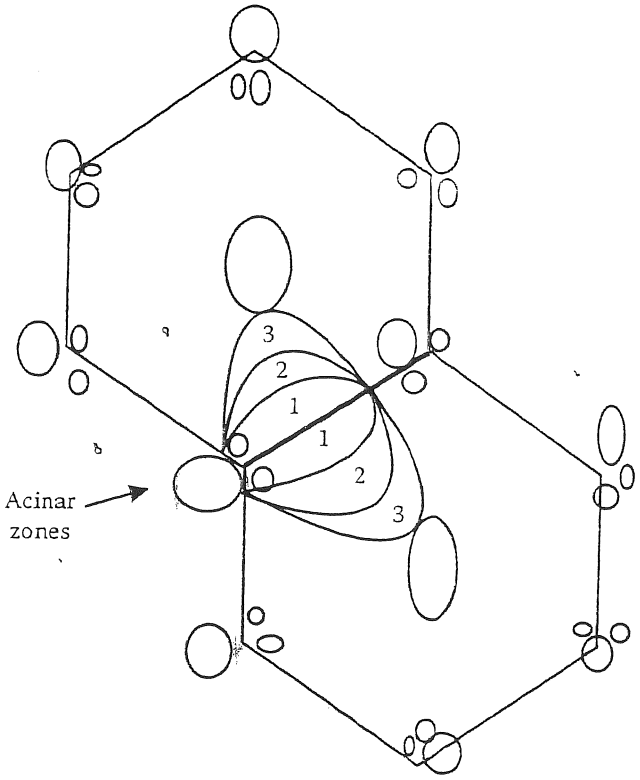

FIGURE 11.3
The hepatic acinus and surrounding zones.

vessels. Cells in *zone 1* show a high activity of enzymes involved in respiration; zone 1 also seems to be the site where regeneration and replacement of liver cells begins (the new cells then migrate outward through zones 2 and 3). Cells in *zone 3*, on the other hand, show high cytochrome P450 activity. An alternative descriptive scheme (based on the earlier lobule model) describes cells as being *centrilobular* (near the central vein, roughly corresponding to zone 3), *periportal* (near the portal area, corresponding to parts of zones 1, 2, and 3), or *midzonal* (along the edge between lobules, corresponding to the remainder of zone 1).

Function of the Liver

The liver is an organ with an important role in many metabolic processes, as well as being a critical organ in toxicology. One main function of the liver is, of course, to assist in the absorption, metabolism, and storage of nutrients. Nutrient-containing blood from the gastrointestinal tract travels first to the liver (via the portal vein), where carbohydrates, lipids, and vitamins are removed. When blood glucose levels rise (following a meal, for example), hepatocytes are capable of converting sugars, fats, and amino acids into

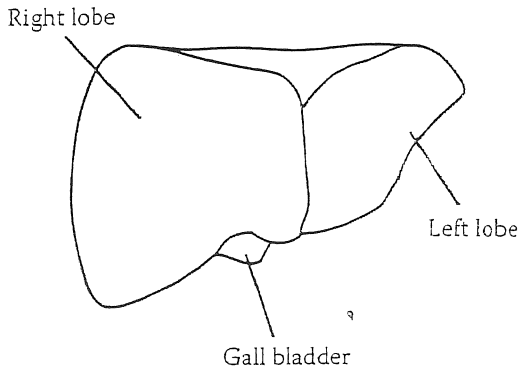

FIGURE 11.1
The anatomy of the liver.

