Toxic Responses of the Liver

The liver is the main organ where exogenous chemicals are metabolized and eventually excreted.

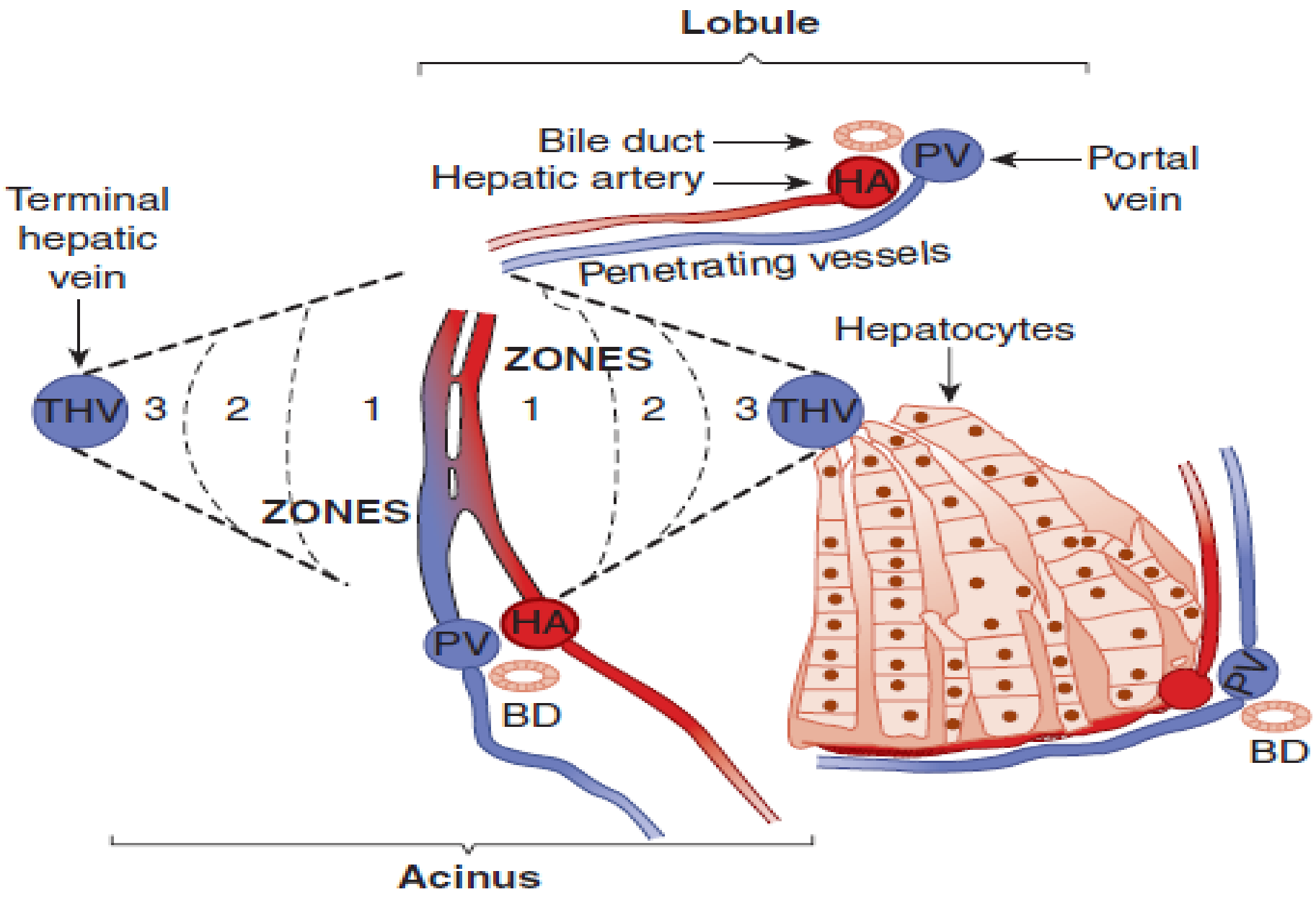
As a consequence, liver cells are exposed to significant concentrations of these chemicals, which can result in liver dysfunction, cell injury, and even organ failure.

