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اسم المادة باللغة العربية: السموم العامة

اسم المادة باللغة الإنكليزية: **General toxicology**

المرحلة: الرابعة

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عنوان المحاضرة باللغة العربية: الاستجابات السامة للكبد

عنوان المحاضرة باللغة الإنكليزية: **Toxic Responses of the Liver**

Toxic Responses of the Liver

The liver is the main organ where exogenous chemicals are metabolized and eventually excreted. As a consequence, liver cells are exposed to significant concentrations of these chemicals, which can result in liver dysfunction, cell injury, and even organ failure.

Hepatic Functions:

Liver is the first organ to encounter ingested nutrients, vitamins, metals, drugs, and environmental toxicants as well as waste products of bacteria that enter portal blood. The Venous blood from the stomach and intestine flows into the portal vein and then through the liver before entering the systemic circulation.

All the major functions of the liver can be detrimentally altered by acute or chronic exposure to toxicants (Table 1).
Loss of function also occurs when toxicants kill an appreciable number of cells and when chronic insult leads to replacement of cell mass by nonfunctional scar tissue. Alcohol abuse is the major cause of liver disease in most western countries; thus ethanol provides a highly relevant example of a toxicant with multiple functional consequences. Early stages of ethanol abuse are characterized by lipid accumulation (fatty liver) due to diminished use of lipids as fuels and impaired ability to synthesize the lipoproteins that transport lipids out of the liver.

Structural Organization

Two concepts exist for organization of the liver into operational units, namely, the lobule and the acinus. Classically, the liver was divided into hexagonal lobules oriented around terminal hepatic venules (also known as central veins).
At the corners of the lobule are the portal triads (or portal tracts), containing a branch of the portal vein, a hepatic arteriole, and a bile duct (Figure 1). Blood entering the portal

Major Functions of Liver and Consequences of Impaired Hepatic Functions

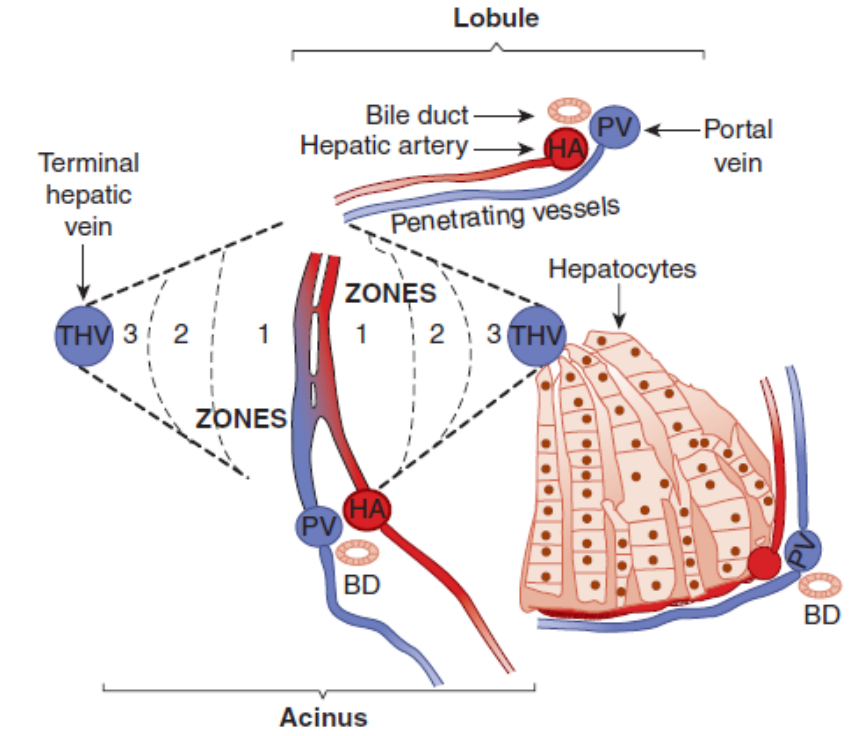
TYPE OF FUNCTION	EXAMPLES	CONSEQUENCES OF IMPAIRED FUNCTIONS
Nutrient homeostasis	Glucose storage and synthesis Cholesterol uptake	Hypoglycemia, confusion Hypercholesterolemia
Filtration of particulates	Products of intestinal bacteria (eg, endotoxin)	Endotoxemia
Protein synthesis	Clotting factors Albumin Transport proteins (eg, very low density lipoproteins)	Excess bleeding Hypoalbuminemia, ascites Fatty liver
Bioactivation and detoxification	Bilirubin and ammonia Steroid hormones Xenobiotics	Jaundice, hyperammonemia-related coma Loss of secondary male sex characteristics Diminished drug metabolism Inadequate detoxification
Formation of bile and biliary secretion	Bile acid-dependent uptake of dietary lipids and vitamins Bilirubin and cholesterol Metals (eg, Cu and Mn) Xenobiotics	Fatty diarrhea, malnutrition, Vitamin E deficiency Jaundice, gallstones, hypercholesterolemia Mn-induced neurotoxicity Delayed drug clearance

