CHAPTER 1
Anatomy and Function

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Learning points

• The liver is derived from a foregut endodermal bud which develops in the third week of gestation and divides into two parts: hepatic and biliary.
• The Couinaud classification subdivides the liver into eight segments (segments I–IV in the left lobe, segments V–VIII in the right lobe) based on vascular and biliary anatomical landmarks.
• The lobule described by Kiernan is the most widely used unit of liver microanatomy, consisting of a hexagon-like region of liver parenchyma with a central vein as its hub and portal tracts located in the periphery of the hexagon.
• Hepatocytes are functionally heterogeneous within the lobular parenchyma, whereby centrilobular cells subserve different functions (e.g. drug metabolism) from periportal cells (e.g. bile salt-dependent bile formation).
• Uncomplicated regeneration of hepatocytes and/or bile duct epithelium usually occurs by cell division of the indigenous cells; however, when normal regenerative capacity is overwhelmed there may be activation of progenitors cells located in the region of the canals of Hering.

Development of the liver and bile ducts

The liver begins as a hollow endodermal bud from the foregut (duodenum) during the third week of gestation. The bud separates into two parts—hepatic and biliary. The hepatic part contains bipotential progenitor cells that differentiate into hepatocytes or ductal cells, which form the early primitive bile duct structures (bile duct plates). Differentiation is accompanied by changes in cytokeratin type within the cell [1]. Normally, this collection of rapidly proliferating cells penetrates adjacent mesodermal tissue (the septum transversum) and is met by ingrowing capillary plexuses from the vitelline and umbilical veins, which will form the sinusoids. The connection between this proliferating mass of cells and the foregut, the biliary part of the endodermal bud, will form the gallbladder and extrahepatic bile ducts. Bile begins to flow at about the 12th week. Connective tissue cells of portal tracts are derived from the mesoderm of the septum transversum. Kupffer cells derive from circulating monocytes and possibly yolk sac macrophages. Hepatic stellate cells appear to be mesodermal derivatives from submesothelial cells located beneath the surface of the developing liver [2]. The fetal liver is the main site of haemopoiesis by the 12th week; this subsides in the fifth month coincident with the onset of bone marrow haemopoietic activity, so that only a few haemopoietic cells remain at birth.

Anatomy of the liver

The liver, the largest organ in the body, weighs 1200–1500 g and comprises one-fiftieth of the total adult body weight. It is relatively larger in infancy, comprising one-eighth of the birth weight. This is mainly due to a large left lobe.

Sheltered by the ribs in the right upper quadrant, the upper border lies approximately at the level of the nipples. There are two anatomical lobes, the right being about six times the size of the left (Figs 1.1–1.3). Lesser segments of the right lobe are the caudate lobe on the posterior surface and the quadrate lobe on the inferior surface. The right and left lobes are separated anteriorly by a fold of peritoneum called the falciform ligament, posteriorly by the fissure for the ligamentum venosum and inferiorly by the fissure for the ligamentum teres.

The liver has a double blood supply. The portal vein brings venous blood from the intestines and spleen and the hepatic artery, coming from the coeliac axis, supplies the liver with arterial blood. These vessels enter the liver through a fissure, the porta hepatis, which lies far back on the inferior surface of the right lobe. Inside the porta, the portal vein and hepatic artery divide into branches to the right and left lobes, and the right and left hepatic
bile ducts join to form the common hepatic duct. The hepatic nerve plexus contains fibres from the sympathetic ganglia T7–T10, which synapse in the coeliac plexus, the right and left vagi and the right phrenic nerve. It accompanies the hepatic artery and bile ducts into their finest ramifications, even to the portal tracts and hepatic parenchyma [3].

The ligamentum venosum, a slender remnant of the ductus venosus of the fetus, arises from the left branch of the portal vein and fuses with the inferior vena cava at the entrance of the left hepatic vein. The ligamentum teres, a remnant of the umbilical vein of the fetus, runs in the free edge of the falciform ligament from the umbilicus to the inferior border of the liver and joins the left branch of the portal vein. Small veins accompanying it connect the portal vein with veins around the umbilicus. These become prominent when the portal venous system is obstructed inside the liver.

The venous drainage from the liver is into the right and left hepatic veins which emerge from the back of the liver and at once enter the inferior vena cava very near its point of entry into the right atrium.

Lymphatic vessels terminate in small groups of glands around the porta hepatis. Efferent vessels drain into glands around the coeliac axis. Some superficial hepatic lymphatics pass through the diaphragm in the falciform ligament and finally reach the mediastinal glands. Another group accompanies the inferior vena cava into the thorax and ends in a few small glands around the intrathoracic portion of the inferior vena cava.

The inferior vena cava makes a deep groove to the right of the caudate lobe about 2 cm from the midline.

The gallbladder lies in a fossa extending from the inferior border of the liver to the right end of the porta hepatis.

The liver is completely covered with peritoneum, except in three places. It comes into direct contact with the diaphragm through the bare area which lies to the right of the fossa for the inferior vena cava. The other areas without peritoneal covering are the fossae for the inferior vena cava and gallbladder.

