Toxic Responses of the Liver

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❑ Background:

The liver performs many functions that are critical to life.

- ✓ Among these are the processing of foods and other substances absorbed from the intestinal tract and the subsequent delivery of processed nutrients to other organs in the body.
- ✓ The liver is also an integral contributor of immunity that protects mammals from harmful pathogens.
- ✓ In addition to its immunological roles, it is the main organ where exogenous chemicals are metabolized, a process that hastens their excretion into bile and urine.

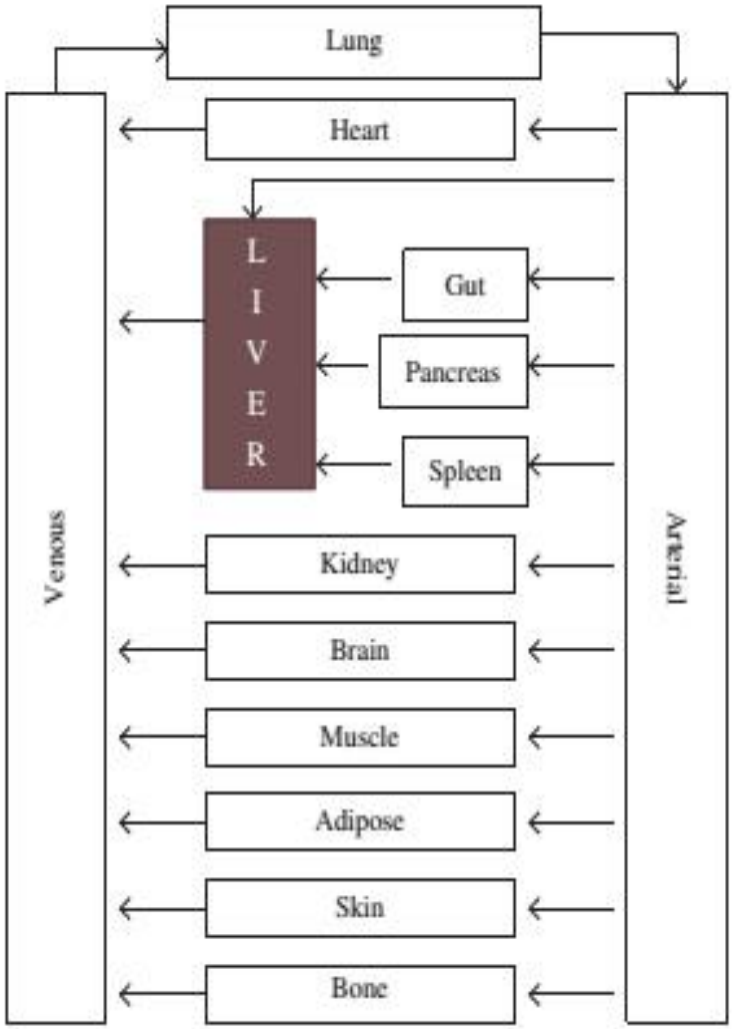
- **As a consequence**, liver cells are exposed to significant concentrations of these chemicals and their metabolites, some of which can cause liver dysfunction.
- Many industrial chemicals, plant toxins, environmental pollutants, food-borne agents, herbal remedies, and drugs (both pharmaceutical and recreational) are known to be hepatotoxic.
- In the pharmaceutical industry, **adverse effects on the liver are one of the most frequent reasons for discontinuing the development of drug candidates and withdrawal of drugs from the market.**

- **The liver comprises several cell types**, each with different functions, and it is nourished by a blood supply that is unique in the body.
- Chemical-induced liver injury is typically initiated by one or more
  critical events, such as formation of a toxic metabolite, which trigger intracellular responses that can progress to dysfunction or death of hepatic parenchymal cells (i.e., HPCs, hepatocytes). These intrahepatocellular events can in turn prompt secondary events involving activation of non-parenchymal cells that magnify or attenuate the initial injury

Liver Anatomy and Physiology

Hepatic Functional Anatomy

- Livers of mammals typically have **two** (humans) or more (rodents) lobes into which blood vessels enter and exit. The liver is unusual among organs in that it has a dual blood supply (Fig. 13-1).
- As is typical of other organs, the **liver has an arterial supply via the hepatic artery**, which provides a minority of blood entering the liver (**about 1/3 in humans**, less in rodents). The major blood supply to the liver arises from the hepatic portal vein, which comprises venous drainage from the stomach and intestine.
- This unique anatomy positions the liver to **have first contact with food-borne xenobiotic agents absorbed into the blood from the gastrointestinal (GI) tract**, but it also means that the liver receives blood from which much **oxygen has been removed after nourishing the GI tract**.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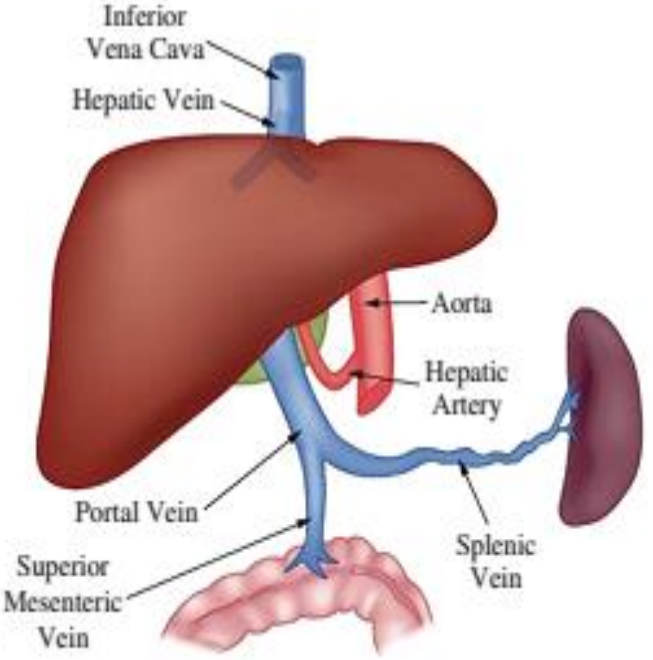
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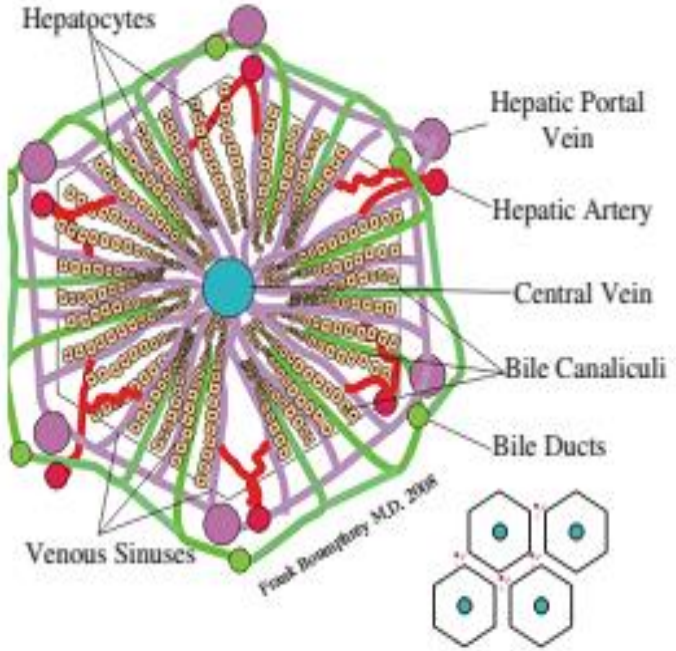
Hepatocytes
Hepatic Portal
Vein
Hepatic Artery
Central Vein
Bile Canaliculi
Bile Ducts
Venous Sinuses
Frank Bunnyboy M.D. 2008