Table 1

tract via the portal vein and hepatic artery is mixed in the penetrating vessels, enters the sinusoids, and percolates along the cords of parenchymal cells (hepatocytes), eventually flows into terminal hepatic venules, and exits the liver via the hepatic vein. The lobule is divided into three regions known as centrilobular, midzonal, and periportal. The acinus is the preferred concept for a functional hepatic unit. The terminal branches of the portal vein and hepatic artery, which extend out from the portal tracts, form the base of the acinus. The acinus has three zones: zone 1 is closest to the entry of blood, zone 3 abuts the terminal hepatic vein, and zone 2 is intermediate. Acinar zonation is of considerable functional consequence regarding gradients of components both in blood and in hepatocytes. Blood entering the acinus consists of oxygen-depleted blood from the portal vein (60%–70% of hepatic blood flow) plus oxygenated blood from the hepatic artery (30%–40%). Enroute to the terminal hepatic venule, oxygen rapidly leaves the blood to meet the high metabolic demands of the parenchymal cells. Approximate oxygen concentrations in zone 1 are 9% to 13%, compared with only 4% to 5% in zone 3. Therefore, hepatocytes in zone 3 are exposed to substantially lower concentrations of oxygen than hepatocytes in zone 1. In comparison to other tissues, zone 3 is hypoxic. Another well-documented acinar gradient is that of bile salts. Physiological concentrations of bile salts are efficiently extracted by zone 1 hepatocytes with little bile acids left in the blood that flows past zone 3 hepatocytes.

There is difference in bile acid transporter expression between different zones.

Figure 1: Schematic of liver operational units, the classic lobule and the acinus. The lobule is centered around the terminal hepatic vein (central vein), where the blood drains out of the lobule. The acinus has as its base the penetrating vessels, where blood supplied by the portal vein and hepatic artery fl ows down the acinus past the cords of hepatocytes. Zones 1, 2, and 3 of the acinus represent metabolic regions that are increasingly distant from the blood supply.



