studies of rodents suggested the presence of alternative pathways, including a putative progenitor cell compartment. Animal models in which the growth stimulus from partial hepatectomy was combined with growth arrest of fast-replicating mature hepatocytes using toxins such as 2-acetylaminofluorene or 5-aminouracil led to the proliferation of pluripotent so-called "oval cells" in the canals of Hering.[51,52] The phenotype of these putative hepatic progenitor cells, the oval cell, and the existence of a stem cell compartment was accepted for animals. The presence of similar oval cells in humans was more difficult to demonstrate, partly because of the inability to transfer the experimental model into an acceptable human study and

partly because of phenotypic differences between humans and mice. The finding of the hemopoietic stem cell marker and proto-oncogene c-*kit* in certain biliary cells from diseased pediatric liver was one of the first steps in demonstrating the presence of this type of stem cell compartment in humans.[53] An understanding of the physiology of liver regeneration was improved by animal studies conducted by Petersen *et al.*,[54] which were confirmed in humans by Theise *et al.*[55] They demonstrated the presence of Y chromosome–positive hepatocytes and biliary epithelium in female recipients of a therapeutic bone-marrow transplant from male donors, confirming the ability of human bone marrow–derived stem cells to differentiate into the hepatic cell lineages. However, clinically, bone marrow–derived cells are only marginally involved in physiological repair, since in acute liver failure regeneration occurs via proliferation of hepatic progenitor cells, whereas following partial hepatectomy it is restored by replication of normally quiescent hepatocytes.

Liver regeneration is now known to vary in accordance with circadian rhythms and metabolic requirements. Increased metabolic demands and proinflammatory cytokines are likely to be essential. Effects of cytokines (tumor necrosis factor-α, interleukin-6) and growth factors (hepatocyte growth factor) are probably linked by the effect of the tissue-bound metalloproteinases. Experiments with hepatocytes and cocultured biliary epithelium have shown that the degradation of extracellular matrix by metalloproteinases to release growth factors is an essential step in hepatocyte proliferation.

The limitations of understanding of hepatic regeneration have led to persistent problems in clinical hepatocyte transplantation. Therapeutic liver repopulation is an attractive option in a number of metabolic conditions and has been successfully performed in a small number of patients. It remains problematic to achieve persistent engraftment of adequate numbers of cells, because it has not been possible to create an environment that allows preferential replication of transplanted cells. It is possible that significant medical and surgical hepatic preconditioning, similar to myeloablation in bone-marrow transplantation, will be necessary to provide an appropriate environment for persistent engraftment.[56] Clinical studies in this field are limited by the need to match the long-term outcome with orthotopic liver transplantation, at around 90%.

Liver fibrosis

The outcome of most disease processes in the liver is fibrosis. In hepatic fibrogenesis, stellate cells produce an excess of type I and III collagen, which replaces the normal extracellular matrix. Activation of these cells by injured hepatocytes, biliary cells, or inflammatory stimuli leads to the conversion of quiescent vitamin A–storing cells into proliferative, contractile, and fibrogenic myofibroblasts. The dogma that hepatic fibrosis is irreversible is increasingly being challenged, and an understanding of stellate-cell activation is an essential step forward here.[57] Different metalloproteinases that cleave collagens are mainly involved in matrix degradation of the liver, although neutrophils, macrophages, and stellate cells also contribute to this process. Tissue inhibitors of matrix metalloproteinases (TIMPs) are the key regulators in determining the reversal of fibrosis. Sustained TIMP-1 expression inhibits protease activity for matrix degradation and blocks apoptosis of activated stellate cells. A number of phase I clinical trials are being planned to investigate the use of these drugs to prevent or reverse fibrosis.[58]

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