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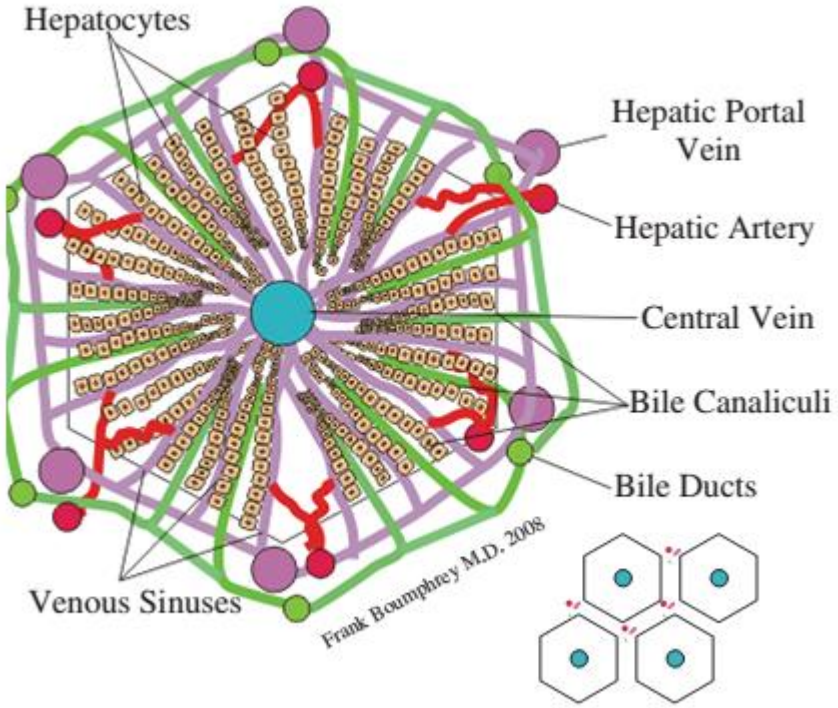
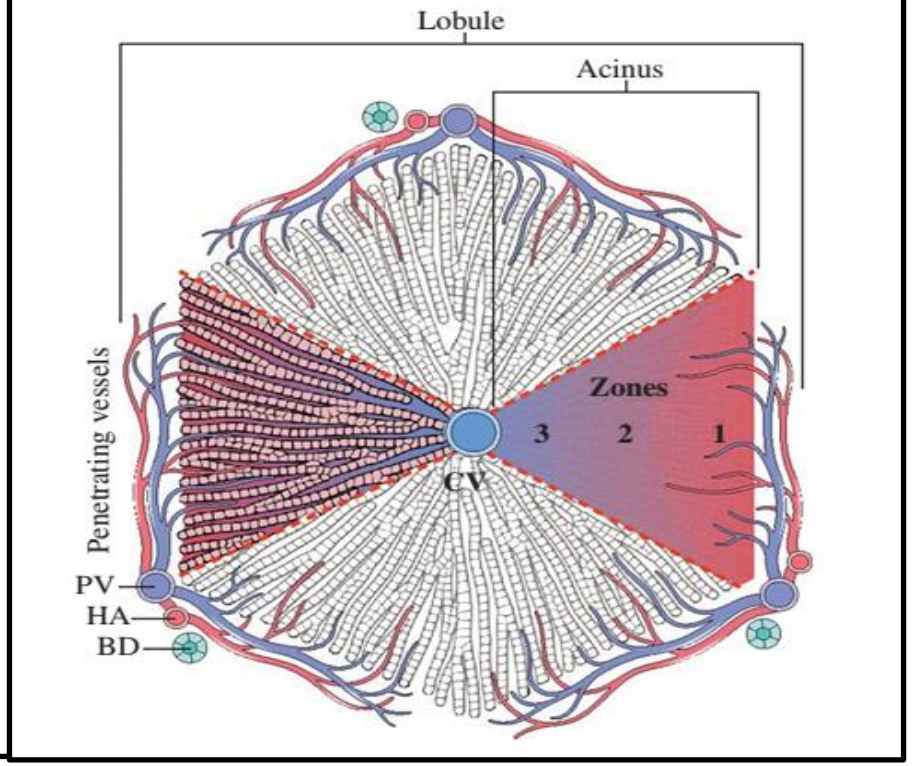
- Much oxygen has been removed after nourishing the GI tract.
- These two blood supplies in part determine how liver is organized into smaller anatomical substructures within each lobe.
- Two concepts exist for organization of operational units, termed either **"lobules"** or **"acini"** .
- According to the **classical lobular concept**, the liver is organized into **hexagonal lobules** **that are more or less apparent histologically.**

- Each lobule is oriented around a **central vein** (also known as a **terminal hepatic venule**).
- At the corners of the lobule are **portal triads (also known as portal tracts)**.
- As the name implies, each of these contains a branch of the portal vein (portal venule), a hepatic arteriole, and one or more small bile ducts (Figs. 13-1C and 13-2).





- The capillary equivalents in liver lobules are called "**sinusoids**." Blood entering the portal tract via the portal vein and hepatic artery is mixed in the penetrating vessels, enters the sinusoids, and percolates along the chords of parenchymal cells (HPCs).
- The blood then collects into terminal hepatic venules (central veins), which coalesce to form the hepatic vein that connects to the vena cava (Figs. 13-1B and 13-2).
- Keeping in mind this general structure, the lobule is viewed as having three regions known as **periportal** (nearest portal triad), **centrilobular (surrounding the central vein)**, and **midzonal** (**between periportal and centrilobular**)

- Another way of viewing the functional unit of the liver is the **acinus**. This concept is preferred from the standpoint that it better reflects the manner in which blood flows into the **sinusoids**; that is, some of the blood entering via the portal venule and hepatic arteriole mixes, then some flows laterally (between portal triads) before entering the sinusoid.
- The terminal branches of the **portal vein and hepatic artery** form the base of the acinus, which has three zones: zone 1 is closest to the entry of blood (i.e., cells near the portal triad), zone 3 abuts the central vein, and zone 2 is in between (Figs. 13-2 and 13-3). These zones correspond roughly to **periportal, centrilobular, and midzonal** areas of the classical lobule, respectively, but more closely align with the manner in which blood is delivered to the sinusoids.

- Despite the greater functional accuracy of the acinar concept, lobular terminology is still used to describe location of pathological lesions of hepatic parenchyma.

- Acinar/lobular zonation is of considerable functional consequence regarding gradients of components
both in blood and in HPCs.

- For example, as noted above blood entering the acinus comprises mostly blood from the portal vein that is poorly oxygenated relative to the blood entering from the hepatic artery. Enroute the central vein, oxygen rapidly leaves the blood to meet the high metabolic demands of the HPCs.
- Therefore, **HPCs in zone 3 are exposed to substantially smaller concentrations of oxygen than those in zone 1. These anatomical substructures are also important because liver lesions caused by chemical exposure usually appear preferentially in one of them**.

- The **sinusoids** are **endothelium-lined channels between cords of HPCs through which blood flows on its way to the central vein**. The sinusoidal microvasculature of the liver differs in important ways from capillaries in other organs. Sinusoids are larger and more irregular than typical capillaries.
- **Three major** types of cells in the sinusoids are sinusoidal endothelial cells (SECs), Kupffer cells, and stellate cells (HSCs) (Fig. 13-3) .In addition, the liver contains dendritic cells and a substantial number of lymphocytes (PIT cells) , especially natural killer (NK) and NKT cells.
- Each of these **non-parenchymal** cell types performs important functions.

❑ Liver Cells and Their Functions:

- ❖ The liver's location in the circulation between the intestinal tract and the rest of the body facilitates its performance **as a primary contributor to maintaining metabolic homeostasis of the body**.
- ❖ Because venous blood from the stomach and intestine flows into the portal vein and then through the liver before entering the systemic circulation, it is the first **organ to encounter ingested nutrients, vitamins, drugs, and environmental toxicants, as well as bacterial products that enter the portal blood after being absorbed in the gastrointestinal tract.**
- ❖ **Efficient scavenging or uptake** processes extract these absorbed materials from the blood for processing, storage, and/or excretion into bile.

❑ Hepatic Parenchymal Cells:

- ❖ Hepatic parenchymal cells (**HPCs**), also known as **hepatocytes**, are large epithelial cells that account for about 60% of the cells in the liver and about 80% of the liver volume. These cells occur in cords along the microvasculature of the liver (Fig. 13-3), with microvilli on their basal surface in apposition with the endothelium (Fig. 13-4).
- ❖ The apical aspect of these cells is in contact with neighboring HPCs, which are joined by tight junctions. Here a channel, or **canaliculus**, is formed into which bile is secreted by HPCs (Fig. 13-3); this bile ultimately flows into bile ducts and from there into the small intestine.

