CHAPTER 12
BIOCHEMICAL AND METABOLIC PRINCIPLES

Kurt C. Kleinschmidt and Kathleen A. Delaney

Xenobiotics are compounds that are foreign to a living system. Toxic xenobiotics interfere with critical metabolic processes, causing structural damage to cells or altering the cellular genetic material. The specific biochemical sites of actions that disrupt metabolic processes are well characterized for many xenobiotics although mechanisms of cellular injury are not. This chapter reviews the biochemical principles that are relevant to an understanding of the damaging effects of toxic xenobiotics and the biotransformation enzymes and their clinical implications.

The capacity of a xenobiotic to produce injury is affected by many factors including its absorption, distribution, elimination, site of activation or detoxification, site of action, and capability of cross membranes to access a particular organ. Sites of action include the active sites of enzymes, DNA, and lipid membranes. The route of exposure to a xenobiotic may confine damage primarily to one organ, for example, pulmonary injury that follows inhalation or GI injury that follows a caustic ingestion. Hepatocellular injury results when a toxic xenobiotic is delivered to the liver, either by the portal venous system following ingestion or by the hepatic artery carrying blood with xenobiotics absorbed from other sites of exposure. Various factors affect the ability of a xenobiotic to access a particular organ. For example, many potentially toxic xenobiotics fail to produce CNS injury because they cannot cross the blood–brain barrier. The negligible CNS effects of the mercuric salts when compared with organic mercury compounds are related to their inability to penetrate the CNS. Two potent biologic xenobiotics—ricin (from *Ricinus communis*) and α-amanitin (from *Amanita phalloides*)—block protein synthesis through the inhibition of RNA polymerase. However, they cause different clinical effects because of access to different tissues. Ricin has a special binding protein that enables it to gain access to the endoplasmic reticulum in GI mucosal cells, where it inhibits cellular protein synthesis and causes severe diarrhea.[4] α-Amanitin is transported into hepatocytes by bile salt transport systems, where inhibition of protein synthesis results in cell death.[50,55] The electrical charge on a toxin also affects its ability to enter a cell. Unlike the ionized (charged) form of a xenobiotic, the uncharged form is lipophilic and passes easily through lipid cell membranes to enter the cells. The pK_a of an acidic xenobiotic ($HA \leftrightarrow A^- + H^+$) is the pH at which 50% of the molecules are charged (A^- form) and 50% are uncharged (HA form). A xenobiotic with a low pK_a is more likely to be absorbed in an acidic environment where the uncharged form predominates.

GENERAL ENZYME CONCEPTS

The capability to detoxify and eliminate both endogenous toxins and xenobiotics is crucial to the maintenance of physiologic homeostasis and normal metabolic functions. A simple example is the detoxification of cyanide, a potent cellular poison that is common in the environment and is also a product of normal metabolism. Mammals have evolved the enzyme rhodanese, which combines cyanide with thiosulfate to create the less toxic, renally excreted compound thiocyanate.[6]

Most xenobiotics have lipophilic properties that facilitate absorption across cell membranes in organs that are portals of entry into the body: the skin, GI tract, and lungs. The liver has the highest concentration of enzymes that metabolize xenobiotics. Enzymes found in the liquid matrix of hepatocytes, the cytosol, that are specific for alcohols, aldehydes, esters, or amines act on many different substrates within these broad chemical classes. Enzymes that act on more lipophilic xenobiotics, including the cytochrome P450 (CYP) enzymes, are embedded in the lipid membranes of the cytosol-based endoplasmic reticulum. When cells are mechanically disrupted and centrifuged, these membrane bound enzymes are found in the pellet, or microsomal fraction, hence they are called *microsomal enzymes*. Enzymes located in the liquid matrix of cells are called *cytosolic enzymes* and are found in the supernatant when disrupted cells are centrifuged.[19]

BIOTRANSFORMATION OVERVIEW

The study of xenobiotic metabolism was established as a scientific discipline by the seminal publication of Williams in 1949.[94] Biotransformation is the physiochemical alteration of a xenobiotic, usually as a result of enzyme action. Most definitions also include that this action converts lipophilic substances into more polar, excretable substances.[56,87] The chemical nature of the xenobiotic determines whether it will undergo biotransformation; however, most undergo some degree of biotransformation. The hydrophilic nature of ionized compounds such as carboxylic acids enables the kidneys to rapidly eliminate them. Very volatile compounds, such as enflurane, are expelled promptly via the lungs. Neither of these groups of xenobiotics undergo significant enzymatic metabolism.

