Anatomy and Function

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Abbreviations

AFP	Alpha fetoprotein
cAMP	cyclic adenosine 3′,5′-monophosphate
cGMP	cyclic guanosine 3′,5′-monophosphate
Cx	Connexin
EGFR	Epidermal growth factor receptor
GVHD	Graft-versus-host disease
HCV	Hepatitis C virus
IL-6	Interleukin-6
JAMs	Junctional adhesion molecules
PDZ	Postsynaptic density 95; Discs large, zonula occludens
PKC	Protein kinase C
PSC	Primary sclerosing cholangitis
SR-BI	Scavenger receptor BI
TNF	Tumor necrosis factor
ZO	Zonula occludens

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1.1 Anatomy of the Liver

The liver weighing 1200–1500 g is the largest organ in the human adult and occupies about 2% of body weight. There are two anatomical lobes in the liver, right and left, with the right lobe six times in volume than the left lobe. The right and left lobes are separated anteriorly by the falciform ligament, posteriorly by ligamentum venosum, and inferiorly by ligamentum teres. The Couinaud classification [1] defines eight segments of the liver, and the Bismuth classification [2] divides it into four sectors; they are subdivided into right anterior (V and VIII), right posterior (VI and VII), left medial (IV), or left lateral (II and III) segment and caudate lobe (I) (Fig. 1.1).

The liver receives double blood supply from the portal vein and hepatic artery. The portal vein brings about 65% of hepatic blood flow to the liver from the intestine and spleen, while the hepatic artery brings the remainder 35% from the celiac axis. These vessels enter the liver through the porta hepatis. Inside

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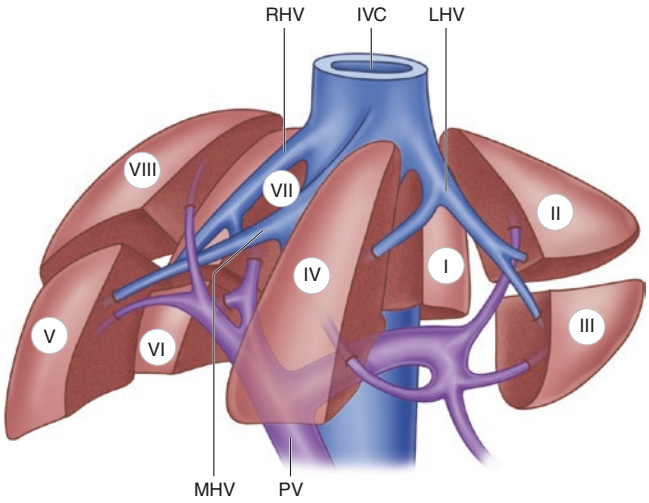


Fig. 1.1 Schematic demonstration of the vascular relations with the segments of the liver (Bismuth classification). The right anterior sector contains segments V and VIII; right posterior sector, segments VI and VII; left medial sector, segment IV; left lateral sector, segments II and III; caudate lobe, segment I. *IVC* inferior vena cava, *LHV* left hepatic vein, *MHV* middle hepatic vein, *RHV* right hepatic vein, *PV* portal vein

the porta hepatis, the portal vein and hepatic artery branch into the right and left lobes. Venous blood from the liver drains into the right and left hepatic veins and enters the inferior vena cava very near to the entry of the right atrium. Lymphatic channels are divided into deep and superficial networks. The former runs parallel to the branches of the portal vessels and hepatic veins, while the latter is found in the capsule, with numerous anastomoses among these networks. The right and left hepatic bile ducts join to form the common hepatic duct. The hepatic nerve plexus contains fiber from the synaptic ganglia, and it accompanies the hepatic artery and bile ducts in the portal tracts. A few fibers enter at the porta hepatis, and arteries are innervated by sympathetic fibers. The bile ducts are innervated by both sympathetic and parasympathetic fibers (Fig. 1.2a). Nerve fibers are present in the portal tract (Fig. 1.2b), and these unmyelinated sympathetic fibers innervate the hepatic parenchyma. Most hepatic nerve fibers are aminergic or peptidergic. Vasoactive intestinal peptide, neuropeptide Y, glucagon, somatostatin, neurotensin, and calcitonin gene-related peptide are present in hepatic nerve fibers (Fig. 1.2c) [3].

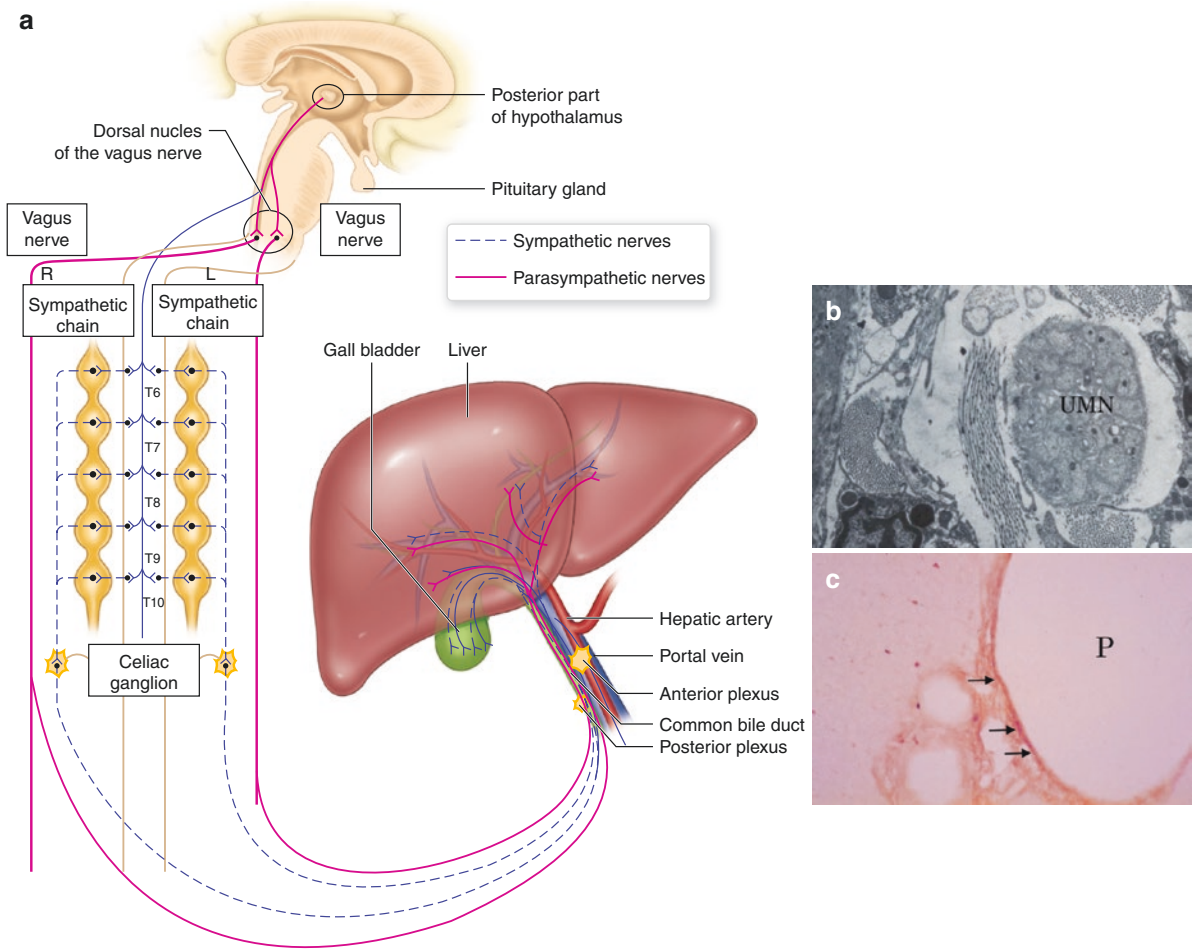


Fig. 1.2 Hepatic nerves. (**a**) Parasympathetic nerve fibers are derived from the dorsal nucleus of vagus nerve, while sympathetic nerve fibers are derived from spinal segments of T6 or 7–T10. They form intercommunicating plexuses around the hepatic artery and portal vein. (**b**) Unmyelinated nerve (UMN) bundle is seen in portal tract of rat liver. (**c**) Glucagon-immunoreactivity (arrow) is seen on nerve fibers in the wall of rat portal vein (P). (**b**, **c**); reuse of Iwai M, et al. Immunoreactive glucagon in rats with normal or regenerative livers induced by galactosamine. Biomedical Res. 1988;9:85–92, with permission of its chief editor

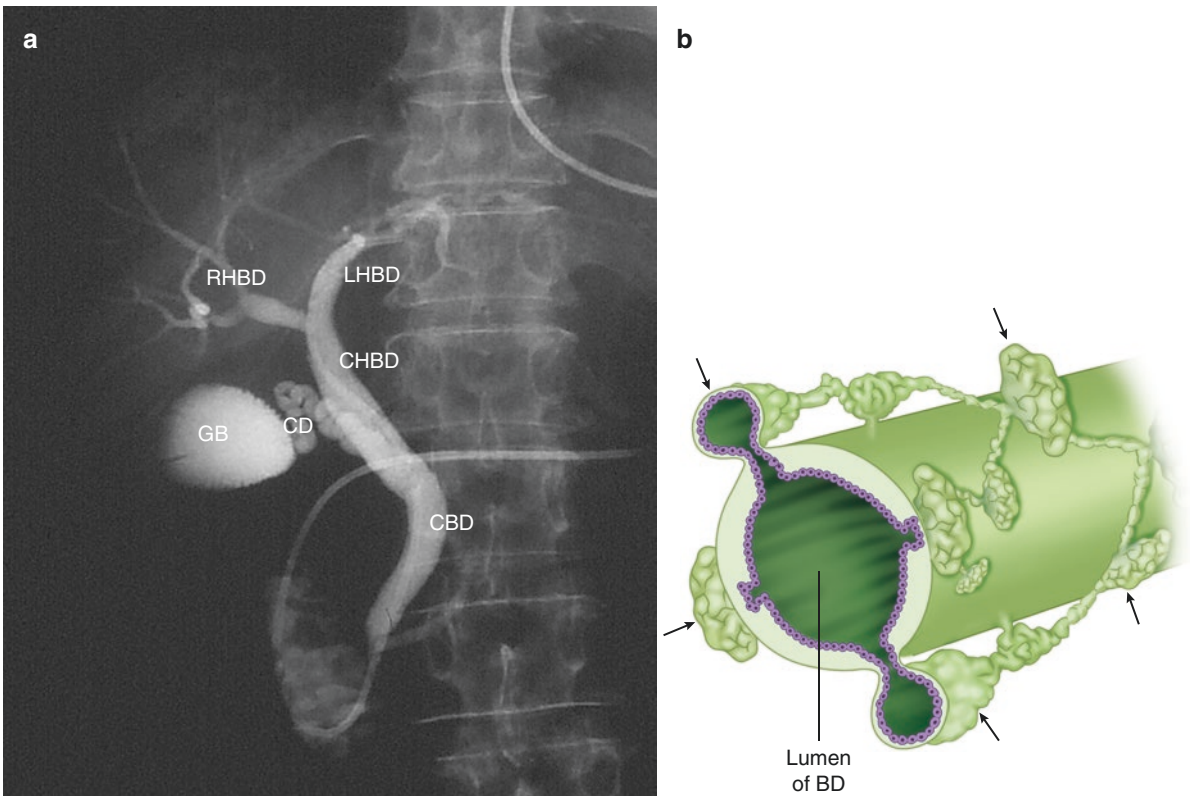


Fig. 1.3 Anatomy of bile duct (BD) and peribiliary gland. (**a**) Cholangiography under therapy of endoscopic biliary drainage shows common bile duct (CBD), common hepatic bile duct (CHBD), right or left hepatic bile duct (RHBD or LHBD), and gall bladder (GB) with cystic duct (CD). (**b**) Peribiliary glands (arrow) are communicating with large bile ducts

The liver takes important roles for metabolism of amino acid/protein, carbohydrate, lipid/lipoprotein, bile acid, bilirubin, hormones, vitamins, and porphyrin. Biotransformation and detoxification of trace elements, acid-base balance, and alcohol degradation also occur in the liver. The morphological and functional integrity of the liver is vital to the health of body, and disturbance of their integrity is closely associated with pathogenesis of liver diseases.