- HPCs perform most of the many critical metabolic functions of the liver (Table 13-1). They process dietary carbohydrates by converting monosaccharides into energy through glycolysis and mitochondrial metabolism.
- HPCs are also involved in anabolic processes, such as the synthesis of glycogen for carbohydrate storage.
- They respond to energy needs of other organs by releasing stored glucose into the circulation. Similarly, HPCs are a central hub for the regulation of lipids. They are capable of synthesizing lipids and removing them from the blood. HPCs can then process these lipids for energy production or for export into the circulation as lipoproteins for use by other organs.
- HPCs are also the source of many circulating proteins, such as plasma albumin and other binding proteins, coagulation factors, and complement proteins. In addition, HPCs detoxify circulating substances produced during metabolism by other organs.
- For example, ammonia is a toxic product of protein catabolism, and HPCs detoxify it by forming urea, which is eliminated by the kidneys. They also synthesize heme and are involved with the reutilization of iron.

- Additionally, HPCs synthesize bile acid and that aid in the absorption and processing of dietary lipids.

- Another important function of these cells is clearing the circulation of many endogenous, biologically active substances (e.g., hormones, neurotransmitters, steroids) as well as xenobioticagents such as drugs, environmental toxicants, and dietary toxins.

- This is accomplished primarily by oxidative and conjugative metabolism of such substances following uptake from plasma into the cells, sometimes by active transport processes.

Table 13-1

General Functions of Liver

- Processing of foods
  - Monosaccharides → glycogen or energy
  - Gluconeogenesis
  - Lipids → processing, energy
- Synthesis of circulating lipids
- Uptake of dietary lipids (e.g., cholesterol) and vitamins from blood
- Degradation of cholesterol and steroids
- Protein synthesis—for intrinsic and extrinsic proteins (e.g., albumin, coagulation, and complement factors, lipoproteins)
- Ammonia detoxification (urea formation)
- Heme synthesis
- Elimination of bilirubin
- Iron reutilization
- Xenobiotic metabolism (drugs, food-borne agents, etc.)
- Excretion via biliary tract (drugs, metals, etc.)
- Elimination of particulates and bacterial products from blood

- **HPCs** contain **large concentrations of phase I enzymes**, which typically produce metabolites that are more polar and easily excreted by the kidneys than the parent compound.

- They also have a wide variety of **phase II enzymes** that conjugate chemicals or their metabolites with endogenous molecules.

- Phase II metabolism typically reduces toxicity and markedly increases polarity, which facilitates excretion by biliary and renal routes.

- Some of the **HPC functions render the liver a target organ for many toxicants**. For example, metabolism of some xenobiotic agents by HPCs can result in toxic metabolites that directly injure these cells (examples with acetaminophen and other).
- In addition to HPCs, the liver contains other cell types that **perform important physiological functions but that also can participate in pathogenesis following exposure to toxicants**.

- It follows that **damage to HPCs** and other liver cells from chemical exposure can **lead not only to liver dysfunction but to dysfunction of other tissues (e.g., hepatic encephalopathy, coagulopathies**.

❑Sinusoidal Endothelial Cells:

- Sinusoids are lined by thin, discontinuous endothelial cells with numerous fenestrae (or pores; Fig. 13-5) that allow molecules smaller than 250 kDa to cross the interstitial space (known as the space of Disse) between the endothelium and HPCs.

- In the space of Disse, sinusoidal endothelial cells (SECs) are separated from the HPCs by a basement membrane-like matrix, which is attenuated relative to basement membranes in other tissues . However, this subendothelial extracellular matrix is important for the normal function of all resident liver cells

- **The numerous fenestrae** and the attenuated basement membrane facilitate exchange of fluids and molecules, such as albumin, between the sinusoid and HPCs, but hinder movement of particles larger than chylomicron remnants.

- **Endothelial cells are important in the scavenging of lipoproteins via the apolipoprotein E receptor and of denatured proteins and advanced glycation end products by scavenger receptors.**

- Hepatic endothelial cells also secrete biologically active molecules such as **cytokines, prostanoids, nitric oxide, and endothelins** and express intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on the cell surface. These SEC-derived substances play imporant roles in liver injury and repair.

Kupffer Cells:

- Are the **resident macrophages** of the liver and constitute approximately 80% of the fixed macrophages in the body .
- They are situated within the lumen of the sinusoid, in apposition to SECs and with processes that extend through fenestrae to contact HPCs (Figs. 13-3 and 13-4). A primary function of Kupffer cells is to ingest and degrade particulate matter (e.g., bacteria).
- Also, Kupffer cells are important players in immune responses; as such, **they are a major source of cytokines, eicosanoids, and reactive oxygen species (ROS) and can act as antigen-presenting cells**.

Hepatic Stellate Cells:

- Hepatic stellate cells **(HSCs)** are located between endothelial cells and HPCs (Figs. 13-3 and 13-4).
- They are also known as **Ito cells** or by the more descriptive term of **fat-storing cells**. Stellate cells are the **major sites for vitamin A storage in the body** .Indeed, early responses to high-dose vitamin **A therapy are HSC engorgement, activation, increase in number, and protrusion into the sinusoid.**
- These cells express **smooth muscle actin(α-SMA)** ; they are contractile and appear to control local flow of blood in the sinusoids.
- When activated, especially during chronic injury to the liver, stellate cells can assume a **myofibroblastic phenotype**, **synthesizing and secreting collagen** and other extracellular matrix proteins and thereby can initiate liver fibrosis.

Other Immune Cells of Liver Sinusoids:

- In addition to resident liver macrophages (Kupffer cells), the liver is **home to a population of immune cells of diverse lineage and function**.
- Specific immune cell content and organization within the **liver sinusoid makes the liver a true immune organ**, capable of mounting both innate and adaptive immune responses both under normal conditions and during disease .
- Immune cells contribute to liver homeostasis and immunity. Thus, these cells may become engaged in the progression of liver damage driven by inflammation and/or become activated in response to injury-associated changes, including inflammatory stimuli .

- An important role of various immune cells is to mount a specific response to injury or disease. For example, *dendritic cells* (DCs; Fig. 13-3) and ***plasmacytoid DCs*** **play a key role in antigen processing and in presentation of antigens to other immune cells along with production of inflammatory cytokines, such as interferons .**
- Notably, although the cell surface protein CD11c is often considered a DC marker, an increasing body of knowledge of DC and macrophage heterogeneity suggests more comprehensive cell surface marker analysis is required to identify DCs definitively.
- Interestingly, **DCs may play a protective role in certain experimental settings, including in resolution of fibrosis and in acetaminophen hepatotoxicity** .

❑ Natural Killer (NK) Cells and Natural Killer T (NKT) Cells:

- These are **subsets of lymphocytes** that accumulate in liver in response to injury and inflammatory challenges; they **produce a variety of cytokines** that modify the activity of several other cell types in the liver.

- Both NK and NKT cells can display quite **dichotomous (dual) functions** in various disease or injury settings.

- Invariant NKT cells have been shown to drive alcohol-induced liver injury in rodent model and several studies showed that **NK cells have both inflammatory and anti-fibrotic activity**.

- Although some studies note a damaging role for NK and NKT cells in acetaminophen hepatotoxicity, other studies have challenged this finding .
- Indeed, other studies indicate that these same cell types play a protective role; differences in these results might **relate to differences in the solvents used for acetaminophen**.

- A set of recently described resident liver immune cells are new members of the **innate lymphoid cell (ILC)** family.
- Although functional **overlap is observed between subtypes of ILCs and T lymphocytes**, the stimuli required to activate and engage ILCs in local inflammatory responses are different. **ILCs do not respond to specific antigens but rather lie waiting in the liver (and other tissues) to receive a variety of signals cued up by local inflammation or injury .**
- For example, IL-33 has been identified as an inflammatory cue (q) released by injured HPCs that drives **ILC activation and subsequent liver fibrosis** .

