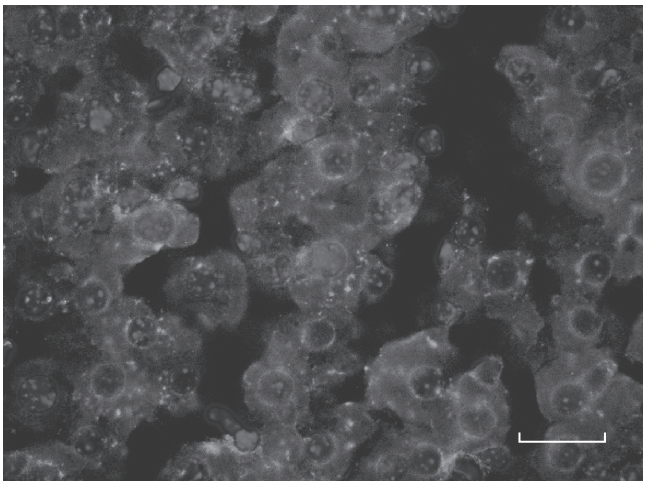
17

LIVER REGENERATIVE ENGINEERING



The presence of c-Kit-positive cells in the mouse liver. C-Kit is a cell membrane protein that is expressed in stem cells found in the embryo, fetus, and adult bone marrow. Red: c-Kit. Green: albumin. Blue: cell nuclei. Scale: 10 μm. See color insert.

Bioregenerative Engineering: Principles and Applications, by Shu Q. Liu

ANATOMY AND PHYSIOLOGY OF THE LIVER

Structure [17.1]

The liver is located in the upper right abdominal cavity. This organ is composed of a large number of functional units, known as hepatic lobules. Each *hepatic lobule* is a cylindrical structure of several millimeters in length and about 1 mm in diameter, and is composed of hepatic cell plates and several tubular systems, including the central vein, portal vein, hepatic artery, lymphatic vessels, and bile ducts. The *portal vein* in each unit is a branch of the major hepatic portal vein and conducts blood from the gastrointestinal tracts into the central vein through a structure between the hepatic cell plates known as *hepatic sinusoid*. The *central vein* in each unit converges blood into the hepatic vein and then into the inferior vena cava. The *hepatic artery* in each unit is a branch of the major hepatic artery and supplies oxygenated blood to the hepatic unit. The *lymphatic vessels* collect and conduct excessive fluids from the interstitial tissue to the large lymphatic vessels and then to the vena cava. The *bile ducts* collect and conduct bile to larger bile ducts and then to the duodenum.

The liver consists of several types of cell, including the hepatocytes, Küpffer cells, Ito cells, epithelial cells, and endothelial cells. *Hepatocytes* are the largest cell population in the liver, are major constituents for the functional units of the liver, and are characterized by the expression of albumin (Chapter 17 opening figure). *Küpffer cells* are macrophages and are found in the hepatic sinusoids. These cells are responsible for the destruction and clearance of microorganisms present in the blood. *Ito cells* are found in the hepatic sinusoids and are responsible for the generation of extracellular matrix components. *Epithelial cells* are constituents of the bile ducts. *Endothelial cells* are found in blood vessels and lymphatic vessels and are responsible for the transport of molecules and electrolytes.

Functions [17.1]

The liver is a vital organ that conducts several essential functions, including nutrient metabolism, detoxification, blood filtration and storage, and bile excretion. The liver possesses a large capacity of functional reserve that is not used under physiological conditions. About one-third of the total liver is sufficient for the maintenance of metabolic homeostasis. The reserve capacity is developed during evolution for sudden changes in metabolic demand under unusual conditions, such as ingestion of a large amount of toxins, liver trauma, and liver infection. Here, the basic functions of the liver are briefly discussed.

Metabolism. The liver is responsible for the metabolism of the three major types of nutrient: carbohydrates, lipids, and proteins. *Carbohydrates* include glucose, galactose, and fructose. These substances are absorbed from the small intestine to the blood, and transported to and processed in the liver. Glucose is the most important carbohydrate for energy production. The liver is responsible for the control of a stable level of blood glucose at ~80 mg/dL. When the level of blood glucose is increased (for instance, immediately after a meal), the hepatocytes are able to synthesize glycogen, a glucose polymer, from glucose molecules via a process known as *glycogenesis*, thus reducing the blood glucose level. In contrast, when the level of blood glucose is decreased in the fasting state, the hepatocytes can hydrolyze glycogen to produce glucose via a process called glycogenolysis, bringing back the blood glucose level. Other types of carbohydrate, such as galactose and fructose,

can be converted to glucose in the liver, contributing to the accumulation of blood glucose. In addition, under conditions with a very low level of blood glucose, the liver is capable of converting amino acids and glycerol into glucose, a process called gluconeogenesis. The maintenance of the blood glucose level is essential for the function of vital organs and tissues, including the brain, heart, and skeletal muscles.

The liver conducts several functions related to lipid metabolism, including energy generation from fatty acids, synthesis of phospholipids and lipoproteins, and conversion of amino acids and carbohydrates to fatty acids. *Fatty acids* can be oxidized to form acetylcoenzyme A, which is further oxidized to generate energy. Hepatocytes are responsible for the synthesis of *cholesterols*, which are released to the blood, transported to cells in other organs, and used for the construction of cell membrane. Cholesterols are also used to constitute bile, which is released into the duodenum via the bile ducts. Hepatocytes can synthesize phospholipids, which are utilized to construct cell membranes. The liver is a major organ that synthesizes and processes lipoproteins, which are responsible for the transport of lipids and cholesterols between the blood, liver, and other organs. The liver is also responsible for the conversion of amino acids and carbohydrates to fatty acids, which are stored in the fat cells or adipocytes.

The liver is the most important organ in the body for protein metabolism. Hepatocytes conduct several protein-related metabolic functions, including the formation of urea, synthesis of proteins, formation of amino acids, and transamination and deamination of amino acids. Protein metabolism generates highly toxic ammonia, which is converted to urea in the hepatocytes. Urea is released into the blood and removed from the kidney. Hepatocytes can synthesize a number of plasma proteins, including albumin, fibrinogen, heparin, globulins, and blood coagulation factors. These factors play critical roles in many important cellular activities. Hepatocytes are capable of forming a number of amino acids, known as nonessential amino acids (meaning that it is not necessary to ingest these amino acids from diets). In addition, hepatocytes are able to carry out transamination and deamination of amino acids. Transamination is a process by which an amino group is transferred from an amino acid to a keto acid by an aminotransferase. After such a process, amino acids can participate in the metabolism of the citric acid cycle and generate energy. Deamination is a process by which the amino group of amino acids is removed by deaminases. Such a process generates ammonia, a waste product of protein metabolism.

Detoxification. The processes of metabolism as described above generate waste substances, most of which are toxic. In addition, various types of toxic substances, such as medicines and chemicals, may be ingested on a daily basis. Hepatocytes are able to chemically modify toxic substances, reducing their toxicity and rendering the substances less toxic and removable. For instance, biological agents, such as penicillin and ampicillin, are processed in the liver, released into the bile, and transported to the intestinal system, where the substances are removed. Excessive hormones, such as cortisol and estrogen, are modified and detoxified in the liver.

Blood Filtration and Storage. In the small intestine, bacterial and toxic particles can be transported into the blood during nutrient absorption. These toxic particles are transported to the liver through the portal vein. The Küpffer cells (specialized reticuloendothelial cells) can endocytose bacterial and toxic particles so that portal blood can be cleansed. In addition, the liver can serve as an organ for blood storage. Under physiological conditions, about 0.5 L of blood can be stored in the hepatic sinusoids and blood vessels. The

blood storage function is important when heart failure occurs. In such a case, excessive blood can be stored in the liver to prevent systemic edema.