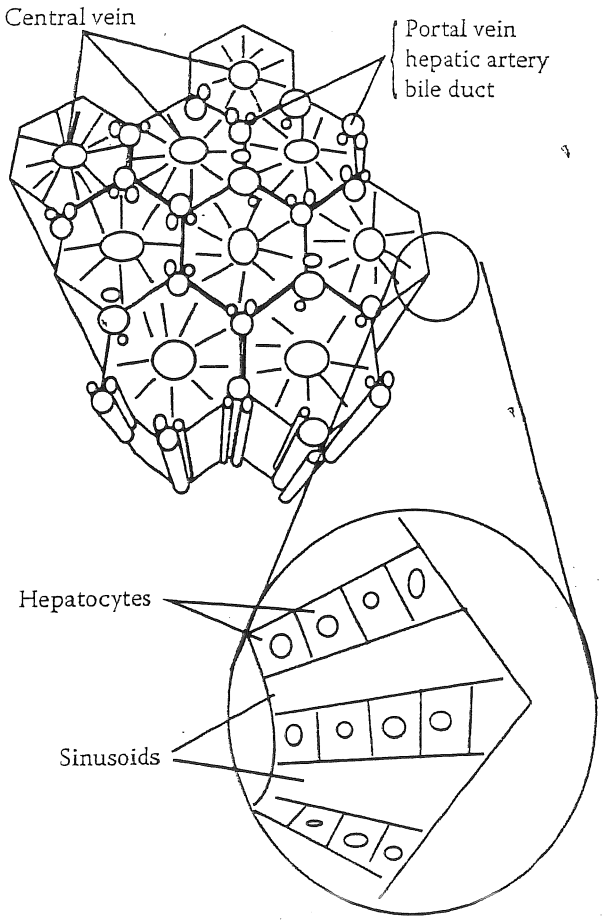

FIGURE 11.2
The hepatic lobule, showing the arrangement of central vein, portal triads, hepatocytes, and sinusoids.

glucose and then storing glucose in the form of the polysaccharide glycogen. Alternatively, excess glucose can also be converted to *triglycerides*. Conversely, when blood glucose levels fall, hepatocytes break down glycogen to release glucose into the bloodstream.

Hepatocytes also play a critical role in protein metabolism by modifying and breaking down amino acids, converting the amine group into ammonia and then into urea for elimination. The amine group can also be used to synthesize other amino acids and used in protein synthesis (such as in making the protein component of lipoproteins).

The liver is particularly important in lipid metabolism. Hepatocytes synthesize and secrete a substance called *bile*, which contains water, ions, cholesterol derivatives known as *bile acids*, or *bile salts*, and *bile pigments* such as *bilirubin*, which is released when hemoglobin is broken down (the liver is important in maintenance of proper blood volume and composition, storing blood, and phagocytizing damaged red blood cells). Bile is stored in the gall bladder and secreted into the small intestine, where it plays an important role in aiding in digestion and absorption lipids (about 80% of the bile acids are reabsorbed by the small intestine), as well as excretion of bile pigments and other wastes (which are not reabsorbed). Hepatocytes can break down, synthesize, and store fats, and can package lipids together with proteins to form the lipoproteins that transport lipids through the bloodstream to other cells.

Lipid Peroxidation
See also:
Cellular sites of action *Ch. 4, p. 65*
Hepatotoxicity *Ch. 11, p. 225*

Excess levels of bilirubin may arise in the case of either increased production (such as what might happen with large-scale destruction of blood cells) or impaired excretion (due to hepatic dysfunction). This will cause *jaundice*, a yellow discoloration of the skin that is particularly common in newborns, due to a rate of blood cell turnover that is much higher than in adults. Newborn jaundice is generally mild and can be treated by *phototherapy*, which consists of exposing the infant to bright lights (in the blue wavelengths), which will convert bilirubin into a form that is more easily excreted. Severe cases can be treated with transfusion. Left untreated, high levels of bilirubin can be neurotoxic to infants, resulting in *kernicterus*, which is characterized by brain damage to the basal ganglia. Recent evidence, however, has also argued for a protective effect of low levels of bilirubin, which can act as an antioxidant to protect cells against free radicals.