Biotransformation usually results in "detoxification," a reduction in the toxicity, by the conversion to hydrophilic metabolites of the xenobiotic that can be renally eliminated.[56] However, this is not always the case. Many parent xenobiotics are inactive and must undergo "metabolic activation," a classic concept introduced in 1947.[59] When metabolites are more toxic than the parent xenobiotic, biotransformation has resulted in "toxification."[87] Biotransformation via acetylation or methylation may enhance the lipophilicity of a xenobiotic. Biotransformation is done by impressively few enzymes, reflecting broad substrate specificity. The predominant pathway for the biotransformation of an individual xenobiotic is determined by many factors including the availability of cofactors, changes in the concentration of the enzyme caused by induction, and the presence of inhibitors. The predominant pathway is also affected by the rate of substrate metabolism, reflected by the K_m (Michaelis-Menten dissociation constant) of the biotransformation enzyme.[87] (Chap. 8)

Biotransformation is often divided into phase I and phase II reactions, terminology first introduced in 1959.[95] Phase I reactions prepare lipophilic xenobiotics for the addition of functional groups or actually add the groups, converting them into more chemically reactive metabolites. This is usually followed by phase II synthetic reactions that conjugate the reactive products of phase I with other molecules that render them more water soluble, further detoxifying the xenobiotics and facilitating their elimination. However, biotransformation often does not follow this stepwise process and it has been suggested that phase I and II terminology be eliminated.[42] Some xenobiotics undergo only a phase I or a phase II reaction prior to elimination. Phase II reactions can precede phase I. While virtually all phase II synthesis reactions cause inactivation, a classic exception is fluoroacetate being metabolized to fluorocitrate, a potent inhibitor of the tricarboxylic acid cycle.[70]

Biotransformed xenobiotics cannot be eliminated until they are moved back across cell membranes, out of the cells. Membrane transporters are proteins that move agents across the membranes without

altering their chemical compositions, a process called a phase III reaction because it typically occurs after biotransformation.[42] However, membrane transport does not always occur after phase I or II reactions. Some parent compounds are transported across membranes without any biotransformation at all.

PHASE I BIOTRANSFORMATION REACTIONS

Oxidations are the predominant phase I reactions, adding reactive functional groups suitable for conjugation during phase II. These groups include hydroxyl (–OH), sulfhydryl (–SH), amino ($–NH_2$), aldehyde (–COH), or carboxyl (–COOH) moieties. Noncarbon elements such as nitrogen, sulfur, and phosphorus are also oxidized in phase I reactions. Other phase I reactions include hydrolysis (the splitting of a large molecule by the addition of water that is divided among the 2 products), hydration (incorporation of water into a complex molecule), hydroxylation (the attachment of –OH groups to carbon atoms), reduction, dehalogenation, dehydrogenation, and dealkylation.[56,87]

The CYP enzymes are the most numerous and important of the phase I enzymes. A common oxidation reaction catalyzed by CYP enzymes is illustrated by the hydroxylation of a xenobiotic R–H to R–OH (Fig. 12–1).[25] Membrane-bound flavin monooxygenase (FMO), an NADPH-dependent oxidase located in the endoplasmic reticulum, is an important oxidizer of amines and other compounds containing nitrogen, sulfur, or phosphorus.[56]

The alcohol, aldehyde, and ketone oxidation systems use predominantly cytosolic enzymes that catalyze these reactions using NADH/NAD^+.[53,87] Two classic phase I oxidation reactions are the metabolism of ethanol to acetaldehyde by alcohol dehydrogenase (ADH) followed by the metabolism of acetaldehyde to acetic acid by aldehyde dehydrogenase (ALDH) (Fig. 12–2). Alcohol dehydrogenase, which oxidizes many different alcohols, is found in the liver, lungs, kidney, and gastric mucosa.[53] Women have less ADH in their gastric mucosa than men. This results in decreased first-pass metabolism of alcohol and increased alcohol absorption. Some populations, particularly Asians, are deficient in ALDH, resulting in increased acetaldehyde concentrations and symptoms of the acetaldehyde syndrome[53] (Chap. 79).