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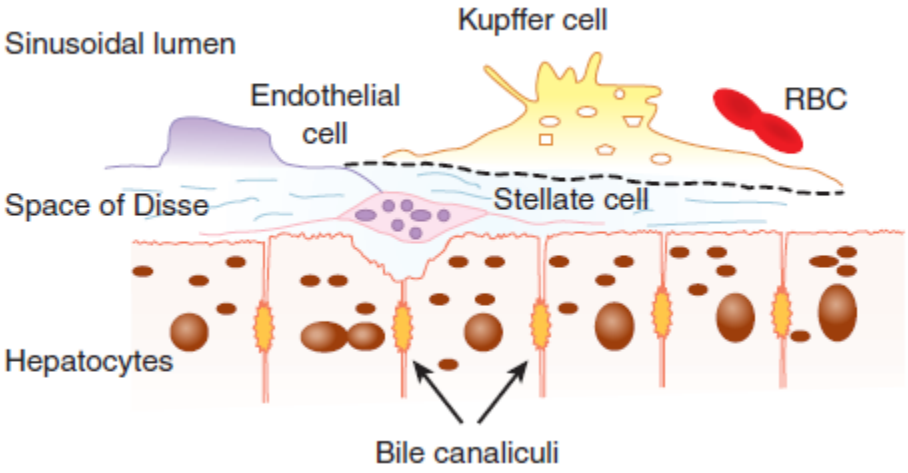
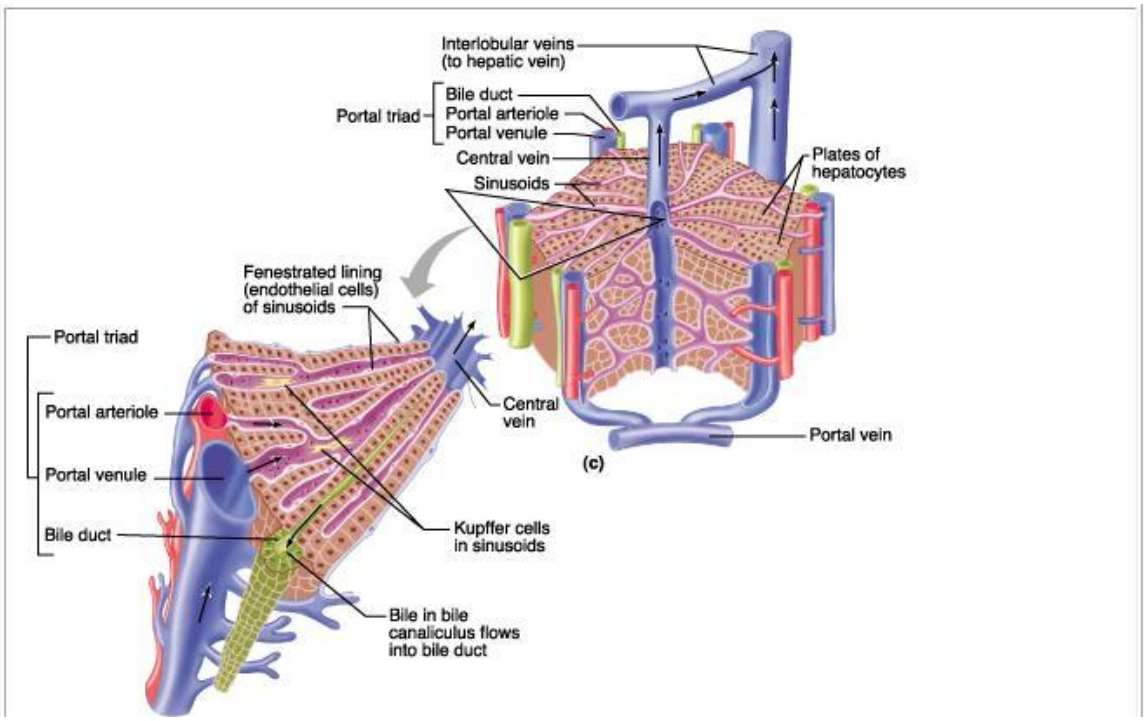


Figure 2:Schematic of liver sinusoidal cells. Note that the Kupffer cell resides within the sinusoidal lumen. The stellate cell is located in the space of Disse between the thin, fenestrated endothelial cells, and the cord of hepatocytes.

Hepatocytes in the mitochondria-rich zone 1 are predominant in fatty acid oxidation, gluconeogenesis, and ammonia detoxification to urea. Gradients of enzymes involved in the bioactivation and detoxification of xenobiotics have been observed along the acinus by immunohistochemistry. Hepatic sinusoids are the channels between cords of hepatocytes

where blood percolates on its way to the terminal hepatic vein. Sinusoids are larger and more irregular than normal capillaries.



The three major types of cells in the sinusoids are endothelial cells, Kupffer cells, and stellate cells (Figure 2). Sinusoids are lined by thin, discontinuous endothelial cells with numerous fenestrae (or pores) that allow molecules smaller than 250 kDa to cross the interstitial space (known as the space of Disse) between the endothelium and hepatocytes. The numerous fenestrae and the lack of basement membrane facilitate exchanges of fluids and molecules, such as albumin, between the sinusoid and hepatocytes, but hinder movement of particles larger than chylomicron remnants. Kupffer cells are the resident macrophages of the liver and constitute approximately 80% of the fixed macrophages in the body. Kupffer cells are situated within the lumen of the sinusoid. The primary function of Kupffer cells is to ingest and degrade particulate matter. Also, Kupffer cells are a major source of cytokines and eicosanoids and can act as antigen-presenting cells. Hepatic stellate cells (HSCs; also known as Ito cells or by the more descriptive terms of fat-storing cells) are located between endothelial cells and hepatocytes. Stellate cells are the major sites for vitamin A storage in the body. Upon activation, these cells can synthesize and excrete collagen and other extracellular matrix proteins and express smooth muscle actin.