The liver is kept in position by peritoneal ligaments and by the intra-abdominal pressure transmitted by the tone of the muscles of the abdominal wall.
Functional liver anatomy: sectors and segments

Based on the external appearances described above, the liver has a right and left lobe separated along the line of insertion of the falciform ligament. This separation, however, does not correlate with blood supply or biliary drainage. A functional anatomy is now recognized based upon vascular and biliary anatomy. The Couinaud classification [4] defines eight segments (segments I-IV in the left lobe, V-VIII in the right lobe), while the Bismuth classification [5] divides the liver into four sectors. These can be correlated with results seen with imaging techniques.

The main portal vein divides into right and left branches and each of these supplies two further subunits (variously called sectors). The sectors on the right side are anterior and posterior and, in the left lobe, medial and lateral—giving a total of four sectors (Fig. 1.4). Using this definition, the right and left side of the liver are divided not along the line of the falciform ligament, but along a slightly oblique line to the right of this, drawn from the inferior vena cava above to the gallbladder bed below. The right and left side are independent with regard to portal and arterial blood supply, and bile drainage. Three planes separate the four sectors and contain the three major hepatic vein branches.

Closer analysis of these four hepatic sectors produces a further subdivision into segments (Fig. 1.5). The right anterior sector contains segments V and VIII; right posterior sector, VI and VII; left medial sector, IV; left lateral sector, II and III. There is no vascular anastomosis between the macroscopic vessels of the segments but communications exist at the sinusoidal level. Segment I, the equivalent of the caudate lobe, is separate from the other segments and does not derive blood directly from the major portal branches or drain by any of the three major hepatic veins.

This functional anatomical classification allows interpretation of radiological data and is of importance to the
surgeon planning a liver resection. There are wide variations in portal and hepatic vessel anatomy which can be demonstrated by spiral computed tomography (CT) and magnetic resonance imaging (MRI) reconstruction [6].

**Anatomical abnormalities of the liver**

These are being increasingly diagnosed with more widespread use of CT and ultrasound scanning.

**Accessory lobes.** The livers of the pig, dog and camel are divided into distinct and separate lobes by strands of connective tissue. Occasionally, the human liver may show this reversion and up to 16 lobes have been reported. This abnormality is rare and without clinical significance. The lobes are small and usually on the undersurface of the liver so that they are not detected clinically but are noted incidentally at scanning, operation or necropsy. Rarely they are intrathoracic [7]. An accessory lobe may have its own mesentery containing hepatic artery, portal vein, bile duct and hepatic vein. This may twist and demand surgical intervention.

**Ectopic liver.** Small nodules of normal liver derived from the embryologic hepatic bud may be found in less than 1% of laparoscopies and autopsies near the gallbladder, hepatic ligaments, gastrorenal ligament, omentum, retroperitoneum and thorax. These may give rise to hepaticocellular carcinoma [8,9].

**Riedel’s lobe.** This is fairly common and is a downward tongue-like projection of the right lobe of the liver [10]. It is a simple anatomical variation; it is not a true accessory lobe. The condition is more frequent in women. It is detected as a mobile tumour on the right side of the abdomen which descends with the diaphragm on inspiration. It may come down as low as the right iliac region. It is easily mistaken for other tumours in this area, especially a visceroptotic right kidney. It does not cause symptoms and treatment is not required. Rarely, it is a site for metastasis or primary hepatocellular carcinoma. Scanning may be used to identify Riedel’s lobe and other anatomical abnormalities.

**Cough furrows on the liver.** These are vertical grooves on the convexity of the right lobe. They are one to six in number and run anteroposteriorly, being deeper posteriorly. These represent diaphragmatic sulci and fissures produced by pressure exerted by diaphragmatic muscle on peripheral structurally weak liver parenchymal zones associated with watershed vascular distribution [11]. Chronic cough produces such pressure.

**Corset liver.** This is a horizontal fibrotic furrow or pedicle on the anterior surface of one or both lobes of the liver just below the costal margin [12]. The mechanism is unknown, but it affects elderly women who have worn corsets for many years. It presents as an abdominal mass in front of and below the liver and is isodense with the liver. It may be confused with a hepatic tumour.

**Lobar atrophy.** Interference with the portal supply or biliary drainage of a lobe may cause atrophy. There is usually hypertrophy of the opposite lobe. Left lobe atrophy found at post-mortem or during scanning is not uncommon and is probably related to reduced blood supply via the left branch of the portal vein. The lobe is decreased in size with thickening of the capsule, fibrosis and prominent biliary and vascular markings. The vascular problem may date from the time of birth. Loss of left lobe parenchyma in this instance develops by the process of ischaemic extinction due to impaired flow from the affected large portal vein branch. Replacement fibrosis ensues. This large vessel extinction process should be distinguished from cirrhosis in which the entire liver is affected by numerous intrahepatic and discrete extinction lesions, which affect small hepatic veins and portal vein branches during the course of inflammation and fibrosis. Hence, in cirrhosis the entire liver surface is diffusely converted to regenerative parenchymal nodules surrounded by fibrosis.

Obstruction to the right or left hepatic bile duct by benign stricture or cholangiocarcinoma is now the most common cause of lobar atrophy [13]. The alkaline phosphatase is usually elevated. The bile duct may not be dilated within the atrophied lobe. Relief of obstruction may reverse the changes if cirrhosis has not developed. Distinction between a biliary and portal venous aetiology may be made using technetium-labelled iminodiacetic acid (IDA) and colloid scintiscans. A small lobe with normal uptake of IDA and colloid is compatible with a portal aetiology. Reduced or absent uptake of both isotopes favours biliary disease.