OXIDATION OVERVIEW

Biotransformation often results in the oxidation or reduction of carbon. A substrate is oxidized when it transfers electrons to an electron-seeking (electrophilic or oxidizing) molecule, leading to reduction of the electrophilic molecule. These oxidation-reduction reactions are usually coupled to the cyclical oxidation and reduction of a cofactor, such as the pyridine nucleotides, nicotinamide adenine dinucleotide phosphate (NADPH/$NADP^+$) or nicotinamide adenine dinucleotide (NADH/NAD^+). The nucleotides alternate between their reduced (NADPH, NADH) and oxidized ($NADP^+$, NAD^+) forms. Since xenobiotic oxidation is the most common phase I reaction, the reduced cofactors must have a place to unload their electrons; otherwise, biotransformation ends. The electron transport chain is the major electron recipient.

Electrons resulting from the catabolism of energy sources are extracted primarily by NAD^+, forming NADH. Within the mitochondria,

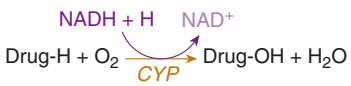

FIGURE 12–1. A common oxidation reaction catalyzed by CYP enzymes: the hydroxylation of Drug-H to Drug-OH.

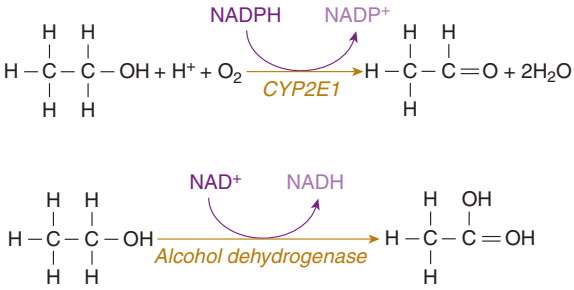

FIGURE 12–2. Conversion of ethanol to acetaldehyde by CYP2E1 that uses NADPH and oxygen and by alcohol dehydrogenase that uses NAD^+. This illustrates how NAD and NADP can function in oxidation reactions in both their oxidized and reduced forms. Alcohol dehydrogenase has a low K_m for ethanol and is the predominant metabolic enzyme in moderate drinkers.

NADH transports its electrons to the cytochrome-mediated electron transport chain. This results in the production of adenosine triphosphate (ATP), the reduction of molecular oxygen, and the regeneration of NAD^+—all parts critical to the maintenance of oxidative metabolism. NADPH, created within the hexose monophosphate shunt, is used in the synthetic (anabolic) reactions of biosynthesis (especially fatty acid synthesis). NADPH is also coupled to the reduction of glutathione, which plays an important role in the protection of cells from oxidative damage.

The oxidation state of a specific carbon atom is determined by counting the number of hydrogen and carbon atoms to which it is connected. The more reduced a carbon, the higher the number of connections. For example, the carbon in methanol (CH_3OH) has three carbon-hydrogen bonds and is more reduced than the carbon in formaldehyde ($H_2C=O$), which has two. Carbon–carbon double bonds count as only one connection.

CYTOCHROME ENZYMES—AN OVERVIEW

Cytochromes are a class of hemoprotein enzymes whose function is electron transfer, using a cyclical transfer of electrons between oxidized (Fe^{3+}) or reduced (Fe^{2+}) forms of iron. One type of cytochrome is cytochrome P450 (CYP) whose nomenclature derives from the spectrophotometric characteristics of its associated heme molecule. When bound to carbon monoxide, the maximal absorption spectrum of the reduced CYP (Fe^{2+}) enzyme occurs at 450 nm.[63] CYP enzymes, which incorporate one atom of oxygen into the substrate and one atom into water, were once called *mixed-function oxidases*. This activity is now referred to as a *microsomal monooxygenation reaction*.[63,87]