1.2 Anatomy of the Biliary Tract

The right and left hepatic bile ducts (diameter: >800 μm) emerge from the liver and unite at the porta hepatis to form the common hepatic duct (diameter: 0.4 mm–1.3 cm). They are joined by the cystic duct from the gall bladder to form the common bile duct (Fig. 1.3a). Large bile ducts are lined by columnar epithelium with thicker fibrous walls. In primary sclerosing cholangitis (PSC) and IgG4-related cholangitis, the large bile ducts or extrahepatic bile duct is stenotic.

Small intrahepatic bile ducts are classified into septal (diameter >100 μm) and interlobular bile ducts (diameter 40–100 μm). The interlobular bile ducts are connected to the bile canalicular network by ductules and the canals of Hering (diameter: <15 μm). Small bile ducts less than 100 μm are the focus of examination in chronic allograft rejection, GVHD, and Alagille syndrome and are severely damaged in primary biliary cholangitis.

Peribiliary glands are seen within the walls of extrahepatic bile ducts and along the large intrahepatic bile ducts and drain secretory component into the bile duct lumen via their own conduit [4] (Fig. 1.3b). They are thought to have absorptive and secretory activities and may be a site of biliary epithelial regeneration. Cystic lesion, hyperplasia, adenoma, and adenocarcinoma can arise from peribiliary glands.

1.3 Development of the Liver

The human liver arises from hepatic diverticulum of the foregut during the third or fourth week of gestation (Fig. 1.4a). The left and right vitelline veins around the foregut communicate to each other and form sinusoids [5]. The umbilical veins pass on each side of the liver and connect to the hepatic sinusoids (Fig. 1.4b). As the embryo develops, blood, supplying this region, delivers nutrients from the yolk sac, placenta, and gut [6]. Hepatocyte precursors, hepatoblasts, arise from endodermal cells at the front of the diverticulum and invade the mesoderm of septum transversum. The confluence of endodermal cells from the hepatic diverticulum that grows

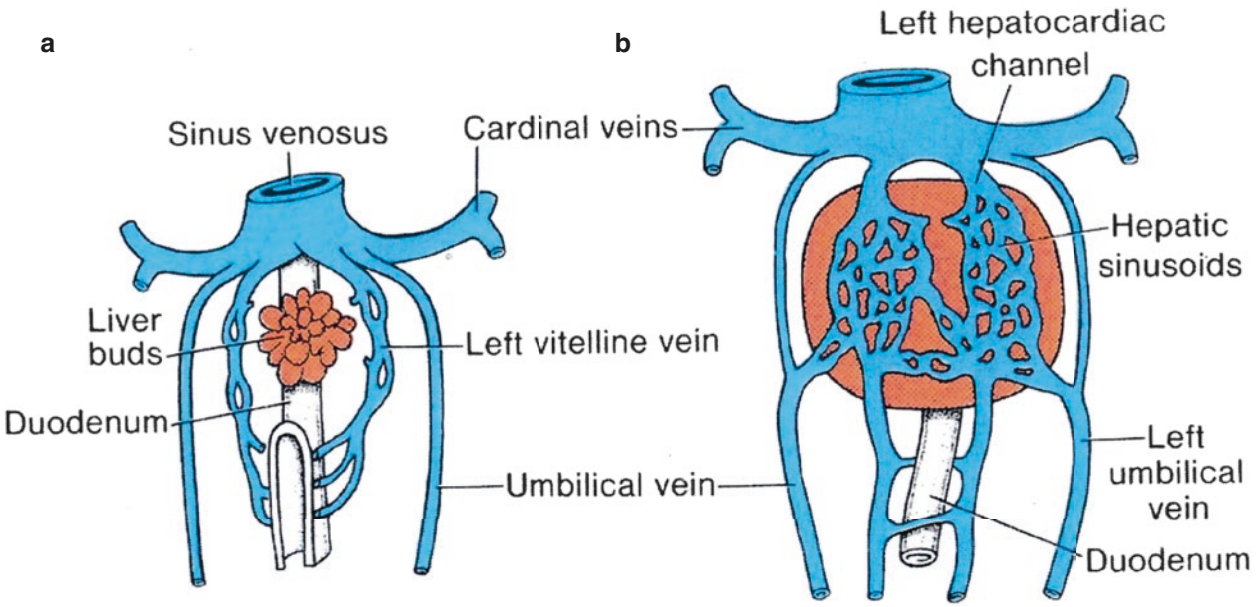


Fig. 1.4 Development of the vitelline and umbilical veins during the fourth (**a**) and fifth (**b**) week. Note the plexus around the duodenum, formation of the hepatic sinusoids, and initiation of left-to-right shunts between the vitelline veins. (**a**) Liver buds arise from the ventral wall of the future duodenum. (**b**) Blood from umbilical veins drains to the sinusoidal plexus and passes through symmetrical right and left hepatocardiac channels, to enter the sinus venous. (Reproduced from Sadler's, Langman's Medical Embryology, tenth edition with permission from Wolters Kluwer)

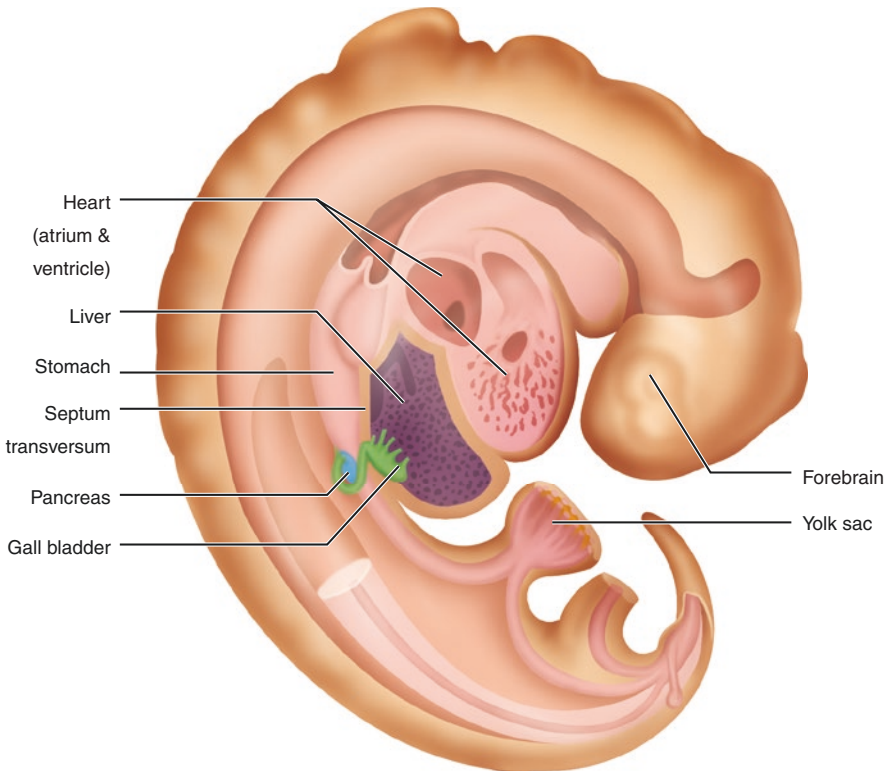


Fig. 1.5 Sagittal section of a human embryo at approximately 32 days of development. The confluence of endodermal cells from hepatic diverticulum invades septum transversum

into host mesenchyme creates the solid organ destined to be liver, while hepatoblasts form trabecular structures (Fig. 1.5). The vitelline veins traverse the region, bridging blood from the yolk sac and digestive tube to the heart. As hepatoblasts invade the mesenchyme, they disrupt the vitelline veins of which segments become the portal veins. The hepatic bud is subdivided into cords by new capillaries of sinusoids, and the sinusoidal flow coalesces into three major hepatic veins.