**Agenesis of the right lobe** [14]. This rare lesion may be an incidental finding associated, probably coincidentally, with biliary tract disease and also with other congenital abnormalities. It can cause presinusoidal portal hypertension. The other liver segments undergo compensatory hypertrophy. It must be distinguished from lobar atrophy due to cirrhosis or hilar cholangiocarcinoma.

**Situs inversus (SI).** In the exceedingly rare SI totalis or abdominalis the liver is located in the left hypochondrium and may be associated with other anomalies including biliary atresia, polysplenia syndrome, aberrant hepatic artery anatomy and absent portal vein. Hepatic surgery (partial hepatectomy, liver transplantation) is feasible, but complex. Other conditions associated with displacement of the liver from its location in
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less than 11 mm, although after cholecystectomy it may be more in the absence of obstruction.

The duodenal portion of the common bile duct is surrounded by a thickening of both longitudinal and circular muscle fibres derived from the intestine. This is called the sphincter of Oddi (c. 1887).

The gallbladder is a pear-shaped bag 9 cm long with a capacity of about 50 mL. It always lies above the transverse colon, and is usually next to the duodenal cap overlying, but well anterior to, the right renal shadow. The fundus is the wider end and is directed anteriorly; this is the part palpated when the abdomen is examined. The body extends into a narrow neck which continues into the cystic duct. The valves of Heister are spiral folds of mucous membrane in the wall of the cystic duct and neck of the gallbladder. Hartmann’s pouch is a saccula-
tion at the neck of the gallbladder; this is a common site for a gallstone to lodge.

The mucosa is in delicate, closely woven folds; instead of glands there are indentations of mucosa which usually lie superfi cial to the muscle layer. Increased intralumi-
nal pressure in chronic cholecystitis results in formation of branched, diverticula-like invaginations of the mucosa which reach into the muscular layer, termed Rokitansky–Aschoff sinuses. There is no submucosa or muscularis mucosae. The gallbladder wall consists of a loose connective tissue lamina propria and muscular layer containing circular, longitudinal and oblique muscle bundles without defi nite layers, the muscle being particularly well developed in the neck and fundus. The outer layers are the subserosa and serosa. The distensible normal gallbladder fi lls with bile and bile acids secreted by the liver, concentrates the bile with meals contracts under the infl uence of cholecystokinin (acting through preganglionic cholinergic nerves) to empty bile into the duodenum.

Blood supply. The gallbladder receives blood from the cystic artery. This branch of the hepatic artery is large, tortuous and variable in its anatomical relationships. Smaller blood vessels enter from the liver through the gallbladder fossa. The venous drainage is into the cystic vein and thence into the portal venous system. Attention to the vascular-biliary anatomy in the reference area known as Calot’s triangle (bordered by the cystic duct, common hepatic duct and lower edge of the liver) reduces the risk of vascular injuries and potential biliary strictures. Most bile duct injuries occur at cholecystectomy (incidence of <1.3% for either open or laparoscopic cholecystectomy). After liver transplantation 10–33% of patients may develop biliary complications, of which biliary stricture is the most important.

The arterial blood supply to the supraduodenal bile duct is generally by two main (axial) vessels which run the right upper quadrant include congenital diaphragmatic hernias, diaphragmatic eventration and omphalocoele.

Anatomical abnormalities of the gallbladder and biliary tract are discussed in Chapter 12.

Anatomy of the biliary tract (Fig. 1.6)

The right and left hepatic ducts emerge from the liver and unite in the porta hepatis to form the common hepatic duct. This is soon joined by the cystic duct from the gallbladder to form the common bile duct.

The common bile duct runs between the layers of the lesser omentum, lying anterior to the portal vein and to the right of the hepatic artery. Passing behind the first part of the duodenum in a groove on the back of the head of the pancreas, it enters the second part of the duodenum. The duct runs obliquely through the pos-
teromedial wall, usually joining the main pancreatic duct to form the ampulla of Vater (c. 1720). The ampulla makes the mucous membrane bulge inwards to form an eminence, the duodenal papilla. In about 10–15% of sub-
jects the bile and pancreatic ducts open separately into the duodenum.

The dimensions of the common bile duct depend on the technique used. At operation it is about 0.5–1.5 cm in diameter. Using ultrasound the values are less, the common bile duct being 2–7 mm, with values greater than 7 mm being regarded as abnormal. Using endoscop ic cholangiography, the duct diameter is usually

Fig. 1.6. Gallbladder and biliary tract.
an obese subject it may be difficult to identify the outer border of the rectus sheath and the gallbladder may then be located by the Grey–Turner method. A line is drawn from the left anterior superior iliac spine through the umbilicus; its intersection with the right costal margin indicates the position of the gallbladder. These guidelines depend upon the individual’s build. The fundus may occasionally be found below the iliac crest.

**Methods of examination**

**Liver.** The lower edge should be determined by palpation just lateral to the right rectus muscle. This avoids mistaking the upper intersection of the rectus sheath for the liver edge.

The liver edge moves 1–3 cm downwards with deep inspiration. It is usually palpable in normal subjects beside the bile duct. These are supplied predominantly by the retroduodenal artery from below, and the right hepatic artery from above, although many other vessels contribute. This pattern of arterial supply would explain why vascular damage results in bile duct stricture [15].