Cytochrome enzymes perform many functions. The biotransformation CYP enzymes are bound to the lipid membranes of the smooth endoplasmic reticulum. They execute 75% of all xenobiotic metabolisms and most phase I oxidative biotransformations of xenobiotics.[34] A second role for CYP enzymes is synthetic: biotransforming endobiotics (chemicals endogenous to the body) to cholesterol, steroids, bile acids, fatty acids, prostaglandins, and other important lipids. Cytochromes also act as electron transfer xenobiotics within the mitochondrial electron transport chain.[36,63]

While over 6000 CYP genes exist in nature, the human genome project completed in 2003 set the number of human CYP genes at 57.[63] CYP enzymes are categorized according to the similarities of their

amino acid sequences. They are in the same "family" if they are more than 40% comparable and same "subfamily" if they are more than 55% similar. Families are designated by an Arabic numeral, subfamilies by a capital letter, and each individual enzyme by another numeral, resulting in the nomenclature CYPnXm for each enzyme. For example, CYP3A4 is enzyme number 4 of the CYP3 family and of the CYP3A subfamily.[32,61] Most xenobiotic metabolism is done by the CYP1, CYP2, and CYP3 families, with a small amount done by the CYP4 family.[12,92] While 15 CYP enzymes metabolize xenobiotics,[69] nearly 90% is done by 6 CYP enzymes: 1A2, 2C9, 2C19, 2D6, 2E1, and 3A4 (Table 12–1).[63]

Most CYP enzymes are found in the liver, where they comprise 2% of total microsomal protein.[69] High concentrations are also in extrahepatic tissues, particularly the gastrointestinal tract and kidney.[21,64] The lungs,[97] heart,[66] and brain[22] have the next highest amounts. Each tissue has a unique profile of CYP enzymes that determines its sensitivity to different xenobiotics.[21] The CYP enzymes in the enterocytes of the small intestine actually contribute significantly to "first-pass" metabolism of some xenobiotics.[44,63] Corrected for tissue mass, the CYP enzyme system in the kidneys is as active as that in the liver. The activity of the renal CYP enzymes is decreased in patients with chronic renal failure, with relative sparing of CYPs 1A2, 2C19, and 2D6 compared with 3A4 and 2C9.[12]

CYTOCHROME P450 ENZYME SPECIFICITY FOR SUBSTRATES

In vitro models have been used to define the specificities of CYP enzymes for their substrates and inhibitors. However, activity in a test tube does not always correlate with that in a cell. These models use substrate and inhibitor concentrations that are much higher than would be encountered in vivo, and the mathematical models that extrapolate to clinically relevant processes yield conflicting results. This has resulted in discrepancies in reported substrates, inhibitors, and inducers of specific CYP enzymes.[93]

The substrate specificity of a CYP enzyme greatly affects its role in biotransformation.[34] CYP enzymes involved in endobiotic biotransformation are highly selective. For example, CYP1A2 specifically catalyzes only the 21-hydroxylation of progesterone, an important step in steroid synthesis.[35] Most CYP enzymes involved in xenobiotic biotransformation have broad substrate specificity and can metabolize many xenobiotics.[34] This is fortunate because the number of xenobiotic substrates may exceed 200,000.[52] Broad substrate specificity often results in multiple CYP enzymes being able to biotransform a xenobiotic. This enables the ongoing biotransformation despite an inhibition or deficiency of an enzyme. When a substrate can be biotransformed by more than one enzyme, the one that has the highest affinity for the substrate usually predominates at low substrate concentrations, while enzymes with lower affinity may be very important at high concentrations. This transition is usually concomitant with, but not dependent on, the saturation of the catalytic capacity of the primary enzyme as it reaches its maximum rate of activity.[34] The K_m, which is defined as the concentration of enzyme that results in 50% of maximal enzyme activity, describes this property of enzymes. For example, ADH in the liver has a very low Km for ethanol, making it the primary metabolic enzyme for ethanol when concentrations are low.[53] Ethanol is also biotransformed by the CYP2E1 enzyme, which has a high K_m for ethanol and only functions when concentrations are high. The CYP2E1 enzyme metabolizes little ethanol in moderate drinkers but accounts for significantly more

