46 Liver Metabolism

*The liver is strategically interposed between the general circulation and the digestive tract. It receives 20 to 25% of the volume of blood leaving the heart each minute (the cardiac output) through the portal vein (which delivers absorbed nutrients and other substances from the gastrointestinal tract to the liver) and through the hepatic artery (which delivers blood from the general circulation back to the liver). Potentially toxic agents absorbed from the gut or delivered to the liver by the hepatic artery must pass through this **metabolically active organ** before they can reach the other organs of the body. The liver's relatively large size (approximately 3% of total body weight) allows extended residence time within the liver for nutrients to be properly metabolized as well as for potentially harmful substances to be detoxified and prepared for excretion into the urine or feces. Among other functions, therefore, the liver, along with the kidney and gut, is an **excretory organ**, equipped with a broad spectrum of detoxifying mechanisms. It has the capacity, for example, to carry out **metabolic conversion pathways** as well as **secretory systems** that allow the excretion of potentially toxic compounds. Concurrently, the liver contains highly specific and selective **transport mechanisms** for essential nutrients that are required not only to sustain its own energy but to provide physiologically important substrates for the systemic needs of the organism. In addition to the myriad of transport processes within the sinusoidal and canalicular plasma membrane sheets (see below), intracellular hepatocytic transport systems exist in organelles such as **endosomes, mitochondria, lysosomes**, as well as the **nucleus**. The sequential transport steps carried out by these organelles include (1) **uptake**, (2) **intracellular binding and sequestration**, (3) **metabolism**, (4) **sinusoidal secretion**, and (5) **biliary excretion**. The rate of hepatobiliary transport is determined, in part, by the rate of activity of each of these steps. The overall transport rate is also determined by such factors as hepatic blood flow, plasma protein binding, and the rate of canalicular reabsorption. The various aspects of the major metabolic processes performed by the liver have been discussed in greater detail elsewhere in this text. These sources are referred to as the broad spectrum of the liver's contributions to overall health and disease are described.*

THE WAITING ROOM

Jean Ann Tonich's family difficulties continued, and, in spite of a period of sobriety lasting 6 months, she eventually started drinking increasing amounts of gin again in an effort to deal with her many anxieties. Her appetite for food declined slowly as well. She gradually withdrew from much of the

social support system that her doctors and friends had attempted to build during her efforts for rehabilitation. Upper mid-abdominal pain became almost constant, and she noted an increasing girth and distention of her abdomen. Early one morning, she was awakened in excruciating pain in her upper abdomen. She vomited dark-brown "coffee ground" material followed by copious amounts of bright red blood. She called a friend, who rushed her to the hospital emergency room.

Amy Biasis, a 23-year-old missionary, was brought to the hospital emergency room complaining of the abrupt onset of fever, chills, and severe pain in the right upper quadrant of her abdomen. The pain was constant in nature and radiated to her right shoulder top. She vomited undigested food twice in the hour before arriving at the emergency room. This did not relieve her pain.

Her medical history indicated that, while serving as a missionary in western Belise, Central America, 2 months earlier, she had a 3-day illness that included fever, chills, and mild but persistent diarrhea. A friend of Amy's there, a medical missionary, had given her an unidentified medication for 7 days. Amy's diarrhea slowly resolved, and she felt well again until her current abdominal symptoms began.

On physical examination, she appeared toxic and had a temperature of 101°F. She was sweating profusely. Her inferior anterior liver margin was palpable three fingerbreadths below the right rib cage, suggestive of an enlarged liver. The liver edge was rounded and tender. Gentle first percussion of the lower posterior right rib cage caused severe pain. Routine laboratory studies were ordered, and a computed tomogram (CT) of the upper abdomen was scheduled to be done immediately.

Fig. 46.1. Schematic view of liver anatomy.

I.   LIVER ANATOMY

The human liver consists of two lobes, each containing multiple lobules and sinusoids. The liver receives 75% of its blood supply from the portal vein, which carries blood returning to the heart from the small intestine, stomach, pancreas and spleen. The remaining 25% of the liver's blood supply is arterial, carried to the liver by the hepatic artery.

Blood from both the portal vein and hepatic artery empty into a common conduit, mixing their contents as they enter the liver sinusoids (Fig. 46.1). The sinusoids are expandable vascular channels that run through the hepatic lobules. They are lined with endothelial cells that have been described as "leaky" because, as blood flows through the sinusoids, the contents of the plasma have relatively free access to the hepatocytes, which are located on the other side of the endothelial cells.

The liver is also an exocrine organ, secreting bile into the biliary drainage system. The hepatocytes secrete bile into the bile canniculus, whose contents flow parallel to that in the sinusoids but in the opposite direction. The canniculi empty into the bile ducts. The lumina of the bile ducts then fuse, forming the common bile duct. The common duct then releases bile into the duodenum. Some of the liver's effluent is stored in the gallbladder and discharged into the duodenum postprandially to aid in digestion.

The entire liver surface is covered by a capsule of connective tissue that branches and extends throughout the liver. This capsule provides support for the blood vessels, lymphatic vessels, and bile ducts that permeate the liver. In addition, this connective tissue sheet subdivides the liver lobes into the smaller lobules.

II.   LIVER CELL TYPES

The primary cell type of the liver is the hepatocyte. Hepatocytes, also known as the hepatic parenchymal cells, form the liver lobules. Eighty percent of the liver volume is composed of hepatocytes, but only 60% of the total number of cells in the liver
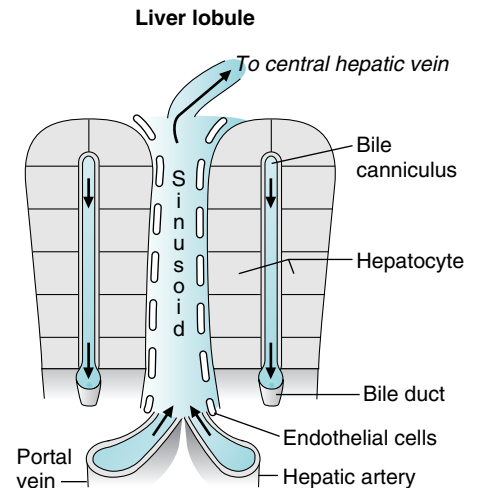
are hepatocytes. The other 40% of the cells are the nonparenchymal cells, which constitute the lining cells of the walls of the sinusoids. The lining cells comprise the endothelial cells, Kupffer cells, and hepatic stellate cells. In addition, intrahepatic lymphocytes, which include pit cells (liver-specific natural killer cells) are also present in the sinusoidal lining.

A. Hepatocytes

The hepatocyte is the cell that carries out the many functions of the liver. Almost all pathways of metabolism are represented in the hepatocyte and these pathways are controlled through the actions of hormones that bind to receptors located on the plasma membrane of their cells. Although normally quiescent cells with low turnover and a long life span, hepatocytes can be stimulated to grow if damage occurs to other cells in the liver. The liver mass has a relatively constant relationship to the total body mass of adult individuals. Deviation from the normal or optimal ratio (caused, for example, by a partial hepatectomy or significant hepatic cell death or injury) is rapidly corrected by hepatic growth caused by a proportional increase in hepatocyte replication.

The reports of **Amy Biasis'** initial laboratory studies showed an elevation in her serum hepatic transaminases, her serum alkaline phosphatases, as well as her serum total bilirubin level.

B. Endothelial Cells

The sinusoidal endothelial cells constitute the lining cells of the sinusoid. Unlike endothelial cells in other body tissues, these cells contain fenestrations with a mean diameter of 100 nm. They do not, therefore, form a tight basement membrane barrier between themselves and the hepatocytes. In this way, they allow for free diffusion of small molecules to the hepatocytes but not of particles the size of chylomicrons (chylomicron remnants, however, which are smaller than chylomicrons, do have free passage to the hepatocyte). The endothelial cells are capable of endocytosing many ligands and also may secrete cytokines when appropriately stimulated. Because of their positioning, lack of tight junctions, and absence of a tight basement membrane, the liver endothelial cells do not present a significant barrier against the movement of the contents of the sinusoids into hepatocytes. Their fenestrations or pores further promote the free passage of blood components through this membrane into the liver parenchymal cells.

C. Kupffer Cells

These cells are located within the sinusoidal lining. They contain almost one quarter of all the lysosomes of the liver. The Kupffer cells are tissue macrophages with both endocytotic and phagocytic capacity. They phagocytose many substances such as denatured albumin, bacteria, and immune complexes. They protect the liver from gut-derived particulate materials and bacterial products. On stimulation by immunomodulators, these cells secrete potent mediators of the inflammatory response and play a role in liver immune defense through the release of cytokines that lead to the inactivation of substances considered foreign to the organism. The Kupffer cells also remove damaged erythrocytes from the circulation.

D. Hepatic Stellate Cells

The stellate cells are also called perisinusoidal or Ito cells. There are approximately 5 to 20 of these cells per 100 hepatocytes. The stellate cells are lipid-filled cells (the primary storage site for vitamin A). They also control the turnover of hepatic connective tissue and extracellular matrix and regulate the contractility of the sinusoids. When cirrhosis of the liver is present, the stellate cells are stimulated by various signals to increase their synthesis of extracellular matrix material. This, in turn, diffusely infiltrates the liver, eventually interfering with the function of the hepatocytes.

E. Pit Cells

The hepatic pit cells, also known as liver-associated lymphocytes, are natural killer cells, which are a defense mechanism against the invasion of the liver by potentially toxic agents, such as tumor cells or viruses.