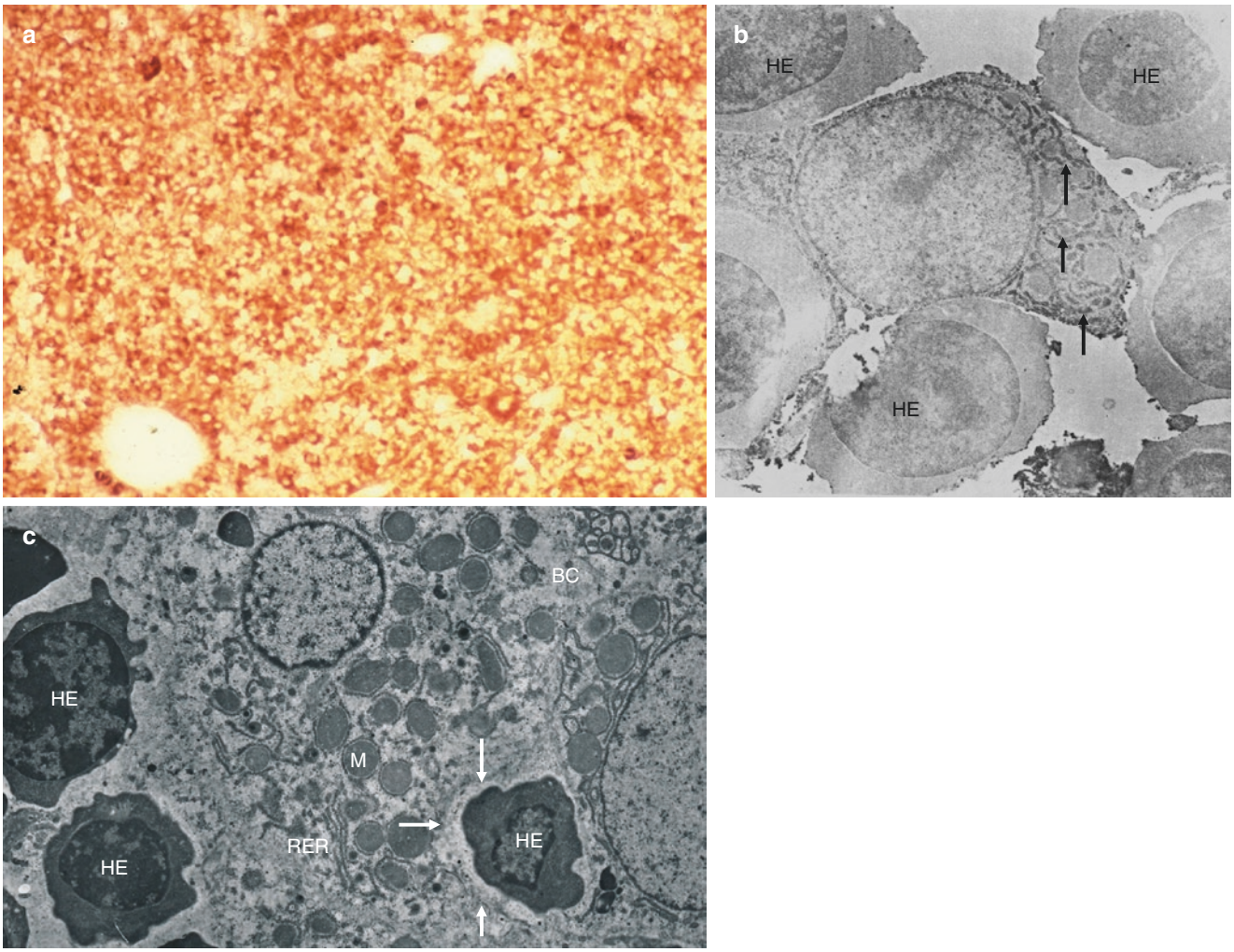


Fig. 1.6 AFP-positive hepatocytes in prenatal liver of rat an Immunohistochemistry for AFP in the liver of prenatal period. (**a**) AFP immunoreactivity is present in all hepatocytes. (**b**) Immunoelectron microscopy of AFP. AFP immunoreactivity (arrow) is detected in poor lamellar structure of rough endoplasmic reticulum. AFP-positive cell is in contact with hematopoietic cells (HE) and is oppressed by HE. (**c**) Electron microscopy shows hepatocytes surrounded by HE, and they have poorly structured rough endoplasmic reticulum (RER) and mitochondria (M), and microvilli (arrow) and bile canaliculi (BC) are visible

Hepatoblast cords develop into anastomosing tubular structures with central bile canaliculi that eventually communicate with the bile ducts. Most hepatoblasts produce AFP (Fig. 1.6a, b) and differentiate into hepatocytes. Numerous hematopoietic cells are found in the sinusoids even at birth and surround immature hepatocytes (Fig. 1.6c), but they are largely gone from the liver by 4 weeks of age.

Hepatoblasts adjacent to the portal mesenchyme differentiate into a layer of duct progenitors called the ductal plate [7]. The ductal plate gradually becomes bilayer and forms ductal segments with lumina. These ductal segments migrate away from the limiting plate to a more central location in the portal tracts near the portal veins. Portion of the ductal plate is resorbed, leaving a complex anastomosing network of ducts which continues to simplify in the weeks after birth. Abnormal remodeling of the embryonic ductal plate causes neonatal biliary atresia [8], congenital hepatic fibrosis, Caroli's disease, microhamartoma, choledochal cyst, and polycystic disease in which there is a genetic abnormality in cholangiocytic cilia leading to disruption of fluid transport and cholangiocyte proliferation [9]. The common bile duct, left and right hepatic ducts, and gallbladder develop in the stalk region of the hepatic diverticulum. These ducts are connected with the ductal plate at the cranial end of the diverticulum.

The liver occupies most of the abdominal cavity in the third month of gestation, in part because of large masses of sinusoidal hematopoietic cells. Thereafter, the right lobe grows faster than the left lobe but less than the growth rate of the rest of the body. The liver cell cords remain tubular until birth when they begin to remodel into double-cell plates and eventually into single-cell plates by 5 years of age.

1.4 Functional Heterogeneity of the Liver

Rappaport advocates a series of functional acini, each centered on the portal tract with its terminal branch of portal vein, hepatic artery and bile duct (Fig. 1.7a), [10] and fibrous tissue supports the structure of the portal tract and central vein (Fig. 1.7b). Hepatocytes show different structural and functional characteristics depending on their acinar location. The relative functions of cells in acini area adjacent to terminal hepatic veins (zone 3) are different from those in the area of zone 1. The zonation is related to the lobular/acinar oxygen gradient and to signaling via Wnt/beta-catenin pathway [11]. Glycogenesis, fatty acid metabolism, and protein, albumin, or fibrinogen synthesis are more active in zone 1 than in zone 3 hepatocytes (Fig. 1.8a, b). Urea synthesis and glutaminase are found in the highest concentration in zone 1, whereas glutamine synthetase is more active at perivenular zone 3. The drug-metabolizing P450 enzymes are present in greater amounts in zone 3, which is the site of detoxification and biotransformation of drugs. The cells in zone 3 receive their oxygen supply last and are particularly prone to anoxic liver injury. Sharply defined

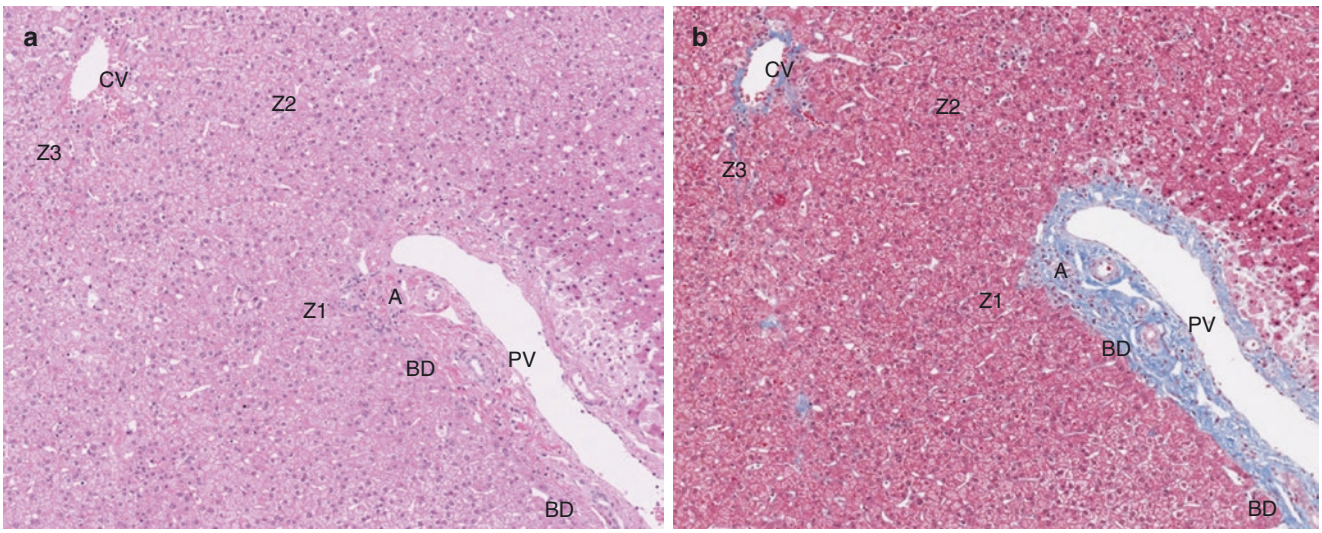


Fig. 1.7 Normal architecture of hepatic acini in adult liver. (**a**) Cords of liver cells are radiating from a central vein (CV) and interlaced in ordered fashion by sinusoids, and at the periphery a portal tract contains arteriole (A), bile duct (BD), and portal vein (PV). Z1, 2, and 3; zone 1, zone 2, and zone 3. H&E staining. (**b**) Portal tract and central vein are supported by fibrous tissue, which is scarcely seen along hepatocytes. Masson trichrome staining. (Courtesy of Dr. Y Harada)

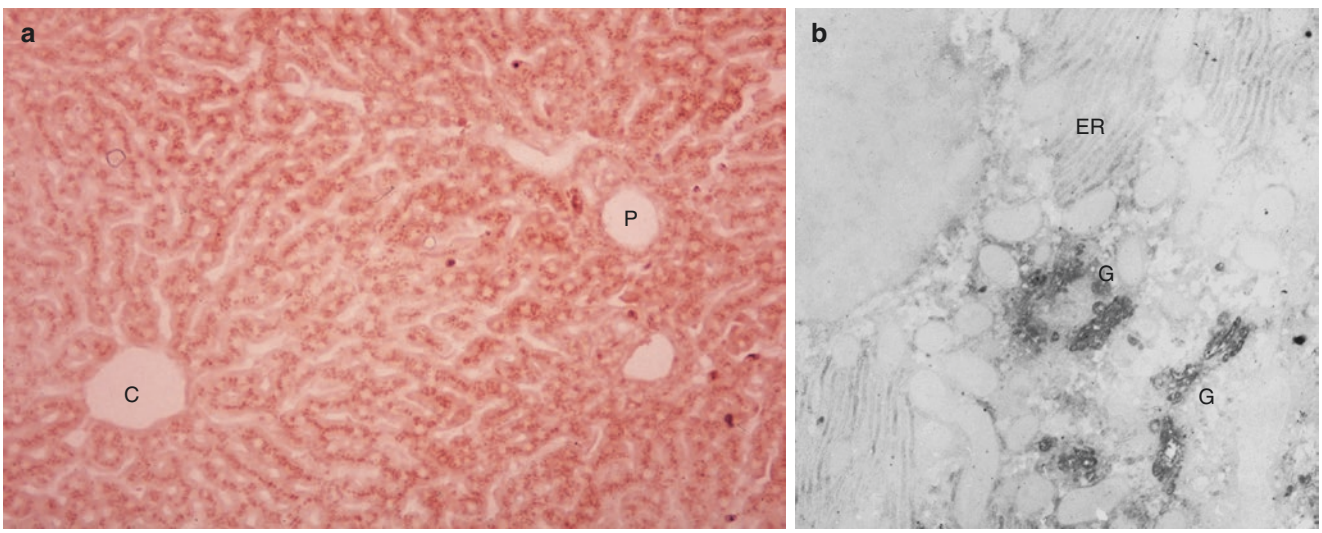


Fig. 1.8 Immunohistochemistry of albumin-positive hepatocytes in a lobule of adult rat and ultrastructural finding of albumin immunoreactivity. (**a**) All hepatocytes contain albumin immunoreactivity, and its intensity is stronger in periportal area than in pericentral one (C). *P* portal vein; *C* central vein. (**b**) Albumin immunoreactivity is visible in rough endoplasmic reticulum (ER) and Golgi (G) apparatus

Table 1.1 Functional and ultrastructural heterogeneity of hepatocytes in acini

	Zone 1	Zone 3
Organelle	Golgi complex; rich Mitochondria; numerous, larger, more inner membranes	Smooth ER; rich Mitochondria; smaller, less inner membranes
Protein synthesis (albumin fibrinogen)	3+	1+
Carbohydrate	Gluconeogenesis	Glycolysis
Glutathione	2+	1+
Ammonia metabolism	1+	2+
Cytochrome 450	+	3+
Oxygen supply	3+	+
Bile formation		
Bile salt dependent	2+	1+
Non-bile salt dependent	1+	2+

zone 3 necrosis is characteristic of toxicity from acetaminophen, pyrrolizidine alkaloids, and various hydrocarbons such as halothane and carbon tetrachloride. Zone 1 necrosis has been found with allyl alcohol, phosphorus, and high-dose iron ingestion (Table 1.1).