Bile Excretion. Bile is produced by hepatocytes, released into the bile ducts, and transported into the duodenum. A human liver can produce about 1,000 ml bile per day. Bile is composed of bile salts, bilirubin, cholesterol, electrolytes, and water. The bile salts are produced from cholesterol and possess two important functions: (1) emulsifying fat diets in the small intestine into minute particles that can be digested by pancreatic enzymes and (2) facilitating the transport and absorption of fat particles through the intestinal epithelial cells. Bilirubin is a metabolic product of hemoglobin, is produced in the hepatocytes, and is transported to the bile ducts and then to the duodenum, where the substance is removed. The accumulation of bilirubin, in the case of liver dysfunction, induces jaundice. Excessive cholesterol molecules are also removed through bile formation and excretion.

Liver Regeneration [17.2]

Organ regeneration is a process of organ self-reconstruction by cell differentiation and proliferation as well as matrix production following organ injury or partial removal. The capacity of regeneration varies among different organs. For instance, the brain and heart have very a low capacity of regeneration, whereas digestive organs are able to regenerate extensively following organ injury. In particular, the liver has a very high capacity of regeneration. It can completely regenerate itself when it is partially removed. No other organs in the human body have the regeneration capacity of the liver. Such a phenomenon has been documented since year the 1890. For more than a century, liver regeneration has fascinated physicians and scientists. For the past several decades, extensive investigations have been carried out for the mechanisms of liver regeneration. These investigations have provided a foundation for today's liver regenerative engineering and reconstruction. See page 399 for detailed discussion about liver regeneration.

HEPATIC DISORDERS

Acute Viral Hepatitis and Liver Failure

Pathogenesis, Pathology, and Clinical Features [17.3]. *Acute viral hepatitis* is a liver infectious disorder induced predominantly by hepatitis A virus and hepatitis B virus. Hepatitis is one of the most popular diseases in the world. About 33% of the human population are infected by hepatitis viruses. This disorder is characterized by inflammatory reactions, edema, cell necrosis, and hyperplasia of Küpffer cells in the liver. The clinical manifestations and consequences of hepatitis vary widely, ranging from mild asymptomatic infections without any noticeable pathological changes in the liver to fatal acute liver failure with massive hepatocyte necrosis. Some patients can completely recover from acute hepatitis, whereas others exhibit persistent infections, which eventually develop into chronic hepatitis and cirrhosis. The outcome of the disease is largely dependent on the responsiveness or sensitivity of the immune system as well as the age of individual patients. The occurrence of chronic hepatitis from the population with acute hepatitis decreases with age. While chronic hepatitis may be found in about 30% of children at the age of <5, the disorder may be found in only about 2% of the adult population with acute hepatitis. The mechanisms for the age related epidemiology remain poorly understood.

Hepatitis A virus is a virus (~26 nm in diameter) that invades the digestive system and blood of humans. This virus is often transmitted via the oral route. Poor hygiene and overpopulation are factors that facilitate the transmission of the virus. Hepatitis A virus can be found in the liver, bile, and stools from patients who carry the virus. Antibodies against hepatitis virus A (IgM class) can be detected in the serum of patients and is often used for the diagnosis of the disease. *Hepatitis B virus* is transmitted via several routes, including oral ingestion, blood infusion, intimate contact, and perinatal transmission. In patients with hepatitis B, viral antigens and corresponding antibodies can be detected in almost all body fluids, including the saliva, tears, serum, gastric fluid, and urine. The presence of hepatitis B antigens and antibodies indicates the infection of the virus.

The pathogenic mechanisms of hepatocyte injury and necrosis in response to the stimulation of hepatitis virus A and B remain poorly understood. It has been thought that the host cell-initiated immune responses may play a role in the development of infectious reactions. The invasion of hepatitis viruses stimulates the host immune system to produce antibodies, which form complexes with the viral antigens. These complexes may sensitize cytotoxic T cells, which recognize hepatitis antigens as well as certain host hepatic molecules that are similar to the viral antigens in structure. The cytotoxic T cells may in turn attack the host liver cells, inducing cell injury and necrosis.

In acute infection of hepatitis A and B viruses, *pathological examinations* often reveal several changes. These include infiltration of mononuclear cells into the parenchyma of the liver, hepatocyte degeneration and necrosis, and edema, which are often associated with hepatocyte proliferation. Immunohistochemical examinations demonstrate the presence of hepatitis viral antigens in the cytoplasm and plasma membrane of hepatocytes. In severe cases, massive hepatocyte necrosis occurs, resulting in acute hepatic atrophy and failure. Acute liver failure is accompanied with rapid jaundice, imbalance of fluid electrolytes, and accumulation of toxins, which cause symptoms such as anorexia, vomiting, fever, fatigue, and headache. Hepatitis induced by hepatitis viruses may contribute to the development of hepatoma. The incidence of hepatoma in patients with hepatitis is considerably higher than that of the general population.

Conventional Treatment [17.4]. Viral hepatitis can be effectively prevented by vaccination with specific vaccines. The effectiveness of vaccination can usually reach about 95%. However, once hepatitis occurs, there are few effective approaches for the treatment of the disorder. For patients with symptoms such as nausea and vomiting, bed rest may help to relieve the symptoms. Patients should avoid taking drugs that are metabolized and reduced in the liver. Hypoglycemia and imbalance of fluids and electrolytes, if any, should be corrected immediately via venous infusion of glucose and physiological fluids. Protein-rich diets should be limited to reduce the workload for the liver. Most patients can be self-cured without clinical consequences.

Certain drugs have been developed and used for suppressing the activities of hepatitis viruses and treat hepatitis. These drugs are primarily nucleoside analogs, including lamivudine and adefovir dipivoxil, which are analogues for deoxycytidine and deoxyadenosine, respectively. These nucleoside analogues can integrate into the viral genome during DNA replication, stop viral DNA elongation, and suppress viral amplification. Thus, a treatment with these nucleoside analogues reduces pathological changes in hepatitis.

In the case of acute liver failure or liver atrophy with complete loss of liver function, it is necessary to conduct allogenic liver transplantation. Since allogenic liver cells induce

immune rejection responses, it is necessary to administrate immune suppressors for protecting the transplanted liver from acute rejection. Although liver cells have high capacity of regeneration, it is impossible to generate a new liver within a short period. Furthermore, in acute liver failure, almost all hepatocytes are necrotic or injured. It is difficult to collect sufficient healthy hepatocytes that can be used for liver regeneration.

Molecular Regenerative Therapies. The strategies of molecular treatment are similar among acute and chronic hepatitis as well as cirrhosis, including suppressing viral activities, inhibiting inflammatory reactions, preventing fibrosis, and enhancing hepatocyte proliferation. Several approaches have been developed and used to achieve these goals. These include gene transfer, antisense oligonucleotide delivery, and genetic vaccination.

Suppression of Viral Activities [17.5]. Certain types of cytokines exert antiviral effects. A typical cytokine that inhibits the activity of hepatitis viruses is interferon α. As described above, a treatment with interferon α results in reduced activities of hepatitis viruses and improved hepatic function in chronic hepatitis. However, interferon α protein undergoes rapid degradation. It is difficult to induce long-term effects by protein delivery. The transfer of the interferon α gene represents a potential approach for overcoming such a problem. Experimental investigations have demonstrated that the transfer of the interferon α gene into the liver can induce a sustained increase in the level of interferon α. Such a gene transfer approach results in a reduction in fibrogenesis, improvement of hepatic function, and prevention of the development of hepatoma.