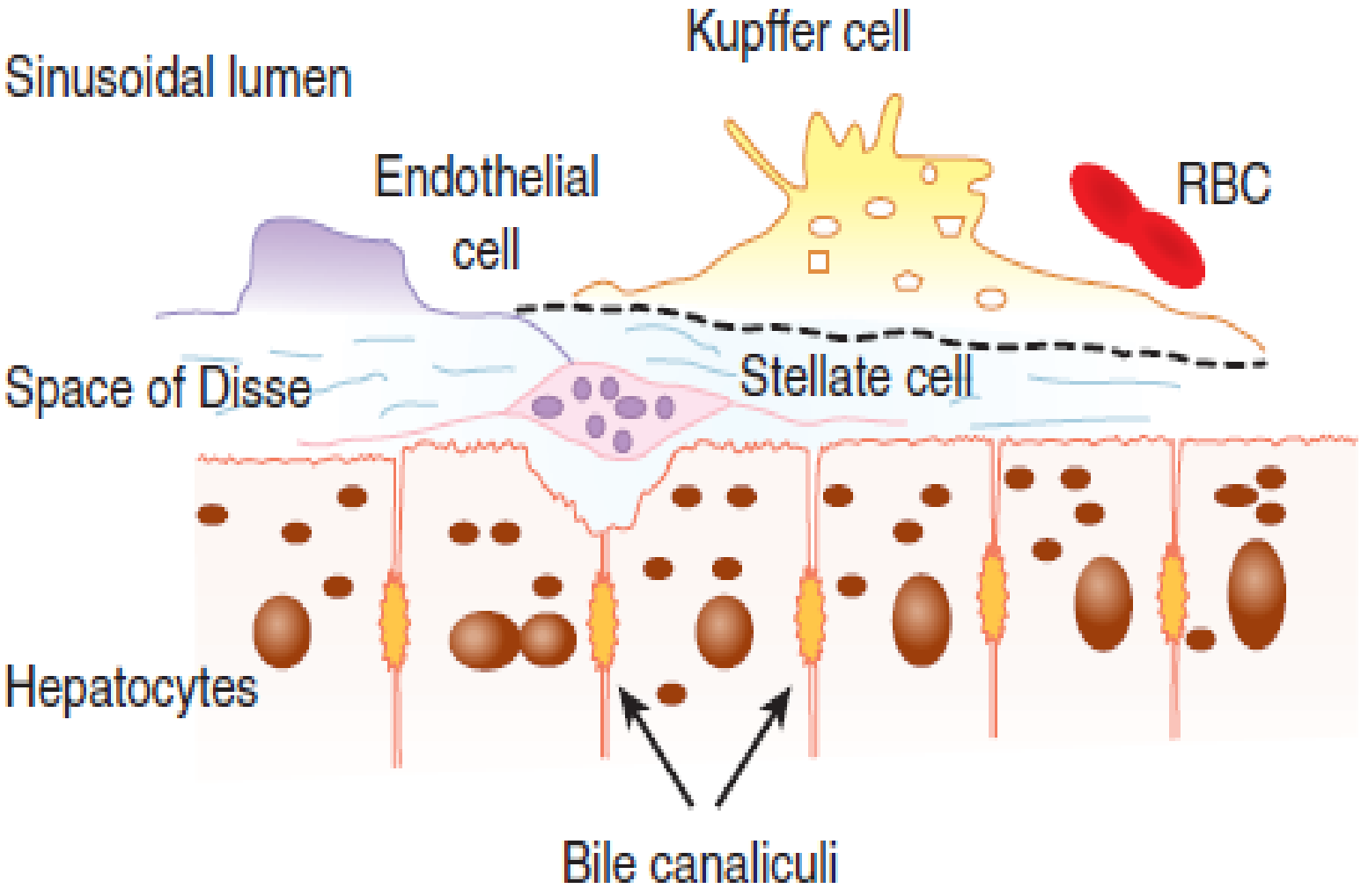
Major Functions of Liver and Consequences of Impaired Hepatic Functions