Critical Factors in Toxicant-Induced Liver Injury

Why is the **liver the target site for many chemicals of diverse structure**? Why do many hepatotoxicants preferentially damage one type
of liver cell?:

- **Location and specialized processes for uptake and biliary secretion produce higher exposure levels in the liver than in other tissues of the body, and strikingly high levels within certain types of liver cells.**
- **Then, the abundant capacity for bioactivation reactions influences the rate of exposure to proximate toxicants.**
- **Subsequent events in the pathogenesis appear to be critically influenced by responses of sinusoidal cells and the immune system.**

Table 13-3

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

Examples of Hepatotoxicants and their Mechanisms of Action

- **Acetaminophen:**
  One of the most widely used analgesics, acetaminophen (*N*-acetyl*p*-aminophenol; **APAP**) is a safe drug when used at therapeutically recommended doses.
- However, an overdose can cause severe liver injury and even liver failure in experimental animals and in humans
- About half of all overdose cases are caused by suicide attempts, but an increasing number of cases are reported with unintentional overdosing (.Although the toxicity is rare relative to the millions of patients taking the drug daily, APAP mediated liver injury represents a significant clinical problem.
- During the last two decades, APAP-induced hepatotoxicity became **the most frequent cause of acute**, drug-induced liver failure in the UK.

- APAP poisoning results in **centrilobular hepatocellular necrosis** (Fig. 13-12) .
- At therapeutic doses, approximately 90% of APAP is conjugated with sulfate or glucuronide and excreted. This limits formation by CYPs of a reactive, toxic metabolite, *N*-acetyl*p*-benzoquinone imine (NAPQI).
- Most of the NAPQI is detoxified by conjugation with glutathione (GSH), thereby limiting its covalent binding to cellular proteins, which is the initiating event for HPC damage.
- In addition, the low levels of protein adducts formed after therapeutic doses are removed by autophagy . Thus, therapeutic doses of APAP pose minimal risk for liver injury.
- Consistent with these findings, long-term studies with APAP in osteoarthritis patients did not reveal evidence of liver dysfunction or **cell injury even in patients consuming the maximal recommended daily dose of APAP for 12 months**.

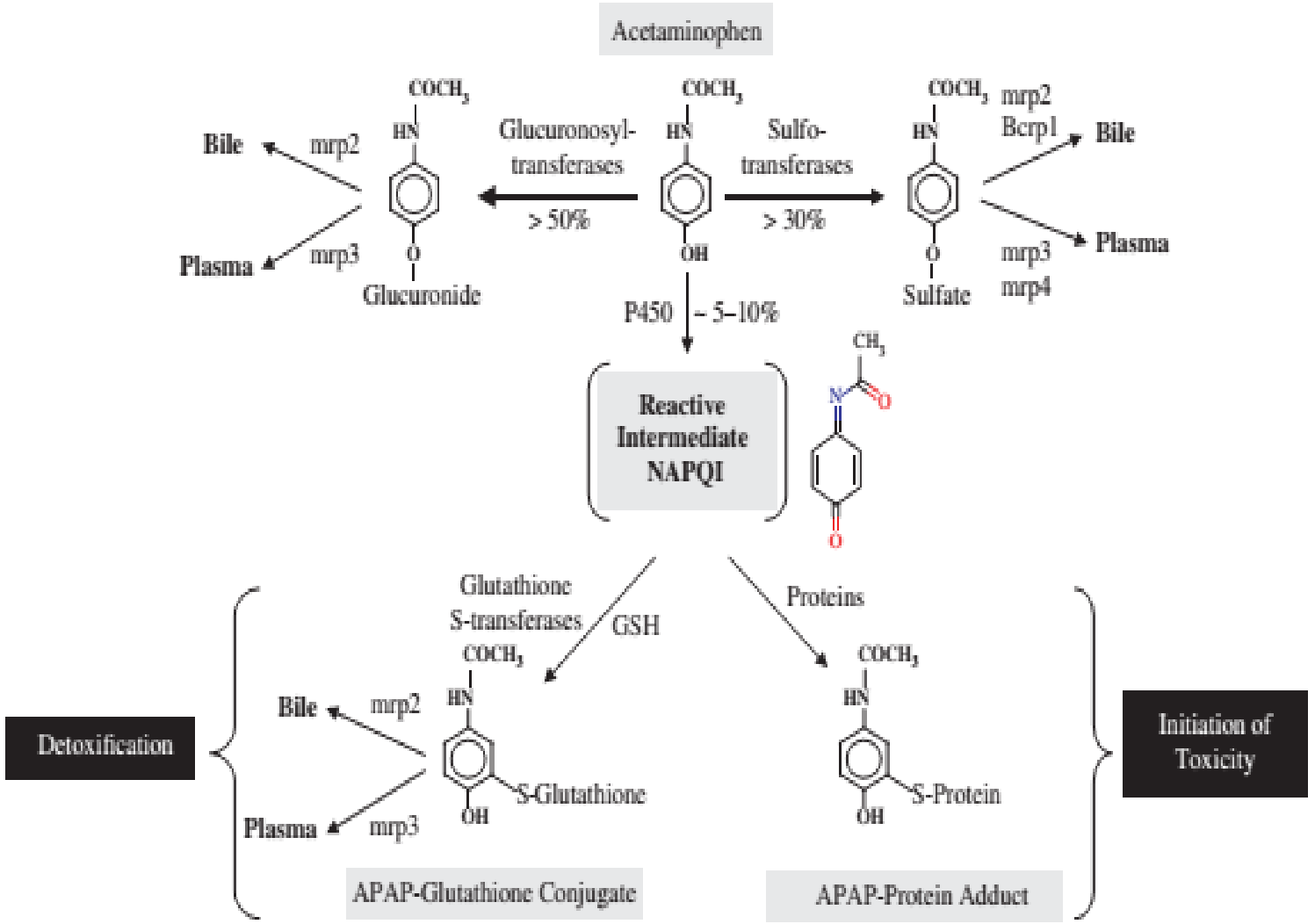


Figure 13-13. *Hepatic metabolism of acetaminophen.*

- In contrast, after an overdose, overwhelmed sulfate and glucuronide conjugation pathways lead to break-through formation of large amounts of NAPQI, resulting in severe depletion of cellular GSH stores needed for NAPQI inactivation and thereby allowing extensive covalent binding of NAPQI to intracellular proteins.
- The generally greater concentration of bioactivating CYPs combined with the lesser GSH concentration in centrilobular HPCs are the main reasons for the predominantly centrilobular necrosis observed after APAP poisoning.
- Consistent with the critical role of protein binding for cell injury are the findings that APAP protein adducts are located mostly in centrilobular HPCs undergoing necrosis and that no APAP hepatotoxicity is observed without protein binding.
- Because protein binding can be prevented by conjugation of NAPQI with GSH, any manipulation that reduces hepatic GSH levels, for example, fasting or protein malnutrition, enhances the toxicity of APAP.
- **In contrast, interventions such as the supply of cysteine, the rate-limiting amino acid for GSH synthesis, promote the detoxification of NAPQI and limit cell injury .Based on this fundamental insight into the mechanism of APAP hepatotoxicity, *N*-acetylcysteine (NAC) was introduced into the clinic as intervention therapy.**

- This highly successful therapeutic approach, which saved the lives of many patients who consumed an APAP overdose, is still the most effective treatment available .
- More recent evidence indicates that NAC treatment not only promotes cytosolic GSH synthesis to detoxify NAPQI but also replenishes the depleted mitochondrial GSH, which scavenges reactive oxygen and peroxynitrite.
- In addition, excess NAC is degraded and supports mitochondrial ATP generation.

- Mitochondrial protein binding of NAPQI causes several untoward events, including inhibition of mitochondrial respiration, mitochondrial oxidant stress and peroxynitrite formation, and declining ATP concentration in the liver (Fig. 13-14).
- Additional support for the central role of mitochondrial dysfunction comes from the observation that removal of damaged mitochondria by autophagy limits APAP-induced liver injury in vivo and in cultured cells .Autophagy appears to be most relevant in preventing cell death at the periphery of centrilobular lesions .