**Lymphatics.** There are many lymphatic vessels in the submucous and subperitoneal layers. These drain through the cystic gland at the neck of the gallbladder to glands along the common bile duct, where they anastomose with lymphatics from the head of the pancreas.

**Nerve supply.** The gallbladder and bile ducts are liberally supplied with nerves, from both the parasympathetic and the sympathetic system.

**Surface marking** (Figs 1.7, 1.8)

**Liver.** The upper border of the right lobe is on a level with the 5th rib at a point 2 cm medial to the right midclavicular line (1 cm below the right nipple). The upper border of the left lobe corresponds to the upper border of the 6th rib at a point in the left midclavicular line (2 cm below the left nipple). Here only the diaphragm separates the liver from the apex of the heart.

The lower border passes obliquely upwards from the 9th right to the 8th left costal cartilage. In the right nipple line it lies between a point just under to 2 cm below the costal margin. It crosses the midline about midway between the base of the xiphoid and the umbilicus and the left lobe extends only 5 cm to the left of the sternum.

**Gallbladder.** Usually the fundus lies at the outer border of the right rectus abdominis muscle at its junction with the right costal margin (9th costal cartilage) (Fig. 1.8). In

Fig. 1.7. The surface marking of the liver.

Fig. 1.8. Surface markings of the gallbladder. Method I: the gallbladder is found where the outer border of the right rectus abdominis muscle intersects the 9th costal cartilage. Method II: a line drawn from the left anterior superior iliac spine through the umbilicus intersects the costal margin at the site of the gallbladder.
inspiring deeply. The edge may be tender, regular or irregular, firm or soft, thickened or sharp. The lower edge may be displaced downwards by a low diaphragm, for instance in emphysema. Movements may be particularly great in athletes or singers. Some patients with practice become very efficient at ‘pushing down’ the liver. The normal spleen can become palpable in similar fashion. Common causes of a liver palpable below the umbilicus are malignant deposits, polycystic or Hodgkin’s disease, amyloidosis, congestive cardiac failure and gross fatty change. Rapid change in liver size may occur when congestive cardiac failure is corrected, cholestatic jaundice relieved, or when severe diabetes is controlled. The surface can be palpated in the epigastrium and any irregularity or tenderness noted. An enlarged caudate lobe, as in the Budd–Chiari syndrome or with some cases of cirrhosis, may be palpated as an epigastric mass.

Pulsation of the liver, usually associated with tricuspid valvular incompetence, is felt by manual palpation with one hand behind the right lower ribs posteriorly and the other anteriorly on the abdominal wall.

The upper edge is determined by fairly heavy percussion passing downwards from the nipple line. The lower edge is recognized by very light percussion passing upwards from the umbilicus towards the costal margin. Percussion is a valuable method of determining liver size and is the only clinical method of determining a small liver.

The anterior liver span is obtained by measuring the vertical distance between the uppermost and lowermost points of hepatic dullness by percussion in the right midclavicular line. This is usually 12–15 cm. Direct percussion is as accurate as ultrasound in estimating liver span [16].

Friction may be palpable and audible, usually due to recent biopsy, tumour or perihepatitis. The venous hum of portal hypertension is audible between the umbilicus and the xiphisternum. An arterial murmur over the liver may indicate a primary liver cancer or acute alcoholic hepatitis.

**Gallbladder.** The gallbladder is palpable only when it is distended. It is felt as a pear-shaped cystic mass usually about 7 cm long. In a thin person, the swelling can sometimes be seen through the anterior abdominal wall. It moves downwards on inspiration and is mobile laterally but not downwards. The swelling is dull to percussion and directly impinges on the parietal peritoneum, so that the colon is rarely in front of it. Gallbladder dullness is continuous with that of the liver.

Abdominal tenderness should be noted. Inflammation of the gallbladder causes a positive *Murphy’s sign.* This is the inability to take a deep breath when the examining fingers are hooked up below the liver edge. The inflamed gallbladder is then driven against the fingers and the pain causes the patient to catch their breath.

The enlarged gallbladder must be distinguished from a **visceroptotic right kidney.** This, however, is more mobile, can be displaced towards the pelvis and has the resonant colon anteriorly. A regenerative or malignant nodule feels much firmer.

**Imaging.** A plain film of the abdomen, including the diaphragms, may be used to assess liver size and in particular to decide whether a palpable liver is due to actual enlargement or to downward displacement. On moderate inspiration the normal level of the diaphragm, on the right side, is opposite the 11th rib posteriorly and the 6th rib anteriorly.

Ultrasound, CT or MRI can be used to study liver size, shape and content.

**Microanatomy of the liver.**

For over a century, many models of liver substructure have been proposed [17]. The most popular of these is the **lobule** introduced by Kiernan in 1833 as the basic architectural unit, based on pig dissections [18]. He described circumscribed, hexagonal lobules consisting of a central tributary of the hepatic vein (central vein) and at the periphery a portal tract containing the bile duct, portal vein radicle and hepatic artery branch. Cords (plates) of liver cells and blood-containing sinusoids extend between these two systems. The lobule has foundations in pig, camel, raccoon and polar bear livers, in which such hexagonal units are surrounded by interlobular connective tissue septa [19]. Such septa have no counterparts in human liver.