1.5 Microanatomy of the Liver (Fig. 1.9)

Hepatocytes: The life span of liver cells is about 150 days in experimental animals, and hepatocytes comprise about 65% of the liver. They are arranged in plates of one cell in thickness and polygonal in shape, ranging from 30 to 40 μm in diameter. Microvilli project into the perisinusoidal space, providing active secretion or absorption of nutrients from the space of Disse. Hepatocytes are attached to one another by tight junctions, gap junctions, and desmosomes. There are

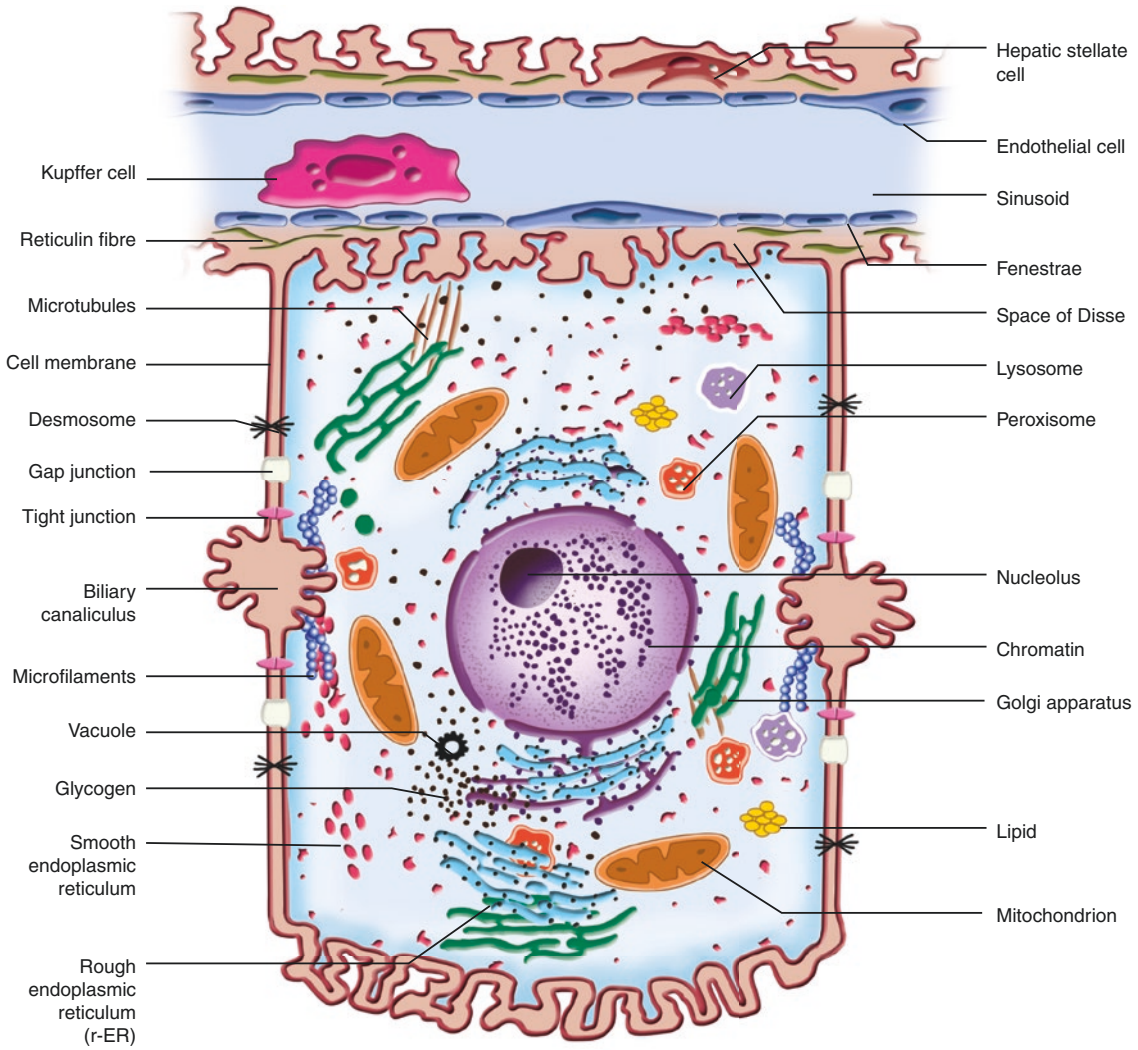


Fig. 1.9 Ultrastructural scheme of hepatocyte and mesenchymal cells within the lobule. Hepatocytes are attached to one another by junctional complex and they have abundant organellae, projecting microvilli in the perisinusoidal space. Sinusoids are composed of endothelial and stellate cells, and infiltrated by Kupffer cells

three surfaces: sinusoidal, basolateral, and canalicular surfaces. The polarity of the cell membrane is maintained by tight junctions [12].

The hepatocytes have single nucleus or, sometimes, multiple nuclei. The cytoplasm is rich in rough or smooth endoplasmic reticulum, mitochondria, peroxisomes, and lysosomes. Cell functions associated with the endoplasmic reticulum include protein synthesis, metabolism of fatty acids, production of cholesterol or triglyceride and bile acids, xenobiotic metabolism, and heme degradation. Golgi complex, lying near the canaliculus, has a role not only for secretion of proteins but also for glycosylation of secretory proteins. Production of energy and oxidative phosphorylation take place in mitochondria. The mitochondria also contain various enzymes useful for cycle of citric acid, beta-oxidation of fatty acid, and heme synthesis. Lysosomes located adjacent to the bile canaliculi contain many hydrolytic enzymes which can destroy the cell. The lysosomes are the site of deposition of ferritin, copper, bile pigment, lipofuscin, and senescent organelles. The peroxisomes are enzyme-rich and oxidative-reactive structures. The enzymes include simple oxidases and those involved in beta-oxidation cycles, glyoxylate cycle, lipid synthesis, and cholesterol biosynthesis.

The cytoskeleton supporting the hepatic structure consists of microtubules, microfilaments, and intermediate filaments [13]. Microtubules that contain tubulin control subcellular motility, vesicle movement, and secretion of plasma protein or glycoprotein. Microfilaments made up of actin are contractile and are important for the motility of bile canaliculus and for the bile flow. Intermediate filaments consisting of cytokeratins are essential for the stability and special organization of hepatocytes.

Endothelial cells: The sinusoidal endothelial cells constitute 70% of sinusoidal cells, and they do not have intercellular junctions or a conventional basement membrane. They act as a sieve between sinusoid and space of Disse due to presence of fenestrations. The fenestrations are larger in zone 1, but smaller and more numerous in zone 3, filtering macromolecules of different sizes [14]. The fenestrations can change in size in response to stimuli of pressure, neural impulses, endotoxins, alcohol, serotonin, and nicotine. They also have specific and non-specific endocytic activities and a variety of receptors. Receptor-mediated endocytosis exists for several molecules including transferrin, ceruloplasmin, and high- or low-density lipoprotein. Sinusoidal endothelial cells can clear small particles (<0.1 μm) from circulation as well as denatured collagen. Unlike vascular endothelial cells elsewhere in the body, sinusoidal endothelial cells do not express CD34 or factor VIII-related antigen. However, in chronic liver disease or liver cirrhosis, they undergo a phenotypic shift to conventional vascular endothelium and are referred as capillarization of sinusoids [15].

Kupffer cells: Kupffer cells are the resident macrophages attached to the endothelial cell lining of the sinusoids and can be mobilized. They are derived from monocytes in bone marrow and are found in greater number in the periportal area. Their cytoplasm contains microvilli, intracytoplasmic-coated vesicles, and dense bodies made up by lysosomal apparatus. They are responsible for removing old blood cells, cellular debris, bacteria, viruses, parasites, and tumor cells by endocytosis through receptor- or non-receptor-mediated mechanisms [16]. They are activated by agents of endotoxin, sepsis, shock, interferon-gamma, arachidonic acid, and tumor necrosis factor. They produce cytokines; hydrogen peroxide; nitric oxide; tumor necrosis factor; interleukin 1, 6, or 10; interferon alpha; transforming growth factor; and prostanoids [17].

Hepatic stellate cells: They are known as fat-storing cells, stellate cells, Ito cells, or lipocytes. They lie within the subendothelial space of Disse. Cytoplasmic droplets contain abundant vitamin A in the form of retinol palmitate [18], which can be identified by their immunoreactivity to smooth muscle actin. When the droplets are scanty, they resemble fibroblasts, which contain actin and myosin and contract in response to endothelin-1 and substance P [19] to regulate blood flow and to influence portal pressure [20]. In hepatocellular injury, hepatic Kupffer cells are activated, releasing many cytokines. Stellate cells transform to myofibroblast-like phenotype, producing collagen types 1, 3, and 4 and laminin. They also release matrix proteinases and their inhibitory molecules. In normal condition or liver injury, hepatic stellate cells play a role in regeneration, induced by hepatic growth factor in response to insulin-like growth factor 2 [21].

Pit cells: Pit cells are natural killer-lymphocytes attached to the sinusoidal surface of the endothelium [22] and are numerous in sinusoids of zone 1. They are short-lived cells that are renewed from circulating large granular lymphocytes. They have characteristic granules which contain perforin injuring cell membrane [22] and have a role for killing tumor cells and virus-infected hepatocytes.

Bile duct epithelial cells: Biliary epithelial cells that are columnar in shape, lining intrahepatic and extrahepatic bile duct system, constitute 3.0–5.0% of all hepatic cells [23]. Compared to hepatocytes, biliary epithelial cells contain fewer mitochondria and less endoplasmic reticulum. They are rich in cytoskeleton and contain prominent Golgi apparatus, numerous vesicles, and short luminal microvilli. Cholangiocytes lining small ducts transport water and organic solutes under hormonal control (secretin and somatostatin). IgA and IgM are secreted through cholangiocytes [24]. Bile acids are absorbed by biliary epithelium and are recirculated by a cholehepatic shunt pathway via the peribiliary plexus, [25] which promotes bile acid-dependent bile flow in the ducts [26].

1.6 Junctional Complex Between Hepatocytes

Hepatocytes are attached to each other by tight junctions, gap junctions, and desmosomes. There are three surfaces of hepatocytes: sinusoidal, basolateral, and canalicular domains. The polarity of cell membrane is maintained by tight junctions [12].