TABLE 12–1. Characteristics of Different Cytochrome P450 Enzymes[1,12,21,25,51,63,67]

Enzyme	1A2	2C9	2C19	2D6	2E1	3A4
Percent of liver CYPs	2%	10%–20%	10%–20%	30%	7%	40%–55%
Contribution to enterocyte CYPs	Minor	Minor	Minor	Minor	Minor	70%
Organs other than liver with enzyme	Lung	Small intestine, nasal mucosa, heart	Small intestine, nasal mucosa, heart	Small intestine, kidney, lung, heart	Lung small intestine, kidney	Much in small intestine; some in kidney, nasal mucosa, lung, stomach
Percent of metabolism of typically used drugs	2%–15%	10%–15%		25%–30%		50%–60%
Polymorphism[a]	No	Yes	Yes	Yes	No	No
Poor metabolizer						
African American		1%–2%	20%	2%–8%		
Asian		1%–2%	15%–20%	>1%		
White		1%–3%	3%–5%	5%–10%		
Ultra extensive metabolizer						
Asian				1%		
Ethiopian				30%		
Northern Europeans				1%–2%		
Southern Europeans				10%		

[a] Enzyme variations exist even in those listed as "No" for polymorphism.

biotransformation in alcoholics. As another example, diazepam is metabolized by both CYP2C19 and CYP3A4 enzymes. However, the affinity of CYP3A4 for diazepam is so low (ie, the K_m is high) that most diazepam is metabolized by CYP2C19.[37]

The substrate selectivity of some CYP enzymes is determined by molecular, and physicochemical properties of the substrates. The CYP1A subfamily has greater specificity for planar polyaromatic substrates such as benzo[*a*]pyrene. The CYP2E enzyme subfamily targets low-molecular-weight hydrophilic xenobiotics, whereas the CYP3A4 enzyme has increased affinity for lipophilic compounds. Substrates of CYP2C9 are usually weakly acidic, whereas those of CYP2D6 are more basic.[51] High specificity can also result from key structural considerations such as stereoselectivity. Some xenobiotics are racemic mixtures of stereoisomers. These may be substrates for different CYP enzymes and have distinct affinities for the enzymes, resulting in different rates of metabolism. For example, R-warfarin is biotransformed by CYP3A4 and CYP1A2, whereas S-warfarin is metabolized by CYP2C9.[78,87]

The CYP enzymes that biotransform a specific xenobiotic cannot be predicted by its drug class. Whereas fluoxetine and paroxetine are both major substrates and potent inhibitors of CYP2D6, sertraline is not extensively metabolized and exhibits minimal interaction with other antidepressants.[5] Most β-hydroxy-β-methylglutarylcoenzyme A (HMG-CoA) reductase inhibitors are metabolized by CYP3A4 (lovastatin, simvastatin, and atorvastatin); however, fluvastatin is metabolized by CYP2D6 and pravastatin undergoes virtually no CYP enzyme metabolism at all.[32] Among angiotensin-II receptor blockers, losartan and irbesartan are metabolized by CYP2C9, while valsartan, eprosartan, and candesartan are not substrates for any CYP enzyme. In addition, losartan is a prodrug whose active metabolite provides most of the pharmacologic activity, while irbesartan is the primary active compound. For these two drugs the inhibition of CYP2C9 is predicted to have opposite effects.[28]

CYTOCHROME P450 AND DRUG–DRUG/ DRUG–CHEMICAL INTERACTIONS

Adverse reactions to medications and drug–drug interactions are common causes of morbidity and mortality in hospitalized patients, the risk of which increases with the number of drugs taken (Chaps. 138 and 139). Fifty percent of adverse reactions may be related to pharmacogenetic factors.[30] The most significant interactions are mediated by CYP enzymes.[63] The impact of genetic polymorphism and enzyme induction or inhibition are addressed below.