Another approach used for the suppression of the viral activities is to deliver antisense oligodeoxynucleotides or genes that encode oligodeoxynucleotides specific to viral mRNA. The delivered or expressed oligodeoxynucleotides bind to viral mRNAs and render the mRNAs incapable of translating necessary proteins, thus suppressing viral activities and replication. During the end stage of cirrhosis, few functional hepatocytes may be found in the liver. In such a case, the therapeutic oligodeoxynucleotides or genes can be used to transfect functional hepatocytes in vitro. The transfected hepatocytes can then be transplanted into the liver. These cells may become virus-resistant cells and may proliferate and repopulate the liver.

Genetic vaccination is an approach by which viral antigen genes are constructed and delivered into the host cells. The gene products, once expressed, may sensitize the host immune cells and induce antibody generation, rendering the cells prepared for further virus invasion. Sensitized cytotoxic T cells can suppress viral activities. A typical viral antigen gene is the gene that encodes the nucleocapsid protein of the woodchuck hepatitis virus. This gene has been used for immunization in animal models. Interestingly, the cotransfer of certain types of cytokine, such as interleukin-12, is required for the activation of the transferred antigen gene. The expression of the viral antigen gene alone does not effectively protect the hepatocytes from virus attacks.

Enhancement of Hepatocyte Proliferation [17.6]. Chronic hepatitis and cirrhosis are both associated with the loss of hepatocytes, a critical pathological change that causes functional deficiency of the liver. Thus, the enhancement of hepatocyte proliferation is an essential strategy for the treatment of these liver disorders. Several growth factors, including hepatocyte growth factor (HGF), interleukin-6 (IL6), epidermal growth factor (EGF), transforming growth factor (TGF)α, and keratinocyte growth factor (KGF) have been

shown to stimulate hepatocyte proliferation. Several other factors, such as insulin, insulin-like growth factors, vasopressin, angiotensin, norepinephrine, and glucagon, can enhance the activity of the growth factors listed above, thus enhancing hepatocyte proliferation. Among these factors, HGF is one of the most potent growth factors that stimulates hepatocyte proliferation and inhibits hepatocyte apoptosis (Fig. 17.1). In addition, HGF exerts inhibitory effects on the activation of Ito cells, the expression of procollagen genes, and the expression of TGFβ, and thus suppressing hepatic fibrogenesis and the progression of cirrhosis. These observations suggest that the HGF gene is a potential therapeutic candidate gene for the treatment of chronic hepatitis and cirrhosis. Experimental investigations have shown that the transfer of the HGF gene into the liver results in an increase in the expression of HGF. The expression of HGF is associated with a number of changes, including (1) tyrosine phosphorylation of the HGF receptor, (2) an elevation in the expression of proliferative cellular nuclear antigen (PCNA), (3) enhancement of hepatocyte proliferation, (4) facilitation of angiogenesis, (5) a decrease in the expression of TGF-β, (6) an reduction in hepatocyte apoptosis; and (7) improvement of hepatic structure and function.

Another hepatic growth promoter is hepatopoietin or augmenter of liver regeneration (ALR), which is a 30-kDa homodimeric protein (see Table 17.1 for characteristics of

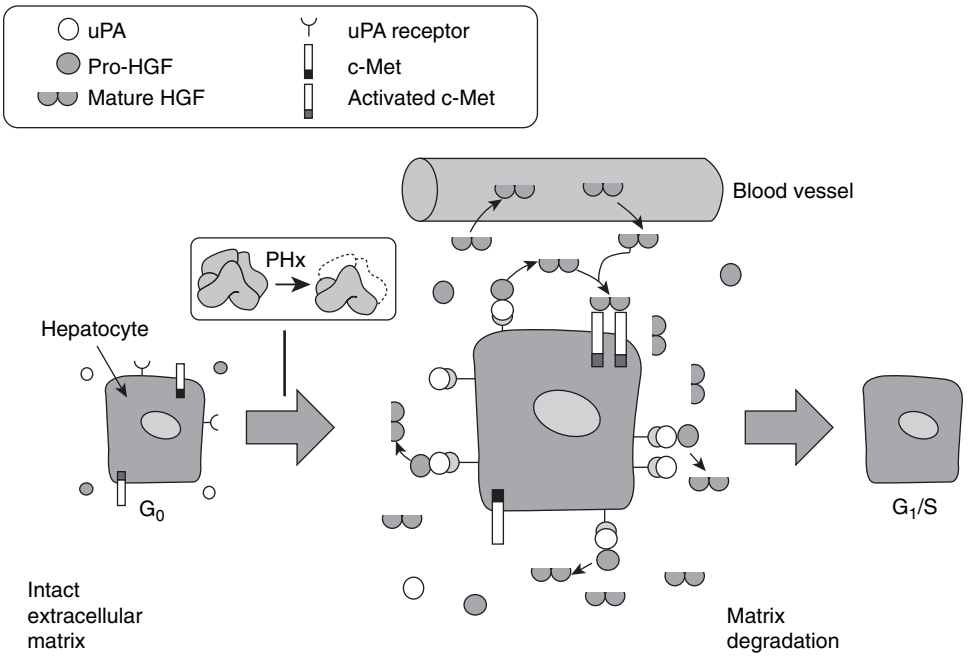


Figure 17.1. Proposed model for the role of HGF in liver regeneration. Rapid upregulation of the uPA receptor leads to activation of uPA within 5 min after PHx. This initiates a protease cascade causing degradation of the scant extracellular matrix surrounding hepatocytes and releasing, among others, matrix-bound inactive pro-HGF. uPA activates pro-HGF into the mature active form. Active HGF is released in the blood and stimulates hepatocyte DNA synthesis by an endocrine or paracrine mechanism by binding to the c-Met receptor. (Reprinted with permission from Michalopoulos GK, DeFrances MC; Liver regeneration, *Science* 276:60–6, copyright 1997 AAAS.)

TABLE 17.1. Characteristics of Selected Therapeutic Proteins for Chronic Hepatitis and Cirrhosis*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Keratinocyte growth factor	KGF, fibroblast growth factor 7 (FGF7)	194	23	Skin, blood vessels, pancreas, uterus, intestine, ovary, skeletal muscle, cornea	A member of the fibroblast growth factor (FGF) family, stimulating the survival and proliferation of epithelial cells and keratinocytes, regulating embryonic development and morphogenesis, promoting tissue repair, and enhancing tumor growth and invasion
Insulin	INS	110	12	Pancreas	Stimulating glucose uptake via interaction with insulin receptor (INSR) and augmenting the activity of growth factors
Insulin-like growth factor	IGFI, somatomedin C	195	22	Brain, blood cells, adrenal gland, bone marrow, intestine, skeletal muscle, uterus	Enhancing the effect of growth hormone (HG) during development, stimulating cell proliferation, and protecting neuronal cells from injury and apoptosis

TABLE 17.1. *Continued*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Vasopressin	VP, antidiuretic hormone (ADH), vasopressin neurophysin II, arginine vasopressin neurophysin II, vasopressin neurophysin II copeptin	164	17	Brain	A posterior pituitary hormone synthesized in the supraoptic nucleus and paraventricular nucleus of the hypothalamus, stimulating cell growth, inducing vasoconstriction by acting on smooth muscle cells, and exerting an antidiuretic effect on the kidney
Glucagon	Glucagon-like peptide 1 (GLP1), glucagon-like peptide 2 (GLP2), glicentin-related polypeptide (GRPP),glucagon preproprotein	180	21	Intestine, pancreas	Increasing the glucose level by stimulating glycogenolysi and gluconeogenesis, enhancing cell proliferation via the G-protein-coupled receptor signaling pathways, and inducing transformation of intestinal epithelial cells to insulin-producing cells
Hepatopoietin	HERV1, augmenter of liver regeneration (ALR), growth factor ERV1-like, hepatic regenerative stimulation substance (HSS)	125	15 (monomer)	Liver, testis	Serving as a hepatotrophic factor that stimulates hepatocyte proliferation and liver regeneration

*Based on bibliography 17.6.

hepatopoietin). This protein has been shown to stimulate hepatocyte proliferation and protect liver cells from injury and apoptosis. The transfer of the hepatopoietin gene into animal model of cirrhosis results in a reduction in the progression of hepatic fibrosis and improvement of hepatic structure and function. Genes that encode other hepatic growth promoters can also be used for therapeutic purposes.