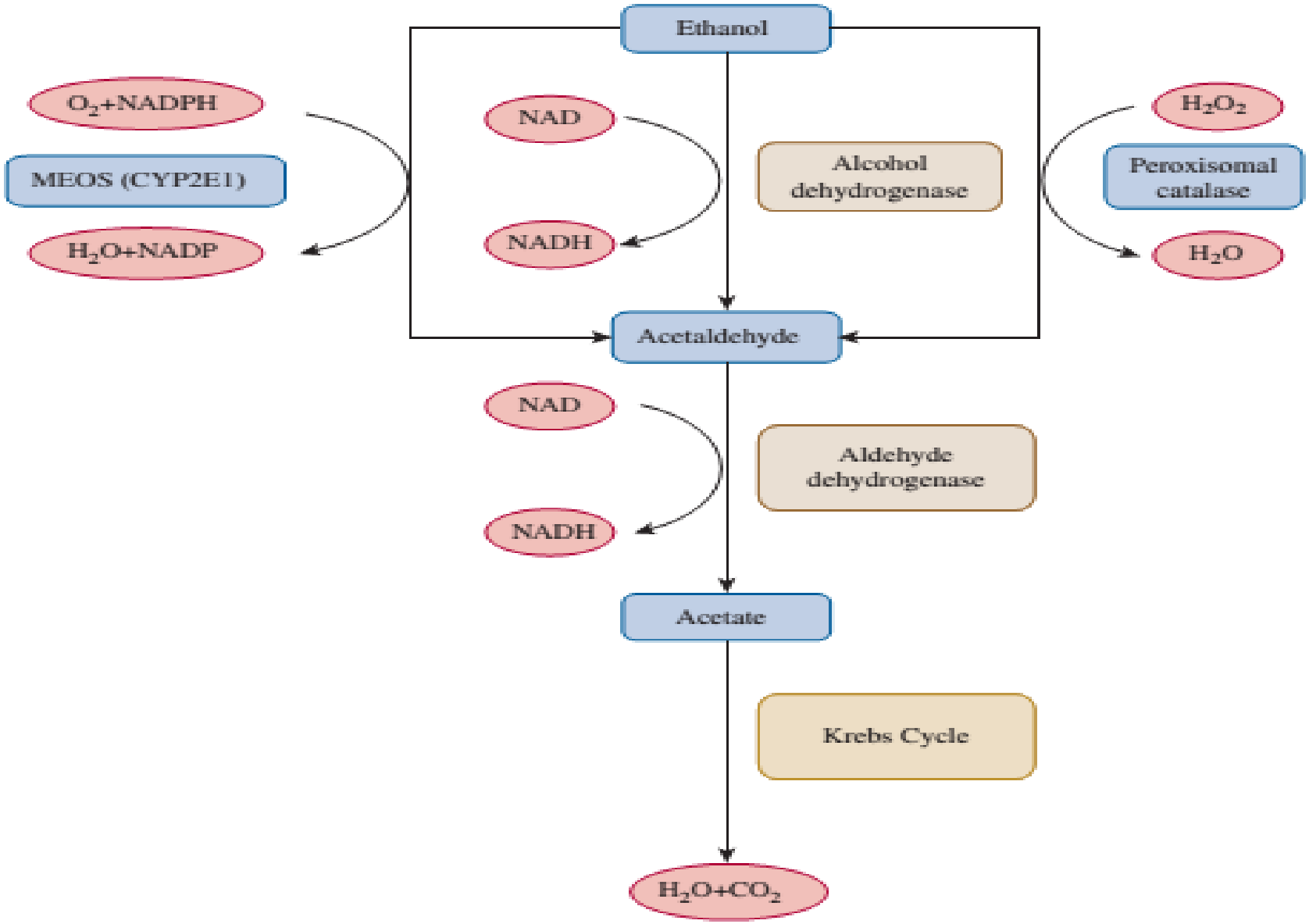
- A significant sensitivity factor in APAP hepatotoxicity can be the consumption of **alcoholic beverages**.
- In addition to potential malnutrition in alcoholic individuals,
- ethanol is a potent inducer of CYP2E1, which is the main enzyme responsible for the metabolic activation of APAP in humans.

- **2- Ethanol**

  Alcohol abuse is among the major causes of liver disease in Western countries .
- The **early stage of ethanol abuse** is associated with hepatic lipid accumulation (steatosis). As alcohol-induced liver disease progresses, appreciable cell death occurs alongside **increasing hepatic inflammation** (i.e., steatohepatitis). If left unchecked, these pathologic processes drive **replacement of functional liver mass with scar tissue**.

- This results in impairment of many functions of liver, including a progressive reduction in capacity for biotransformation of drugs.
- People with hepatic cirrhosis due to chronic alcohol abuse frequently become deficient at detoxifying both the **ammonia formed by catabolism of amino acids and the bilirubin derived from breakdown of hemoglobin**.
- Such hepatic dysfunction combined **with defects in synthesis of key proteins, such as albumin and clotting factors**, **can ultimately drive multiple organ dysfunction and death**. **Thus, ethanol provides a highly relevant example of a toxicant to which exposure contributes significantly to liver-related morbidity**.

- Morbidity and mortality associated with the consumption of alcohol is mainly caused by the toxic effects of ethanol and its metabolites on the liver , although effects of alcohol on other tissues are increasingly appreciated.
- This targeted toxicity is observed because >90% of a dose of ethanol is metabolized in the liver. There are three principal pathways of ethanol **metabolism (Fig. 13-16).**
- Alcohol dehydrogenase **(ADH)** is quantitatively the most important pathway for alcohol metabolism. ADH oxidizes ethanol to acetaldehyde; the electrons are transferred to NAD+, which leads to the production of NADH.
- Acetaldehyde is further oxidized to acetate, also in a NAD+-dependent reaction, by **acetaldehyde dehydrogenase (ALDH)**. This pathway **is mainly limited by the mitochondrial capacity** to utilize NADH and regenerate NAD+



Pathways of ethanol metabolism. See text for abbreviations.

- Both **ADH and ALDH** exhibit genetic polymorphisms and ethnic variations that contribute to risk of alcoholism and ethanol mediated liver damage . One example of a toxicologically important polymorphism involves mitochondrial ALDH2, for which the ALDH2*2 form shows little or no catalytic activity.
- The resultant increased concentration of toxic acetaldehyde present in individuals who carry this polymorphism is thought to cause the uncomfortable **"flushing" syndrome that accompanies ethanol exposure**. This inactive form of ALDH is found in 50% of Asians but is absent in Caucasians.
- The discomfort experienced by people with this polymorphism might contribute the lower incidence of alcoholism in Asia compared to Europe and North America .

- However, heterozygotes of ALDH2*2 were found to develop more severe liver injury in response to lower alcohol consumption, suggesting a higher susceptibility to alcoholic liver disease. Polymorphisms also exist in ADH, some of which could lead to more rapid production of acetaldehyde.
- These findings underscore the importance of acetaldehyde in the pathophysiology of alcohol toxicity

- Although most ethanol is metabolized in the liver by ADH, a second pathway of importance involves the alcohol-inducible enzyme, CYP2E1, which, like ADH, oxidizes ethanol to acetaldehyde (Fig. 13-16).
- This enzyme is located predominantly in HPCs of the centrilobular region and requires both oxygen and NADPH. this reaction is most relevant for large doses of ethanol, and because expression

- The mechanisms of alcohol-induced liver disease are complex and still incompletely understood. **Hepatocellular steatosis is a common feature of chronic alcohol consumption**. **Alcohol-mediated steatosis is caused by the excessive supply of acetate and NADH, which increases fatty acid synthesis.**
- In addition, **both ethanol and acetaldehyde** disrupt constitutive regulation of fatty acid metabolizing enzymes by impairing DNA binding of the transcription factor, peroxisome **proliferator-activated receptor-α (PPAR-α)** .
- In addition to this imbalance favoring increased fatty acid synthesis, ethanol exposure **inhibits the transfer of triglycerides from liver to adipose tissue**.
- Acetaldehyde inhibits the **microsomal triglyceride transfer protein**, which incorporates triglycerides into VLDL and reduces VLDL release from HPCs by interfering with microtubular function . These effects of ethanol and its metabolites can be compounded in the **presence of a high-fat diet**.

- Although steatosis alone is not considered a form of severe liver disease, it represents an early and permissive phase in the spectrum of liver pathologies caused by alcohol exposure .
- In the **two-hit hypothesis** of alcoholic liver disease, steatosis is considered the "**first hit**," which requires a "**second hit**" to drive simple steatosis to the **necroinflammatory change** observed in severe alcoholic liver injury . This should not exclude the possibility that lipotoxicity itself is a critical determinant of disease progression . Moreover, results of recent studies suggest that inflammatory events triggered by ethanol can occur independently of steatosis .