CYP enzymes are involved in many types of drug interactions. The ability of potential new drugs to induce or inhibit enzymes is an important consideration of industry. Drug development focuses on the potential of new xenobiotics to induce or inhibit during the drug discovery phase. Various in vitro models have been created to enable this early determination.[54]

Many xenobiotics interact with the CYP enzymes. St. John's wort, an herb marketed as a natural antidepressant, induces multiple CYP enzymes including 1A2, 2C9, and 3A4. The induction of CYP3A4 by St. John's wort is associated with a 57% decrease in effective serum concentrations of indinavir when given concomittantly.[71] Xenobiotics contained in grapefruit juice, such as naringin and furanocoumarins, are both substrates and inhibitors of CYP3A4. They inhibit the first-pass metabolism of CYP3A4 substrates by inhibiting CYP3A4 activity in both the gastrointestinal tract and the liver.[18] Polycyclic hydrocarbons found in charbroiled meats and in cigarette smoke induce CYP1A2. For smokers who drink coffee, concentrations of caffeine, a CYP1A2 substrate, will be increased following cessation of smoking.[25]

GENETIC POLYMORPHISM

There is much variation in response to xenobiotics and to coadministration of inhibitory or inducing xenobiotics. The translation of DNA sequences into proteins results in the phenotypic expression of the genes. When a genetic mutation occurs, the changed DNA may continue to exist, be eliminated, or propagate into a polymorphism. A polymorphism is a genetic change that exists in at least 1% of the human population.[30] A polymorphism in a biotransformation enzyme may change its rate of activity. The heterogeneity of CYP enzymes contributes to the differences in metabolic activity between patients.[30] Differences in biotransformation capacity that lead to toxicity, once thought to be "idiosyncratic" drug reactions, are likely caused by these inherited differences in the genetic complement of individuals.

The normal catalytic speed of CYP enzyme activity is called *extensive*. There are 2 major metabolizer phenotypes due to polymorphism: poor (slow) and ultraextensive (rapid).[61,30] The CYP2C19 and CYP2D6 genes are highly polymorphic (Table 12–1).[41] The CYP2D6 gene, which has 76 different alleles, is associated with both ultraextensive and poor metabolism. The CYP2C19 and CYP2C9 genes are both associated with poor metabolizers.[12,63]

The clinical implications of polymorphisms are vast. A prodrug may not be bioactivated because the patient is a poor metabolizer. Conversely, a drug may not reach a therapeutic concentration because the patient is an ultraextensive metabolizer.[30]

Polymorphisms exist for enzymes other than CYP enzymes. A classic one is the inheritance of rapid or slow "acetylator" phenotypes. Acetylation is important for the biotransformation of amines ($R–NH_2$) or hydrazines ($NH_2–NH_2$). Slow acetylators are at increased risk of toxicity associated with the slower biotransformation of certain nitrogen-containing xenobiotics such as isoniazid, procainamide, hydralazine, and sulfonamides.[24,80]

Polymorphic genes that code for enzymes in important metabolic pathways affect the toxicity of a xenobiotic by altering the response to or the disposition of the xenobiotic. An example occurs in glucose-6-phosphate dehydrogenase (G6PD) deficiency. G6PD is a critical enzyme in the hexose monophosphate shunt, a metabolic pathway located in the red blood cell (RBC) that produces NADPH, which is required to maintain RBC glutathione in a reduced state. In turn, reduced glutathione prevents hemolysis during oxidative stress.[14] In patients deficient in G6PD, oxidative stress produced by electrophilic xenobiotics results in hemolysis.

INDUCTION OF CYP ENZYMES

Biotransformation by induced CYP enzymes results in either increased activity of prodrugs or enhanced elimination of drugs. Stopping an inducing agent may result in the opposite effects. Either way, maintaining therapeutic concentrations of affected drugs is difficult, resulting in either toxicity or subtherapeutic concentrations. Interestingly, not all CYP enzymes are inducible. Inducible ones include CYP2A, CYP2B, CYP2C, CYP2E, and CYP3A.[54]