Steroscopic reconstructions and scanning electron microscopy have shown the human liver as cords of liver cells radiating from a central vein, and interlaced in orderly fashion by sinusoids (Figs 1.9, 1.10). The terminal branches of the portal vein discharge their blood into the sinusoids and the direction of flow is determined by the higher pressure in the portal vein than in the central vein (or terminal hepatic venule)—see below.

The **portal tracts** are small connective tissue islands containing triads composed of the portal vein radicle, the hepatic arteriole and bile duct (Fig. 1.11). Portal tracts are surrounded by a limiting plate of liver cells. Histological sections of normal liver show portal tracts containing dyads as frequently as triads, with the portal vein being the most frequently absent element. Within each linear centimetre of liver tissue obtained at biopsy there are usually two interlobular bile ducts, two hepatic arteries and one portal vein per portal tract, with six full portal triads [20].

The liver has to be divided *functionally.* Traditionally, the unit is based on a central hepatic vein and its
Fig. 1.9. The structure of the normal human liver.

Fig. 1.10. Normal hepatic histology. H, terminal hepatic vein; P, portal tract. (H & E, ×60.)
The liver cells (hepatocytes) comprise about 60% of the liver. They are polygonal and approximately 30 μm in diameter. The nucleus is single or, less often, multiple and divides by mitosis. The lifespan of liver cells is about 150 days in experimental animals. The hepatocyte has three surfaces: one facing the sinusoid and space of Disse, the second facing the canaliculus and the third facing neighbouring hepatocytes (Fig. 1.14). There is no basement membrane.

The sinusoids are lined by endothelial cells with small pores (fenestrae) for macromolecule diffusion from blood to hepatocytes. On the vascular side of the sinusoids are the phagocytic cells of the reticuloendothelial system (Kupffer cells) and pit cells with natural killer function.

There are approximately $202 \times 10^3$ cells in each milligram of normal human liver, of which $171 \times 10^3$ are parenchymal and $31 \times 10^3$ littoral (sinusoidal, including Kupffer cells).

The space of Disse between hepatocytes and sinusoidal endothelial cells contains a few collagen fibrils and the hepatic stellate cells, which have also been called fat-storing cells, Ito cells and lipocytes. These cells store vitamin A and when activated in disease become collagen-synthesizing myofibroblasts. The hepatic lymphatics are found in the periportal connective tissue and are lined throughout by endothelium. Tissue fluid seeps through the endothelium into the lymph vessels.

The branch of the hepatic arteriole forms a plexus around the bile ducts and supplies the structures in the portal tracts. It empties into the sinusoidal network at different levels. There are no direct hepatic arteriolar-portal venous anastomoses.

The excretory system of the liver begins with the bile canaliculi (Figs 1.14, 1.15). These are formed by...
**Fig. 1.13.** Blood supply of the simple liver acinus, zonal arrangements of cells and the microcirculatory periphery. The acinus occupies adjacent sectors of the neighbouring hexagonal fields. Zones 1, 2 and 3, respectively, represent areas supplied with blood of first, second and third quality with regard to oxygen and nutrient content. These zones centre on the terminal afferent vascular branches, bile ductules, lymph vessels and nerves (PS) and extend into the triangular portal field from which these branches crop out. Zone 3 is the microcirculatory periphery of the acinus since its cells are as remote from their own afferent vessels as from those of adjacent acini. The perivenular area is formed by the most peripheral portions of zone 3 of several adjacent acini. In injury progressing along this zone, the damaged area assumes the shape of a starfish (darker tint around a terminal hepatic venule, THV, in the centre). 1–3, microcirculatory zones; 1′–3′, zones of neighbouring acinus [21].

**Fig. 1.14.** The organelles of the liver cell.
The mitochondria also have a double membrane, the inner being invaginated to form grooves or cristae. An enormous number of energy-providing processes take place within them, particularly those involving oxidative phosphorylation. They contain many enzymes,
enzymes include simple oxidases, β-oxidation cycles, the glyoxalate cycle, ether lipid synthesis, and cholesterol and dolichol biosynthesis. Several disorders of peroxisomal function are recognized of which Zellweger syndrome is one [22]. Endotoxin severely damages peroxisomes [23].

The lysosomes are membrane-bound, electron-dense bodies adjacent to the bile canaliculi. They contain many hydrolytic enzymes which, if released, could destroy the cell. They are the site of deposition of ferritin, lipofuscin, bile pigment, copper and senescent organelles.

The Golgi apparatus consists of a system of particles and vesicles, again lying near the canaliculus. It may be regarded as a ‘packaging’ site before excretion into the bile. This entire group of lysosomes, microbodies and Golgi apparatus is a means of sequestering any material which is ingested and has to be excreted, secreted or stored for metabolic processes in the cytoplasm. The Golgi apparatus, lysosomes and canaliculi are concerned in cholestasis (Chapter 11).

The intervening cytoplasm contains granules of glycogen.

The cytoskeleton supporting the hepatocyte consists of microtubules, microfilaments and intermediate filaments [24]. Microtubules contain tubulin and control subcellular mobility, vesicle movement and plasma protein secretion. Microfilaments are made up of actin, are contractile and are important for the integrity and motility of the canaliculus and for bile flow. Intermediate filaments are elongated branched filaments comprising cytokeratins [1]. They extend from the plasma membrane to the perinuclear area and are fundamental for the stability and spatial organization of the hepatocyte. They become disrupted or lost with hepatocellular injury by alcohol, lipid peroxidation by-products and ischaemia [25].