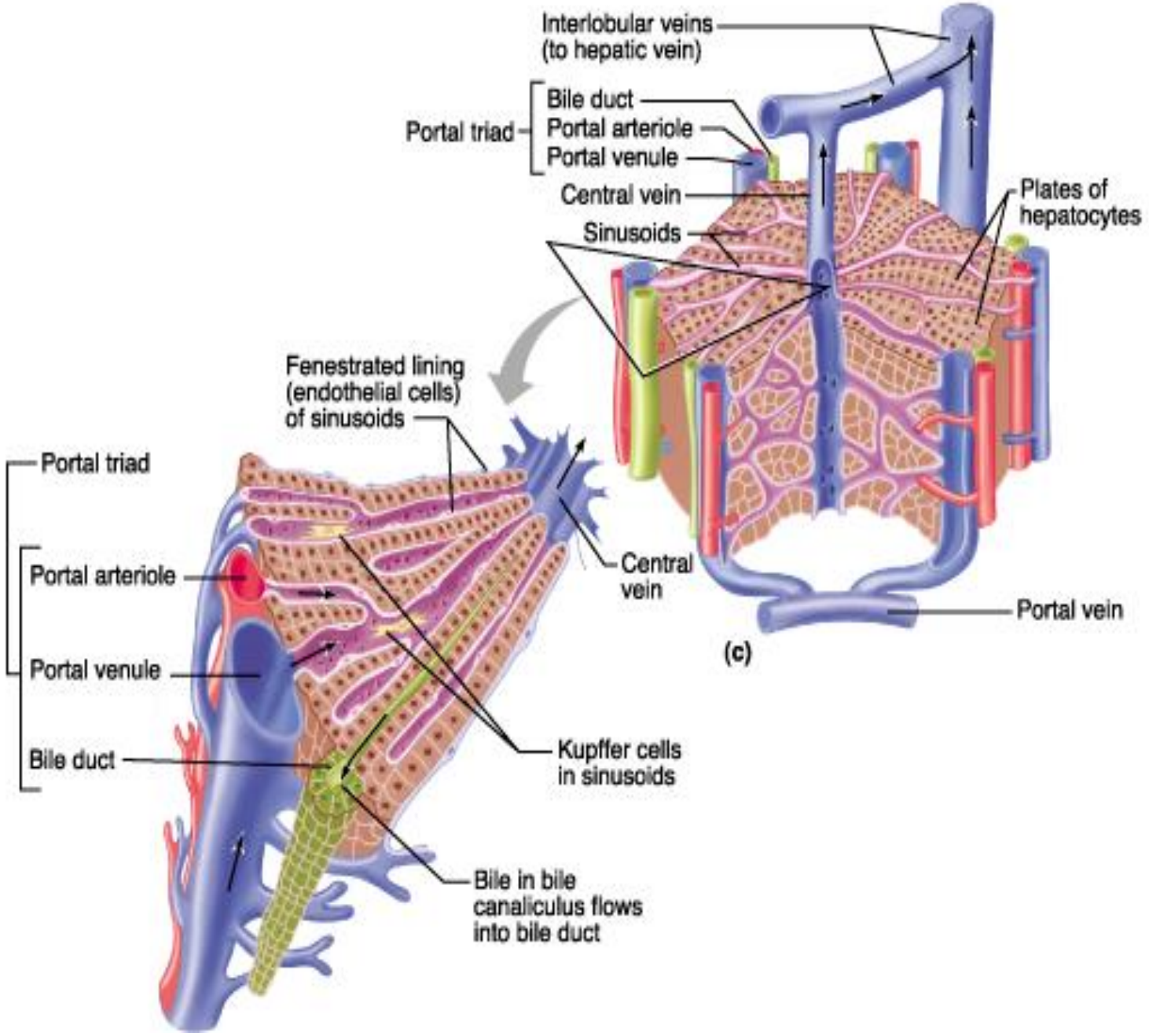
TYPE OF FUNCTION	EXAMPLES	CONSEQUENCES OF IMPAIRED FUNCTIONS
Nutrient homeostasis	Glucose storage and synthesis Cholesterol uptake	Hypoglycemia, confusion Hypercholesterolemia
Filtration of particulates	Products of intestinal bacteria (eg, endotoxin)	Endotoxemia
Protein synthesis	Clotting factors Albumin 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

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.


Lobule
Bile duct
Hepatic artery
PV
HA
Portal vein
Terminal hepatic vein
Penetrating vessels
Hepatocytes
ZONES
THV
3
2
1
1
2
3
THV
ZONES
HA
PV
BD
PV
BD
Acinus


Kupffer cell
Sinusoidal lumen
Endothelial cell
RBC
Space of Disse
Stellate cell
Hepatocytes
Bile canaliculi


Interlobular veins
(to hepatic vein)
Portal triad
Bile duct
Portal arteriole
Portal venule
Central vein
Sinusoids
Plates of
hepatocytes
Fenestrated lining
(endothelial cells)
of sinusoids
Portal triad
Portal arteriole
Portal venule
Bile duct
Central
vein
Portal vein
(c)
Kupffer cells
in sinusoids
Bile in bile
canaliculus flows
into bile duct

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

Mechanisms and Types of Toxicant-Induced Liver Injury

Cell Death

, 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.