III. MAJOR FUNCTIONS OF THE LIVER

A. The Liver Is a Central Receiving and Recycling Center for the Body

The liver can carry out a multitude of biochemical reactions. This is necessary because of its role in constantly monitoring, recycling, modifying, and distributing all of the various compounds absorbed from the digestive tract and delivered to the liver. If any portion of an ingested compound is potentially useful to that organism, the liver will retrieve this portion and convert it to a substrate that can be used by hepatic and nonhepatic cells. At the same time, the liver removes many of the toxic compounds that are ingested or produced in the body and targets them for excretion in the urine or in the bile.

As mentioned previously, the liver receives nutrient-rich blood from the enteric circulation through the portal vein; thus, all of the compounds that enter the blood from the digestive tract pass through the liver on their way to other tissues. The enterohepatic circulation allows the liver first access to nutrients to fulfill specific functions (such as the synthesis of blood coagulation proteins, heme, purines, and pyrimidines) and first access to ingested toxic compounds (such as ethanol) and to such potentially harmful metabolic products (such as NH_4^+ produced from bacterial metabolism in the gut).

In addition to the blood supply from the portal vein, the liver receives oxygen-rich blood through the hepatic artery; this arterial blood mixes with the blood from the portal vein in the sinusoids. This unusual mixing process gives the liver access to various metabolites produced in the periphery and secreted into the peripheral circulation, such as glucose, individual amino acids, certain proteins, iron–transferrin complexes, and waste metabolites as well as potential toxins produced during substrate metabolism. As mentioned, fenestrations in the endothelial cells, combined with gaps between the cells, the lack of a basement membrane between the endothelial cells and the hepatocytes, and low portal blood pressure (which results in slow blood flow) contribute to the efficient exchange of compounds between sinusoidal blood and the hepatocyte and clearance of unwanted compounds from the blood. Thus, large molecules targeted for processing, such as serum proteins and chylomicron remnants, can be removed by hepatocytes, degraded, and their components recycled. Similarly, newly synthesized molecules, such as very-low-density lipoprotein (VLDL) and serum proteins, can be easily secreted into the blood. In addition, the liver can convert all of the amino acids found in proteins into glucose, fatty acids, or ketone bodies. The secretion of VLDL by the liver not only delivers excess calories to adipose tissue for storage of fatty acids in triacylglycerol, but it also delivers phospholipids and cholesterol to tissues that are in need of these compounds for synthesis of cell walls as well as other functions. The secretion of glycoproteins by the liver is accomplished through the liver's gluconeogenic capacity as well as its access to a variety of dietary sugars to form the oligosaccharide chains, as well as its access to dietary amino acids with which is synthesizes proteins. Thus, the liver has the capacity to carry out a large number of biosynthetic reactions. It has the biochemical wherewithal to synthesize a myriad of compounds from a broad spectrum of precursors. At the same time, the liver metabolizes compounds into biochemically useful products. Alternatively, it has the ability to degrade and excrete those compounds presented to it that cannot be further used by the body.

The CT scan of **Amy Biasis'** upper abdomen showed an elevated right hemidiaphragm as well as several cystic masses in her liver, the largest of which was located in the superior portion of the right lobe. Her clinical history as well as her history of possible exposure to various parasites while working in a part of Belize, Central America, that is known to practice substandard sanitation, prompted her physicians to order a titer of serum antibodies against the parasite *Entamoeba histolytica* in addition to measuring serum antibodies against other invasive parasites.

A knowledge of functional characteristics of liver cells has been used to design diagnostic agents that can be used to determine the normalcy of specific biochemical pathways of the hepatocytes. These "tailor-made" pharmaceuticals can be designed to be taken up by one or more of the available transport mechanisms available to the liver. For example, receptor-related endocytic processes can be used as targets to probe specific receptor-mediated transport functions of the liver cells. The asialoglycoprotein receptor, also known as the hepatic-binding protein, has been used in this diagnostic approach. The substrate $^{99}T_c{}^m$-galactosyl-neoglycoalbumin (NGA) was developed as a specific ligand for selective uptake via this specific hepatic receptor. The timing and extent of the assimilation of this probe into the hepatocytes, as determined by imaging the liver at precise intervals after the administration of this isotope, yields an estimate of hepatic blood flow as well as the transport capacity of this specific hepatic transporter protein.

Antibody titers against *Entamoeba histolytica* by use of an enzyme immunoassay were strongly reactive in **Amy Biasis'** blood. A diagnosis of **amoebiasis** was made. Her physicians started nitroimidazole amoebicides intravenously in a dose of 500 mg every 6 hours for 10 days. By the third day of treatment, Amy began to feel noticeably better. Her physicians told her that they expected a full clinical response in 95% of patients with amoebic liver abscesses treated in this way, although her multiple hepatic abscesses adversely affected her prognosis to a degree.

Table 46.1. Examples of Enzymes Used in Biotransformation of Xenobiotic Compounds

Acetyltransferase
Amidase-esterase
Dehydrogenase
Flavin-containing mono-oxygenase
Glutathione-S-transferase
Methyl transferase
Mixed-function oxidase
Reductase
Sulfotransferase
UDP-glucosyltransferase
UDP-glucuronosyltransferase

Each of the liver cells described contains specialized transport and uptake mechanisms for enzymes, infectious agents, drugs, and other xenobiotics that specifically target these substances to certain liver cell types. These are accomplished by linking these agents covalently by way of biodegradable bonds to their specific carrier. The latter then determines the particular fate of the drug by using specific cell recognition, uptake, transport, and biodegradation pathways.

B. Inactivation and Detoxification of Xenobiotic Compounds and Metabolites

Xenobiotics are compounds that have no nutrient value (cannot be used by the body for energy requirements) and are potentially toxic. They are present as natural components of foods or they may be introduced into foods as additives or through processing. Pharmacologic and recreational drugs are also xenobiotic compounds. The liver is the principal site in the body for the degradation of these compounds. Because many of these substances are lipophilic, they are oxidized, hydroxylated, or hydrolyzed by enzymes in phase I reactions. Phase I reactions introduce or expose hydroxyl groups or other reactive sites that can be used for conjugation reactions (the phase II reactions). The conjugation reactions add a negatively charged group such as glycine or sulfate to the molecule. Many xenobiotic compounds will be transformed through several different pathways. A general scheme of inactivation is shown in Figure 46.2.

The conjugation and inactivation pathways are similar to those used by the liver to inactivate many of its own metabolic waste products. These pathways are intimately related to the biosynthetic cascades that exist in the liver. The liver can synthesize the precursors that are required for conjugation and inactivation reactions from other compounds. For example, sulfation is used by the liver to clear steroid hormones from the circulation. The sulfate used for this purpose can be obtained from the degradation of cysteine or methionine.

The liver, kidney, and intestine are the major sites in the body for biotransformation of xenobiotic compounds. Many xenobiotic compounds contain aromatic rings (such as benzopyrene in tobacco smoke) or heterocyclic ring structures (such as the nitrogen-containing rings of nicotine or pyridoxine) that we are unable to degrade or recycle into useful components. These structures are hydrophobic, causing the molecules to be retained in adipose tissue unless they are sequestered by the liver, kidney, or intestine for biotransformation reactions. Sometimes, however, the phase I and II reactions backfire, and harmless hydrophobic molecules are converted to toxins or potent chemical carcinogens.

1.   CYTOCHROME P450 AND XENOBIOTIC METABOLISM

The toxification/detoxification of xenobiotics is accomplished through the activity of a group of enzymes with a broad spectrum of biologic activity. Some examples of enzymes involved in xenobiotic transformation are described in Table 46.1. Of the wide variety of enzymes that are involved in xenobiotic metabolism, only the cytochrome P450–dependent monooxygenase system is discussed here. The cytochrome P450–dependent monooxygenase enzymes are determinants in oxidative, peroxidative, and reductive degradation of exogenous (chemicals, carcinogens, and pollutants, etc.) and endogenous (steroids, prostaglandins,
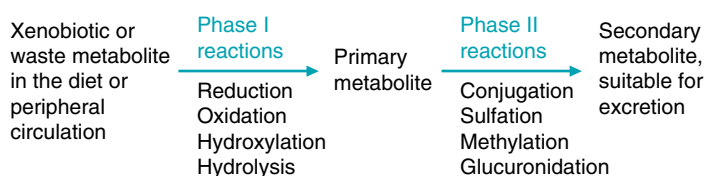

Fig. 46.2. General scheme of xenobiotic detoxification.

retinoids, etc.) substances. The key enzymatic constituents of this system are the flavo protein NADPH-cytochrome P450 oxidoreductase and cytochrome P450 (Fig. 46.3). The latter is the terminal electron acceptor and substrate-binding site of the microsomal mixed-function oxidase complex, a very versatile catalytic system. The system got its name in 1962, when Omura and Sato found a pigment with unique spectral characteristics derived from liver microsome of rabbits. When reduced and complexed with carbon monoxide, it exhibited a spectral absorbance maximum at 450 nm.

The major role of the cytochrome P450 enzymes (see Chapter 25) is to oxidize substrates and introduce oxygen to the structure. Similar reactions can be carried out by other flavin monoxygenases that do not contain cytochrome P450.

The cytochrome P450 enzyme family contains at least 100 to 150 different isozymes with at least 40% sequence homology. These isozymes have different but overlapping specificities. The human enzymes are generally divided into six major subfamilies, and each of these is further subdivided. For example, in the naming of the principal enzyme involved in the oxidation of ethanol to acetaldehyde, CYP2E1, the CYP denotes the cytochrome P450 family, the 2 denotes the subfamily, the E denotes ethanol, and the 1 denotes the specific isozyme.