Suppression of Inflammatory Reactions [17.7]. Inflammatory reactions in chronic hepatitis and cirrhosis often induce fibrogenesis, which prevents hepatocyte regeneration and deteriorates the function of the liver. Thus, one of the strategies for the treatment of chronic hepatitis and cirrhosis is to suppress inflammation. Interleukin (IL)10 is known as an anti-inflammatory cytokine, which suppresses the activity of proinflammatory cytokines. This factor is produced in several types of cells, including lymphocytes, monocytes, and macrophages (see page 634 for the characteristics of IL10). Genetically induced deficiency of IL10 is associated with enhanced hepatic fibrosis and monocyte infiltration in animal models. The transfer of IL10 gene into the liver cells induces a reduction in the activity of the collagen gene promoter, leukocyte infiltration, and fibrosis in experimental models of liver cirrhosis. Thus, the IL10 gene can be considered a potential gene for the treatment of human chronic hepatitis and cirrhosis.

Inhibition of Fibrosis [17.8]. Hepatic fibrosis is often promoted by certain factors. Transforming growth factor (TGF)β is one of such factors. TGFβ stimulates the transformation of Ito cells to fibroblast-like cells, which produce excessive extracellular matrix components, including collagen and fibronectin, and contribute to hepatic fibrosis. Thus, the blockade of the TGFβ signal transduction pathway may exert an inhibitory effect on hepatic fibrosis. One approach to reduce the activity of TGFβ is to modify the structure of the TGFβ receptor. Experimental investigations have demonstrated that the transfer of a truncated dominant-negative TGFβ receptor gene into the liver of experimental cirrhosis results in the inhibition of TGFβ activity, which is associated with a reduction in the production of collagen and fibronectin, monocyte infiltration, activity of the Ito and Küpffer cells, and hepatic fibrosis (Fig. 17.2). The hepatic function is improved accordingly. The truncated TGFβ receptor can compete for the TGFβ ligands with the wild-type TGFβ receptor present in the cells, but cannot transmit the TGFβ signal to the intracellular signaling pathways, thus reducing the effect of TGFβ. A large quantity of the truncated TGFβ receptor gene is usually required to achieve therapeutic effectiveness.

Enhancing the Activity of Telomerase [17.9]. Telomerase is a complex enzyme composed of RNA and two protein subunits and is responsible for the maintenance of telomere integrity and function. Telomere is a cap structure for eukaryotic chromosomes, and consists of a TTAGGG-rich DNA sequence and catalytic protein enzymes. This structure plays a critical role in the maintenance of the stability and function of chromosomes and in the regulation of DNA synthesis and cell mitosis. Hepatic cirrhosis is associated with reduced length and altered function of telomere, which is thought to contribute to the progression of hepatic cell apoptosis and fibrosis. These changes are attributed to alterations in the activity of the telomerase. Thus, the enhancement of the telomerase activity by telomerase gene transfer into the liver may improve the integrity of telomere and reduce hepatocyte apoptosis and hepatic fibrosis. Experimental investigations have provided evidence that supports such a possibility.

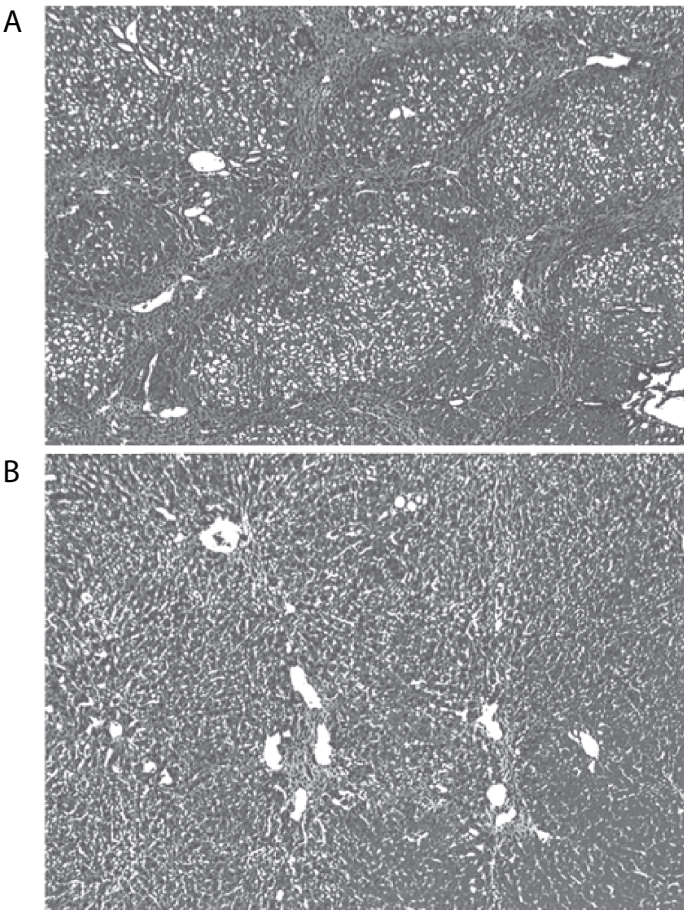


Figure 17.2. Histological micrographs of the rat liver treated with dimethylnitrosamine (DMN), a substance causing persistent liver fibrosis, and a dominant-negative type II TGFβ receptor gene. Rats were infused once via the portal vein with saline (A), or adenoviruses containing a dominant-negative type II TGFβ receptor gene (AdCAT βTR) (B). Both groups of rats were then treated with DMN for 3 weeks. Liver sections were examined histologically by Masson trichrome staining (×200). Note the formation of fibrotic structure in saline-treated liver, but not in the liver transfected with the dominant-negative type II TGFβ receptor gene. (Reprinted with permission from Qi Z et al: *Proc Natl Acad Sci USA* 96:2345–9, copyright 1999 National Academy of Science USA.)

Cell and Tissue Regenerative Engineering [17.10]. The goal of hepatic cell and tissue regenerative engineering is to replace malfunctioned hepatocytes or augment the function of an injured liver by using an engineered liver construct containing necessary liver cells. In principle, a liver construct can be established by assembling liver cells into a liver scaffold, which provides an appropriate environment for liver cell survival, proliferation, and differentiation. Essential criteria are that a liver construct should be implantable and able to conduct essential liver functions. There are a number of issues that should be taken into account for liver reconstruction. These include (1) selection, culture, and manipulation of liver cells; (2) fabrication of liver scaffolds; (3) maintenance of cell viability and

functions; (4) implantation of liver constructs; and (5) test of liver functions. These issues are discussed as follows.