- **CYP2E1** is a source of reactive oxygen formation during ethanol metabolism . The resultant intracellular oxidant stress in HPCs can **ultimately induce mitochondrial dysfunction and cell death of HPCs, but it also activates stellate cells and promotes fibrosis .**
- In addition to the intracellular events, alcohol exposure causes an inflammatory response, which contributes to the oxidant stress.
- **Gut-derived endotoxin** and other bacteria-derived products leaking into the portal circulation from a leaky gut can **activate Kupffer cells through toll-like receptor** activation to produce **reactive oxygen species and cytokines, such as TNF-α** . The formation of these mediators can be amplified by feedback loops, which enhance cytokine and chemokine formation through priming of the redoxsensitive transcription factor, NF-κB, in Kupffer cells .
- In addition, TNF-α can increase expression **of inducible nitric oxide synthase (iNOS, NOS2)** leading to the formation of peroxynitrite, a potent oxidant species capable of nitrating proteins.

- **TNF-α** can also **directly promote cell death by acting on HPCs**, which are rendered sensitive by ethanol-induced depletion of mitochondrial GSH.
- Inhibition of the proteasome pathway, a well-recognized feature of chronic alcohol exposure, can enhance chemokine formation in HPCs and promote inflammatory liver injury .
- Additional pro-inflammatory mediators and immune responses can be triggered by protein adducts of acetaldehyde and by lipid peroxidation products
- as well as by DAMPs released from HPCs and PAMPS (e.g., LPS) translocated from the intestine into the portal circulation (Fig. 13-17).
- Interestingly, **ethanol inhibits hepatic NK cells**. Because NK cells can kill HSCs, the major cell type promoting hepatic fibrosis, ethanol may indirectly support fibrogenesis by preventing the elimination of activated stellate cells . A defense mechanism that is activated during alcoholic liver disease is autophagy, which can remove damaged cell organelles and modified proteins and thereby reduce the activation of the innate immune response.

3- Allyl Alcohol:

- Allyl alcohol is an industrial chemical used in the production of resins, plastics, and fire retardants and has been used as a model hepatotoxicant due to its preferential periportal (zone 1) hepatotoxicity. The alcohol is metabolized by ADH to acrolein, a highly reactive aldehyde (Fig. 13-18), which is then further oxidized by ALDH to acrylic acid.
- The observations that the toxicity depends on depletion of hepatic GSH and is prevented by inhibitors of ADH but enhanced by inhibitors of ALDH suggest that **acrolein** formation is the critical event in liver injury .
- Age and gender differences in allyl alcohol hepatotoxicity can be explained by variations in the balance between ADH and ALDH expression . The occurrence of allyl alcohol injury preferentially in zone 1 HPCs (Fig. 13-18) is caused by the predominant uptake of allyl alcohol in the periportal region and the oxygen dependence of the toxicity .
- Although protein binding of the acrolein and subsequent adduct formation appears to be the main cause of liver cell death , lipid peroxidation can become an important mechanism of cell injury under conditions of a compromised antioxidant status or in the presence of excess iron .
- Lipid peroxidation is caused by a reductive stress during which excessive NADH formation produced by allyl alcohol metabolism leads to mobilization of redox-active iron from storage proteins .

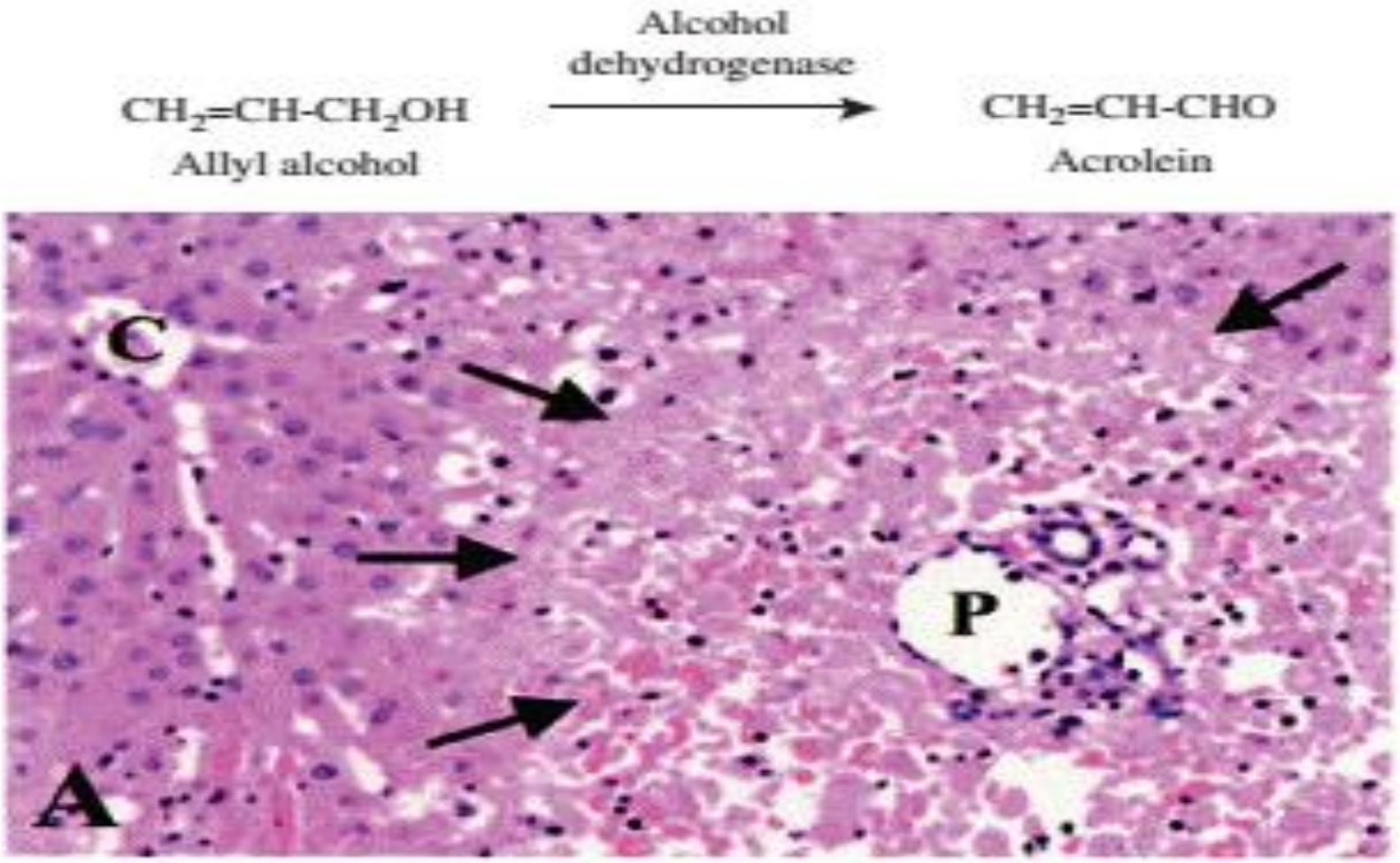


Figure 13-18. *Metabolism and liver injury from allyl alcohol.* Allyl alcohol is bioactivated to acrolein and produces periportal liver injury. Photomicrograph of a rat liver 24 hours after allyl alcohol administration. (From Yin et al. [1999b].)