Bile Formation

Bile is a yellow fluid containing bile acids, GSH, phospholipids, cholesterol, bilirubin and other organic anions, proteins, metals, ions, and xenobiotics. Formation of this fluid is a specialized function of the liver. Adequate bile formation is essential for uptake of lipid

nutrients from the small intestine (Table 1), for protection of the small intestine from oxidative insults, and for excretion of endogenous and xenobiotic compounds. Hepatocytes begin the process of bile formation by transporting bile acids, GSH, and other osmotically active compounds including xenobiotics and their metabolites into the canalicular lumen.

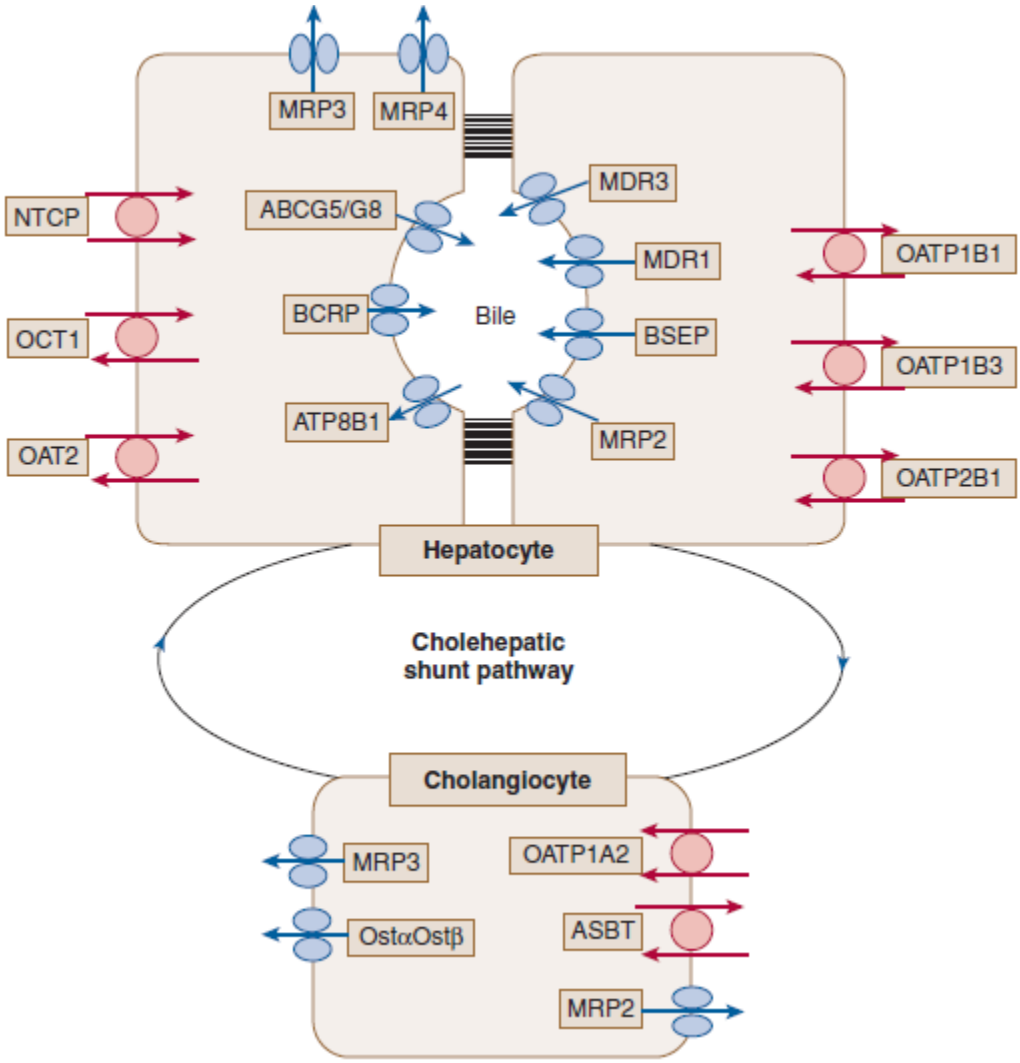
The canaliculi are separated from the intercellular space between hepatocytes by tight junctions, which form a barrier permeable only to water, electrolytes, and to some degree to small organic cations.
Under physiological conditions, tight junctions are impermeable to organic anions allowing the high concentrations of bile acids, GSH, bilirubin diglucuronide, and other organic anions in bile.