Tight junctions constitute the permeability barrier to macromolecules between the bile canaliculus and the rest of the intercellular space, preventing passage of the bile out of the canaliculus. The most apically located intercellular junctional complexes inhibit solute and water flow through the paracellular space (termed the "barrier" function) [27, 28], and they also separate the apical from the basolateral cell surface domains to establish cell polarity (termed the "fence" function)) [29, 30] (Fig. 1.10a). Recent evidence suggests that tight junctions also participate in signal transduction mechanisms that regulate epithelial cell proliferation, gene expression, differentiation, and morphogenesis [31]. The tight junctions are formed by not only the integral membrane proteins claudins, occludins, and JAMs (Fig. 1.10b) but also many peripheral membrane proteins, including the scaffold PDZ-expression proteins, cell polarity molecules, and non-PDZ-expressing proteins (Fig. 1.10c) [32–34]. Tricellulin was identified at tricellular contacts where there are three epithelial cells and has shown to have a barrier function (Fig. 1.10b) [35].

In the human liver, occludin, JAM-A, ZO-1, ZO-2, claudin-1, claudin-2, claudin-3, claudin-7, claudin-8, claudin-12, claudin-14, and tricellulin are detected together in well-developed tight junction structures (Fig. 1.11a, c). Claudin-2 shows a lobular gradient increasing from periportal to pericentral hepatocytes as in the livers of rat and mouse, whereas claudin-1 is expressed in the whole liver lobule (Fig. 1.11b). Tricellulin is detected in the regions of bile canaliculi where tight junctions can be identified as a set of branching intramembranous strands in freeze-fracture replicas (Fig. 1.12a, b) [36]. Claudin-1 and claudin-2 in hepatocytes are regulated by various cytokines and growth factors via distinct signal transduction pathways (Fig. 1.13a, b) [37, 38].

As a genetic disease of human tight junction protein, missense mutations in ZO-2 have been identified in patients with familial hypercholanemia [39]. In neonatal ichthyosis-sclerosing cholangitis (NISCH) syndrome, mutations of claudin-1 may lead to increased paracellular permeability between bile duct epithelial cells [40].

HCV is an enveloped positive-stranded RNA hepatotropic virus. Three host cell molecules are important entry factors or receptors for HCV internalization: scavenger receptor BI (SR-BI), tetraspanin CD81, and claudin-1 [41]. CD81 and claudin-1 act as co-receptors during late stages in the HCV

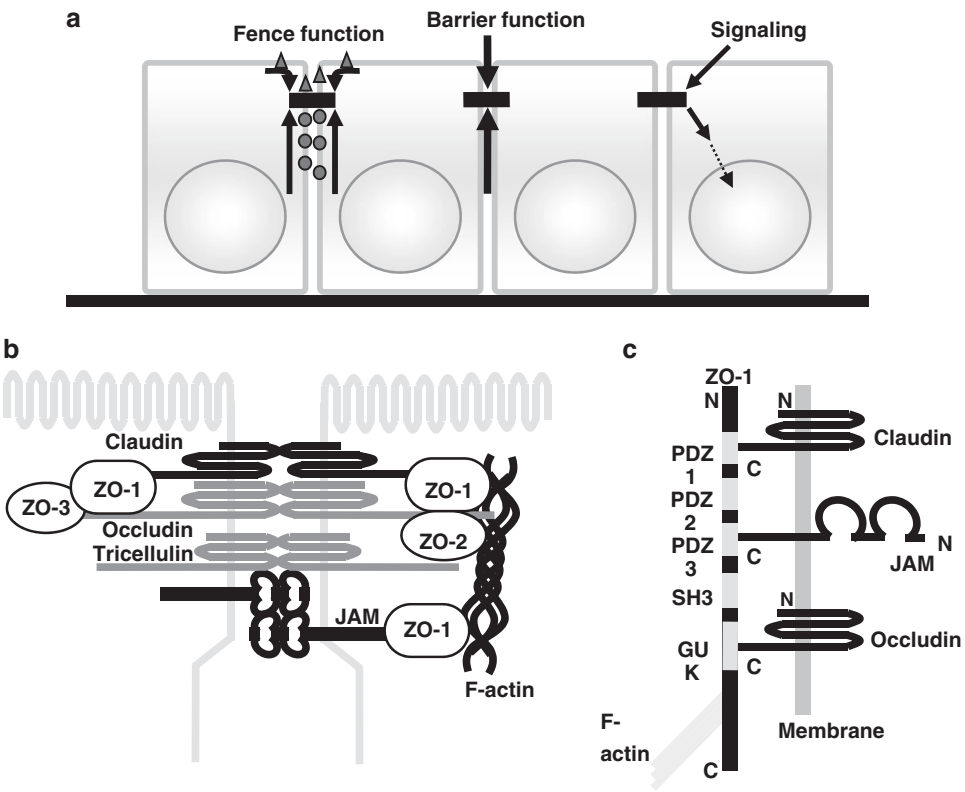


Fig. 1.10 Structure of tight junction. (**a**) Fence or barrier function of tight junction and its signal transduction. (**b**) Molecular structure of tight junction. (**c**) Tight junctional formation in cell membrane in association with actin filament

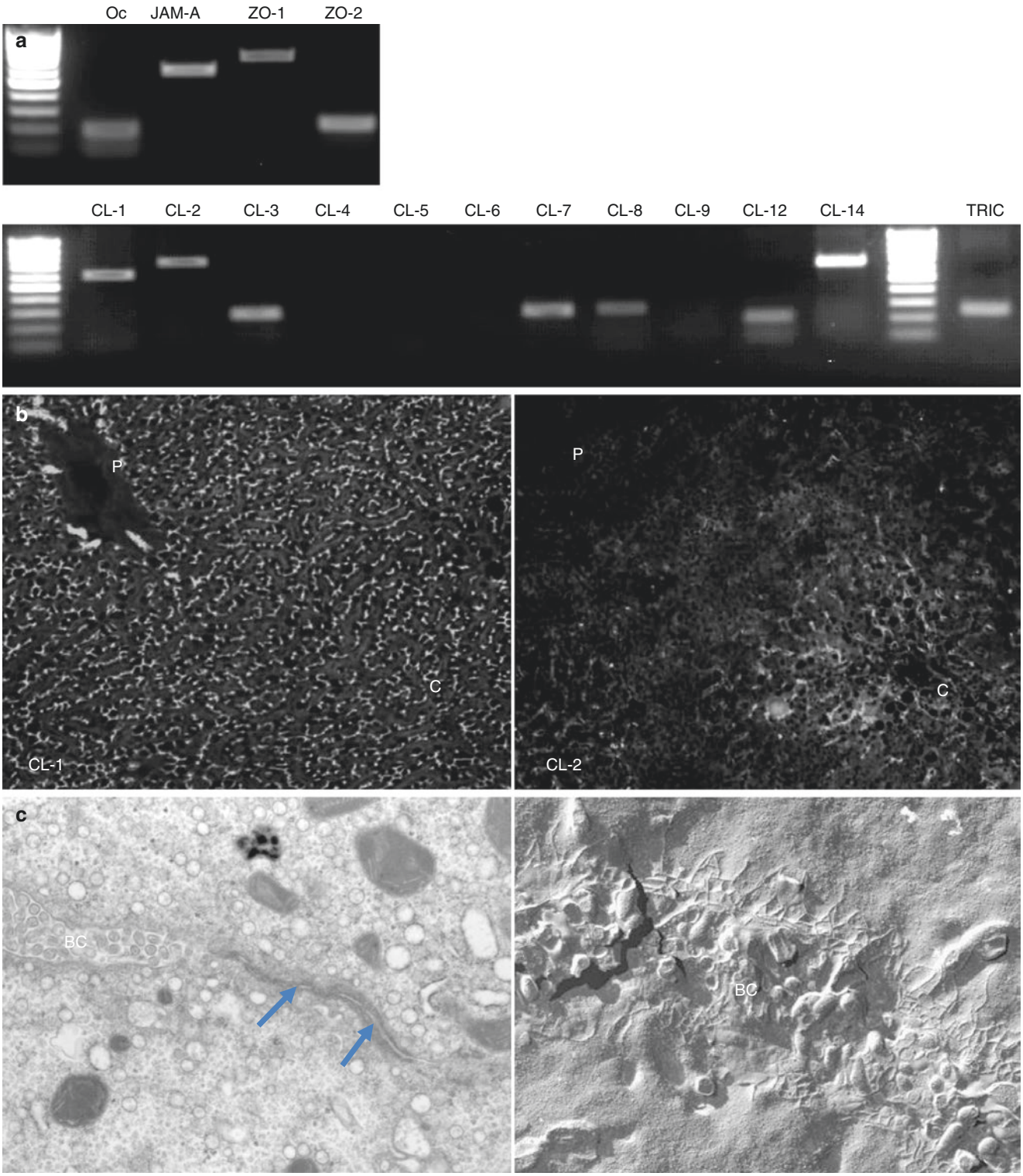


Fig. 1.11 Molecular structure of tight junction. (**a**) Western blot shows expression of JAM, Z-1, ZO-2, CL-1, CL-2, CL3, CL-7, CL-8, CL-12, and CL-14 in human liver. (**b**) Immunohistochemistry of CL-1 and CL-2. CL-1-immunoreactivity is seen diffusely on bile canaliculus of all hepatocytes, and CL-2 is detected on bile canaliculus of hepatocytes in pericentral area. (**c**) TEM and freeze fracture show tight junctional structure (arrow). *BC* bile canaliculus

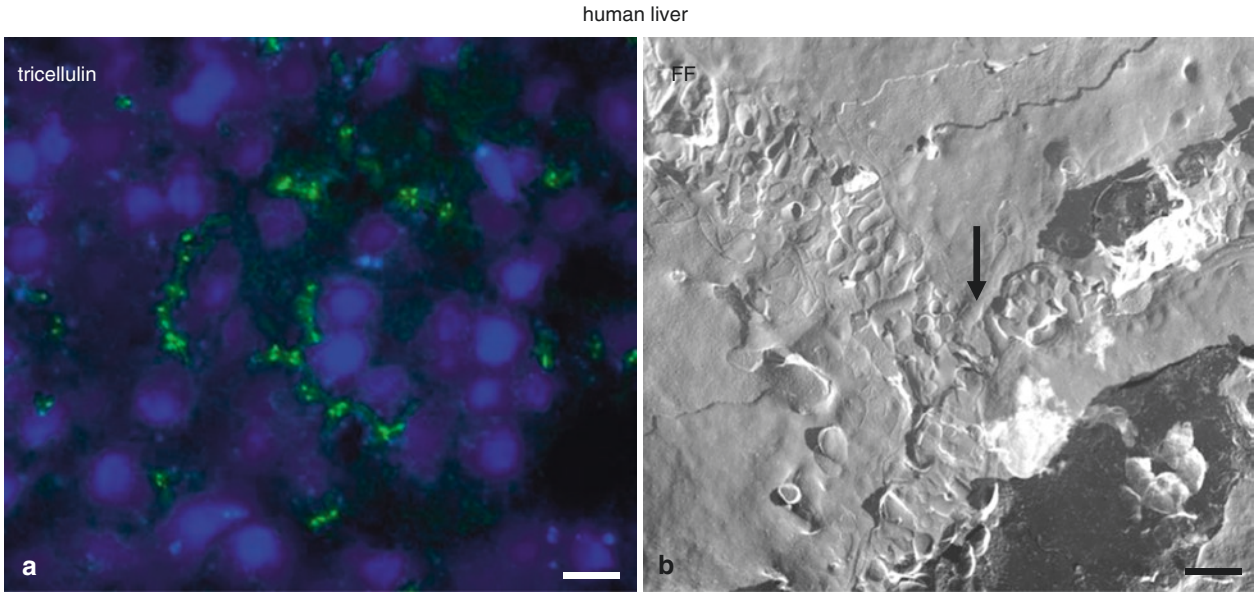


Fig. 1.12 Immunohistochemistry of tricellulin and its observation by freeze-fracture method. (**a**) Tricellulin-positive immunofluorescence is seen on bile canaliculi. (**b**) Freeze-fracture replicas show branched intramembranous strands in tight junction