Sinusoidal cells
The sinusoidal cells (endothelial cells, Kupffer cells, hepatic stellate cells and pit cells) form a functional and histological unit together with the sinusoidal aspect of the hepatocyte [26]. These cells interact via cytokines and other signalling mechanisms [27,28]. The close structural relationship of sinusoidal cells to hepatic cords is evident on transmission (Fig. 1.17) and scanning electron microscopy (Fig. 1.15).

Endothelial cells line the sinusoids and have fenestrae, which provide a graded barrier between the sinusoid and space of Disse (Fig. 1.18). The Kupffer cells anchor on the endothelium by their cytoplasmic projections. The hepatic stellate cells lie in the space of Disse between the hepatocytes and the endothelial cells (Fig. 1.19). Disse’s space contains tissue fluid which flows outwards into lymphatics in the portal zones. When sinu-
soidal pressure rises, lymph production in Disse’s space increases and this plays a part in ascites formation where there is hepatic venous outflow obstruction.

**Endothelial cells.** These cells form a continuous lining to the sinusoids. They differ from endothelial cells elsewhere in not having a regular basement membrane. The endothelial cells act as a sieve between the sinusoid and space of Disse, have specific and non-specific endocytic activity and have a variety of receptors. Their capacity to act as a sieve is due to fenestrae, around 0.15μm in diameter (Fig. 1.18). These make up 6–8% of the total endothelial cell surface, and there are more in the centrilobular zone of the sinusoid than the periportal area. Extracellular matrix affects their function.

Fenestrae are clustered into sieve plates, and act as biofilters and transport pores between sinusoidal blood and the plasma within the space of Disse. They have a dynamic cytoskeleton [29]. This maintains and regulates their size, which can be changed by many influences.
including alcohol, nicotine, serotonin, endotoxin and partial hepatectomy. The fenestrae filter macromolecules of differing size. Particles greater than 0.2 μm in diameter, which includes large triglyceride-rich parent chylomicrons, will not pass. Smaller triglyceride-depleted, cholesterol-rich and retinol-rich remnants can enter the space of Disse [30]. In this way the fenestrae have an important role in chylomicron and lipoprotein metabolism. Open fenestrae are located in the thin cytoplasmic periphery of the endothelial cells while close to the endothelial nuclei fenestrae are multifolded and labyrinth-like [31].

Endothelial cells have a high capacity for endocytosis (accounting for 45% of all pinocytic vesicles in the liver) and are active in clearing macromolecules and small particles from the circulation [32]. Coated and uncoated membrane-bound vesicles on endothelium are present near their nuclei or on non-fenestrated portions of their cytoplasm [31]; these are involved in various endocytic functions. There is receptor-mediated endocytosis for several molecules including transferrin, caeruloplasmin, modified high density lipoprotein (HDL) and very low density lipoprotein (VLDL). Hyaluronan (a major polysaccharide from connective tissue) is taken up and this provides a method for assessing hepatic metabolism. Open fenestrae are located in the thin cytoplasmic periphery of the endothelial cells while close to the endothelial nuclei fenestrae are multifolded and labyrinth-like [31].

**Kupffer cells.** These are highly mobile macrophages attached to the endothelial lining of the sinusoid, in greater numbers in the perportal areas [34]. They have microvilli and intracytoplasmic-coated vesicles and dense bodies which make up the lysosomal apparatus. They proliferate locally but under certain circumstances macrophages can immigrate from an extrahepatic site. They are responsible for removing old and damaged blood cells or cellular debris, also bacteria, viruses, parasites and tumour cells. They do this by endocytosis (phagocytosis, pinocytosis), including absorptive (receptor-mediated) and fluid phase (non-receptor-mediated) mechanisms [35]. Several processes aid this, including cell surface Fc and complement receptors. Coating of the particle with plasma fibronectin or opsonin also facilitates phagocytosis, since Kupffer cells have specific binding sites for fibronectin on the cell surface. These cells also take up and process oxidized LDL (thought to be atherogenic), and remove fibrin in disseminated intravascular coagulation. Alcohol reduces the phagocytic capacity.

Kupffer cells are activated by a wide range of agents, including endotoxin, sepsis, shock, interferon-γ, arachidonic acid and tumour necrosis factor (TNF). The result of activation is the production of an equally wide range of products: cytokines, hydrogen peroxide, nitric oxide, TNF, interleukin (IL) 1, IL6 and IL10, interferon-α and -β, transforming growth factor (TGF-β) and various prostanoids [36]. This whole array acts alone or in combination to stimulate other events in the cytokine cascade, but also increases discomfort and sickness. The Kupffer cell products may be toxic to parenchymal cells and endothelial cells. Kupffer cell-conditioned medium inhibits albumin synthesis in parenchymal cells, as do IL1, IL6 and TNF-α. The toxicity of endotoxin is caused by the secretory products of Kupffer cells since endotoxin itself is not directly toxic.