Selection, Culture, and Manipulation of Liver Cells [17.11]. A critical issue for liver reconstruction is the selection of cell types. Ideally, a liver construct should contain all necessary hepatic cells. The liver construct should be assembled into the form of the natural liver. However, it is difficult to construct a realistic liver with available technologies. The current liver constructs are mostly extracellular matrix- or polymer-based scaffolds containing selected liver or stem cells. Several types of liver cells have been used for such a purpose, including adult hepatocytes, genetically modulated hepatocytes, and hepatoma cells. Stem cells and hepatic progenitor cells derived from the embryo, fetus, and adult bone marrow are also candidates for liver regeneration.

Hepatocytes are the primary choice for liver regeneration since these cells can proliferate rapidly and conduct necessary functions immediately following implantation. Healthy autogenous hepatocytes from the host patient are ideal candidates for liver construction. Such cells do not induce acute immune rejection, which is the most serious problem in cell and tissue transplantation. However, patients who need liver reconstruction may not possess sufficient functional hepatocytes. In such a case, hepatocytes from a close relative may be considered. Other choices of hepatocyte sources may include allogenic and xenogenic livers. Allogenic cells are those collected from different individuals of the same species. Xenogenic cells are from different species. Hepatocytes from pigs are often used for constructing artificial livers in experimental models. Obviously, allogenic and xenogeneic cells induce acute immune rejection responses. Transplanted hepatocytes will be attacked by host immune cells, resulting in cell apoptosis and rejection. Immune suppressor agents should always be used to prevent immune reactions. Since hepatocytes have a high capacity of regeneration, a small biopsy sample of hepatocytes may generate a sufficient number of cells within relatively short period.

Genetically modified immortal hepatic cell lines have been used for liver reconstruction in experimental models. A major feature of these cell lines is that cells are immortalized and can survive in an engineering system, which is difficult to achieve by using primary hepatocytes or stem cells. Immortalized cell lines can be established by viral transformation. For instance, the introduction of simian virus 40 into cultured hepatocytes can transform the cells into an immortal form, which still exhibits certain characteristics of the hepatocytes, such as the generation of albumin and process of bilirubin. Another approach for cell immortalization is to coculture and transform hepatocytes with a different cell type from a different species. An example is the transformation of human hepatocytes by coculturing with the rat liver epithelial cells. The transformed hepatocytes exhibit not only immortal properties, but also certain hepatic characteristics such as the generation of albumin and α-fetoprotein. Hepatoma cell lines may also be considered for the construction of an artificial liver, since these cells are able to survive and keep certain hepatic characteristics. An example is the human hepatoblastoma C3A cell line. Experimental studies have demonstrated that this type of cells can survive in animal transplantation models for a longer time compared to primary cell lines and can produce hepatic proteins. Overall, cell lines are potential cell candidates for liver reconstruction. However, there is a risk of introducing cancers to the host system. In addition, the transformed cells may lose hepatic functions. Further investigations are necessary to clarify these issues.

Stem and progenitor cells derived from the embryo, fetus, and adult bone marrow are potential sources for regenerating functional liver cells, repairing an injured liver, and

reconstructing a malfunctioned liver. Embryonic stem cells are capable of differentiating to all specified cell types. Under an appropriate condition, these cells can differentiate to liver cells and thus can be used for liver reconstruction. Fetal stem and progenitor cells can also be used to regenerate liver cells and reconstruct malfunctioned liver. However, the use of embryonic and fetal cells remains an ethically debating issue, which will likely last for a long time. Alternatively, the adult bone marrow stem and progenitor cells can be used to regenerate liver cells. Several investigations have demonstrated that bone marrow cells can transform to liver cells when the bone marrow cells are delivered to the liver (Fig. 17.3). While the mechanisms of cell transformation remains a research topic, the fusion of bone marrow cells into liver cells has been considered a potential mechanisms for bone marrow cell-based liver regeneration.

Fabrication of Liver Scaffolds and Maintenance of Cell Viability and Function [17.12]. To construct an artificial liver, it is necessary to assemble liver cells in a scaffold. Biological extracellular matrix and synthetic polymers have been used for the construction of such a scaffold. Extracellular matrix components, such as various types of collagens, are natural polymeric materials, which serve as cell substrates and participate in the regulation of cell activities, including cell adhesion, proliferation, and migration. Collagen matrix has been used extensively for the construction of tissue scaffolds. Biodegradable polymers have also been synthesized and used for such a purpose. A unique feature for this type of material is that the scaffold can be gradually degraded in the host system and replaced with cells and natural extracellular matrix.

Several issues should be considered for the construction of hepatic scaffolds:

1. The selected material should be compatible with seeded cells and should not influence the survival and function of the cells. When synthetic polymers are used, biological molecules can be used to coat the surface to which cells attach. A polymeric material should be always tested for toxic effects before being used for constructing tissue scaffolds.
2. A scaffold should be constructed with an appropriate form and structure, factors that may influence the cell function and performance.
3. It is necessary to establish a circulatory system, which introduces blood to the cells seeded in a tissue scaffold.
4. Transplanted cells are subject to an environment that is not natural in a reconstructed liver. Cell apoptosis often occurs within a short period of cell transplantation. It is always a challenge to maintain cell viability when cells are transplanted into a host system in vivo. Thus, cell survival stimulators, such as hepatic growth factor and insulin or their genes, should be applied to liver constructs. These factors play an important role in regulating the survival and proliferation of transplanted liver cells.

Various forms of liver constructs have been established in experimental models. These include scaffolds based on extracellular matrix and polymeric materials, hollow fibers, and microcarriers. These constructs can be used to encapsulate liver cells for transplantation. Experimental investigations and clinical trials have demonstrated the feasibility of using the liver constructs for the treatment of liver failure. It should be noted that all forms of liver constructs established to date are relatively small with respect to the natural liver