Xenobiotic Metabolism
See also:
Biotransformation *Ch. 3, p. 27*

The role of the liver in xenobiotic metabolism and excretion of toxicants is discussed in detail in Chapter 3, so only the basics will be reviewed here. Many hepatocytes contain enzyme systems capable of chemically altering toxic compounds, usually to a less toxic form. There are two basic types of metabolic alterations that can occur, and these are usually referred to as *phase I* and *phase II* reactions.

Phase I reactions involve oxidation or hydrolysis (or sometimes even reduction) of the compound and are carried out by an enzyme system called the *cytochrome P450 system* or by various *hydrolases*. The cytochrome P450 system involves a number of different enzymes, including multiple forms of cytochrome P450 itself. Some of these forms are involved in metabolism of steroids and other endogenous compounds, whereas others metabolize xenobiotics. Two of the major groups of P450 enzymes involved in xenobiotic metabolism are a group of enzymes that is inducible by phenobarbital and a group that is commonly referred to as cytochrome P448 and that is inducible by polycyclic aromatic hydrocarbons (PAHs).

Phase II reactions involve conjugation of the toxicant (or often a metabolite resulting from a phase I reaction) with some other molecule. Generally, this action increases the size and water solubility of the toxicant, leading to enhanced excretion.

Types of Toxicant-Induced Liver Injury

The liver is vulnerable to toxicant-induced injury on several counts. As a site where significant xenobiotic metabolism occurs, liver cells are at risk for exposure to the toxic bioactivated metabolites that result from the metabolism of some toxicants. The direct routing of blood to the liver from the gastrointestinal tract from which ingested xenobiotics are absorbed, as well as the tendency for some compounds to undergo *enterohepatic cycling* (repeated reabsorption from bile and return to the liver), also increases the vulnerability of liver cells to assault from toxicants. Finally, the multiple functional roles of the liver offer multiple potential targets for toxicants. This chapter will focus on the various ways in which toxicants can interact with the liver to produce injury.

Fatty Liver

Since the liver is the site of synthesis, storage, and release of lipids, it stands to reason that interference with these processes could lead to an accumulation of fats in the liver itself. Acute exposure to compounds such as *carbon tetrachloride, ethionine,* and *tetracycline* (an antibiotic) or chronic exposure to *ethanol* can block the secretion of a type of lipid called *triglycerides,* leading to the development of *hepatic steatosis,* or what is most commonly called a *fatty liver.* In this condition,

Carbon Tetrachloride

See also:

Biotransformation *Ch. 3, p. 41*
Cardiovascular toxicology *Ch. 9, p. 167*
Neurotoxicology *Ch. 10, p. 211*
Hepatotoxicology *Ch. 11, pp. 225, 228*
Renal toxicology *Ch. 12, p. 241*
Halogenated hydrocarbons *Appendix, p. 341*

which affects around 30 million individuals in the U.S. alone, anywhere from 5 to 50% of the liver's weight is fat.

Ethanol
See also:
Reproductive toxicology and teratology *Ch. 7, p. 135*
Cardiovascular toxicology *Ch. 9, p. 168*
Neurotoxicology *Ch. 10, p. 211*
Hepatotoxicology *Ch. 11, pp. 226, 227*
Forensic toxicology *Ch. 16, p. 297*
Ethanol *Appendix, p. 340*

Knockout Mice
See also:
Genomics *Ch. 5, p. 76*

Cytochrome P450
See also:
Biotransformation *Ch. 3, p. 33*

The mechanisms by which fatty liver is produced are not completely clear, but most likely involve interference with the normal regulation of lipoprotein synthesis. In this process, the liver takes free fatty acids and, by combining them with glycerol, synthesizes triglycerides. (The chemical reaction of an acid with an alcohol like glycerol to produce an ester plus water is called *esterification*.) These triglycerides are then combined with phospholipids, cholesterol, and proteins to form *very low density lipoproteins* (VLDLs). VLDLs then enter the bloodstream, carrying triglycerides to other cells.