While varied mechanisms of induction exist, the most common and significant is nuclear receptor (NR)-mediated increase in gene transcription.[54] Nuclear receptors are the largest group of transcription factors, proteins that switch genes on or off.[90] They regulate reproduction, growth, and biotransformation enzymes, including CYP enzymes.[88] Nuclear receptors exist mostly within the cytoplasm of cells. The CYP families 2 and 3 both have gene activation triggered through the nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR). The CYP 1A subfamily uses the aryl hydrocarbon receptor (AhR) as its NR. Ligands, molecules that bind to and affect the reactivity of a central molecule, are typically small and lipophilic,

enabling them to enter cells. Many xenobiotics are ligands. Ligands bind the NRs, resulting in structural changes that enable the NR-ligand complexes to be translocated into the cell nucleus. Within the nucleus, NR-ligand complexes bind to a heterodimerization partner such as retinoid X receptor (RXR), shared by PXR and CAR, or AhR nuclear translocator (Arnt), by AhR. This new complex then interacts with specific response elements of DNA, initiating the transcription of a segment of DNA, and resulting in the phenotypic expression of the respective CYP enzyme.

The ligand binding domain of the PXR receptor is very hydrophobic and flexible, enabling this pocket to bind many substrates of varied sizes and reflecting why PXR can be activated by such a broad group of ligands.[67,88] For example, xenobiotic ligands that bind the NR PXR that targets the *CYP3A4* gene include rifampin, omeprazole, carbamazepine, and troleandomycin. Phenobarbital, a classic inducing agent, is a ligand that binds CAR.[90] The induction of CYP1A subfamily enzymes is through the interaction with the NR AhR. Exogenous AhR ligands are hydrophobic, cyclic, planar molecules. Classic AhR ligands include polycyclic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and benzo[*a*]pyrene.[67]

Induction requires time to occur because it involves de novo synthesis of new proteins. Similarly, withdrawal of the inducer results in a slow return to the original enzyme concentration.[54] Polyaromatic hydrocarbons (PAHs) result in CYP1A subfamily induction within 3 to 6 hours with maximum effect within 24 hours.[84] The inducer rifampin does not affect verapamil trough concentrations maximally until 1 week; followed by a 2-week return to baseline steady state after withdrawal of rifampin.[54] Xenobiotics with long half-lives require longer periods to reach steady-state concentrations that maximize induction. Phenobarbital or fluoxetine, which have long half-lives, may fully manifest induction only after weeks of exposure. Conversely, xenobiotics with short half-lives, such as rifampin or venlafaxine, can reach maximum induction within days.[12,32]

Inconsistency in CYP induction exists between individuals. This variability exists for CYP enzymes in all organs.[54,67] In an in vitro study of inducers on 60 livers, differences in enzyme induction ranged from 5-fold for CYP3A4 and CYP2C up to more than 50-fold for CYP2A6 and CYP2D6.[54] The inconsistency likely results from multiple environmental factors including diet, tobacco, and pollutants.[54] There is variation in the extent to which inducers can generate new CYP enzymes. Identical dosing regimens with rifampin have resulted in induction of in vivo hepatic CYP3A4 with up to 18-fold differences between subjects.[54] There is an inverse correlation of the degree of inducibility of an enzyme and the baseline enzyme concentration. Patients with a relatively low baseline concentration of a CYP enzyme will be more inducible than those with a high baseline concentration. Interestingly, the maximum concentrations of CYP enzymes seem to be quantitatively similar among individuals, suggesting a limit to which enzymes can be induced.[54]

While the focus of this section is on CYP enzymes, it appears that all phases of xenobiotic metabolism are regulated by nuclear receptors.[9] Also, just as genetic polymorphisms exist for CYP enzymes, they exist for nuclear receptors including AhR, CAR, and PXR. This results in varied sensitivities to the ligands that complex with the nuclear receptors, ultimately resulting in differences in CYP enzyme induction.[54,90]

INHIBITION OF CYP ENZYMES

CYP enzyme inhibition can result in increased bioavailability of a drug or decreased activity of a prodrug that is no longer able to be metabolically activated.[69] Inhibition of CYP enzymes is the most common cause of harmful drug–drug interactions.[69] Inhibition of CYP enzymes by coadministered xenobiotics has resulted in the removal of many medications from the market in recent years including terfenadine, mibefradil, bromfenac, astemizole, cisapride, cerivastatin, and nefazodone.[93] The appendix at the end of this chapter includes a comprehensive listing of cytochrome P450 substrates, inhibitors, and inducers.