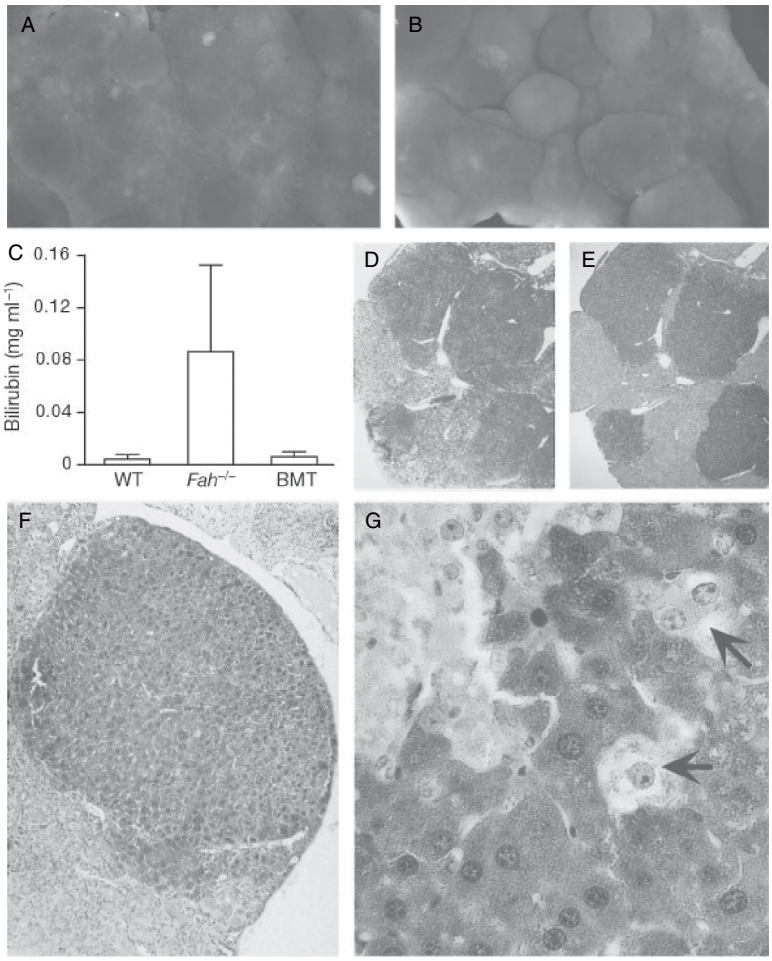


Figure 17.3. Formation of functional fumarylacetoacetate hydrolase-positive (Fah^{+}) liver nodules in $Fah^{-/-}$ mice by transplantation of $Fah^{+/+}$ wildtype bone marrow cells. (A, B) Gross liver specimens from transplant recipients showing embedded (A) and protruding (B) nodules photographed on a dissecting microscope. (C) Total serum bilirubin levels (mean +/−s.d., $n >= 4$) from wildtype (WT) mice, $Fah^{-/-}$ mice maintained without NTBC for >4 weeks, and $Fah^{-/-}$ mice after wildtype bone marrow transplantation (BMT). (D, E) Serial liver sections from transplant recipients stained with haematoxylin and eosin (D) or with an anti-Fah antibody to stain expressing cells brown (E–G). Images were photographed with ×2.5 (D, E), ×10 (F), or ×40 (G) objectives. Arrows indicate the locations of nonexpressing cells at the edge of an Fah^{+} nodule. These observations show that transplanted $Fah^{+/+}$ wildtype bone marrow cells can engraft to the liver and differentiate into hepatocytes with fah function in transgenic $Fah^{-/-}$ mice. (Reprinted by permission from Macmillan Publishers Ltd.: Vassilopoulos G, Wang PR, Russell DW: *Nature* 422:901–4, copyright 2003.)

because of difficulties in the construction of a vascular system. These models are briefly discussed as follows.

For the *scaffold model*, hepatocytes can be seeded in an extracellular matrix or polymeric scaffold, which provides a substrate for cell attachment and assembly. The cell-seeded scaffold can be then enclosed within a semipermeable membrane system

(Fig. 17.4). Several types of material, including polysaccharide hydrogels, hydroxyethyl methacrylate-methyl methacrylate matrix, calcium alginate, and collagen matrix, have been used for constructing liver scaffolds. The semipermeable membrane can be constructed with polymeric materials, such as cellulose and polysulfone. This membrane separates the enclosed hepatocytes from the host tissue and thus prevents leukocyte infiltration and immune rejection responses, when allogenic or xenogenic cells are used. At the same time, the semipermeable membrane allows the release of proteins produced by the enclosed liver cells from the scaffold to the surrounding tissue. The semipermeable membrane can also restrain the transplanted cells within the scaffold, preventing potentially harmful effects, such as carcinogenesis when immortal cells are used.

A liver construct can be implanted into the abdominal cavity of the host. Ideally, the liver construct should be connected to the host circulatory system so that liver-produced proteins can be release into the blood (see next section). However, it is often difficult to establish blood circulation in an artificial liver. Alternatively, multiple small liver scaffolds can be constructed and implanted into the abdominal cavity without connecting to the host circulatory system. When the scaffolds are sufficient small, oxygen and nutrients can diffuse to the cells seeded in the scaffolds.

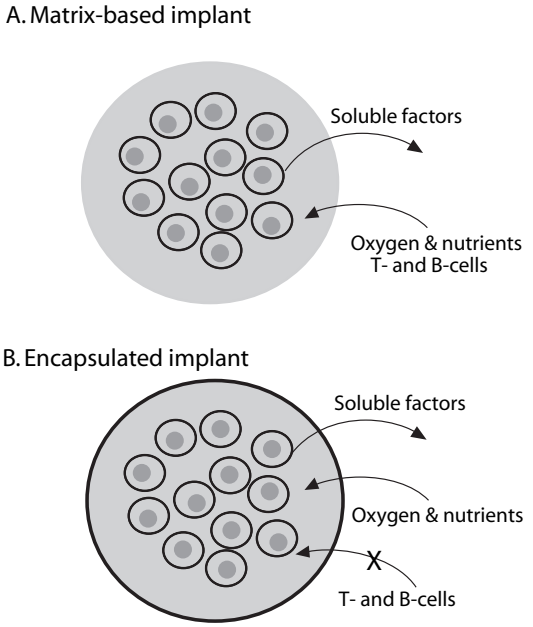


Figure 17.4. Types of implantable devices with hepatocytes. (A) Cells are in an open matrix, which is often biodegradable. Surrounding tissue, including blood vessels, can grow into the implanted matrix. This provides no protection from the immune system from the host. (B) Cells are encapsulated so that they are protected by a barrier that prevents immune cells and factors from reaching the cells while allowing small (usually <50 kDa) metabolites to transport from the capsule to surrounding tissue. Metabolite transport to and from the nearest vascular bed is chiefly by diffusion and may be adversely affected by the presence of a fibrotic layer, which often develops around such implants. (Reprinted from Chan C et al: Hepatic tissue engineering for adjunct and temporary liver support: Critical technologies, *Liver Transplant* 10:1331–42, copyright 2004 by permission of John Wiley & Sons, Inc.)

For the *microcarrier model*, polymeric materials can be used to construct bead-like carriers. The carriers can be coated with extracellular matrix molecules, such as collagen and fibronectin, which enhance cell attachment and survival. Hepatocytes can be collected and cultured on the carrier beads. When cells reach confluence, the carriers can be implanted into the abdominal cavity. In this model, the transplanted cells are directly exposed to the host system. Immune rejection reactions will occur when allogenic and xenogeneic cells are used.

For the *hollow fiber model*, polymeric hollow fibers can be filled with a gel of extracellular matrix component, such as collagen, mixed with hepatocytes (Fig. 17.5). The collagen gel serves as a matrix for the attachment and assembly of the seeded hepatocytes.

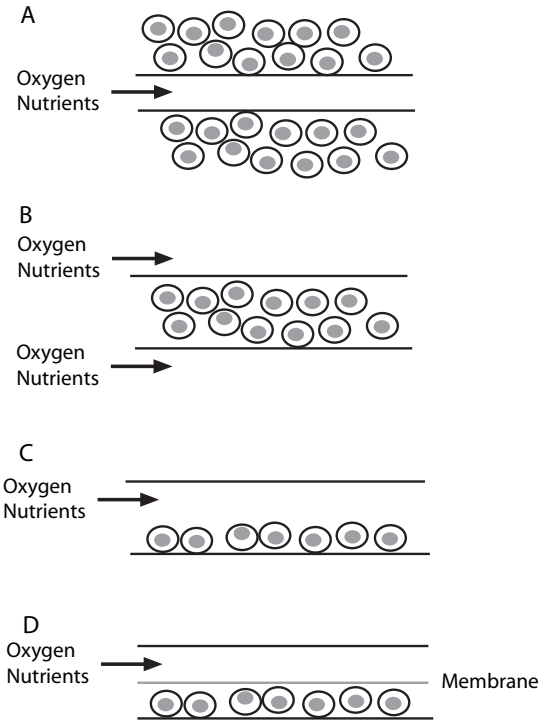


Figure 17.5. Common bioreactor designs for bioartificial livers. (A) Hepatocyte aggregates on microcarriers are placed on the outside of hollow fibers. Oxygenated plasma is flown through the hollow fibers. (B) Hepatocyte aggregates in a supporting matrix are inside hollow fibers and oxygenated plasma is flown outside the hollow fibers. (C) Similar to panel A, although separate hollow fibers are used to deliver hepatocyte culture medium, plasma, and oxygen into the system. Circle with O_2 is a hollow fiber perpendicular to the plane of the paper. (D) Hepatocyte aggregates are in a supporting matrix next to hollow fibers that deliver oxygen. Oxygenated plasma is flown in the space outside of the hollow fibers and percolates through the matrix–hepatocyte network. (E) Hepatocytes are seeded as a monolayer on the bottom surface of a flat plate and placed within a parallel-plate flow chamber. Oxygenated plasma is flown directly above the cells. (F) System is similar to that shown in panel E, except that oxygen is delivered through a permeable membrane directly above the flow channel with the hepatocytes. (Reprinted from Chan C et al: Hepatic tissue engineering for adjunct and temporary liver support: Critical technologies, *Liver Transplant* 10:1331–42, copyright 2004 by permission of John Wiley & Sons, Inc.)