The CYP3A4 isoform accounts for 60% of CYP450 enzymes in the liver and 70% of cytochrome enzymes in gut wall enterocytes. It metabolizes the greatest number of drugs in humans. Specific drugs are substrates for CYP3A4. The concomitant ingestion of two CYP3A4 substrates could potentially induce competition for the binding site, which, in turn, could alter the blood levels of these two agents. The drug with the highest affinity for the enzyme would be preferentially metabolized, whereas the metabolism (and degradation) of the other drug would be reduced. The latter drug's concentration in the blood would then rise.

Moreover, many substances or drugs impair or inhibit the activity of the CYP3A4 enzyme, thereby impairing the body's ability to metabolize a drug. The lipid-lowering agents known as the statins (HMGCoA reductase inhibitors) require CYP3A4 for degradation. Appropriate drug treatment and dosing takes into account the normal degradative pathway of the drug. However, grapefruit juice is a potent inhibitor of CYP3A4-mediated drug metabolism. Evidence suggests that if a statin is regularly taken with grapefruit juice, its level in the blood may increase as much as 15-fold. This marked increase in plasma concentration could increase the muscle and liver toxicity of the statin in question. because side effects of the statins appear to be dose-related.

The cytochrome P450 isozymes all have certain features in common:

1. They all contain cytochrome P450, oxidize the substrate, and reduce oxygen.
2. They all have a flavin-containing reductase subunit that uses NADPH, and not NADH, as a substrate.
3. They are all found in the smooth endoplasmic reticulum and are referred to as microsomal enzymes (for example, CYP2E1 is also referred to as the microsomal ethanol oxidizing system, MEOS).
4. They are all bound to the lipid portion of the membrane, probably to phosphatidylcholine.
5. They are all inducible by the presence of their own best substrate and somewhat less inducible by the substrates for other P450 isozymes.
6. They all generate a reactive free radical compound as an intermediate in the reaction.

2. EXAMPLES OF CYTOCHROME P450 DETOXIFICATION REACTIONS

i. Vinyl Chloride

The detoxification of vinyl chloride provides an example of effective detoxification by a P450 isozyme (ethanol detoxification was previously discussed in Chapter 25). Vinyl chloride is used in the synthesis of plastics and can cause angiosarcoma in the liver of exposed workers. It is activated in a phase I reaction to a reactive epoxide by
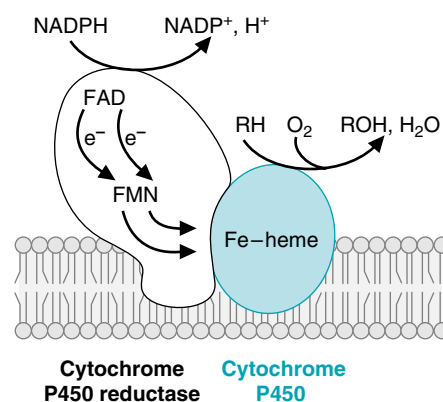

Fig. 46.3. General structure of the P450 enzymes. O_2 binds to the P450 Fe-heme in the active site and is activated to a reactive form by accepting electrons. The electrons are donated by the cytochrome P450 reductase, which contains an FAD plus an FMN or Fe-S center to facilitate the transfer of single electrons from NADPH to O_2. The P450 enzymes involved in steroidogenesis have a somewhat different structure. For CYP2E1, RH is ethanol (CH_3CH_2OH), and ROH is acetaldehyde (CH_3COH).
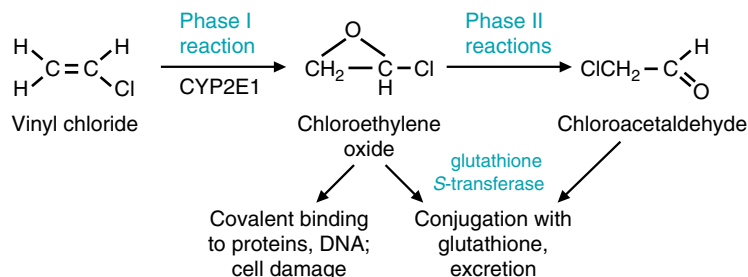
Fig. 46.4. Detoxification of vinyl chloride.

a hepatic P450 isozyme (CYP2E1), which can react with guanine in DNA or other cellular molecules. However, it also can be converted to chloroacetate, conjugated with reduced glutathione, and excreted in a series of phase II reactions (Fig. 46.4).

ii. Aflatoxin B1

Aflatoxin B1 is an example of a compound that is made more toxic by a cytochrome P450 reaction (CYP2A1). Current research suggests that ingested aflatoxin B1 in contaminated food (it is produced by a fungus [*Aspergillus flavus*] that grows on peanuts that may have been stored in damp conditions) is directly involved in hepatocarcinogenesis in humans by introducing a G > T mutation into the *p53* gene. Aflatoxin is metabolically activated to its 8,9 epoxide by two different isozymes of cytochrome P450. The epoxide modifies DNA by forming covalent adducts with guanine residues. In addition, the epoxide can combine with lysine residues within proteins and thus is also a hepatotoxin.

iii. Acetaminophen

Acetaminophen (Tylenol) is an example of a xenobiotic that is metabolized by the liver for safe excretion; however, it can be toxic if ingested in high doses. The pathways for acetaminophen metabolism are shown in Fig. 46.5 As shown in the
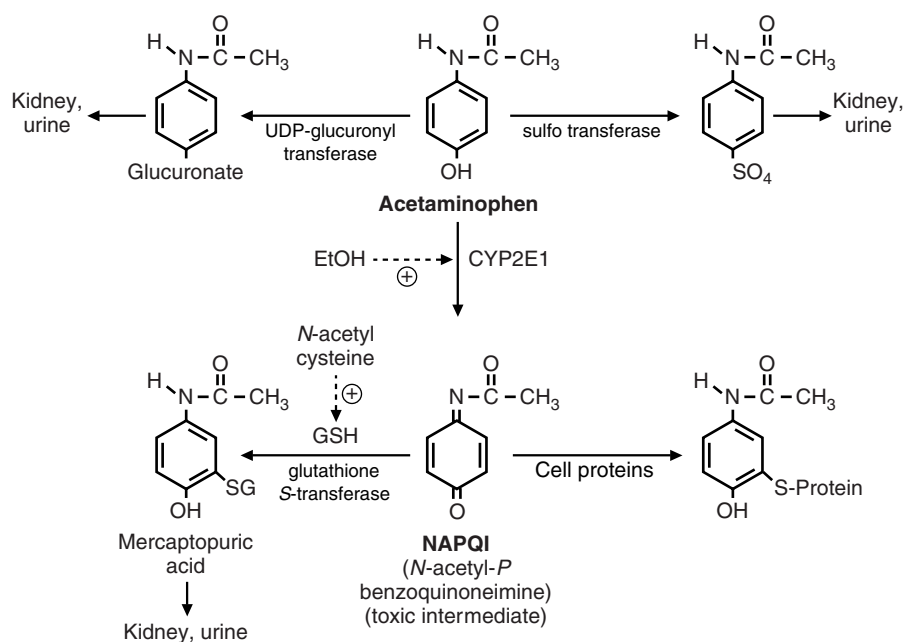

Fig. 46.5. Pathways of acetaminophen detoxification. N-acetyl cysteine stimulates the production of glutathione, thereby reducing the levels of NAPQI, which can damage cellular proteins. Ethanol upregulates CYP2E1 activity (the MEOS).

figure, acetaminophen can be glucuronylated or sulfated for safe excretion by the kidney. However, a cytochrome P450 enzyme produces the toxic intermediate N-acetyl-p-benzoquinoneimine (NAPQI), which can be excreted safely in the urine after conjugation with glutathione.

NAPQI is a dangerous and unstable metabolite that can damage cellular proteins and lead to death of the hepatocyte. Under normal conditions, when acetaminophen is taken in the correct therapeutic amounts, less than 10% of the drug forms NAPQI, an amount that can be readily handled by the glutathione detoxifying system (phase II reactions). However, when taken at doses that are potentially toxic, the sulfotransferase and glucuronyl transferase systems are overwhelmed, and more acetaminophen is metabolized through the NAPQI route. When this occurs, the levels of glutathione in the hepatocyte are insufficient to detoxify NAPQI, and hepatocyte death can result.

The enzyme that produces NAPQI, CYP2E1, is induced by alcohol (see Chapter 25, MEOS). Thus, individuals who chronically abuse alcohol have an increased sensitivity to acetaminophen toxicity, because a higher percentage of acetaminophen metabolism is directed toward NAPQI, as compared with an individual with low levels of CYP2E1. Therefore, even recommended therapeutic doses of acetaminophen can be toxic to these individuals.

An effective treatment for acetaminophen poisoning involves the use of N-acetyl cysteine. This compound supplies cysteine as a precursor for increased glutathione production, which, in turn, enhances the phase II reactions, which reduces the levels of the toxic intermediate.

C. Regulation of Blood Glucose Levels

One of the primary functions of the liver is to maintain blood glucose concentrations within the normal range. The manner in which the liver accomplishes this has been the subject of previous chapters (26, 31, and 36). In brief, the pancreas monitors blood glucose levels and secretes insulin when blood glucose levels rise and glucagon when such levels decrease. These hormones initiate regulatory cascades that affect liver glycogenolysis, glycogen synthesis, glycolysis, and gluconeogenesis. In addition, sustained physiologic increases in growth hormone, cortisol, and catecholamine secretion help to sustain normal blood glucose levels during fasting.