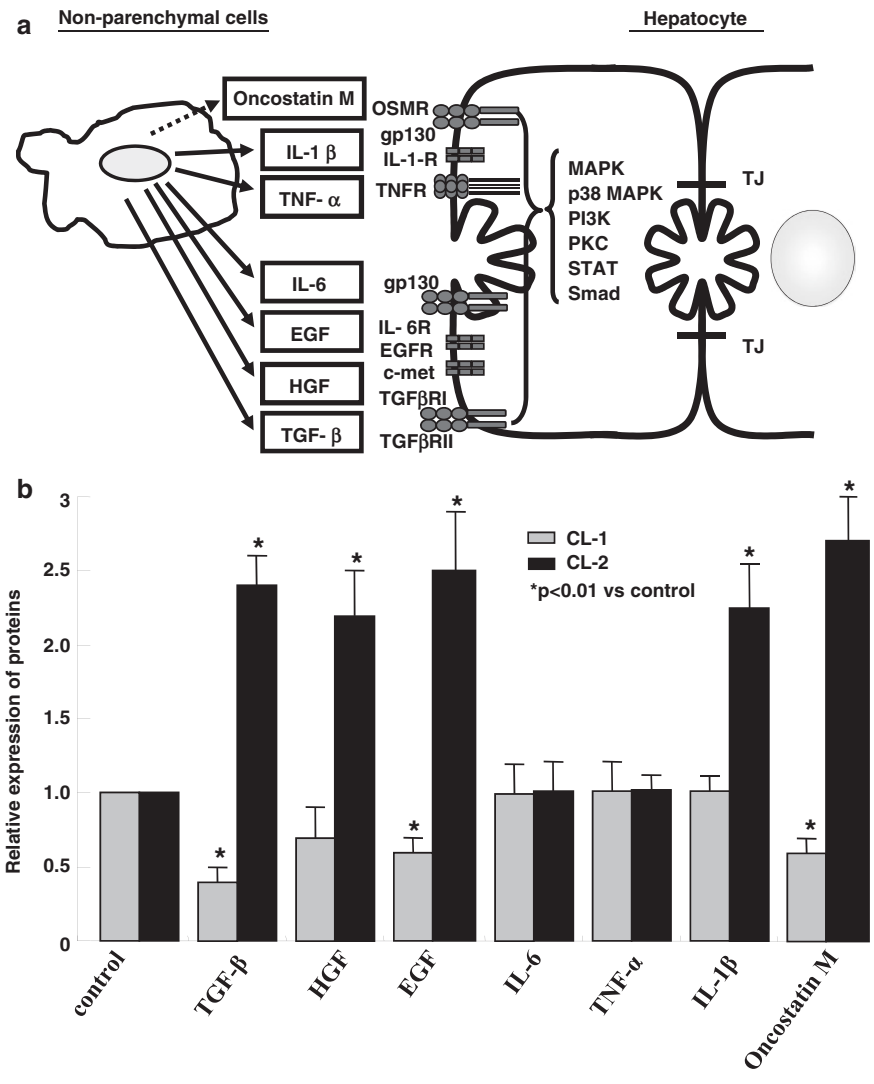


Fig. 1.13 Relation between tight junction and various cytokines. (**a**) Association of tight junction and oncostatin, IL-1beta, TNF alpha, IL-6, EGF, HGF, and TGF beta produced by non-parenchymal cells. (**b**) Oncostatin, EGF, and TGF beta suppress Claudin-1 expression in hepatocytes. Oncostatin, IL-1 beta, EGF, HGF, and TGF beta can induce expression of Claudin-2

entry process. The first extracellular loop of claudin-1 in the liver is critical for the entry [42]. Occludin is also reported to be required for HCV entry [43, 44]. The tight junction proteins, claudin and occludin, are novel key factors for HCV infection. Both are potential targets for antiviral drugs.

Gap junctions are composed of 12 subunits (connexin) (Fig. 1.14a, b), six of which are contributed by each neighboring cell; the six-subunit assembly contributed by each cell is called a connexon or hemichannel (Fig. 1.14a, c) [45]. Intercellular communications occur at the gap junctions, involving calcium ion, second messenger RNA, and nerve impulses from zone 1–3. Connexin (Cx) 32 are permeable to both cAMP and cGMP, whereas heteromeric connexons composed of Cx32 and Cx26 lose permeability to cAMP but not to cGMP (Fig. 1.14d) [46]. The gap junctions also play an important role in liver homeostasis, [47] liver development [48], cancer, [49–52] and non-tumor liver diseases (hepatitis, liver fibrosis, cirrhosis, cholestasis, hepatic ischemia, and reperfusion injury) [47]. Hemichannels of connexon are made up of Cx32 in pericentral area of adult liver (Fig. 1.15a), and they are mixed with Cx32 and Cx26 in periportal area (Fig. 1.15b, c) [53]. The cells of the liver capsule, Ito (fat-storing) cells, cholangiocytes, and endothelial cells express Cx43 as a major gap junction protein (Fig. 1.15d) [46].

In freeze-fracture images, hepatic gap junctions are recognizable as plaques of approximately 8- to 9-nm intramembranous particles present in the P fracture face in vertebrate tissues; complementary pits appear on the E fracture face (Fig. 1.16a). These plaques are generally round or oval and can be quite large in hepatocytes. Furthermore, small gap junction plaques are associated with tight junction strands in some cell types including hepatocytes (Fig. 1.16b), and Cx32 is partly colocalized with occludin and claudin-1 forming tight junction structures [54]. In Cx32-transfectanted immortalized mouse hepatocytes, which lack endogenous Cx32 and Cx26, induction of tight junction strands, and the integral tight junction proteins, occludins and claudins are observed,

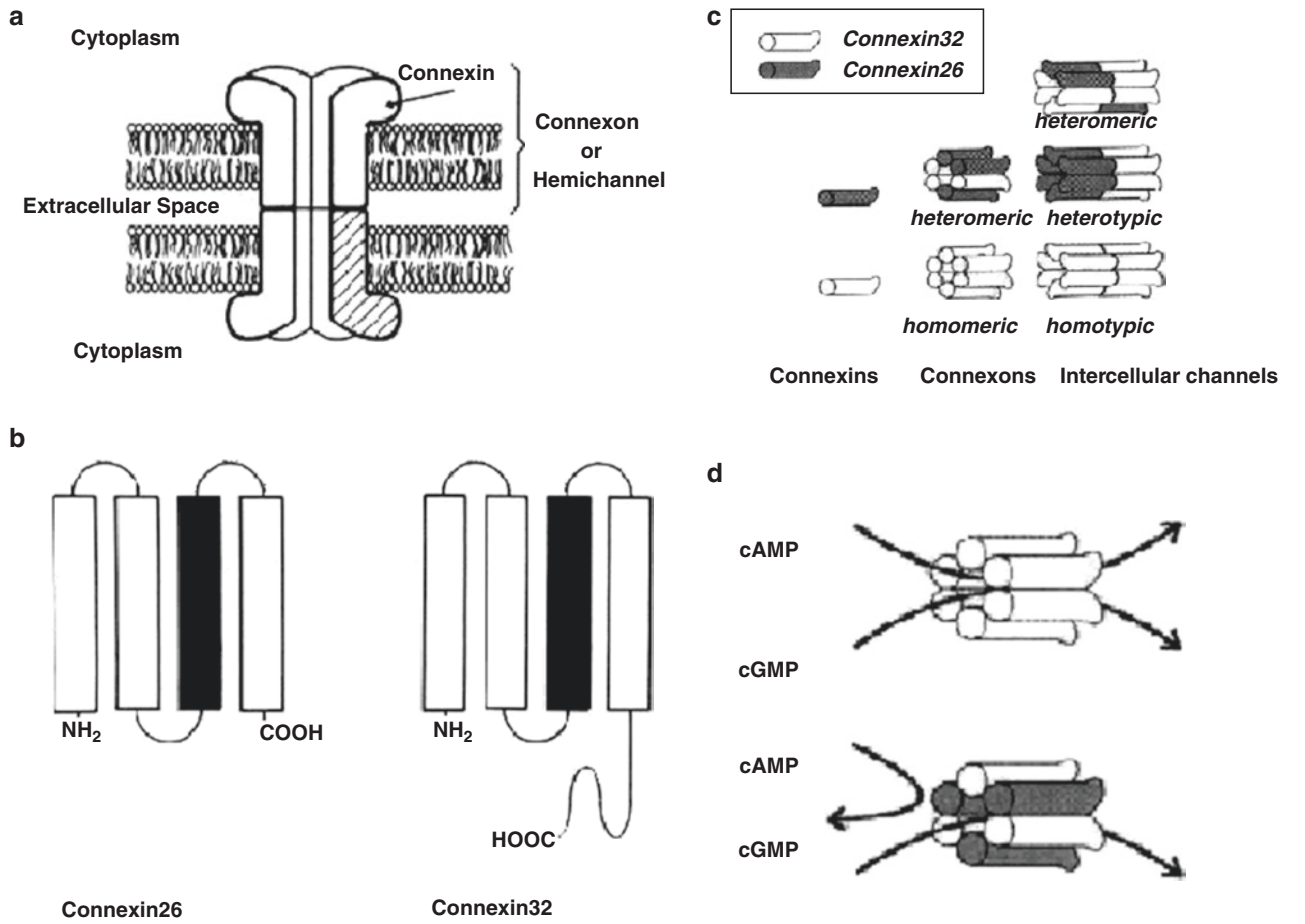
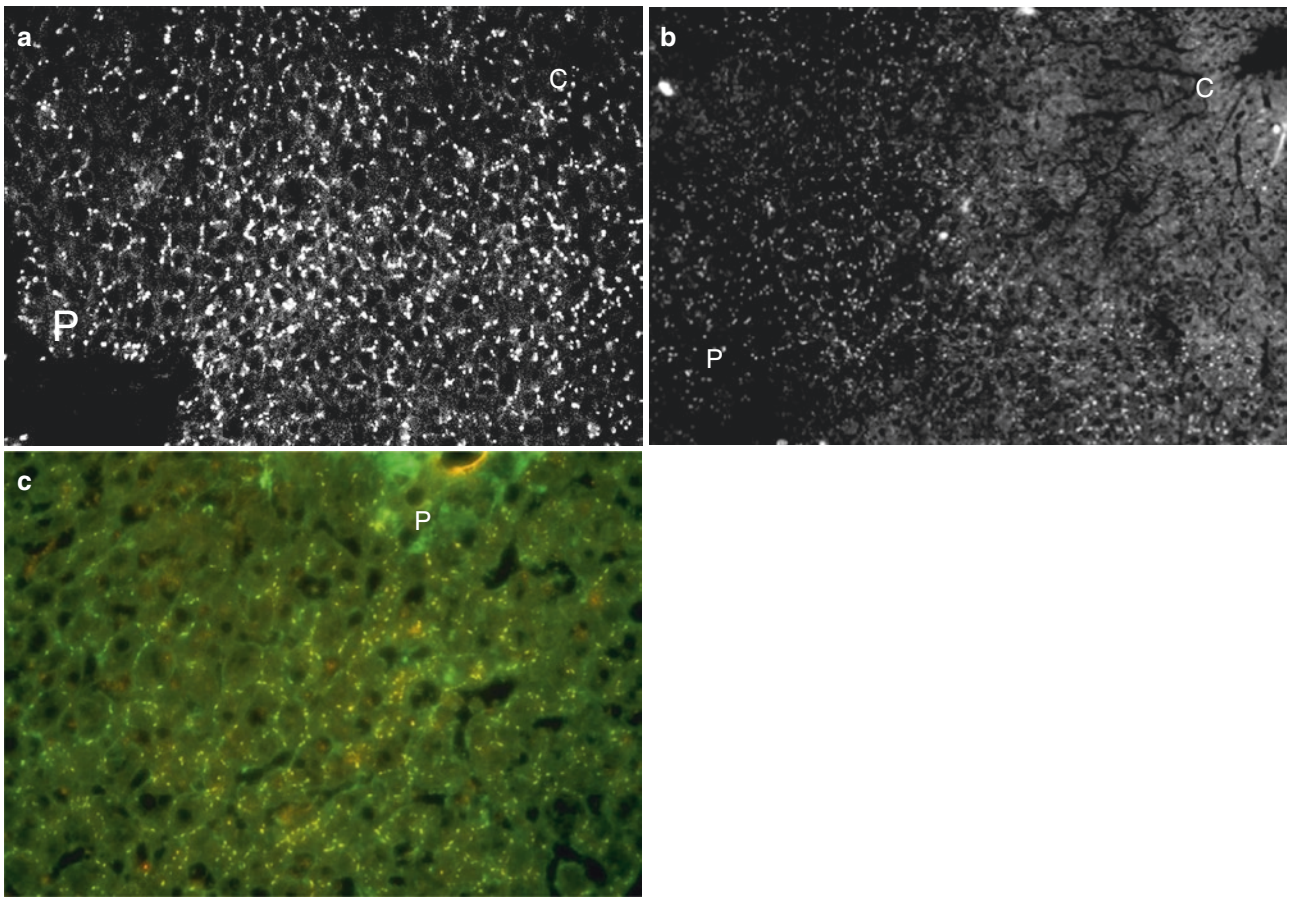