The large extrahepatic bile ducts merge into the common bile duct. Bile can be stored and concentrated in the gallbladder before its release into the duodenum. On the basal (sinusoidal) side of the hepatocytes, there are sodium-dependent and sodium-independent uptake systems. Most conjugated bile acids (taurine and glycine conjugates) and some of the unconjugated bile acids are transported into hepatocytes by sodium/taurocholate cotransporting polypeptide (NTCP) (Fig. 3). Sodium-independent uptake of conjugated and unconjugated bile acids is performed by members of the organic anion transporting polypeptides (OATPs). OATP1B1 and OATP1B3 are predominantly expressed in liver and are capable of transporting conjugated and unconjugated bile acids and steroids, bromosulfophthalein, and many other organic anions.

Furthermore, the OATPs are transporting numerous drugs and also some hepatotoxins, for example, phalloidin, microcystin, and amanitin.
Bile acid excretion is a major driving force of bile formation (bile salt-dependent bile flow). Other constituents of bile are transported by members of the multidrug resistance

(MDR) P-glycoprotein family such as MDR3 (ABCC2), which transports phospholipids, and the heterodimeric transporters ABCG5/ABCG8, which transport cholesterol and plant sterols into bile. In addition, MRP2 (a member of the multidrug resistance- associated proteins) transports GSH, which is the main compound responsible for the bile salt-independent bile flow, as well as sulfated and glucuronidated bile acids, glutathione disulfide and glutathione conjugates, bilirubin diglucuronide, and many other conjugated drugs and chemicals. Other transport systems of the canalicular membrane include the breast cancer resistance protein (BCRP; ABCG2), which can contribute to the biliary excretion of bile acids and xenobiotics. Biliary excretion is important in the homeostasis of multiple metals, notably copper, manganese, cadmium, selenium, gold, silver, and arsenic.

Figure 3:Transport proteins in human hepatocytes and cholangiocytes. Efflux transporters (blue symbols) and Uptake transporters (red symbols).



Mechanisms and Types of Toxicant-Induced Liver Injury

Cell Death

Based on morphology, liver cells can die by two different modes, oncotic necrosis ("necrosis") or apoptosis. Necrosis is characterized by cell swelling, leakage of cellular contents, nuclear disintegration (karyolysis), and an influx of inflammatory cells. Because necrosis is generally the result of an exposure to a toxic chemical or other traumatic conditions, for example, ischemia, large numbers of contiguous hepatocytes and nonparenchymal cells may be affected. Thus, an ongoing oncotic necrotic process can be identified by the release of liver-specific enzymes such as alanine (ALT) or aspartate (AST) aminotransferase into the plasma and by histology, where areas of necrosis with loss of nuclei and inflammatory infiltrates are easily detectable in H&E sections. In contrast, apoptosis is characterized by cell shrinkage, chromatin condensation, nuclear

fragmentation, formation of apoptotic bodies, and, generally, a lack of inflammation. The characteristic morphological features of apoptosis are caused by the activation of caspases, which trigger the activation of enzymes such as caspase-activated DNase (CAD) responsible for internucleosomal DNA fragmentation. In addition, caspases can directly cleave cellular and nuclear structural proteins. Under these conditions, apoptotic bodies are phagocytosed by Kupffer cells or taken up by neighboring hepatocytes. In recent years, signaling mechanisms of apoptosis were elucidated in great detail. In the extrinsic pathway of apoptosis, ligands (eg, Fas ligand, TNF-α) bind to their respective death receptor (Fas receptor, TNF receptor type I), which triggers the trimerization of the receptor followed by recruitment of various adapter molecules and procaspases to the cytoplasmic tail of the receptor. The assembly of this death-inducing signaling complex (DISC) leads to the activation of initiator caspases (caspase-8 or -10). In contrast to the extrinsic pathway, the intrinsic or mitochondrial pathway of apoptosis is initiated independent of the TNF receptor family, caspase-8 activation, and formation of the DISC.

Despite the upstream differences, the postmitochondrial effects are largely similar to the extrinsic pathway. The intrinsic pathway is generally triggered by a cytotoxic stress or DNA damage, which activates the tumor suppressor p53.

Canalicular Cholestasis

This form of liver dysfunction is defined physiologically as a decrease in the volume of bile formed or an impaired secretion of specific solutes into bile. Cholestasis is characterized biochemically by elevated serum levels of compounds normally concentrated in bile, particularly bile salts and bilirubin. When biliary excretion of the yellowish bilirubin pigment is impaired, this pigment accumulates in skin and eyes, producing jaundice, and spills into urine, which becomes bright yellow or dark brown. Because drug-induced jaundice reflects a more generalized liver dysfunction, it is considered a more serious warning sign in clinical trials than mild elevations of liver enzymes. Toxicant-induced cholestasis can be transient or chronic. Many different types of chemicals, including metals, hormones, and drugs, cause cholestasis (Table 2). The molecular mechanisms of cholestasis are related to expression and function of transporter systems in the basolateral and canalicular membranes.