4- Carbon Tetrachloride:

- CCl4 was once a widely used solvent, but human exposure to it has been restricted due to recognition that it is a potent hepatotoxicant. Acute exposure to CCl4 causes **centrilobular necrosis** (Fig. 13-19).
- It has been used widely to model liver injury in animals, with its current use heavily weighted toward studying fibrosis (Fig. 13-19).
- Cytochrome P450-dependent conversion of CCl 4 to trichloromethyl free radical (•CCl3) and then to the trichloromethyl peroxyl radical (CCl3OO•) is a classic example of xenobiotic bioactivation to a free radical capable of initiating lipid peroxidation by abstracting hydrogen atoms from polyunsaturated fatty acids in phospholipid membranes (Fig. 13-19).
- Metabolic activation of CCl4 in vivo involves primarily CYP2E1 as indicated by the absence CCl 4 hepatotoxicity in CYP2E1 knockout mice .
- CCl4-induced lipid peroxidation increases the permeability of the plasma membrane to Ca2+, leading to severe disturbances in calcium homeostasis and consequent necrotic cell death.
- CCl4 also induces significant mitochondrial damage, which is dependent on CYP2E-mediated metabolism and lipid peroxidation . In addition, the •CCl3 radical can bind directly to tissue macromolecules, as can some of the lipid peroxidation products such as 4-hydroxynonenal, which can form adducts with cellular proteins

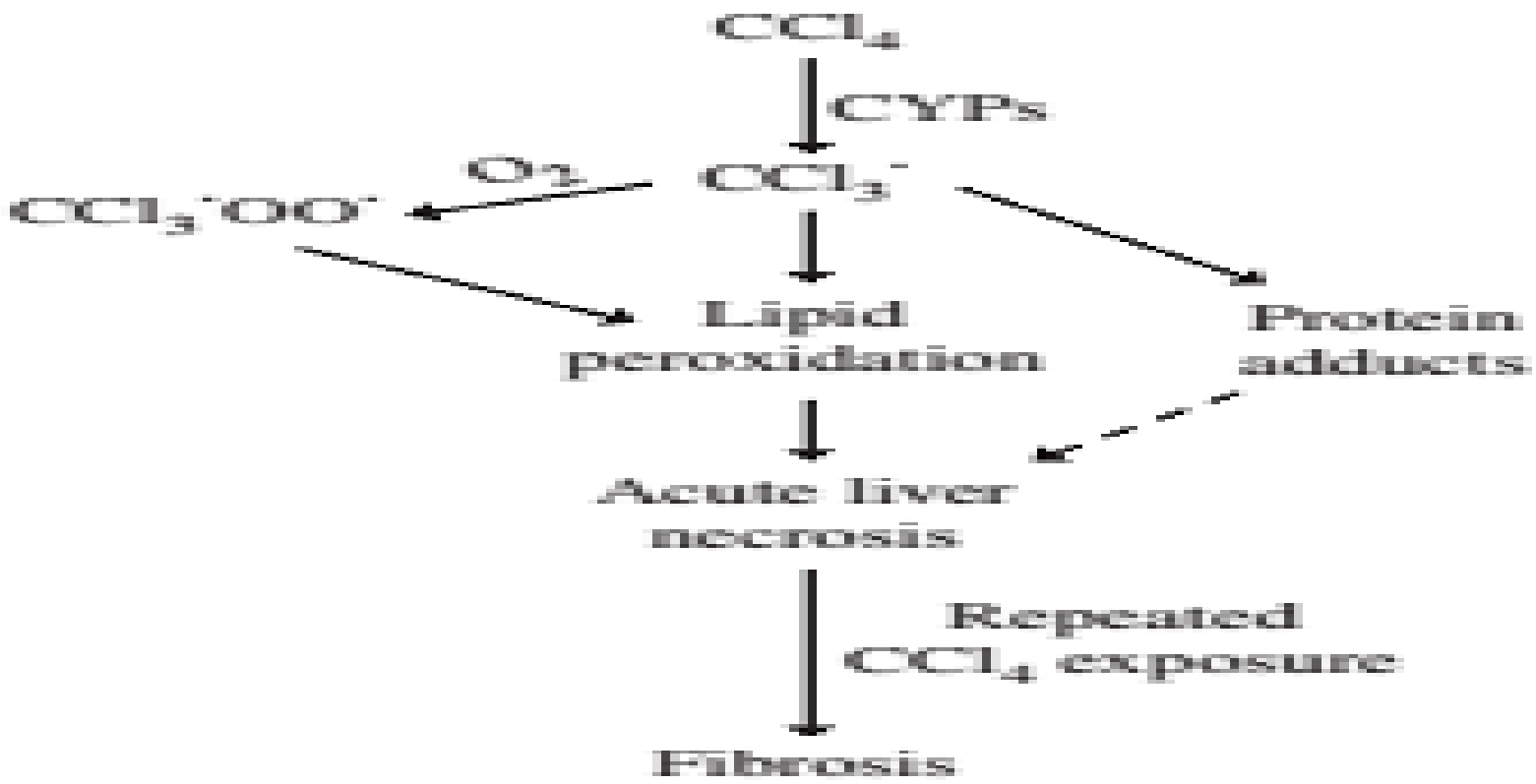
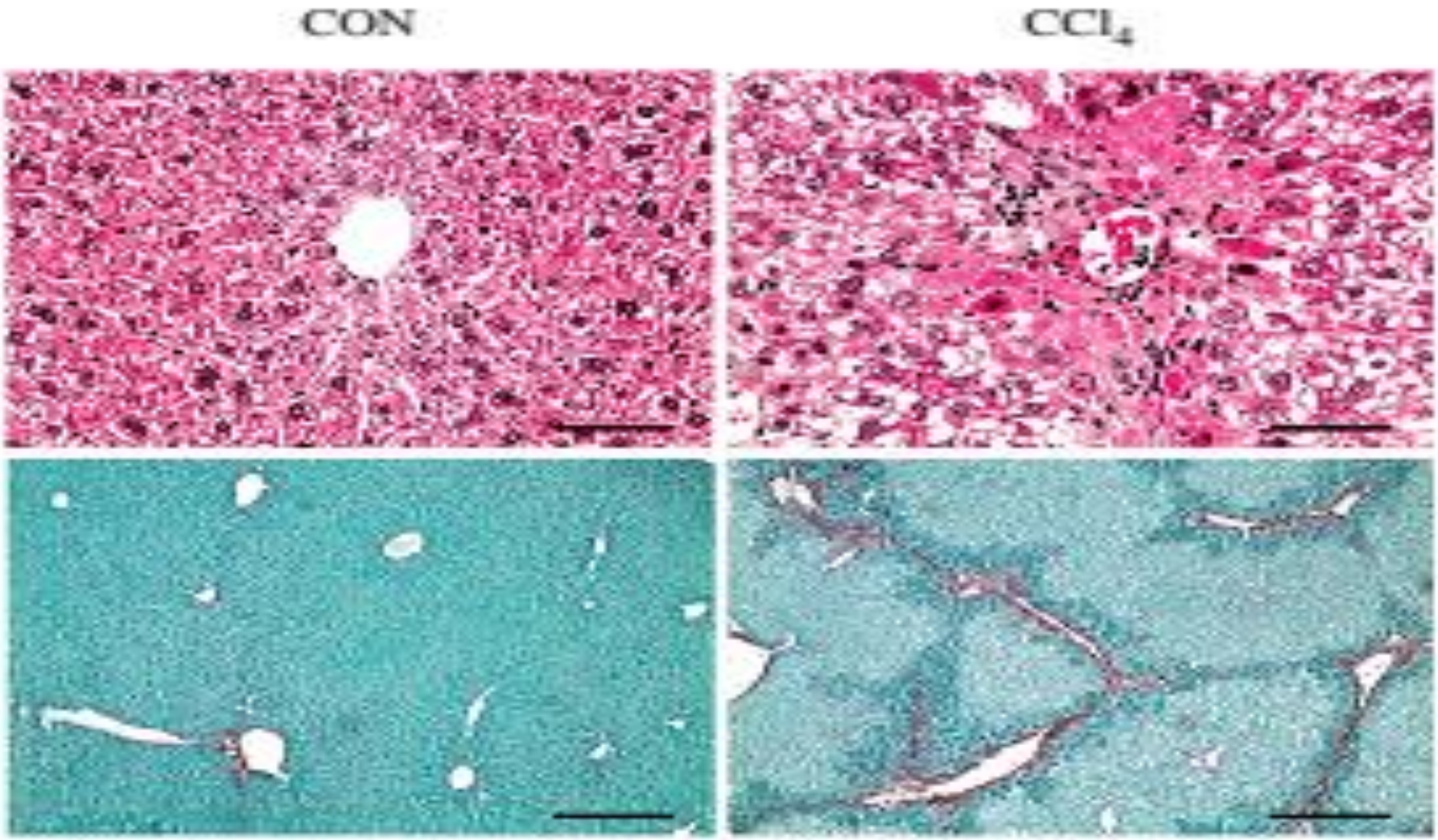