Numerous other factors, beside insulin and glucagon, can affect liver glucose metabolism, as has been described in Chapter 43.

When blood glucose levels drop, glycolysis and glycogen synthesis are inhibited, and gluconeogenesis and glycogenolysis are activated. Concurrently, fatty acid oxidation is activated to provide energy for glucose synthesis. During an overnight fast, blood glucose levels are primarily maintained by glycogenolysis and, if gluconeogenesis is required, the energy (6 ATP are required to produce one molecule of glucose from two molecules of pyruvate) is obtained by fatty acid oxidation. On insulin release, the opposing pathways are activated such that excess fuels can be stored either as glycogen or fatty acids. The pathways are regulated by the activation or inhibition of two key kinases, the cyclic adenosine monophosphate (cAMP)-dependent protein kinase, and the AMP-activated protein kinase (see Fig. 36.11 for a review of these pathways). Recall that the liver can export glucose because it is one of only two tissues that express glucose-6-phosphatase.

D. Synthesis and Export of Cholesterol and Triacylglycerol

When food supplies are plentiful, hormonal activation leads to fatty acid, triacylglycerol, and cholesterol synthesis. A high dietary intake and intestinal absorption of cholesterol will compensatorily reduce the rate of hepatic cholesterol synthesis, in which case the liver acts as a recycling depot for sending excess dietary cholesterol to the peripheral tissue when needed as well as accepting cholesterol from these tissues when required. The pathways of cholesterol metabolism were discussed in Chapter 34.

E. Ammonia and the Urea Cycle

The liver is the primary organ for synthesizing urea and, as such, is the central depot for the disposition of ammonia in the body. Ammonia groups travel to the liver on glutamine and alanine, and the liver converts these ammonia nitrogens to urea for excretion in the urine. The reactions of the urea cycle were discussed in Chapter 38.

Table 46.2 lists some of the important nitrogen-containing compounds that are primarily synthesized or metabolized by the liver.

F. Ketone Body Formation

The liver is the only organ that can produce ketone bodies, yet it is one of the few that cannot use these molecules for energy production. Ketone bodies are produced when the rate of glucose synthesis is limited (i.e., substrates for gluconeogenesis are limited), and fatty acid oxidation is occurring rapidly. Ketone bodies can cross the blood-brain barrier and become a major fuel for the nervous system under conditions of starvation. Ketone body synthesis and metabolism have been described in Chapter 23.

G. Nucleotide Biosynthesis

The liver can synthesize and salvage all ribonucleotides and deoxyribonucleotides for other cells to use. Certain cells have lost the capacity to produce nucleotides de

Table 46.2. Nitrogen-Containing Products Produced by the Liver

Product	Precursors	Tissues	Function
Creatine	Arginine, glycine, and S-adenosyl methionine (SAM)	Liver	Forms creatine phosphate in muscle for energy storage. Excreted as creatinine.
Glutathione	Glutamate, cysteine, glycine	All tissues but highest use in the liver	Protection against free radical injury by reduction of hydrogen peroxide and lipid peroxides. In liver and kidney forms mercapturic acids.
Purines	Glycine, glutamine, aspartate, carbon dioxide, tetrahydrofolate, PRPP	Liver, small amounts in brain and cells of the immune system	Adenine and guanine nucleosides and nucleotides. DNA, RNA, and coenzymes, and energy-transferring nucleotides.
Pyrimidines	Aspratate, glutamine, carbon dioxide	Liver, small amounts in brain and cells of the immune system	Uracil, thymine and cytosine
Sialic acid (NANA), other amino sugars	Glutamine	Most cells	In the liver, synthesis of oligosaccharide chains on secreted proteins. Most cells, glycoproteins, proteoglycans, and glycolipids.
Sulfated compounds	Cysteine	Liver and kidney produce sulfate	Many cells use sulfate in blood for formation of PAPS, which transfers sulfate to proteoglycans, drugs, and xenobiotics
Taurine	Cysteine	Liver	Conjugated bile salts
Glycocholic acid, and glycocheno-Deoxycholic acid	Glycine, bile salts	Liver	Conjugated bile salts are excreted into the bile and assist in the absorption of lipids and fat-soluble vitamins through the formation of micelles
Sphingosine	Serine and palmitoyl CoA	Liver, brain, and other tissues	Precursor of sphingolipids found in myelin and other membranes
Heme	Glycine and succinyl CoA	Liver, bone marrow	Heme from liver is incorporated into cytochromes. Heme from bone marrow is incorporated into hemoglobin.
Glycine conjugates of xenobiotic compounds	Glycine, medium-size hydrophobic carboxylic acids	Liver, kidney	Inactivation and targeting toward urinary excretion
Niacin	Tryptophan, glutamine	Liver	NAD, NADP coenzymes for oxidation reactions
One-carbon methyl donors for tetrahydrofolate and SAM	Glycine, serine, histidine, methionine	Most cells, but highest in liver	Choline, phosphatidylcholine, purine and pyrimidine synthesis, inactivation of waste metabolites and xenobiotics through methylation.

novo but can use the salvage pathways to convert free bases to nucleotides. The liver can secrete free bases into the circulation for these cells to use for this purpose. Nucleotide synthesis and degradation are discussed in Chapter 41.

H. Synthesis of Blood Proteins

The liver is the primary site of the synthesis of circulating proteins such as albumin and the clotting factors. When liver protein synthesis is compromised, the protein levels in the blood are reduced. Hypoproteinemia may lead to edema because of a decrease in the protein-mediated osmotic pressure in the blood. This, in turn, causes plasma water to leave the circulation and enter (and expand) the interstitial space, causing edema.

Most circulating plasma proteins are synthesized by the liver. Therefore, the hepatocyte has a well-developed endoplasmic reticulum, Golgi system, and cellular cytoskeleton, all of which function in the synthesis, processing, and secretion of proteins. The most abundant plasma protein produced by the liver is albumin, which represents 55 to 60% of the total plasma protein pool. Albumin serves as a carrier for a large number of hydrophobic compounds, such as fatty acids, steroids, hydrophobic amino acids, vitamins, and pharmacologic agents. It is also an important osmotic regulator in the maintenance of normal plasma osmotic pressure. The other proteins synthesized by the liver are, for the most part, glycoproteins. They function in hemostasis, transport, protease inhibition, and ligand binding, as well as secretogogues for hormone release. The acute phase proteins that are part of the immune response and the body's response to many forms of "injury" are also synthesized in the liver. Table 46.3 lists some of the proteins and their functions.

I.   The Synthesis of Glycoproteins and Proteoglycans

The liver, because it is the site of synthesis of most of the blood proteins (including the glycoproteins), has a high requirement for the sugars that go into the oligosaccharide portion of glycoproteins (The synthesis of glycoproteins is discussed in Chapter 30.). These include mannose, fructose, galactose, and amino sugars.

One of the intriguing aspects of the hepatic biosynthetic pathways that use carbohydrate in the synthesis of these compounds is that the liver is not dependent on either dietary glucose or hepatic glucose to generate the precursor intermediates for these pathways. This is because the liver can generate carbohydrates from dietary amino acids (which enter gluconeogenesis generally as pyruvate or an intermediate of the TCA cycle), lactate (generated from anaerobic glycolysis in other tissues), and glycerol (generated by the release of free fatty acids from the adipocyte). Of course, if dietary carbohydrate is available, the liver can use that source as well.

Most of the sugars secreted by the liver are O-linked, that is, the carbohydrate is attached to the protein at its anomeric carbon through a glucosidic link to the –OH of a serine or a threonine residue. This is in contrast to the N-linked arrangement in which there is an N-glycosyl link to the amide nitrogen of an asparagine residue (Fig. 46.6). A particularly important O-linked sugar is N-acetylneuraminic acid (NANA or sialic acid), a nine-carbon sugar that is synthesized from fructose-6-phosphate and phosphoenolpyruvate (see Fig. 30.8). As circulating proteins age,

Cirrhosis of the liver results in portal hypertension, which because of increasing back pressure into the esophageal veins promotes the development of dilated thin-walled esophageal veins (varices). At the same time, synthesis of blood coagulation proteins by the liver and required vitamin K–dependent reactions are greatly diminished (resulting in a prolonged prothrombin time; which, in turn, increases clotting time). When the esophageal varices rupture, massive bleeding into the thoracic or abdominal cavity as well as the stomach may occur. Much of the protein content of the blood entering the gastrointestinal tract is metabolized by intestinal bacteria, releasing ammonium ion, which enters the portal vein. Because hepatocellular function has been compromised, the urea cycle capacity is inadequate, and the ammonium ion enters the peripheral circulation, thereby contributing to hepatic encephalopathy (brain toxicity due to elevated ammonia levels).