The hepatotoxicity of phalloidin, microcystin, and amanitin is facilitated by the uptake through OATPs. Furthermore, there is a growing list of drugs including rifampicin, bosentan, and troglitazone, which are known to directly inhibit BSEP. However, estrogen and progesterone metabolites inhibit BSEP from the canalicular side after excretion by MRP2. A substantial inhibition of bile salt excretion can lead to accumulation of these compounds in hepatocytes and may directly cause cell injury. However, more recent findings indicate that most of the bile acids accumulating in the liver after obstructive cholestasis are nontoxic and instead of cell death cause pro-inflammatory gene expression in hepatocytes. Thus, liver injury after obstructive cholestasis is caused mainly by inflammatory cells. Bile acids are substrates for the nuclear receptor farnesoid X receptor

(FXR). FXR activation stimulates the small heterodimeric partner 1 (SHP1), which downregulates NTCP and limits bile acid uptake. In addition, FXR activation causes the increased expression of BSEP and MDR3, which enhances the transport capacity for bile acids and phospholipids, respectively, at the canalicular membrane.

Sinusoidal Damage

The sinusoid is, in effect, a specialized capillary with numerous fenestrae for high permeability. The functional integrity of the sinusoid can be compromised by dilation or blockade of its lumen or by progressive destruction of its endothelial cell wall. Progressive destruction of the endothelial wall of the sinusoid will lead to gaps and then ruptures of its barrier integrity, with entrapment of red blood cells. These disruptions of the sinusoid are considered the early structural features of the vascular disorder known as veno-occlusive disease. Well established as a cause of veno-occlusive disease are the pyrrolizidine alkaloids (eg, monocrotaline, retrorsine, and seneciphylline) found in some plants used for herbal teas and in some seeds that contaminate food grains. Numerous episodes of human and animal poisoning by pyrrolizidine alkaloids have been reported around the world, including massive problems affecting thousands of people in Afghanistan in 1976 and 1993. (1974–1976 – Afghanistan: widespread poisoning (an estimated 7800

people affected with hepatic veno-occlusive disease (liver damage) and about 1600 deaths) was attributed to wheat contaminated with weed seeds known as charmac (Heliotropium popovii. H Riedl) that contain pyrrolizidine alkaloids.)

Types of Hepatobiliary Injury

TYPE OF INJURY OR DAMAGE	REPRESENTATIVE TOXINS
Fatty liver	Amiodarone, CCl_4, ethanol, fialuridine, tamoxifen, valproic acid
Hepatocyte death	Acetaminophen, allyl alcohol, Cu, dimethylformamide, ethanol
Immune-mediated response	Diclofenac, ethanol, halothane, tienilic acid
Canalicular cholestasis	Chlorpromazine, cyclosporin A, 1,1-dichloroethylene, estrogens, Mn, phalloidin
Bile duct damage	Alpha-naphthylisothiocyanate, amoxicillin, methylenedianiline, sporidesmin
Sinusoidal disorders	Anabolic steroids, cyclophosphamide, microcystin, pyrrolizidine alkaloids
Fibrosis and cirrhosis	CCl_4, ethanol, thioacetamide, vitamin A, vinyl chloride
Tumors	Aflatoxin, androgens, arsenic, thorium dioxide, vinyl chloride

Table 2.

Veno-occlusive disease is also a serious complication in about 15% of the patients given high doses of chemotherapy (eg, cyclophosphamide) as part of bone-marrow transplantation regimen. Selective depletion of GSH within sinusoidal endothelial cells and activation of matrix metalloproteinases are critical events in the mechanism of endothelial cell injury in the pathophysiology of veno-occlusive disease.

Fatty Liver

Fatty liver (steatosis) is defined biochemically as an appreciable increase in the hepatic lipid (mainly triglyceride) content, which is <5 wt% in the normal human liver. Currently, the most common cause of hepatic steatosis is insulin resistance due to central obesity and sedentary lifestyle.