**Hepatic stellate cells** (fat-storing cells, lipocytes, Ito cells). These cells lie within the subendothelial space of Disse. They have long cytoplasmic extensions, some giving close contact with parenchymal cells, and others reaching several sinusoids, where they may regulate blood flow and hence influence portal hypertension [37]. In normal liver they are the major storage site of retinoids, giving the morphological characteristic of cytoplasmic lipid droplets. When empty of these droplets, they resemble fibroblasts. They contain actin and myosin and contract in response to endothelin-1 and substance P [38]. With hepatocyte injury, hepatic stellate cells lose their lipid droplets, proliferate, migrate to zone 3 of the acinus, change to a myofibroblast-like phenotype, and produce collagen type I, III and IV and laminin [39]. Stellate cells also release matrix proteinases and inhibitory molecules of matrix proteinases [40] (tissue inhibitor of metalloproteinases, TIMP) (Chapter 6). Collagenization of the space of Disse results in decreased access of protein-bound substrates to the hepatocyte.

**Pit cells.** These are highly mobile, liver-specific, natural killer lymphocytes attached to the sinusoidal surface of the endothelium [36,41]. They are short-lived cells and are renewed from circulating large granular lymphocytes, which differentiate within the sinusoids. They have characteristic granules and rod-cored vesicles. Pit cells show spontaneous cytotoxicity against tumour- and virus-infected hepatocytes. There are complex interactions between Kupffer and endothelial cells, as well as sinusoidal cells and hepatocytes [27]. Kupffer cell activation by lipopolysaccharide suppresses hyaluronan uptake by endothelial cells, an effect probably mediated by leukotrienes [42]. Cytokines produced by sinusoidal cells can both stimulate and inhibit hepatocyte proliferation [28].

In or around the space of Disse, all major constituents of a basement membrane can be found including type
proteins which, if mutated, lead to fibropolycystic diseases [46] (Chapter 14).

**Functional heterogeneity of the liver** (Fig. 1.20)

Hepatocytes show different structural and functional characteristics depending on their acinar location [47]. The relative functions of cells in the circulatory periphery of acini (zone 3) adjacent to terminal hepatic veins are different from those in the circulatory area adjacent to terminal hepatic arteries and portal veins (zone 1). This zonation is related to the lobular/ acinar oxygen gradient [48] and to signalling via the Wnt/β-catenin pathway [49].

Krebs’ cycle enzymes (urea synthesis and glutaminase) are found in the highest concentration in zone 1, whereas glutamine synthetase is perivenular (Fig. 1.20). Cells in zone 3 receive their oxygen supply last and are particularly prone to anoxic liver injury.

The drug-metabolizing P450 enzymes are present in greater amounts in zone 3. This is particularly so after enzyme induction, for instance with phenobarbital. Hepatocytes in zone 3 receive a higher concentration of any toxic product of drug metabolism. They also have a reduced glutathione concentration. This makes them particularly susceptible to hepatic drug reactions as exemplified by the centrilobular necrosis produced by ‘predictable’ or ‘direct’ hepatotoxins such as paracetamol (acetaminophen, Tylenol) and carbon tetrachloride.

<table>
<thead>
<tr>
<th>Zone 1</th>
<th>Zone 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carbohydrates</strong></td>
<td>Glycogen synthesis</td>
</tr>
<tr>
<td>Proteins</td>
<td>Albumin synthesis</td>
</tr>
<tr>
<td>Cytochrome P450 after phenobarbital</td>
<td>Fibrinogen synthesis</td>
</tr>
<tr>
<td>Glutathione</td>
<td>++++</td>
</tr>
<tr>
<td>Ammonia metabolism: glutamine synthetase</td>
<td>++</td>
</tr>
<tr>
<td>Oxygen supply</td>
<td>+</td>
</tr>
<tr>
<td>Bile formation</td>
<td>-</td>
</tr>
<tr>
<td>* bile-salt dependent*</td>
<td>+++</td>
</tr>
<tr>
<td>* non-bile-salt dependent*</td>
<td>--</td>
</tr>
</tbody>
</table>

**Fig. 1.20.** The functional heterogeneity of hepatocytes in perivenular (acinar zones 3) versus periporal (acinar zones 1) regions affects many synthetic and metabolic processes. Below, a portion of a lobule immunostained with antibody to glutamine synthetase shows localization of the enzyme to several layers of hepatocytes surrounding the central vein (CV). Negative staining is present elsewhere. PT, portal tract.
Hepatocytes in zone 1 receive blood with a high bile salt concentration and, therefore, are particularly important in bile-salt-dependent bile formation. Hepatocytes in zone 3 are important in non-bile-salt-dependent bile formation. There are also zonal differences in the hepatic transport rate of substances from the sinusoid to canaliculus.

The cause of the metabolic difference between the zones varies. For some functions (gluconeogenesis, glycolysis, ketogenesis) it appears to be dependent upon the direction of blood flow along the sinusoid. For others (cytochrome P450) the gene transcription rate differs between perivenular and periportal hepatocytes. The differential expression of glutamine synthetase across the acinus is already established in fetal liver.

**Dynamics of the hepatic microenvironment in physiology and disease** (Fig. 1.21)

The sinusoidal plasma membrane of the hepatocyte is a receptor-rich and metabolically dynamic domain which is separated from the bile canaliculus by a lateral domain which participates in cell–cell interactions. Toll-like receptors on the hepatocyte surface react with microbial substances such as lipopolysaccharide (LPS) of Gram-negative bacteria resulting in a wave of intracellular signalling [50]. Receptor-mediated endocytosis is responsible for the transfer of large molecules such as glycoproteins, growth factors and carrier proteins (transferrin [51]). These ligands bind to receptors on the sinusoidal membrane, the occupied receptors cluster into a coated (clathrin) pit and endocytosis proceeds. The fate of the ligand within the cell varies according to the molecule involved, and the pathways are complex. Certain ligands, once bound to cell surface receptors, are then transferred for further interaction with claudins and occluden proteins located in tight junctions prior to clathrin-pit endocytosis. This is true of hepatitis C virus entry into liver cells [52]. Many ligands terminate in lysosomes where they are broken down while the receptor returns to the sinusoidal plasma membrane to perform again. Some ligands such as copper pass by vesicular transport across the cell to be discharged into the bile canaliculus.