There are a number of ways in which toxicants may produce alterations in this process. For example, there is some experimental evidence that exposure to ethanol leads to an increase in triglyceride synthesis. One possible mechanism for this effect would be through interaction with regulatory proteins such as *sterol regulatory element-binding protein-1c* (SREBP-1c). This protein, along with other transcription factors (such as *carbohydrate response element-binding protein* (ChREBP) and *PPAR-γ*), regulates synthesis of triglycerides by activating genes that code for the enzymes in those synthetic pathways. This same regulatory protein also blocks oxidation of fatty acids by mitochondria, thus potentially increasing lipid levels through two mechanisms. The use of knockout mice to study the role of these proteins in the development of fatty liver has been particularly useful.

Induction of one form of cytochrome P450, CYP2E1, has also been implicated in the pathogenesis of fatty liver. CYP2E1 is induced following exposure to ethanol and is also upregulated in obesity and diabetes, perhaps as a result of the increased triglyceride levels that are also associated with those conditions. Increased levels of the CYP2E1 enzyme may potentially lead to an increase in production of free radicals and other potentially reactive metabolites that might contribute to lipid peroxidation and membrane damage. Damage to endoplasmic reticulum could then lead to inhibition of protein synthesis, and thus VLDL synthesis. Other evidence has indicated that inhibition of release of the lipoprotein may also be a factor.

Finally, inflammation may also play a role in fatty liver. The cytokine *tumor necrosis factor alpha* (TNF-α), which is involved in the inflammatory response,

can trigger release of oxygen radicals from mitochondria, as well as promote apoptosis. Evidence indicates that increase in TNF-α activity is associated with fatty liver, and that blockade of TNF-α activity may be effective in treating patients with the condition.

Interestingly enough, the presence of the excess fat in the liver does not necessarily affect the functioning of the hepatocytes. Steatosis may, however, in some cases progress to cirrhosis (see later in this chapter) and more serious problems.

Liver Cell Death: Necrosis and Apoptosis

A number of compounds have been reported to cause hepatic *necrosis*, or cell death. Necrosis of hepatocytes is characterized by accumulation of vacuoles in the cytoplasm, damage to endoplasmic reticulum, swelling of mitochondria, destruction of the nucleus, and disruption of the plasma membrane. Necrosis is often described as being *focal* (confined to a limited area), *zonal* (occurring in a particular zone, usually zone 3), *diffuse* (scattered throughout the liver), or *massive*, and its location is frequently described using the descriptive terms (introduced earlier) centrilobular, midzonal, or periportal.

One possible cause of hepatic necrosis is *lipid peroxidation*. Compounds such as *carbon tetrachloride*, *chloroform*, *bromobenzene*, and other *halogenated hydrocarbons* are metabolized by cytochrome P450 to form free radicals, reactive metabolites that can bind to and damage macromolecules. Unsaturated fatty acids in membranes are particularly vulnerable to attack by free radicals. Carbon tetrachloride exposure has been shown to produce damage to hepatocyte membranes, including smooth and rough endoplasmic reticulum, thus reducing xenobiotic-metabolizing ability as well as reducing protein synthesis. In fact, a small initial dose of carbon tetrachloride protects against injury from a later larger dose, probably by destroying P450 and limiting the ability of the liver to bioactivate the later dose. Further evidence that carbon tetrachloride produces lipid peroxidation is found in the increased production of molecules called *conjugated dienes*, which are fre-

Necrosis
See also:
Cellular sites of action *Ch. 4, p. 68*

Lipid Peroxidation
See also:
Lipid peroxidation *Ch. 4, p. 65*
Hepatotoxicity *Ch. 11, p. 222*

Carbon Tetrachloride
See also:
Biotransformation *Ch. 3, p. 41*
Cardiovascular toxicology *Ch. 9, p. 167*
Neurotoxicology *Ch. 10, p. 211*
Hepatotoxicology *Ch. 11, p. 228*
Renal toxicology *Ch. 12, p. 241*
Halogenated hydrocarbons *Appendix, p. 341*

quently used to monitor the occurrence of lipid peroxidation. Administration of *antioxidants* (to reduce or prevent lipid peroxidation) prevented some but not all toxic effects of carbon tetrachloride, indicating that even though lipid peroxidation is a factor, other mechanisms are probably also involved. Endogenous enzymes such as *superoxide dismutase* may also play a role in limiting peroxidation *in vivo*.