Fig. 1.14 Structure of gap junctions. (**a**) Connexon or hemichannel of gap junction is composed of six subunits of connexin. (**b**) There are two types of connexin in hepatocytes, and connexin 26 or 32 is a penetrating protein in cell membrane. (**c**) There are homomeric or heteromeric hemichannels, and there are homotypic or heterotypic intercellular channels. (**d**) Homomeric channels of Cx32 are permeable to both cAMP and cGMP, whereas heteromeric connexons lose permeability to cAMP but not to cGMP



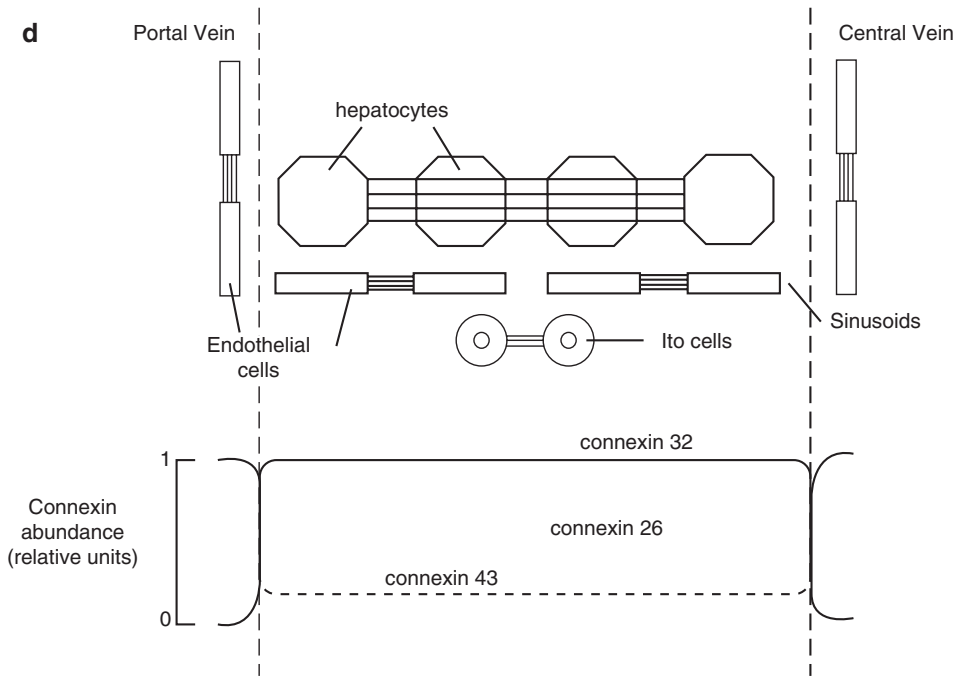


Fig. 1.15 Expression of connexin 26, 32, and 43. (**a**) Immunofluorescence of connexin 32. Connexin 32-positive fluorescence is seen on cell membrane of all hepatocytes. (**b**) Immunofluorescence of connexin 26. Connexin 26-positive fluorescence is present on the cell membrane of periportal hepatocytes. (**c**) Double immunocytochemistry of Cx32 and Cx26. Cx32 labeled with fluorescence and Cx26 labeled with rhodamine are seen in simultaneous dots on cytoplasmic membrane of periportal area (orange color), and fluorescence-positive dots of Cx32 are seen in pericentral area (green color) (rat liver). (**d**) Cx43 are expressed in endothelial cells or Ito cells, and Cx26 expression is gradually decreased in zone 2 to zone 3, and Cx32 are diffusely expressed in a lobule. *P* portal tract, *C* pericentral area

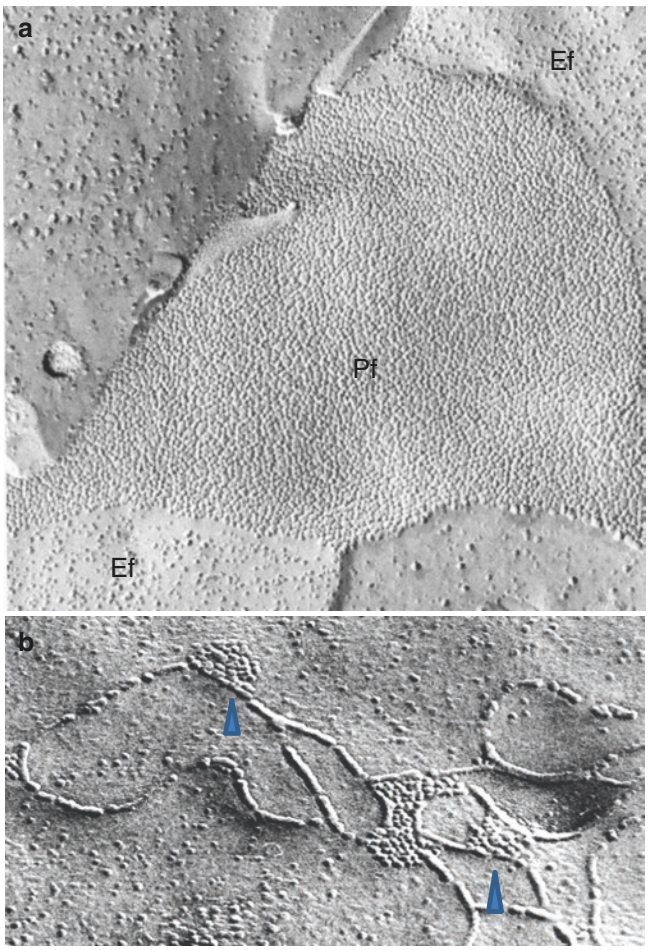


Fig. 1.16 Freeze-fracture images. (**a**) Hepatic gap junctions are recognizable as plaques of approximately 8- to 9-nm intramembranous particles present in the P fracture face (Pf), and complementary pits appear on the E fracture (Ef) face. (**b**) Small gap junction plaques are associated with tight junctional strands (arrow head)

and the induced endogenous occludin protein in the transfectants is found to bind to the exogenously expressed Cx32 protein [55, 56]. Gap junction and tight junction expression are closely correlated in hepatocytes, and gap junction expression may play a crucial role in the establishment of cell polarity via regulation of tight junction proteins. Studies of protein-protein interactions and coordinate/subordinate regulation of gene families are soon expected to disclose the intricacies of inter- and intracellular signaling and growth control at gap junctions and the regulatory mechanisms of the "blood-biliary barrier" formed by tight junctions [57].

1.7 Regeneration

Normal liver structure and function depend upon a balance between cell death and proliferation, [58] and the liver converts to a proliferative organ after surgical resection or massive injury and recovers its mass slowly and can store three-quarters of its mass within 6 months after surgical resection, but it has not been clearly understood from where or how parenchymal cell proliferate in partial hepatectomized liver and injured liver.

Protein kinase C (PKC) alpha type is reported to take an important role in early event of liver regeneration, not only phosphorylating Raf and mitogens but also activating proto-oncogene [59]. Our experimental study using combined technique of immunocytochemistry for PKC alpha and autoradiography with ^{3}H-thymidine reveals that it is expressed in periportal area 6 h after 2/3 hepatectomy and that proliferating hepatocytes appear around portal tract at the same time and PKC alpha expression and proliferating hepatocytes reach a peak 12 and 48 h (Fig. 1.17a, c), then PKC alpha may take a role for early event of liver regeneration after hepatectomy, and DNA synthesis begins in hepatocytes of periportal area after stimulation of PKC alpha. Progenitor cells or proliferating hepatocytes around a portal tract spread to central area in the hepatectomized liver (Fig. 1.17b).

Liver structure is restored by regeneration of parenchymal and mesenchymal cells after liver injury. AFP, which is considered to be a regenerating marker, [60–63] is elevated in serum just after a peak of s-GOT in rat with acute liver injury (Fig. 1.18a), and AFP-positive cells are detected in surrounding area of central necrosis (Fig. 1.18b, c). Proliferating parenchymal cells labeled with ^{3}H-thymidine are found not only in surrounding area of central necrosis but also in periportal area (Fig. 1.19), and then restoration of parenchymal cells after liver injury occurs not only in vicinity of necrosis but also in periportal tract.