Table 46.3. A Partial List of Proteins Synthesized in the Liver

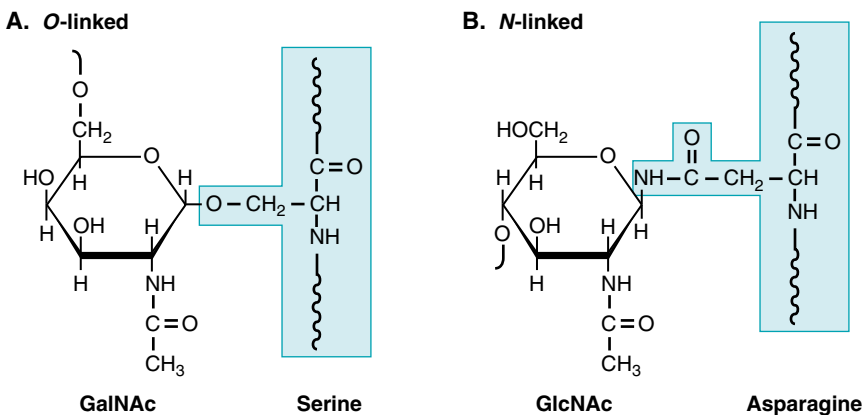| Type of Protein	Examples
Blood coagulation	Blood coagulation factors: fibrinogen, prothrombin, Factors V, VII, IX and X. Also α-2 macroglobulin.
Metal-binding proteins	Transferrin (iron), ceruloplasmin (copper), haptoglobin (heme), hemopexin (heme)
Lipid transport	Apoprotein B-100, apoprotein A-1
Protease inhibitor	α1-Antitrypsin

Fig. 46.6. The general configuration of O-linked and N-linked glycoproteins.

NANA (sialic acid) residues are lost from the serum proteins. This change signals their removal from the circulation and their eventual degradation. An asialoglyco-protein receptor on the liver cell surface binds such proteins, and the receptor–ligand complex is endocytosed and transported to the lysosomes. The amino acids from the degraded protein are then recycled within the liver.

J. The Pentose Phosphate Pathway

The major functions of the pentose phosphate pathway (see Chapter 29) are the generation of NADPH and five-carbon sugars. All cell types, including the red blood cell, can carry out this pathway because they need to generate NADPH so that the activity of glutathione reductase, the enzyme that catalyzes the conversion of oxidized glutathione (GSSG) back to reduced glutathione (GSH) can be maintained. Without the activity of this enzyme, the protection against free radical injury is lost. All cells also need this pathway for the generation of ribose, especially those cells that are dividing rapidly or have high rates of protein synthesis.

The liver has a much greater demand for NADPH than do most other organs. It uses NADPH for the biosynthesis of fatty acids and cholesterol, which the liver must make to produce phospholipids, and for the synthesis of VLDL and bile salts. It also uses NADPH for other biosynthetic reactions, such as that of proline synthesis. NADPH is also used by mixed-function oxidases such as cytochrome P450 that are involved in the metabolism of xenobiotics and of a variety of pharmaceuticals. Because the liver participates in so many reactions capable of generating free radicals, the liver uses more glutathione and NADPH to maintain glutathione reductase and catalase activity than any other tissue. Consequently, the concentration of glucose-6-phosphate dehydrogenase (the rate-limiting and regulated enzyme in the pentose phosphate pathway) is high in the liver, and the rate of flux through this pathway may be as high as 30% of the rate of flux through glycolysis.

IV. FUELS FOR THE LIVER

The reactions used to modify and inactivate dietary toxins and waste metabolites are energy requiring, as are the reactions used by anabolic (biosynthetic) pathways such as gluconeogenesis and fatty acid synthesis. Thus, the liver has a high energy requirement and consumes approximately 20% of the total oxygen used by the body. The principle forms in which energy is supplied to these reactions is the high-energy phosphate bonds of adenosine triphosphate (ATP), uridine triphosphate (UTP), and guanosine triphosphate (GTP), reduced NADPH, and acyl-CoA thioesters. The energy for the formation of these compounds is obtained directly

Table 46.4. Major Fates of Carbohydrates in the Liver

- Storage as Glycogen
- Glycolysis to pyruvate
  - Followed by oxidation to carbon dioxide in the TCA cycle
  - Precursors for the synthesis of glycerol-3-phosphate (the backbone of triacylglycerols and other glyceolipids), sialic acid, and serine
  - Entry into the TCA cycle and exit as citrate, followed by conversion to acetyl CoA, malonyl CoA, and entry into fatty acid synthesis and secretion as VLDL
    - Synthesis of phospholipids and other lipids from triacylglycerols
- Conversion to mannose, sialic acid, and other sugars necessary for the synthesis of oligosaccharides for glycoproteins, including those secreted into blood
- Synthesis of acid sugars for proteoglycan synthesis and formation of glucuronides
- Oxidation in the pentose phosphate pathway for the formation of NADPH (necessary for biosynthetic reactions such as fatty acid synthesis, glutathione reduction, and other NADPH-utilizing detoxification reactions)

from oxidative metabolism, the TCA cycle, or the electron transport chain and oxidative phosphorylation. After a mixed meal containing carbohydrate, the major fuels used by the liver are glucose, galactose, and fructose. If ethanol is consumed, the liver is the major site of ethanol oxidation, yielding principally acetate and then acetyl CoA. During an overnight fast, fatty acids become the major fuel for the liver. They are oxidized to carbon dioxide or ketone bodies. The liver also can use all of the amino acids as fuels, converting many of them to glucose. The urea cycle disposes of the ammonia that is generated from amino acid oxidation.

A. Carbohydrate Metabolism in the Liver

After a carbohydrate-containing meal, glucose, galactose, and fructose enter the portal circulation and flow to the liver. This organ serves as the major site in the body for the utilization of dietary galactose and fructose. It metabolizes these compounds by converting them to glucose and intermediates of glycolysis. Their fate is essentially the same as that of glucose (Table 46.4).

B. Glucose as a Fuel

The entry of glucose into the liver is dependent on a high concentration of glucose in the portal vein after a high-carbohydrate meal. Because the K_m for both the glucose transporter (GLUT2) and glucokinase is so high (approximately 10 mM), glucose will enter the liver principally after its concentration rises to 10 to 40 mM in the portal blood and not at the lower 5-mM concentration in the hepatic artery. The increase in insulin secretion that follows a high-carbohydrate meal will promote the conversion of glucose to glycogen. In addition, the rate of glycolysis will be increased (PFK-2 is active; thus, PFK-1 is activated by fructose-2, 6 bisphosphate) such that acetyl CoA can be produced for fatty acid synthesis (acetyl CoA carboxylase will be activated by citrate; see Chapter 33). Thus, after a high-carbohydrate meal, the liver uses glucose as its major fuel, while activating the pathways for glycogen and fatty acid synthesis.

The rate of glucose utilization by the liver is determined, in part, by the level of activity of glucokinase. Glucokinase activity is regulated by a glucokinase regulatory protein (RP, Fig. 46.7), which is located in the nucleus. In the absence of glucose, glucokinase is partially sequestered within the nucleus, bound to RP, in an inactive form. High concentrations of fructose 6-phosphate promote the interaction of glucokinase with RP, whereas high levels of either glucose or fructose 1-phosphate block glucokinase from binding to RP and promote the dissociation of the complex. Thus, as glucose levels rise in the cytoplasm and nucleus (because of increased blood glucose levels after a meal, for example), there is a significant enhancement of glucose phosphorylation as glucokinase is released from the nucleus, travels to the cytoplasm, and phosphorylates glucose.

Q: Why would you expect fructose 1-phosphate levels to promote the dissociation of glucokinase from regulatory protein (RP)?

The role of glucokinase RP is very complex. Mice that have been genetically engineered to no longer express the RP (a knockout mouse) display reduced levels of total glucokinase activity in the liver. This is attributable to the finding that RP is important in the post-transcriptional processing of the mRNA for glucokinase. In the absence of RP, less glucokinase is produced. These mice, therefore, have no glucokinase in the nucleus, a reduced cytoplasmic glucokinase content, and inefficient glucose phosphorylation in the liver when glucose levels rise.

Fructose-1-phosphate is produced from fructose metabolism. The major dietary source of fructose, the ingestion of which would lead to increased fructose 1-phosphate levels, is sucrose. Sucrose is a disaccharide of glucose and fructose. Thus, an elevation of fructose 1-phosphate usually indicates an elevation of glucose levels as well.
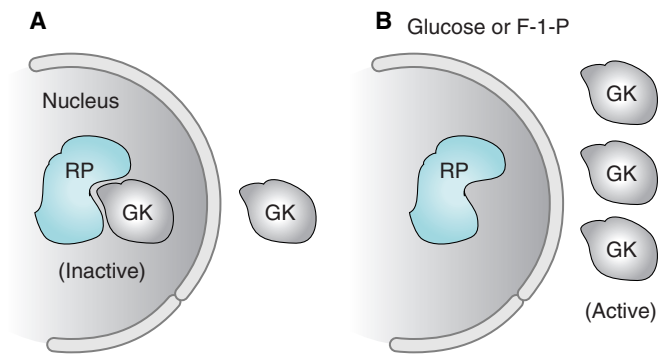

Fig. 46.7. Regulation of glucokinase by regulatory protein (RP). RP is localized to the nucleus, and in the absence of glucose or presence of fructose 6-phosphate, most glucokinase is translocated to the nucleus and binds RP. This leads to the formation of the inactive form of glucokinase. When glucose or fructose-1-phosphate levels rise, glucokinase is released from RP. It then translocates to the cytoplasm and actively converts glucose to glucose 6-phosphate.

The major regulatory step for liver glycolysis is the PFK-1 step. Even under fasting conditions, the ATP concentration in the liver (approximately 2.5 mM) is sufficiently high to inhibit PFK-1 activity. Thus, liver glycolysis is basically controlled by modulating the levels of fructose 2,6-bisphosphate, the product of the PFK-2 reaction. As fructose 2,6-bisphosphate levels increase (which would occur in the presence of insulin) the rate of glycolysis increases; when glucagon levels increase and protein kinase A is activated such that PFK-2 is phosphorylated and inactive, glycolysis will slow down, and gluconeogenesis will be enhanced (see Chapters 22 and 31).