When the fibers are small, oxygen and nutrients can easily diffuse to the enclosed hepatocytes. By using porous polymeric materials with an appropriate pore size, the fiber wall can prevent the invasion of host immune cells and immune rejection responses. Multiple hollow fibers can be grouped and assembled into a large tubular device, which can be used for implantation. Host blood can be introduced to the interfiber spaces via vascular anastomoses (see next section), ensuring sufficient oxygen and nutrient supplies. Alternatively, hepatocytes can be cultured on the exterior surface of semipermeable hollow fibers. Multiple fibers can be assembled within a larger tubular device. Upon implantation, host blood can be introduced into the lumens of the hollow fibers via vascular anastomoses. The hollow fiber model gives a large surface area for molecular diffusion, ensuring efficient release of proteins produced by transplanted hepatocytes. The hollow fiber device can be implanted into the abdominal cavity of the host. Experimental studies have demonstrated the feasibility and usefulness of this model.

Implantation of Liver Constructs [17.13]. Various methods can be used to implant liver constructs, depending on the form of the construct. Microcarriers and capsules can be directly implanted into the abdominal cavity. Since these devices are small, oxygen and nutrients can diffuse from the abdominal serous fluid into the device. For large devices that require blood supply, such as the hollow fiber liver construct, an artery and a vein should be selected and anastomosed to the blood circulatory system of the liver construct. It is important to note that the artery and vein selected for such a purpose should be rich in collateral circulation, so that the use of the blood vessels for the liver construct does not influence blood supply to the distal tissues of the host system. In the abdominal cavity, the small and large intestines are supplied by the superior and inferior mesenteric arteries, which are connected by collateral arteries through the intestinal system. There are also collateral blood vessels for the mesenteric veins. The blockade of the inferior mesenteric artery and vein does not significantly influence the blood supply to the intestines. Thus, the inferior mesenteric artery and vein can be used as a blood supplying system for an implanted liver construct. A common problem for anastomoses with a nonvascular structure is blood coagulation within the liver construct as well as thrombosis and intimal hyperplasia within the anastomotic blood vessels. These pathological changes often result in obstruction of the blood circulation within the implanted liver construct. A persistent administration of anticoagulants and anti-proliferative agents is necessary for preventing thrombosis and intimal hyperplasia.

Testing Liver Function [17.13]. It is important to test the function and durability of the implanted liver construct. There are a number of parameters that are used for testing the liver function. These include the blood concentration of albumin, aminotransferases (aspartate aminotransferases and alanine aminotransferases), clotting factors, and ammonia. *Albumin* is produced by hepatocytes and its blood concentration is a useful index for the assessment of the liver function. The normal serum level of albumin is 3.5–5 g/dL. A significant decrease in the albumin concentration compared to normal controls suggests insufficient function or malfunction of the liver construct. Aspartate aminotransferases and alanine aminotransferases are two enzymes that catalyze the transfer of the γ-amino group from aspartate and alanine to the γ-keto group of ketoglutarate, forming oxaloacetic acid and pyruvic acid. The normal blood level of these enzymes is about 40 IU. Under physiological conditions, these enzymes are degraded in the liver. An increase in the blood level of these enzymes suggests insufficient hepatic function. The liver

synthesizes several blood coagulation factors, including coagulation factor I (fibrinogen), II (prothrombin), V, VII, IX, and X. A reduction in the blood concentrations of these factors indicates insufficient hepatic function. *Ammonia* is a waste product generated by protein metabolism and is transformed into urea in the liver. An elevation in the blood concentration of ammonia strongly suggests deficiency of the hepatic function. Thus, these proteins and substances can be measured and used for assessing the function of an implanted liver construct.

Chronic Hepatitis and Cirrhosis

Pathogenesis, Pathology, and Clinical Features [17.3]. *Chronic hepatitis* is a disorder caused by various pathogens, including hepatitis viruses and chemical toxins, and characterized by continuous inflammatory reactions, hepatocyte necrosis, and fibrosis in the liver. Persistent pathological changes may eventually lead to cirrhosis and liver failure. About 30% of patients with chronic hepatitis have a history of hepatitis B infection. These patients often exhibit positive hepatitis B antigens in their serum. The clinical manifestations of chronic hepatitis vary from mild asymptomatic illness to liver failure. The mechanisms for such a wide range of changes remain poorly understood.

The pathogenesis of chronic hepatitis has been hypothetically related to immune responses in the liver. In many cases, hepatic lesions are associated with T-cell infiltration and activation. Antibodies against host components have been detected in some patients. Other types of autoimmune disorders, such as diabetes and thyroiditis, can be found in some patients with chronic hepatitis. The administration of corticosteroids, which are used to treat autoimmune disorders, is effective for the treatment of chronic hepatitis. These observations support the hypothesis that chronic hepatitis may be induced by autoimmune reactions in the liver.

Chronic hepatitis is usually diagnosed on the basis of biopsy examinations. Typical pathological changes include inflammatory reactions characterized by the presence of dense mononuclear cells, hepatocyte necrosis in peripheral regions of liver lobules, excessive formation of fibrous extracellular matrix, and regeneration of hepatic lobules. A large fraction of patients (up to 50%) are associated with cirrhosis (see next paragraph for details). These pathological changes usually develop within 1–2 years.

Cirrhosis is a hepatic disorder characterized by massive fibrosis, structural distortion, formation of regenerative nodules, and deterioration in the function of the liver. When most hepatocytes are replaced with fibrous tissue, the liver loses its function and dies. A number of factors contribute to the development of cirrhosis. These include alcohol toxicity, hepatitis viral infection, obstruction of biliary ducts, and right heart failure. These factors induce inflammatory reactions in the liver in association with excessive production of extracellular matrix components. Several types of liver cells, including the Kupffer cells, Ito cells, and natural killer cells, can release various cytokines and inflammatory mediators, which may further enhance inflammatory reactions. A typical inflammatory mediator is transforming growth factor β (TGFβ), which stimulates hepatic fibrogenesis and accelerates cirrhosis. Here, the role of alcohol toxicity, hepatitis viral infection, obstruction of biliary ducts, and right heart failure in regulating the development of cirrhosis is briefly discussed.

Alcohol toxicity is a common cause of cirrhosis. Long-term exposure to alcohol induces various changes in the liver. In certain cases, extensive fatty acid deposition occurs in hepatocytes due to the impairment of fatty acid oxidation, leading to fat accumulation in

the liver (Fig. 17.6). This change is associated with enlarged liver, uniform loss of hepatocytes, formation of regenerative nodules, and deposition of extracellular matrix or fibrosis. Alcohol toxicity is often associated with hepatitis, characterized by hepatocyte necrosis, infiltration of leukocytes, and deposition of collagen matrix. During the end-stage, hepatocytes are committed to apoptosis and are replaced with fibroblasts, which produce excessive collagen matrix, leading to the formation of a fibrous network. The liver exhibits structural distortion, extensive fibrosis, and regenerative nodules, in association with progressive shrinkage of the liver. The end-stage disorder is often referred to as alcoholic cirrhosis.