Figure 13-19. ***Liver injury from carbon tetrachloride. Left*****: CCl4 is bioactivated by CYPs to the trichloromethyl free radical and in the presence of oxygen to trichloromethyl peroxyl radical. These radicals initiate lipid peroxidation of cell membranes and consequent hepatocellular necrosis. Protein adducts formed with radicals from CCl 4 and from lipid peroxidation also might contribute to liver injury. Repeated CCl4 exposure leads to fibrosis.**



***Right*: Necrosis and fibrosis in livers of CCl 4-treated mice. Mice were treated twice per week for 10 weeks with CCl4. *Top*: Hematoxylin and eosin staining of livers from control (CON) mice and mice treated with CCl4. In the latter, note the centrilobular hepatocellular necrosis and presence of inflammatory cells within the lesion. *Bottom*: Sirius red staining. Note red staining of excess collagen in septa of lobules in the liver from the CCl4-treated mouse.**

- In addition to the intracellular events, **Kupffer cell activation can contribute to liver injury** . Kupffer cells may enhance the injury by **oxidant stress or TNF-α generation, which can lead to apoptosis** .
- Supporting these different elements of the mechanisms of CCl 4-mediated cell and organ damage, protection from toxicity was conferred by **inhibition of CYPs, preservation of Ca2+ homeostasis, antioxidants, and anoxia .**
- In contrast, treatment with chemicals **such as ethanol that induce CYP2E1 enhance injury**. This was supported in humans by a case report showing greater vulnerability of workers with a history of alcohol abuse to CCl4 vapors compared to similarly exposed, moderately drinking co-workers.

5- aflatoxins:

- Fungi of various species synthesize metabolites known as **mycotoxins** of several types that cause injury to numerous organs.
- Aflatoxins are mycotoxins produced by *Aspergillus* molds that grow on nuts and crops such as corn, wheat, and rice (Fig. 13-20)and consumption of these foods results in exposure to humans and animals .
- Aflatoxins have also been detected in milk of animals that consume contaminated crops, and this can lead to exposure of young children .

- All aflatoxins are metabolized in liver by oxidation, hydrolysis, reduction, and conjugation reactions, some of which lead to toxic products .
- **AFB1** is oxidized by various isoforms of CYPs. Although most of the products lack significant toxicity, oxidation of the furan ring leads to a **reactive epoxide that alkylates DNA** and that can be further metabolized to a **dialdehyde that alkylates proteins** (Fig. 13-20).
- The **DNA adducts are thought to initiate liver cancer**, **whereas protein adducts appear to cause acute hepatotoxicity . Conjugation with GSH is an important pathway for detoxification of the reactive epoxide, which is also hydrolyzed both spontaneously and enzymically by epoxide hydrolases.**
- The relative activities of the bioactivating and detoxifying enzymes appear to be an important determinant of DNA adduct formation and, by extension, to sensitivity to the mutagenic and carcinogenic effects of AFB1.
- In this regard, combinations of polymorphisms in several genes encoding enzymes that metabolize AFB1 are associated with increased risk of developing hepatocellular carcinoma . In addition, there exists a pronounced synergy between AFB exposure and infection with hepatitis B virus in causing hepatocellular carcinoma in humans .

6- Pyrrolizidine Alkaloids:

- Are toxins produced by a wide variety of plant species around the world. They have been responsible for poisoning of livestock grazing on poor pastureland and have intoxicated people who consumed pyrrolizidine alkaloids in herbal teas, contaminated grains, or medicinal remedies .
- Retrorsine, seneciphylline, and monocrotaline are examples of toxic pyrrolizidine alkaloids, the latter being the most well studied.
- **Monocrotaline is taken into HPCs at least in part by OATP1 and is bioactivated by CYPs to monocrotaline pyrrole, which is a bifunctional alkylating agent capable of binding covalently to DNA and proteins to initiate hepatotoxicity.** (Fig. 13-21).
- Acute administration of monocrotaline to rodents results in **centrilobular megalocytosis** (cell enlargement) and death of HPCs, which succumb from both apoptosis and oncotic necrosis (Fig. 13-21). **The ability of monocrotaline pyrrole to cross-link DNA might be responsible for blockade of cell division at, failure to proliferate, and consequent increase in cell size** .
- A prominent feature of pyrrolizidine alkaloid hepatotoxicity is the pronounced destruction of SECs as well as endothelial cells of central venules . **The SEC destruction leads to marked hemorrhage (Fig. 13-21) and is associated with centrilobular tissue hypoxia .**
- Monocrotaline pyrrole is **detoxified primarily by conjugation with GSH** , and it is likely that monocrotaline bioactivation and profound GSH depletion in endothelial cells renders them highly sensitive to injury from monocrotaline

7- Mushroom Toxins:

- There are several toxic, **cyclic peptides that occur in various species of wild mushrooms**. These mushrooms have been mistaken for edible species, and consumption of them has been responsible for numerous poisonings and deaths over the years, especially in Europe but also in North America.
- Of these mushroom species, *Amanita phalloides* ("death cap") has been of greatest concern due to its ability to cause life-threatening toxicity (Fig. 13-23). This mushroom produces several bicyclic heptapeptides known as phallotoxins and octapeptides known as amatoxins. Of these, **phalloidin and α-amanitin** ,respectively, have received the most attention as hepatotoxin.

- **Phalloidin** is taken into HPCs by transporters of the OATP family (and causes **cholestasis and hemorrhagic necrosis in livers after intraperitoneal administration to rodents** .
- Although phalloidin is a potent hepatotoxin, it is **poorly absorbed after oral administration and might therefore contribute minimally to mushroom poisoning in humans** .
- Tight binding of phalloidin **to actin filaments in HPCs prevents** the **disassembly phase** of the normally dynamic rearrangement of the **actin filament** constituent of the cytoskeleton.
- This leads to striking alterations in the actin-rich web of cytoskeleton adjacent to the **canalicular membrane of HPCs**; the actin web becomes accentuated and the canalicular lumen dilates .In isolated HPCs, phalloidin increases **intracellular free calcium, which can lead to alterations in hepatocellular homeostasis**

- Unlike phalloidin, α-amanitin is absorbed from the gastrointestinal tract and is rapidly eliminated in the urine . As with other cyclic peptides, α-amanitin enters HPCs via OATPs, especially OATP1B3, on the basal membrane . **Centrilobular hepatocellular necrosis** and hemorrhage accompanied by a **hepatorenal syndrome** characterizes severe poisoning in humans, and the latter portends a poor prognosis for survival .
- The lethal dose in humans has been estimated to be 0.1 mg/kg, an amount that can occur in one night cap mushroom .
- A primary mechanism of toxicity of α-amanitin derives from its ability to bind to nuclear RNA polymerase II, thereby inhibiting RNA elongation with resultant impaired protein synthesis and cell death .
- presented evidence that enhanced TNF expression occurs in mice treated with α-amanitin and is involved in the progression of injury. Some evidence suggests that oxidative stress is also involved, perhaps arising from free radical formation in α-amanitin itself.

- **8- Metals**

  Metals are excreted into bile by a series of processes that include (1) uptake across sinusoidal membranes of HPCs by membrane transporters or receptor-mediated endocytosis; (2) storage in binding proteins or lysosomes; and (3) canalicular secretion via lysosomes, a GSH-coupled event, or by specific canalicular membrane transport, for example via MRP2 .
- Biliary excretion is important in the homeostasis of several metals, notably copper, manganese, cadmium, selenium, gold, silver, and arsenic
- Species differences are known for biliary excretion of several toxic metals; for example, dogs excrete arsenic into bile much more slowly than rats.

- Inability to export *copper* into bile is a central problem in Wilson's disease, **an autosomal recessive inherited disorder characterized by a defect in or the absence of a copper transporting P-type ATPase (ATP7B)**.
- This carrier is located in the trans-Golgi network within HPCs and transports copper into the secretory pathway for binding to ceruloplasmin and then excretion into bile .Because biliary excretion is the only way to eliminate copper, a defect in ATP7B results in excessive copper accumulation in HPCs, which causes chronic hepatitis and cirrhosis .
- Copper toxicity involves production of reactive oxygen species that initiate liver damage characterized by **centrilobular necrosis and cholestasis**. However, activation of Kupffer cells by copper exposure and a consequent inflammatory response contribute to liver injury.


THANK YOU
FOR YOUR
ATTENTION