C. Lipid Metabolism

Long-chain fatty acids are a major fuel for the liver during periods of fasting, when they are released from adipose tissue triacylglycerols and travel to the liver as fatty acids bound to albumin.

Within the liver, they bind to fatty acid–binding proteins and are then activated on the outer mitochondrial membrane, the peroxisomal membrane, and the smooth endoplasmic reticulum by fatty acyl CoA synthetases. The fatty acyl group is transferred from CoA to carnitine for transport through the inner mitochondrial membrane, where it is reconverted back into fatty acyl CoA and oxidized to acetyl CoA in the β-oxidation spiral (see Chapter 23).

The enzymes in the pathways of fatty acid activation and β-oxidation (the synthetases, the carnitine acyltransferases, and the dehydrogenases of β-oxidation) are somewhat specific for the length of the fatty acid carbon chain. The chain length specificity is divided into enzymes for long-chain fatty acids (C20 to approximately C12), medium-chain (approximately C12 to C4), and short-chain (C4–C2). The major lipids oxidized in the liver as fuels are the long-chain fatty acids (palmitic, stearic, and oleic acids), because these are the lipids that are synthesized in the liver, are the major lipids ingested from meat or dairy sources, and are the major form of fatty acids present in adipose tissue triacylglycerols. The liver, as well as many other tissues, uses fatty acids as fuels when the concentration of the fatty acid–albumin complex is increased in the blood.

1. MEDIUM-CHAIN LENGTH FATTY ACID OXIDATION

The liver and certain cells in the kidney are the major sites for the oxidation of medium-chain-length fatty acids. These fatty acids usually enter the diet of infants

in maternal milk as medium-chain-length triacylglycerols (MCT). In the intestine, the MCT are hydrolyzed by gastric lipase, bile salt–dependent lipases, and pancreatic lipase more readily than long-chain triacylglycerols. Within the enterocytes, they are neither reconverted to triacylglycerols nor incorporated into chylomicrons. Instead, they are directly released into the portal circulation (fatty acids of approximately 8-carbon chain lengths or less are water-soluble). In the liver, they diffuse through the inner mitochondrial membrane and are activated to acyl CoA derivatives by medium-chain-length fatty acid activating enzyme (MMFAE), a family of similar isozymes present only in liver and kidney. The medium-chain fatty acyl-CoA is then oxidized by the normal route, beginning with medium-chain-length acyl CoA dehydrogenase (MCAD; see Chapter 23).

2.   PEROXISOMAL OXIDATION OF VERY-LONG-CHAIN FATTY ACIDS

Peroxisomes are present in greater number in the liver than in other tissues. Liver peroxisomes contain the enzymes for the oxidation of very-long-chain fatty acids such as C24:0 and phytanic acid, for the cleavage of the cholesterol side chain necessary for the synthesis of bile salts, for a step in the biosynthesis of ether lipids, and for several steps in arachidonic acid metabolism. Peroxisomes also contain catalase and are capable of detoxifying hydrogen peroxide.

Very-long-chain fatty acids of C20 to C26 or greater are activated to CoA derivatives by very-long-chain acyl CoA synthetase present in the peroxisomal membrane. The very-long-chain acyl CoA derivatives are then oxidized in liver peroxisomes to the 8-carbon octanoyl CoA level. In contrast to mitochondrial β-oxidation, the first enzyme in peroxisomal β-oxidation introduces a double bond and generates hydrogen peroxide instead of FAD(2H). The remainder of the cycle, however, remains the same, releasing NADH and acetyl CoA. Peroxisomal catalase inactivates the hydrogen peroxide, and the acetyl CoA can be used in biosynthetic pathways such as those of cholesterol and dolichol synthesis.

The octanoyl CoA that is the end-product of peroxisomal oxidation leaves the peroxisomes and the octanoyl group is transferred through the inner mitochondrial membrane by medium-chain-length acylcarnitine transferase. In the mitochondria, it enters the regular β-oxidation pathway, beginning with medium-chain-length acyl CoA dehydrogenase (MCAD).

3.   PEROXISOME PROLIFERATOR ACTIVATED RECEPTORS

The peroxisome proliferator activated receptors (PPAR) play an important role in liver metabolism. These receptors obtained their name from the finding that certain agonists were able to induce the proliferation of peroxisomes in liver. These agonists included hypolipidemic agents, nonsteroidal anti-inflammatory agents, and environmental toxins. The receptors that bind these agents, the PPAR, are members of a nuclear receptor family and, when activated, stimulate new gene transcription. Within the liver, the major form of PPAR directly regulates the activity of genes that are involved in fatty acid uptake and β- and ω-oxidation of fatty acids.

There are three major PPAR isoforms, α, δ/β, and γ,. The major form found in the liver is the α form. Fatty acids are an endogenous ligand for PPAR α, such that when the level of fatty acids in the circulation is increased (with a concurrent increase in the fatty acid content of hepatocytes), there is increased gene transcription for those proteins involved in regulating fatty acid metabolism (Table 46.5). Genetically altered mice have been generated that lack PPAR α. These "knockout" mice exhibit no abnormal phenotype when fed a normal diet. When fasted, however, or when fed a high-fat diet, these mice develop severe fatty infiltration of the liver. The inability to increase the rate of fatty acid oxidation in this organ leads to excessive fatty acid buildup in the hepatocytes. It also leads to an insufficient energy supply with which to make glucose (leading to hypoglycemia) as well as an inability to

Medium-chain triglycerides (MCT) are important components of nutritional supplements used in patients with digestive disorders. They therefore can be employed as an easily absorbed source of calories in patients who have a gastrointestinal (GI) disorder that may result in malabsorption of nutrients. These diseases include pancreatic insufficiency, intraluminal bile salt deficiency due to cholestatic liver disease, biliary obstruction, ileal disease or resection, and disease causing obstruction of intestinal lymphatics. Remember, however, that MCT do not contain polyunsaturated fatty acids that can be used for synthesis of eicosanoids (see Chapter 35).

Zellweger's (cerebrohepatorenal) syndrome occurs in individuals with a rare inherited absence of peroxisomes in all tissues. Patients accumulate C26-C38 polyenoic acids in brain tissue owing to defective peroxisomal oxidation of the very-long-chain fatty acids synthesized in the brain for myelin formation. In liver, bile acid and ether lipid synthesis are affected, as is the oxidation of very-long-chain fatty acids.

Table 46.5. Genes Regulated by Activation of PPAR-α

Fatty acid transport proteins
The mitochondrial and peroxisomal enzymes
 of fatty acid oxidation
Carnitine palmitoyl transferase I
HMG-CoA synthase
Apoprotein CIII (suppression)

The fibrates (e.g., clofibrate) are a class of drugs that bind to PPARs to elicit changes in lipid metabolism. They are typically prescribed for individuals with elevated triglyceride levels because they increase the rate of triglyceride oxidation. This, in turn, leads to a reduction in serum tri-acylglycerol levels. Fibrates, through PPAR-α stimulation, also suppress apoprotein CIII synthesis and stimulate LPL activity. Apo CIII normally inhibits LPL activity, so by reducing CIII synthesis overall, LPL activity is increased. Apo CIII also blocks apoprotein E on IDL particles, causing the IDL particles to accumulate because they cannot be taken up by the apo E receptor in the liver. The sup-pression of apo CIII levels allows more IDL to be endocytosed, thereby also reducing circu-lating triacylglycerol levels.

Reye's syndrome is characterized clinically by vomiting with signs of progressive central nervous system damage. In addition, there are signs of hepatic injury and hypoglycemia. There is mitochondrial dysfunction with decreased activity of hepatic mitochondrial enzymes. Hepatic coma may occur as serum ammonia levels rise. It is epidemiologically associated with the consumption of aspirin by children during a viral illness, but it may occur in the absence of exposure to salicylates. The inci-dence in the United States has decreased dramatically since the 1980s, when parents were made aware of the dangers of giving aspirin to children to reduce fever. Reye's syndrome is not necessarily confined to chil-dren. In patients who die of this disease, the liver at autopsy shows swollen and disrupted mitochondria and extensive accumulation of lipid droplets with fatty vacuolization of cells in both the liver and the renal tubules.

produce ketone bodies. In normal fasted mice or mice fed a high-fat diet, fatty acids would eventually stimulate their own oxidation, via peroxisome proliferation and by induction of other enzymes needed for their oxidation. The knockout mice cannot make these compensations.