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**Fig. 1.21.** Hepatocellular uptake and transport processes include: (1) surface Toll-like receptors (TLRs) for binding microbes and their constituent products; (2) endocytosis into clathrin-coated pits of ligands bound to cell surface receptors results in clathrin uncoating, ligand release from the endosome and further intracellular trafficking of the ligand; (3) certain receptor-ligand moieties (e.g. HCV entry into cells) require further interaction with claudins and occluden in tight junctions; and (4) bile transport proteins on the bile canalicular membrane (see text for further detail). CLDN, claudin; OCC, occluden; BC, bile canaliculus; L, ligand; R, cell surface receptor; TJ, tight junction; OATP, organic anion transport protein; BSEP, bile salt export pump; MDR3, multidrug resistance protein 3; FIC1, familial intrahepatic cholestasis 1.
Anatomy and Function

17

Pathological processes can alter the cellular mechanisms involved in apoptosis, leading to disease [56,57]. Increased apoptosis affecting cholangiocytes may lead to ductopenia. Apoptosis is increased in alcoholic and non-alcoholic fatty liver disease [59–61]. If cells containing a mutation predisposing to malignant change do not undergo apoptosis, malignant transformation is enhanced.

The pathway to apoptotic cellular destruction is complex, and can be described in morphological and biochemical terms. Once the process is initiated a cascade of changes occurs, which may be irreversible after a particular stage is reached. There is great interest in the development of agents that interfere with the apoptotic process, since these may have a therapeutic place in diseases where apoptosis is increased or decreased.

**Hepatocyte death and regeneration** (Fig. 1.22)

Normal liver structure and function depends upon a balance between cell death and regeneration [54,55].

**Cell death**

Hepatocytes die as a result of either necrosis or apoptosis. The characteristic of necrosis is loss of plasma membrane integrity with release of the cellular contents locally which elicit an inflammatory response. This may potentiate the disease process and lead to further cell death. Ischaemia results in necrosis.

**Apoptosis** is the mechanism by which cells, damaged, senescent or excess to requirement, self-destruct with the least production of inflammatory products [56]. There is DNA fragmentation; organelles remain viable. Thus in comparison with necrotic cells, there is minimal release of injurious products, although there may still be a fibrotic reaction. Equilibrium within normal tissue depends upon the mitotic rate equalling the rate of apoptosis. Cytokine release from lymphocytes and other immune cells also causes apoptosis [57]. The classical example of immunologically-mediated apoptosis is the apoptotic body (apoptotic hepatocyte) found in perportal regions of interface hepatitis (piecemeal necrosis) in chronic hepatitis [58].

**Regeneration**

When there is a need for additional hepatocytes, patches of quiescent cells [62] are stimulated by mediators (primers), including cytokines, to move into a primed state (G_0 → G_1), when growth factors can stimulate DNA synthesis and cellular replication (Fig. 1.22). Priming activates transcription factors including NFkB and STAT 3. Regeneration may be rapid, as seen after partial hepatectomy.

If hepatocytes are damaged so that this response is impaired, hepatocytes may be derived from progenitor/stem cells (‘oval cells’ in rodents) located in the vicinity of the canals of Hering and nearby small bile ductules [63]. In the fetus such stem cells are near ductal plates [64]. Hepatocytes may also be derived from extrahepatic stem cells, probably of bone marrow origin [65,66]. The specificity of hepatocellular or bile duct epithelial differentiation by progenitor cells is encoded by progenitor cell transcription factors, which can be reprogrammed according to the cell type required. Hepatocyte nuclear transport proteins are present on hepatocyte basolateral and apical (canalicular) membranes for uptake of organic acids and bile salt export [53] (see Chapter 11). Organic acid transport protein (OATP), bile salt export pump (BSEP), familial intrahepatic cholestasis 1 (FIC1) and multidrug resistance protein 3 (MDR3) are examples (Fig. 1.21). Jaundice and histological cholestasis may result from inhibition (e.g. BSEP by drugs and LPS) or mutation (e.g. FIC1 in Byler’s disease; MDR3 in intrahepatic cholestasis of pregnancy) of transport proteins.

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**Fig. 1.22.** Liver cell death and regeneration. Hepatocytes are lost either through apoptosis or necrosis. The liver normally regenerates through cellular replication. Priming is necessary for hepatocytes to respond to growth factors. If hepatocyte loss is massive or the toxic attack persists, cellular replication may not be possible. Liver cells may then be derived from progenitor/stem cells either from within the liver or from the bone marrow.
factor 1α (HNF1α) and HNF4α regulate gene transcription for hepatocyte lineage, while HNF1β and HNF6 mediate development of the gallbladder and bile ducts [67]. NOTCH signalling in cooperation with the transforming growth factor-β (TGF-β)/activin pathway further specifies bile duct tubulogenesis [68].

References