Polypeptide growth factors like hepatocyte growth factor [64], epidermal growth factor [65], transforming growth factor [66], heparin-binding EGR-like growth factor, [67] and insulin-like growth factor [68] are known to be capable of inducing hepatocyte replication at the beginning of regenerative process. Regenerating liver requires nutrition and various hormones of insulin, glucagon, thyroid and adrenal cortical, parathyroid, prolactin, vasopressin, prostaglandin, or catecholamines, and sex hormones[69].

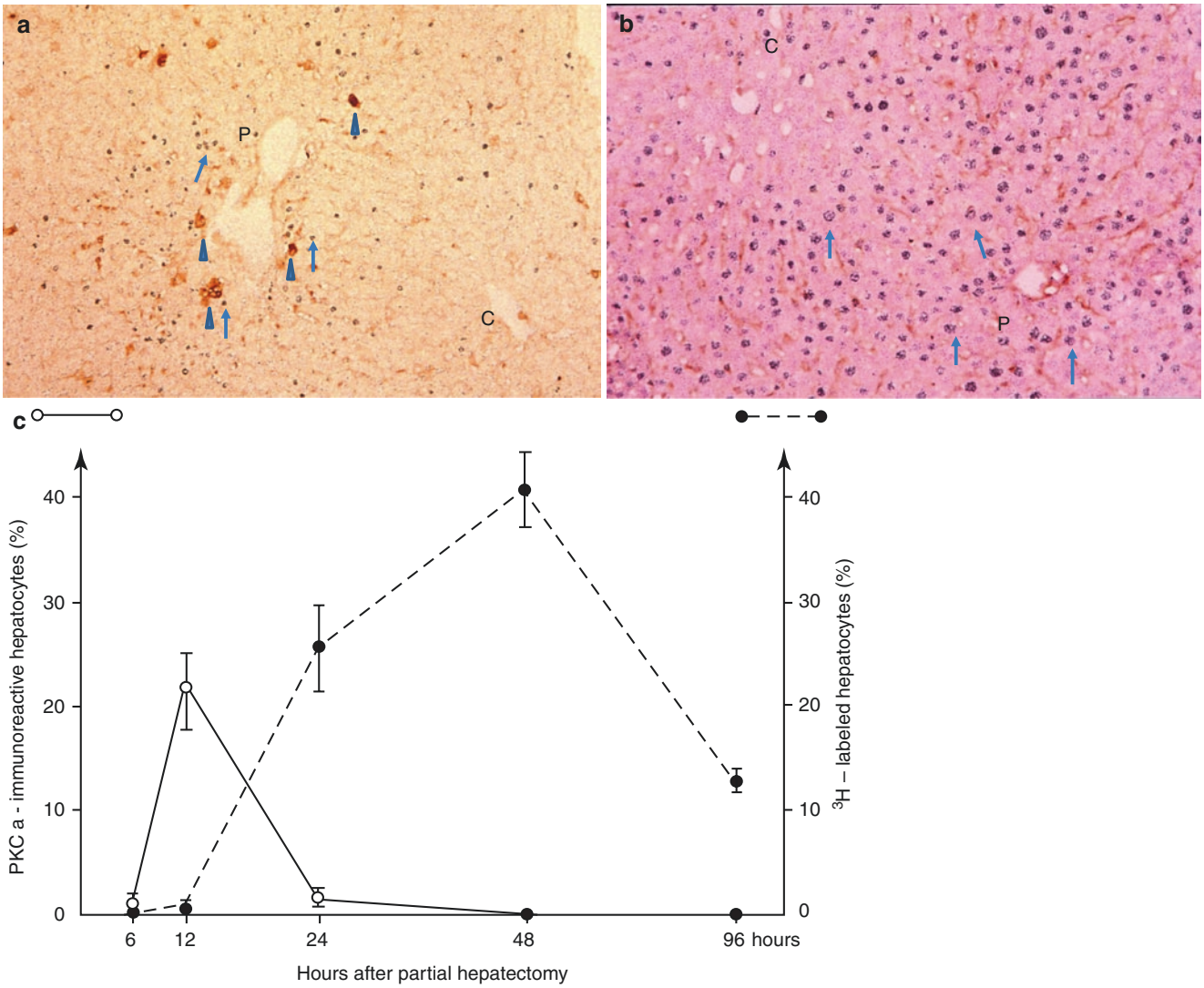
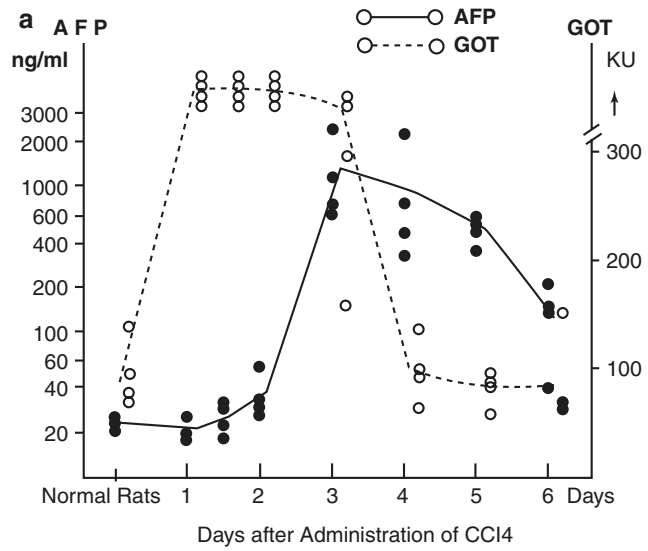
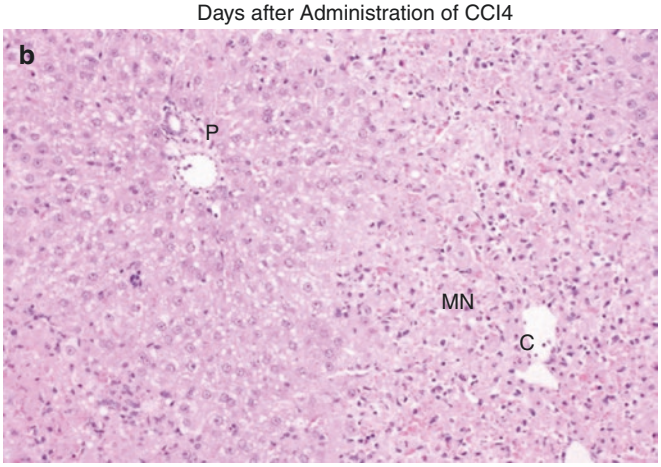


Fig. 1.17 Relation between expression of PKC alpha and presence of ^{3}H-thymidine-labeled hepatocytes in rat liver after 2/3 partial hepatectomy. (**a**) Combined technique of immunohistochemistry for PKC alpha and autoradiography with ^{3}H-thymidine. PKC alpha (arrow head) is expressed from periportal area 9 h after hepatectomy, and ^{3}H-thymidine-labeled hepatocytes (arrow) are first seen in periportal area. (**b**) Combined technique of immunohistochemistry and autoradiography. PKC alpha-immunoreactivity is invisible in 48 h, and ^{3}H-labeled hepatocytes are spread from periportal tract to pericentral area. (**c**) Expression of PKC alpha and ^{3}H-labeled hepatocytes after hepatectomy Expression of PKC alpha reaches a peak in 12 h, and labeled hepatocytes are in a peak in 48 h. *P* portal tract, *C* central vein. (Redrawn from Ishii Y. Expression and significance of PKC alpha in regenerating liver of rats after partial hepatectomy and CCL_4 administration. Jpn J Gastroenterol. 1996;93:717–24, with permission of Japanese Society of Gastroenterology)

Cytokines of IL-6 and TNF alpha play a critical role in the regulation of liver regeneration. Further studies are required to answer remaining questions on liver regeneration [70–72] so that we may treat patients with acute or chronic liver failure effectively. What cells are involved in the liver regeneration after its injury or partial hepatectomy? How are the architecture and function of the liver retained during its regeneration? Which signals are responsible for the turning off of the growth response once the mass of the liver is reconstituted?





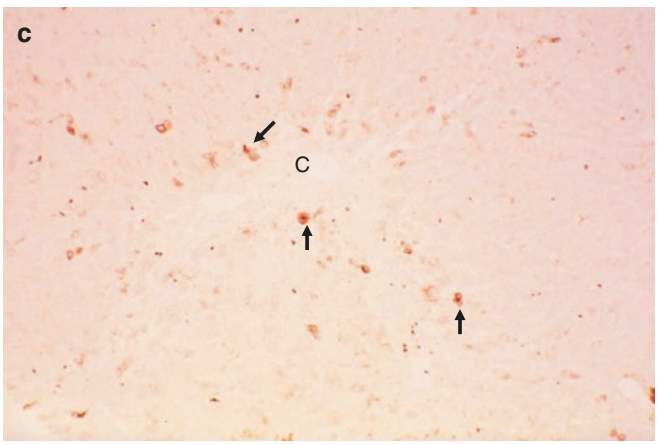


Fig. 1.18 Acute liver injury of rat induced by CCL_4. (**a**) Change of serum GOT and AFP after administration of CCL_4. Serum value of GOT is highest day 1 or 2 and AFP is highest day 3. (Reuse of a printed figure in Shoukakibyo-Gakkai Zasshi 93: p720 with permission of the Japanese Society of Gastroenterology). (**b**) Massive necrosis (MN) is seen around central vein day 1. (**c**) AFP-positive cells (arrow) are distributed in vicinity of central necrosis day 3. *P* portal tract, *C* central vein

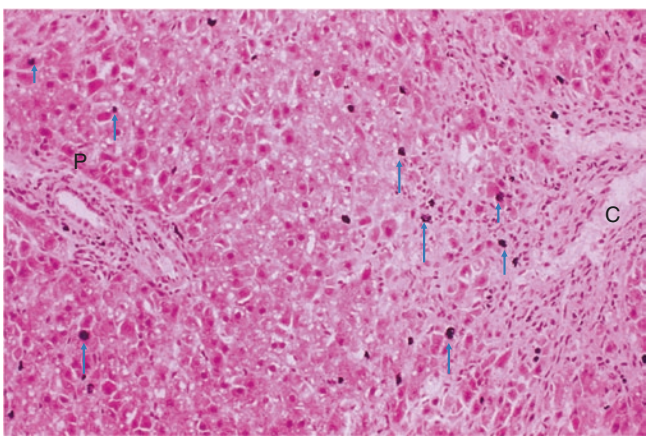


Fig. 1.19 Autoradiographic study of proliferating hepatocytes in acute liver injury induced by CCL_4, using ^{3}H-thymidine ^{3}H-thymidine-labeled hepatocytes (arrow) are seen not only in vicinity of central necrosis day 1 but also in periportal area. *P* portal tract, *C* central vein

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