4.   XENOBIOTICS METABOLIZED AS FATTY ACIDS

The liver uses the pathways of fatty acid metabolism to detoxify very hydrophobic and lipid-soluble xenobiotics that, like fatty acids, either have carboxylic acid groups or can be metabolized to compounds that contain carboxylic acids. Benzoate and salicylate are examples of xenobiotics that are metabolized in this way. Ben-zoate is naturally present in plant foods and is added to foods such as sodas as a pre-servative. Its structure is similar to salicylic acid (which is derived from the degra-dation of aspirin). Salicylic acid and benzoate are similar in size to medium-chain-length fatty acids and are activated to an acyl CoA derivative by MMFAE (Fig. 46.8). The acyl group is then conjugated with glycine, which targets the compound for urinary excretion. The glycine derivatives of salicylate and ben-zoate are called salicylurate and hippurate, respectively. Salicylurate is the major urinary metabolite of aspirin in humans. Benzoate has been administered to treat hyperammonemia associated with congenital defects, because urinary hippurate excretion tends to lower the free ammonia pool. Aspirin cannot be used for this pur-pose because it is toxic in the large doses required

5.   THE METABOLISM OF LIPIDS IN LIVER DISEASE

Chronic parenchymal liver disease is associated with relatively predictable changes in plasma lipids and lipoproteins. Some of these changes are related to a reduction in the activity of lethicin cholesterol acyltransferase (LCAT). This plasma enzyme is synthesized and glycosylated in the liver; then enters the blood, where it catalyzes the transfer of a fatty acid from the 2-position of lecithin to the 3β-OH group of free cholesterol to produce cholesterol ester and lysolecithin. As expected, in severe parenchymal liver disease, in which LCAT activity is decreased, plasma levels of cholesterol ester are reduced and free cholesterol levels normal or increased.
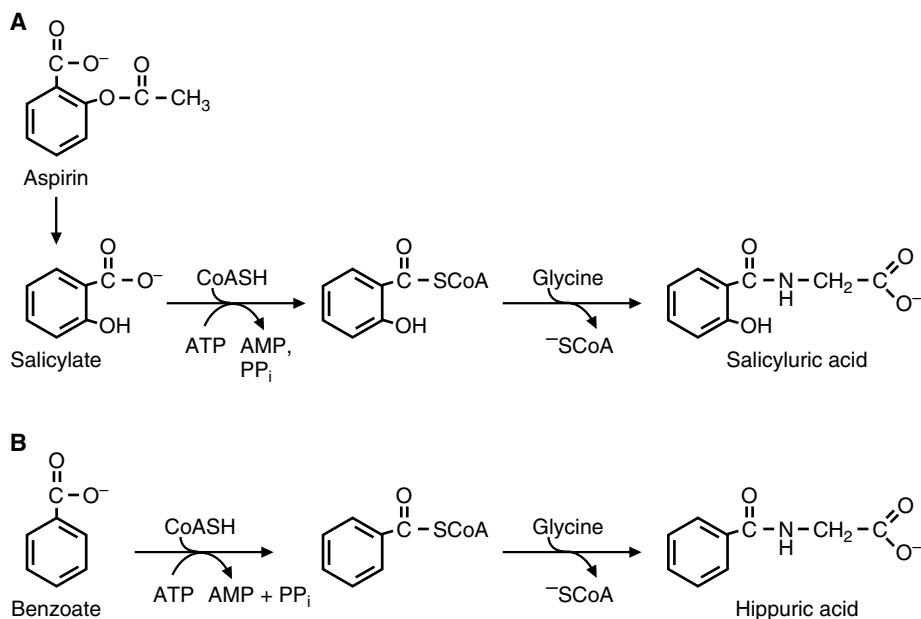

Fig. 46.8. Benzoate and salicylate metabolism.

Plasma triacylglycerols are normally cleared by peripheral lipases (lipoprotein lipase or LPL and hepatic triglyceride lipase or HTGL). Because the activities of both LPL and HTGL are reduced in patients with hepatacellular disease, a relatively high level of plasma triacylglycerols may be found in both acute and chronic hepatitis, in patients with cirrhosis of the liver, and in patients with other diffuse hepatocellular disorders.

With low LCAT activity and the elevated triacylglycerol level described, low-density lipoprotein (LDL) particles have an abnormal composition. They are relatively triacylglycerol rich and cholesterol ester poor.

High-density lipoprotein (HDL) metabolism may be abnormal in chronic liver disease as well. For example, because the conversion of HDL_3 (less antiatherosclerotic) to HDL_2 (more antiatherosclerotic) is catalyzed by LCAT, the reduced activity of LCAT in patients with cirrhosis leads to a decrease in the HDL_2:HDL_3 ratio. Conversely, the conversion of HDL_2 to HDL_3 requires hepatic lipases. If the activity of this lipase is reduced, one would expect an elevation in the HDL_2:HDL_3 ratio. Because the HDL_2:HDL_3 ratio is usually elevated in cirrhosis, the lipase deficiency appears to be the more dominant of the two mechanisms. These changes may result in an overall increase in serum total HDL levels. How this affects the efficiency of the reverse cholesterol transport mechanism and the predisposition to atherosclerosis is not fully understood.

With regard to triacylglycerol levels in patients with severe parenchymal liver disease, the hepatic production of the triacylglycerol-rich, very-low-density lipoprotein (VLDL) particle is impaired. Yet the total level of plasma triacylglycerols remains relatively normal because the LDL particle in such patients is triacylglycerol-rich, for reasons that have not been fully elucidated.

Non-esterified fatty acid (NEFA) levels are elevated in patients with cirrhosis. This change might be expected because basal hepatic glucose output is low in these patients. As a result, more NEFA are presumably required (via increased lipolysis) to meet the fasting energy requirements of peripheral tissues.

D. Amino Acid Metabolism in the Liver

The liver is the principle site of amino acid metabolism in humans. It essentially balances the free amino acid pool in the blood through the metabolism of amino acids supplied by the diet after a protein-containing meal and through metabolism of amino acids supplied principally by skeletal muscles during an overnight fast. In an adult who is no longer growing linearly, the total protein content of the body on a daily basis is approximately constant, such that the net degradation of amino acids (either to other compounds or used for energy) is approximately equal to the amount consumed. The key points concerning hepatic amino acid metabolism are the following:

1. The liver contains all the pathways for catabolism of all of the amino acids and can oxidize most of the carbon skeletons to carbon dioxide. A small proportion of the carbon skeletons are converted to ketone bodies. The liver also contains the pathways for converting amino acid carbon skeletons to glucose (gluconeogenesis) that can be released into the blood.
2. Because the liver is the principle site of amino acid catabolism, it also contains the urea cycle, the pathway that converts toxic ammonium ion to nontoxic urea. The urea is then excreted in the urine.
3. After a mixed or high-protein meal, the gut uses dietary aspartate, glutamate, and glutamine as a fuel (during fasting the gut uses glutamine from the blood as a major fuel). Thus, the ingested acidic amino acids do not enter the general circulation. The nitrogen from gut metabolism of these amino acids is passed to the liver as citrulline or ammonium ion via the portal vein.
4. The branched-chain amino acids (valine, leucine, and isoleucine) can be used by most cell types as a fuel, including cells of the gut and skeletal muscle. After a

high-protein meal, most of the branched-chain amino acids are not oxidized by the liver (because of very low activity of the branched-chain amino acid transaminase) and instead enter the peripheral circulation to be used as a fuel by other tissues or for protein synthesis (these amino acids are essential amino acids). The liver does, however, take up whatever amino acids it needs to carry out its own protein synthesis.

5. Most tissues transfer the amino acid nitrogen to the liver to dispose of as urea. They, therefore, produce either alanine (from the pyruvate–glucose–alanine cycle, in skeletal muscle, kidney, and intestinal mucosa) or glutamine (skeletal muscle, lungs, neural tissues) or serine (kidney), which are released into the blood and taken up by the liver.

6. The liver uses amino acids for the synthesis of proteins that it requires as well as for the synthesis of proteins to be used elsewhere. For example, the liver uses the carbon skeletons and nitrogens of amino acids for the synthesis of nitrogen-containing compounds such as heme, purines, and pyrimidines. The amino acid precursors for these compounds are all nonessential, because they can be synthesized in the liver.

E. Amino Acid Metabolism in Liver Disease

The concentration of amino acids in the blood of patients with liver disease is often elevated. This change is, in part, attributable to a significantly increased rate of protein turnover (general catabolic effect seen in severely ill patients) as well as to impaired amino acid uptake by the diseased liver. It is unlikely that the increased levels are due to degradation of liver protein and the subsequent release of amino acids from the failing hepatocyte into the blood. This is true because the total protein content of the liver is only approximately 300 g. To account for the elevated amino acid levels in the blood, the entire protein content of the liver would have to be degraded within 6 to 8 hours to account for the increased protein turnover rates found. Because 18 to 20 times more protein is present in skeletal muscle (greater mass), the muscle is probably the major source of the elevated plasma levels of amino acids seen in catabolic states such as cirrhosis of the liver.

In cirrhotic patients, such as **Jean Ann Tonich**, the fasting blood α-amino nitrogen level is elevated as a result of reduced clearance. Urea synthesis is reduced as well.

The plasma profile of amino acids in cirrhosis characteristically shows an elevation in aromatic amino acids, phenylalanine and tyrosine, and in free tryptophan and methionine. The latter changes may be caused by impaired hepatic utilization of these amino acids as well as to portosystemic shunting. Although the mechanism is not known, a reduction in fasting plasma levels of the branched-chain amino acids (BCAA) is also seen in cirrhotic patients. These findings, however, must be interpreted with caution because most of the free amino acid pool in humans is found in the intracellular space. Therefore, changes seen in their plasma concentrations do not necessarily reflect their general metabolic fate. Yet the elevation in aromatic amino acids and the suppression of the level of BCAAs in the blood of cirrhotics have been implicated in the pathogenesis of hepatic encephalopathy.