However, acute exposure to many hepatotoxins, for example, carbon tetrachloride and drugs can induce steatosis. Compounds that produce prominent steatosis associated with lethality include the antiepileptic drug valproic acid and the antiviral drug fialuridine. Ethanol is by far the most relevant drug or chemical leading to steatosis in humans and in experimental animals. Often, drug-induced steatosis is reversible and does not lead to death of hepatocytes. Although steatosis alone may be benign, it can develop into steatohepatitis (alcoholic or nonalcoholic), which is associated with significant liver injury. Steatohepatitis can progress to fibrosis and even hepatocellular carcinoma.

Free fatty acids (FFAs) can be newly synthesized in hepatocytes (mainly from carbohydrate-derived acetyl-coenzyme A). FFAs released from adipose tissue can be taken up into hepatocytes, or they are generated in the liver from hydrolysis of absorbed fat (chylomicrons). Once in the cytosol, FFAs can be imported into mitochondria for degradation (β-oxidation), or esterified into triglycerides for incorporation into very low density lipoproteins (VLDL), which transports the FFAs to the peripheral adipose tissue. Thus, FFA synthesis, consumption, and storage are in a state of equilibrium with no relevant accumulation of triglycerides in the liver. However, if there is chronic excess food consumption with obesity and insulin resistance, excess uptake of FFAs derived from adipose tissue and food into hepatocytes leads to an overload of FFAs, which cannot be degraded and are therefore esterified to triglycerides. One part of the excess triglycerides is incorporated into VLDL, and the other part is stored in the liver gradually leading to steatosis. The previously preferred hypothesis of nonalcoholic steatohepatitis (NASH) considered triglyceride accumulation in hepatocytes as the first hit and any additional stress (oxidant stress, lipid peroxidation) as a second hit leading to the progression from steatosis to steatohepatitis. However, more recent data have clearly demonstrated that triglyceride accumulation does neither cause insulin resistance nor cell injury. A new hypothesis postulates that nonalcoholic fatty liver disease (NAFLD) is mainly caused by lipotoxicity of nontriglyceride fatty acid metabolites. Mechanisms of lipotoxicity elucidated in cell culture experiments include endoplasmic reticulum stress, activation of the mitochondrial cell death pathway, and lysosomal dysfunction.