Hepatitis viral infection is a major cause of cirrhosis. In the majority of patients, viral hepatitis can be completely self-cured. However, in a small fraction of patients, such a disorder may develop into chronic hepatitis and eventually into cirrhosis. Pathological

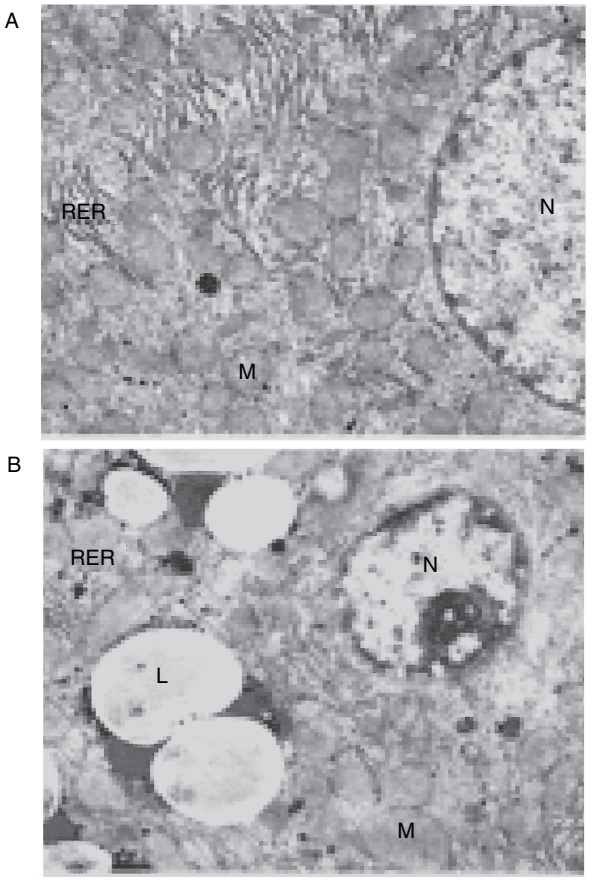


Figure 17.6. Ethanol-induced ultrastructural changes in the hepatocytes of wildtype mice. (A) control; (B) ethanol. Note that presence of lipid droplets in ethanol-treated liver. N, nucleus; M, mitochondria; RER, rough endoplasmic reticulum; L, lipid droplets; FCD, focal cytoplasmic degeneration. Magnification, ×7500. (Reprinted with permission from Zhou Z et al: Metallothionein-independent zinc protection from alcoholic liver injury, *Am J Pathol* 160:2267–74, copyright 2002.)

features in viral hepatitis-induced cirrhosis are similar to those found in alcoholic cirrhosis, except that few fatty acid-filled cells are found. Hepatitis viral infection often causes the obstruction of the biliary ducts. Such an alteration causes bile accumulation, leading to inflammatory reactions and fibrosis in the liver (see below for more details).

Biliary obstruction is another major cause of cirrhosis. There are two types of biliary obstruction: primary and secondary obstruction. Primary obstruction occurs in small intrahepatic biliary ducts. The mechanisms of primary obstruction remain poorly understood. Secondary obstruction is found in large extrahepatic bile ducts and is caused by the compression of gallstones, tumors, and surgical scars. Both types induce similar pathological changes, which include destruction of biliary ducts, infiltration of leukocytes, fibrosis, and progressive loss of hepatocytes, eventually leading to the development of cirrhosis.

Right heart failure is a disorder characterized by reduced performance of the right ventricle and reduced cardiac output. As a result, venous blood pressure increases due to elevated resistance in the right heart, resulting in blood accumulation in the venous system and congestion of the liver. Furthermore, liver ischemia may occur because of heart failure. These changes cause chronic hepatocyte injury and necrosis. Necrotic hepatocytes are often replaced with fibroblasts and fibrous matrix, leading to fibrosis and scar formation. These changes continue unless the right ventricular function is improved. The ultimate consequence is cirrhosis and the death of the liver.

Treatment of Chronic Hepatitis and Cirrhosis [17.4]. Chronic hepatitis and cirrhosis often exhibit similar changes in hepatic structure and function, except that the degree of changes may differ. Chronic hepatitis often develops into cirrhosis, and cirrhosis is considered the final stage of chronic hepatitis. Thus, both disorders are treated with similar approaches. Since chronic viral hepatitis and subsequent cirrhosis are the most common forms of hepatic disorder, we use these disorders as examples for discussing therapeutic strategies. General strategies for treating chronic hepatitis and cirrhosis include the suppression of inflammatory reactions, prevention of fibrosis, and inhibition of viral replication.

Corticosteroids can be used to suppress inflammatory reactions in chronic hepatitis. In about 70% of patients, corticosteroid treatment significantly reduces clinical symptoms, slows down pathological changes, and improves the liver function in chronic hepatitis. Typical signs of improvement include disappearance of fatigue and anorexia, an increase in serum albumin, and a fall in serum bilirubin. Pathological examinations may find a decrease in the infiltration of mononuclear cells and hepatocyte necrosis. However, pathological changes often resume when corticosteroid administration is ceased. Corticosteroid administration may not be effective for the treatment of cirrhosis, since structural changes are often irreversible.

Patients may be administrated with interferon (IFN) α, a cytokine that exerts antiviral, antifibrogenic, and anti-tumoral effects. Such a treatment has been shown to reduce pathological changes and improve hepatic function in chronic hepatitis. Recently, antiviral nucleoside analogues, such as lamivudine and famciclovir, have been used to treat chronic viral hepatitis. These nucleoside analogues can be incorporated into the viral genome and can terminate viral replication. A treatment with these agents results in the suppression of the activity of hepatitis B virus and improvement of hepatic function. The molecular, cell, and tissue regenerative engineering approaches described above for hepatitis and liver failure also apply to the treatment for chronic hepatitis and cirrhosis.

Liver Cancers

Pathogenesis, Pathology, and Clinical Features. The liver may develop two types of cancer: primary carcinoma and metastatic cancer. Primary hepatic carcinoma, also known as *hepatoma*, can initiate from hepatocytes or bile duct epithelial cells. Hepatocyte carcinoma accounts for about 90% of liver carcinomas. Several etiologic factors may contribute to the initiation and development of liver carcinoma, including chronic hepatitis, cirrhosis, viral hepatitis, and exposure to certain carcinogens. The incidence of primary hepatic carcinoma is significantly higher in patients with chronic hepatitis, cirrhosis, and viral hepatitis than in the general population. Such a statistical analysis suggests a causative effect of these disorders on the carcinogenesis of the liver. Carcinogens, such as aflatoxins and formaldehydes, may induce gene mutation or changes in the DNA sequence, contributing to carcinogenesis. Metastatic cancer is a cancer that originated from a different organ and spread to the liver. The liver is susceptible to the invasion of metastatic cancer cells because of its large volume and function as a blood reservoir, which enhance cancer cell accumulation in the liver. The pathogenic mechanisms and pathological changes in cancers will be discussed in detail on page in Chapter 25. These aspects are similar in all types of cancers.

Treatment of Liver Cancers. Liver cancers, including primary and secondary carcinomas, exhibit genetic, biochemical, pathological, and clinical characteristics that are similar to those of other types of cancer. Thus similar therapeutic strategies can be applied to all types of cancer with modifications with regard to anatomical differences. The therapeutic aspect of cancers is discussed in Chapter 25.

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