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 post mitochondrial 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.

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.)

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.

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.

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 fibril forming 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.

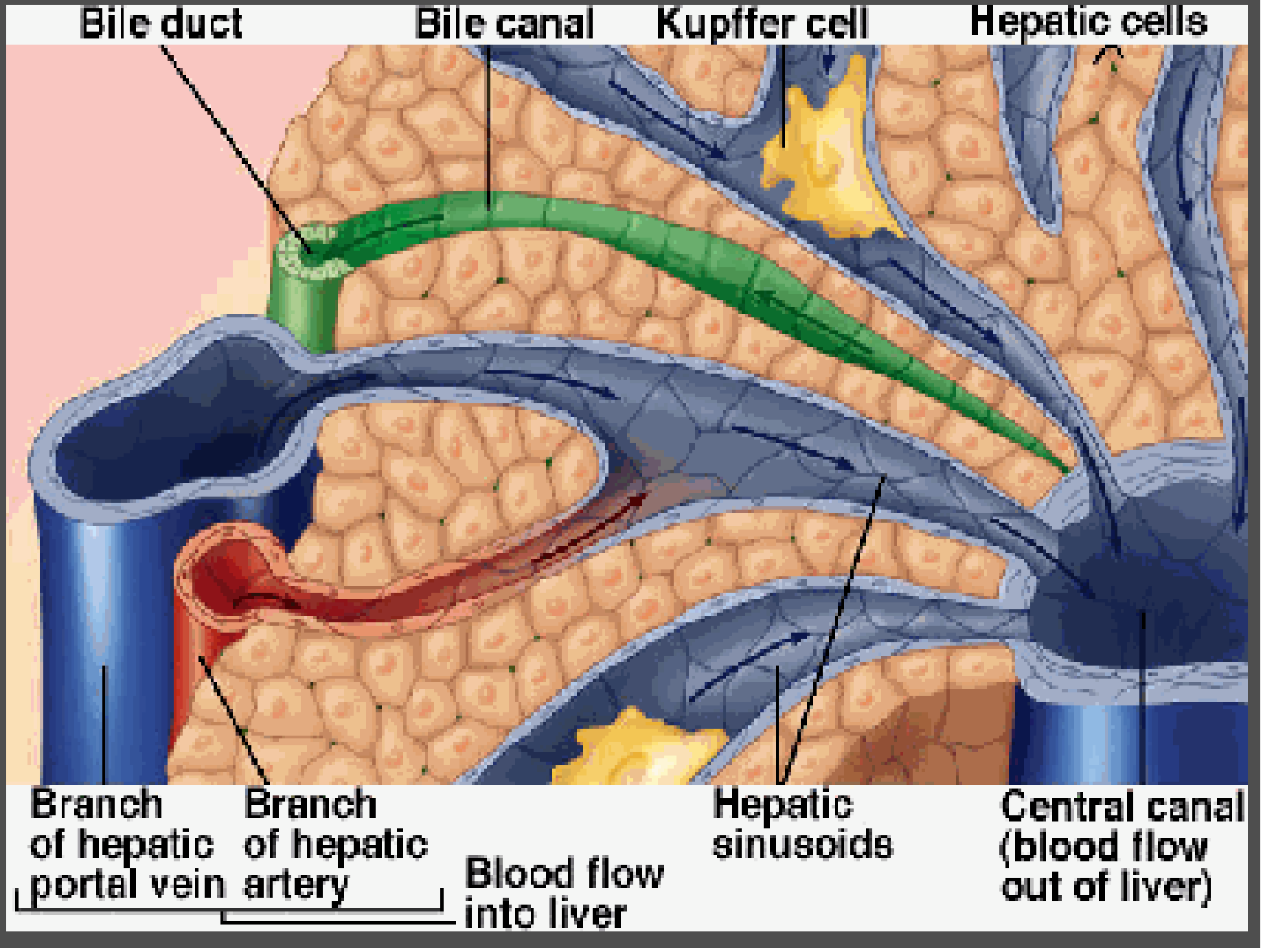
The primary cause of hepatic fibrosis/cirrhosis in humans worldwide is viral hepatitis.