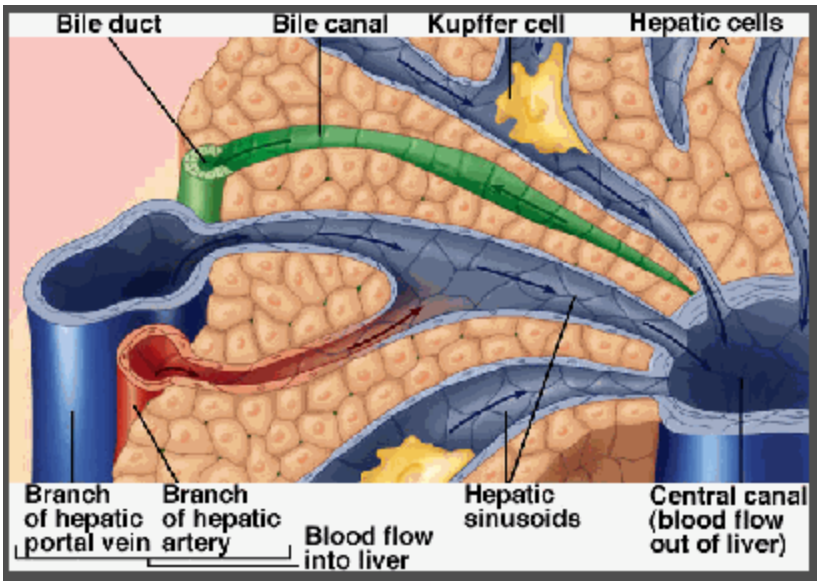
Fibrosis and Cirrhosis

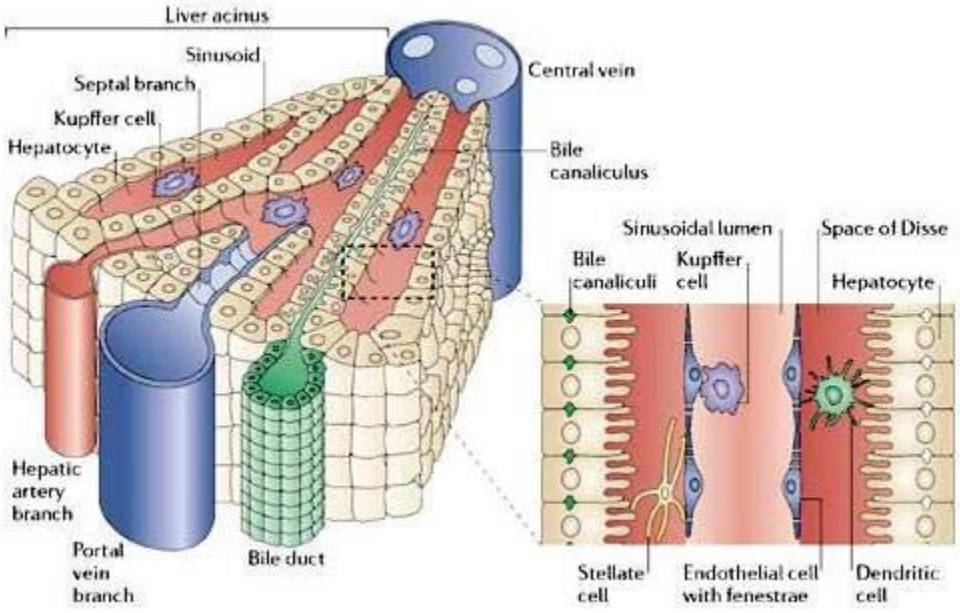
Hepatic fibrosis (scaring) occurs in response to chronic liver injury and is characterized by the accumulation of excessive amounts of fibrous tissue, specifically fibrilforming collagens type I and III, and a decrease in normal plasma membrane collagen type IV. Fibrosis can develop around central veins and portal tracts or within the space of Disse.

With continuing collagen deposition, the architecture of the liver is disrupted by interconnecting fibrous scars. When the fibrous scars subdivide the remaining liver mass into nodules of regenerating hepatocytes, fibrosis has progressed to cirrhosis and the liver has limited residual capacity to perform its essential functions. The primary cause of hepatic fibrosis/cirrhosis in humans worldwide is viral hepatitis. However, biliary obstruction and, in particular, alcoholic and NASH are of growing importance for the development of hepatic fibrosis. In addition, fibrosis can be induced by chronic exposure to drugs and chemicals including ethanol and by heavy metal overload. HSC (Fig. 13-2), which are the main cell type producing extracellular matrix proteins. Products formed during liver cell injury initiate HSC activation. Activating signals can be reactive oxygen species and lipid peroxidation products generated in injured hepatocytes. In addition, Kupffer cells can release reactive oxygen and pro-inflammatory cytokines during the phagocytosis of cell debris or apoptotic bodies, thereby recruiting more inflammatory cells and enhancing the injury and oxidant stress. Damaged sinusoidal endothelial cells contribute to the activation of HSC.

Factors in the Site-Specific Injury of Representative Hepatotoxicants

SITE	REPRESENTATIVE TOXICANTS	POTENTIAL EXPLANATION FOR SITE-SPECIFICITY
Zone 1 hepatocytes (vs zone 3)	Fe (overload) Allyl alcohol	Preferential uptake and high oxygen levels Higher oxygen levels for oxygen-dependent bioactivation
Zone 3 hepatocytes (vs zone 1)	CCl_4 Acetaminophen Ethanol	More P450 isozyme for bioactivation More P450 isozyme for bioactivation and less GSH for detoxification More hypoxic and greater imbalance in bioactivation/detoxification reactions
Bile duct cells	Methylenedianiline, sporidesmin	Exposure to the high concentration of reactive metabolites in bile
Sinusoidal endothelium (vs hepatocytes)	Cyclophosphamide, monocrotaline	Greater vulnerability to toxic metabolites and less ability to maintain glutathione levels
Kupffer cells	Endotoxin, $GdCl_3$	Preferential uptake and then activation
Stellate cells	Vitamin A Ethanol (chronic)	Preferential site for storage and then engorgement Activation and transformation to collagen-synthesizing cell


Bile duct
Bile canal
Kupffer cell
Hepatic cells
Branch of hepatic portal vein
Branch of hepatic artery
Blood flow into liver
Hepatic sinusoids
Central canal (blood flow out of liver)


Liver acinus
Sinusoid
Septal branch
Kupffer cell
Hepatocyte
Central vein
Bile canaliculus
Hepatic artery branch
Portal vein branch
Bile duct
Sinusoidal lumen
Space of Disse
Bile canaliculi
Kupffer cell
Hepatocyte
Stellate cell
Endothelial cell with fenestrae
Dendritic cell