Acetaminophen
See also:
Biotransformation *Ch. 3, p. 44*
Acetaminophen *Appendix, p. 335*

Another potential cause of hepatic necrosis is the production of other types of reactive metabolites. For example, *acetaminophen*, a common over-the-counter analgesic, is metabolized primarily by the CYP2E1 isoform of cytochrome P450 to the active metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI). This metabolite is highly electrophilic and capable of binding to and damaging cellular macromolecules. Acetaminophen has also been shown to stimulate formation of *nitric oxide* in hepatocytes. Nitric oxide, which is also produced by liver cells in response to inflammatory signals, can actually block apoptosis at low levels by inducing stress proteins, promoting the synthesis of cGMP and inhibiting caspase activity. However, at higher concentrations NO may contribute to damage, combining with superoxides to form reactive species such as *peroxynitrite*. In the case of acetaminophen, a combination of glutathione depletion and peroxynitrite production may lead to hepatotoxicity.

Because these toxicants depend on bioactivation to produce their effects, the necrosis that they produce tends to be centrilobular (located in zone 3). This area, as you may recall, is where the greatest P450 activity is located. Also, the toxicity of these compounds can be potentiated by compounds that induce cytochrome P450 activity. Ethanol, for example, potentiates the effects of carbon tetrachloride and other halogenated hydrocarbons, probably through effects on P450.

Phase II Reactions
See also:
Biotransformation *Ch. 3, p. 41*

The ability of the liver to perform phase II reactions is also a significant factor in determining the toxicity of these compounds. For example, many of the reactive metabolites produced in phase I undergo binding to *glutathione* and other phase II cofactors, thus limiting binding to cellular sites. Therefore, competition of other toxicants for binding to these cofactors, or dietary depletion of cofactors may potentiate the toxicity of these reactive metabolites.

Ethanol
See also:
Reproductive toxicology and teratology *Ch. 7, p. 135*
Cardiovascular toxicology *Ch. 9, p. 168*
Neurotoxicology *Ch. 10, p. 211*
Hepatotoxicology *Ch. 11, pp. 224, 227*
Forensic toxicology *Ch. 16, p. 297*
Ethanol *Appendix, p. 340*

Hepatotoxic metabolites can also be produced through enzyme systems other than P450. Ethanol, for example, is metabolized to an aldehyde by *alcohol dehydrogenase*, and then on to acetate by *aldehyde dehydrogenase*. Some individuals possess a slow aldehyde dehydrogenase isoform, resulting in a buildup of acetaldehyde following ethanol exposure and resulting in acute toxicity following ethanol exposure. The metabolism of ethanol also leads to depletion of the cofactor NAD, which can have a negative impact on mitochondrial functioning.

Apoptosis
See also:
Apoptosis *Ch. 4, p. 67*

Which of the many effects of these toxicants is actually responsible for the death of the cells in hepatic necrosis is still a subject of considerable debate. It is probably not inhibition of protein synthesis, because toxicants such as ethionine can do this for hours without killing the cell. Damage to mitochondria has also been suggested as the lethal trigger, but as with inhibition of protein synthesis, ATP depletion can be observed without necrosis. Many theories point to effects on calcium homeostasis, since increased intercellular calcium levels frequently accompany cell death, and damage to membranes (such as the endoplasmic reticulum or mitochondrial membrane) could lead to release of sequestered calcium. Entry of external calcium into the cell through a damaged plasma membrane may or may not be involved, as studies have indicated that an external pool of calcium is not necessary to produce necrosis. It is difficult, however, to determine if the increase in calcium actually causes the death of the cell or if it is a result of it.