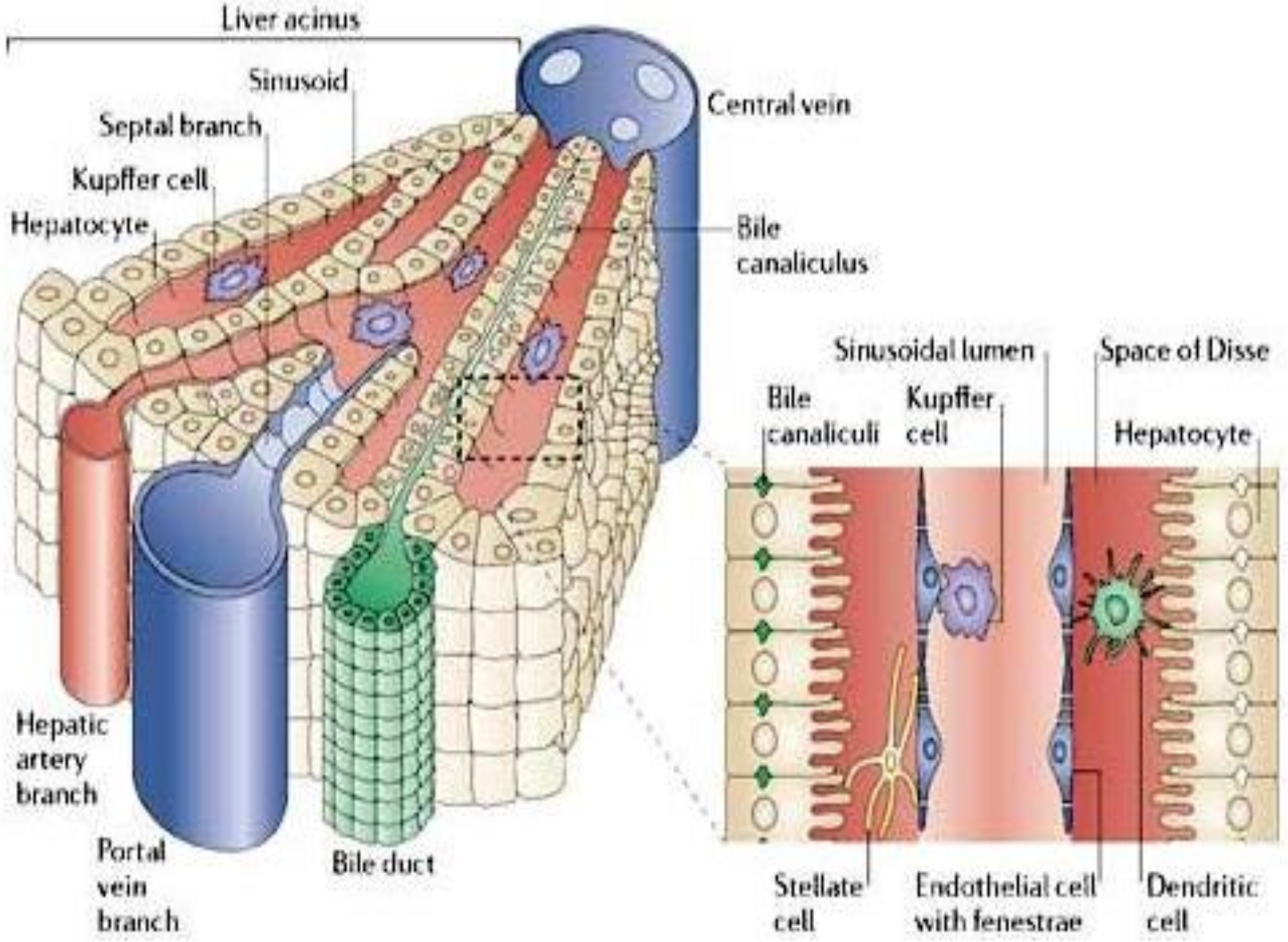
However, biliary obstruction and, in particular, alcoholic and NASH are of growing importance for the development of hepatic fibrosis.

In addition, fibrosis can be induced by chronic exposure to drugs and chemicals including ethanol and by heavy metal overload

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

THANK YOU