V. DISEASES OF THE LIVER

Diseases of the liver can be clinically and biochemically devastating, because no other organ can compensate for the loss of the multitude of functions that the liver normally performs. Alcohol-induced liver disease has been discussed in Chapter 25. A number of diseases can lead to hepatic fibrosis (see Biochemical Comments)

Unlike **Amy Biasis**, whose hepatic amoebic disorder was more localized (abscesses), **Jean Anne Tonich** had a diffuse hepatic disease, known as alcohol-induced cirrhosis (historically referred to as "Laennec's cirrhosis"). The latter is characterized by diffuse fine scarring, a fairly uniform loss of hepatic cells, and the formation of small regenerative nodules (sometimes referred to as "micronodular cirrhosis"). With continued alcohol intake, fibroblasts and activated stellate cells deposit collagen at the site of persistent injury. This leads to the formation of weblike septa of connective tissue in periportal and pericentral zones. These eventually connect portal triads and central veins. With further exposure to alcohol, the liver shrinks and then becomes nodular and firm as "end-stage" cirrhosis develops. Unless successfully weaned from alcohol, these patients eventually die of liver failure. **Amy Biasis,** however, can probably look forward to enjoying normal liver function after successful amoebicidal therapy without evidence of residual hepatic scarring.

and cirrhosis. When this occurs to a great enough extent, liver function becomes inadequate for life. Signs and symptoms of liver disease include elevated levels of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the plasma (due to hepatocyte injury or death with a consequent release of these enzymes into the blood), jaundice (an accumulation of bilirubin in the blood caused by inefficient bilirubin glucuronidation by the liver; see Chapter 45), increased clotting times (the liver has difficulty producing clotting factors for secretion), edema (reduced albumin synthesis by the liver leads to a reduction in osmotic pressure in the blood), and hepatic encephalopathy (reduced urea cycle activity leading to excessive levels of ammonia and other toxic compounds in the central nervous system).

Alanine aminotransferase and aspartate aminotransferase are the modern versions of older names. ALT was formally known as serum glutamic pyruvate transaminase (SPGT), and AST was formally known as serum glutamic oxaloacetic transaminase (SGOT).

CLINCAL COMMENTS

Patients with cirrhosis of the liver who have no known genetic propensity to glucose intolerance, such as **Jean Ann Tonich**, tend to have higher blood glucose levels than do normal subjects in both fasting and fed states. The mechanisms that may increase glucose levels in the fasting state include a reduction in the metabolic clearance rate of glucose by 25 to 40% compared with normal subjects. This reduction in glucose clearance results, in part, from increased oxidation of fatty acids and ketone bodies and the consequent decrease in glucose oxidation by peripheral tissues in cirrhosis patients. This is suggested by the discovery that plasma non-esterified fatty acid (NEFA) levels are high in many patients with hepatocellular dysfunction, in part because of decreased hepatic clearance of NEFA and in part because of increased adipose tissue lipolysis. Another possible explanation for the reduction in whole body glucose utilization in cirrhotic patients relates to the finding that ketone body production is increased in some patients with cirrhosis. This could lead to enhanced utilization of ketone bodies for fuel by the central nervous system in such patients, thereby reducing the need for glucose oxidation by the highly metabolically active brain.

After glucose ingestion (fed state), many patients with liver disease have abnormally elevated blood glucose levels ("hepatogenous diabetes"). Using World Health Organization (WHO) criteria, 60 to 80% of cirrhotic patients have varying degrees of glucose intolerance, and overt diabetes mellitus occurs 2 to 4 times as often in cirrhotics than it does in subjects without liver disease. The proposed mechanisms include a degree of insulin resistance in peripheral tissues; however, as the cirrhotic process progresses, they develop a marked impairment of insulin secretion as well. Although the mechanisms are not well understood, this decrease in insulin secretion leads to increased hepatic glucose output (leading to fasting hyperglycemia) and reduced suppression of hepatic glucose output after meals, leading to postprandial hyperglycemia as well. If the patient has an underlying genetic predisposition to diabetes mellitus, the superimposition of the mechanisms outlined above will lead to an earlier and more significant breakdown in glucose tolerance in these specific patients.

BIOCHEMICAL COMMENTS

Extensive and progressive fibrosis of the hepatic parenchyma leads to cirrhosis of the liver, a process that has many causes. The development of fibrosis requires the activities of hepatic stellate cells, cytokines, proteases, and protease inhibitors.

A major change that occurs when fibrosis is initiated is that the normally "sparse" or "leaky" basement membrane between the endothelial cells and the hepatocyte is replaced with a high-density membrane containing fibrillar collagen. This occurs because of both an increased synthesis of a different type of collagen than is normally produced and a reduction in the turnover rate of existing extracellular matrix components.

The supportive tissues of the normal liver contain an extracellular matrix that, among other proteins, includes type IV collagen (which does not form fibers), glycoproteins, and proteoglycans. After a sustained insult to the liver, a threefold to eightfold increase occurs in extracellular matrix components, some of which contain fibril-producing collagen (types I and III), glycoproteins, and proteoglycans. The accumulation of these fibril-producing compounds leads to a loss of endothelial cell fenestrations and, therefore, a loss of the normal sieve-like function of the basement membranes. These changes interfere with normal transmembrane metabolic exchanges between the blood and hepatocytes.

The hepatic stellate cell is the source of the increased and abnormal collagen production. These cells are activated by growth factors whose secretion is induced by injury to the hepatocytes or endothelial cells. Growth factors involved in cellular activation include TGF-β1 (which is derived from the endothelial cells, Kupffer cells, and platelets) and platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) from platelets. The release of PDGF stimulates stellate cell proliferation and, in the process, increases their synthesis and release of extracellular matrix materials and remodeling enzymes. These enzymes include matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs, as well as converting (activating) enzymes. This cascade leads to the degradation of the normal extracellular matrix and replacement with a much denser and more rigid type of matrix material. These changes are, in part, the result of an increase in the activity of tissue inhibitors of MMP's for the new collagen relative to the original collagen in the extracellular matrix.

One consequence of the increasing stiffness of the hepatic vascular channels through which hepatic blood must flow is a greater resistance to the free flow of blood through the liver as a whole. Resistance to intrahepatic blood flow is also increased by a loss of vascular endothelial cell fenestrations, loss of free space between the endothelial cells and the hepatocytes (space of Disse), and even loss of vascular channels per se. This increased vascular resistance leads to an elevation in intrasinusoidal fluid pressure. When this intrahepatic (portal) hypertension reaches a critical threshold, the shunting of portal blood away from the liver (portosystemic shunting) further contributes to hepatic dysfunction. If the portal hypertension cannot be reduced, portal blood will continue to bypass the liver and return to the heart through the normally low-pressure esophageal veins. When this increasing intraesophageal venous pressure becomes severe enough, the walls of these veins thin dramatically and expand to form varices, which may suddenly burst, causing life-threatening esophageal variceal hemorrhage. This is a potentially fatal complication of cirrhosis of the liver.

Suggested Reading

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✎ REVIEW QUESTIONS—CHAPTER 46

1. Drinking grapefruit juice while taking statins can lead to potentially devastating side effects. This is due to a component of grapefruit juice doing which of the following?

    (A) Interfering with hepatic uptake of statins
    (B) Accelerating the conversion of the statin to a more toxic form
    (C) Inhibiting the inactivation of statins
    (D) Upregulating the HMG CoA reductase
    (E) Downregulating the HMG CoA reductase

2. Which one of the following characteristics of cytochrome P450 enzymes is correct?

    (A) They are all found in the Golgi apparatus and are referred to as microsomal enzymes.
    (B) They all contain a flavin-containing reductase unit that uses NADH and not NADPH as a source of electrons.
    (C) They are all inducible by oxygen, which binds to the iron of the cytochrome.
    (D) They all oxidize the substrate on which they act.
    (E) They all generate a free radical compound as final products of the reaction.

3. Fairly predictable changes occur in the various metabolic pathways of lipid metabolism in patients with moderately advanced hepatocellular disease. Which one of the following changes would you expect to see under these conditions?

    (A) The activity of plasma lecithin cholesterol acyltransferase (LCAT) is increased.
    (B) Serum cholesterol esters are increased.
    (C) Hepatic triglyceride lipase (HTGL) activity is increased.
    (D) Serum triacylglycerol levels are increased.
    (E) Serum nonesterified fatty acid levels are decreased.

4. After a 2-week alcoholic binge, **Jean Ann Tonich** ingested some Tylenol to help her with a severe headache. She took three times the suggested dose because of the severity of the pain. However, within 24 hours Jean Ann became very lethargic, vomited frequently, and developed severe abdominal pain. The symptoms Jean Ann is experiencing are attributable to a reaction to the Tylenol due to which of the following?

    (A) The hypoglycemia experienced by the patient
    (B) Ethanol-induced inhibition of Tylenol metabolism
    (C) The hyperglycemia experienced by the patient
    (D) Ethanol-induced acceleration of Tylenol metabolism
    (E) Acetaminophen inhibition of VLDL secretion by the liver

5. An individual displays impaired glucose tolerance; blood glucose levels remain elevated after a meal for a longer time than is normal, although they do eventually go down to fasting levels. The patient has a normal release of insulin from the pancreas in response to elevated blood glucose levels. Fibroblasts obtained from the patient display normal levels of insulin binding to its receptor, and normal activation of the intrinsic tyrosine kinase activity associated with the insulin receptor. Analysis of glucose 6-phosphate formation within the fibroblasts, however, indicated a much slower rate of formation than in fibroblasts obtained from a normal control. A possible mutation that could lead to these results is which of the following?

    (A) A decrease in the K_m of glucokinase
    (B) An increase in the V_{max} of glucokinase
    (C) A nonfunctional glucokinase regulatory protein
    (D) An increase in hexokinase activity
    (E) A decrease in hexokinase activity