Ethanol
See also:
Reproductive toxicology and teratology *Ch. 7, p. 135*
Cardiovascular toxicology *Ch. 9, p. 168*
Neurotoxicology *Ch. 10, p. 211*
Hepatotoxicology *Ch. 11, pp. 224, 227*
Forensic toxicology *Ch. 16, p. 297*
Ethanol *Appendix, p. 340*

Finally, under some circumstances, hepatocytes can also be induced to undergo *apoptosis*, or programmed cell death. For example, *cholestasis*, or stoppage of bile flow, often results in apoptosis of hepatocytes. Cholestasis occurs in humans following administration of drugs such as *steroids*, *phenothiazines*, and *tricyclic antidepressants*. It is characterized by the development of jaundice, a condition that, as you may recall, is characterized by a yellowish discoloration of the eyes and skin (resulting from the buildup of bile pigments such as bilirubin). The mechanism for the apoptotic effect of cholestasis is not clear, but the trigger is probably the buildup of bile acids in the liver. Bile acids have been shown in cell culture to be hepatotoxic, and experimental evidence indicates that they interact with the *Fas* pathway, one of the major pathways of apoptosis. Other situations that may trigger apoptosis in hepatocytes include treatment with *troglitazone*, a drug that is used to treat type II diabetes as well as viral infection (*viral hepatitis*).

Cirrhosis

Chronic exposure to hepatotoxicants can lead to a condition called *cirrhosis*. A combination of damage to hepatocytes and inadequate regeneration leads to increased activity of fibroblasts and accumulation of collagen in the liver. This results in not only a net loss of functioning hepatocytes, but also in a significant disruption of blood flow in the liver. Chronic exposure to ethanol is a leading cause of cirrhosis in humans, but the mechanism underlying the effect is the subject of considerable debate. Malnutrition frequently accompanies alcoholism, and some investigators hypothesize that it is this factor, rather than the alcohol, that causes the cirrhosis. Evidence has been presented showing that rats that are maintained on an adequate diet can be exposed to ethanol without developing cirrhosis, but other studies have indicated that monkeys develop precirrhotic changes with exposure to ethanol even if no nutritional deficiencies develop. Cirrhosis is irreversible.

Carbon Tetrachloride

See also:
Biotransformation *Ch. 3, p. 41*
Cardiovascular toxicology *Ch. 9, p. 167*
Neurotoxicology *Ch. 10, p. 211*
Hepatotoxicology *Ch. 11, p. 225*
Renal toxicology *Ch. 12, p. 241*
Halogenated hydrocarbons *Appendix, p. 341*

Carcinogenesis

Many hepatotoxicants, including carbon tetrachloride and chloroform, have also been shown to be hepatic carcinogens in laboratory animals. One group of potential hepatic carcinogens is the *aflatoxins*. These toxins are produced by a fungus that grows on grain and other foods. Aflatoxin B1, for example, is metabolized by cytochrome P450 to a reactive epoxide, which then can bind to DNA. Some *polychlorinated biphenyls* (PCBs) may also be hepatic carcinogens. The most well-known human hepatic carcinogen is probably *vinyl chloride*, the monomer used in the manufacture of the polymer polyvinyl chloride (PVC). Its carcinogenic potential was discovered when it became clear that workers exposed to vinyl chloride were developing an unusually large number of cases of the relatively rare type of liver cancer known as *angiosarcoma*.

Miscellaneous Effects

Toxicants can also damage sinusoids, enlarging them so that red blood cells can enter and block the lumen. One drug that can do this is acetaminophen. The *pyrrolizidine alkaloids* can also produce sinusoidal damage.

Exposure to other toxicants (such as the anesthetic *halothane*) can cause a condition resembling viral hepatitis, with headache, nausea, vomiting, dizziness, and jaundice. This effect may be caused at least in part by a reaction of the immune system to the drug.