Inhibition mechanisms include irreversible (mechanism-based inhibition) and the more common reversible processes. The most common type of reversible inhibition is competitive, where the substrate and inhibitor both bind the active site of the enzyme.[69,93] Binding is weak and is formed and broken down easily, resulting in the enzyme becoming available again. It occurs rapidly, usually beginning within hours.[12] Because the degree of inhibition varies with the concentration of the inhibitor, the time to reach the maximal effect correlates with the half-life of the xenobiotic in question.[12] A competitive inhibitor can be overcome by increasing the substrate concentration. Each substrate of a CYP enzyme is an inhibitor of the metabolism of all the other substrates of the same enzyme, thereby increasing their concentrations and half life. Noncompetitive inhibition occurs when an inhibitor binds a location on an enzyme that is not the active site, resulting in a structural change that inhibits the active enzyme site. For example, noncompetitive inhibitors of CYP2C9 include nifedipine, tranylcypromine, and medroxyprogesterone acetate.[79] Another reversible mechanism results from competition between one xenobiotic and a metabolite of a second xenobiotic at its CYP enzyme substrate binding site. For example, the metabolites of clarithromycin and erythromycin produced by CYP3A inhibit further CYP3A activity. The effect is reversible and usually increases with repeated dosing.[79] Some reversible inhibitors bind so tightly to the enzyme that they essentially function as irreversible inhibitors.[69]

Irreversible inhibitors have reactive groups that covalently bind the enzyme, permanently. They display time-dependent inhibition because the amount of active enzyme at a given concentration of irreversible inhibitor will be different depending on how long the inhibitor is preincubated with the enzyme. Because the enzyme will never be reactivated, inhibition lasts until new enzyme is synthesized.[69] A relatively rare form of irreversible inhibition occurs when a reactive metabolite of a xenobiotic inhibits further metabolism of the substrate. This so-called suicide inhibition results in the destruction of the bound CYP enzyme.[28,77]

One measure of inhibitor potency is the inhibitory concentration, K_i, the concentration of the inhibitor that produces 50% inhibition of the enzyme. The more potent the inhibitor, the lower the value.[84] Values below 1 µmol/L are regarded as potent.[65] The azole antifungals are very potent with K_i values of 0.02 µmol/L.[84]

The impact of an inhibitor is also affected by the fraction of the substrate that is cleared by the inhibited, target enzyme. The inhibition of a CYP enzyme will have little impact if the enzyme only metabolizes a fraction of the affected drug.[65] Conversely, drugs that are primarily metabolized by a single CYP enzyme are more susceptible to interactions.[69] Astemizole and simvastatin are mainly biotransformed by CYP3A4. The potent and specific CYP3A inhibitor itraconazole prevents their metabolism, resulting in torsade de pointes dysrhythmias or rhabdomyolysis, respectively.[1]

SPECIFIC CYP ENZYMES

CYP1A1 and 1A2 While 1A1 is located primarily in extrahepatic tissue, 1A2 is a hepatic enzyme and is involved in the metabolism of 10 to 15% of all pharmaceuticals used today.[12,54] They both are very inducible by polycyclic aromatic hydrocarbons including those in cigarette smoke and charred food. They bioactivate several procarcinogens including benzo[*a*]pyrene.[45] Xenobiotics activated by the CYP1 enzyme family in the gastrointestinal tract are linked to colon cancer.[63]

CYP2C9 Approximately one-third of human CYP enzymes are in the CYP2 enzyme family, which, with 76 alleles, exhibits the greatest degree of genetic polymorphism.[25] The CYP2C9 enzyme is the most abundant enzyme of the CYP2C enzyme subfamily, which with CYP2C19, comprises approximately 10%–20% of the CYP enzymes in the liver.[25] This enzyme is associated with polymorphisms (Table 12–1).[12,63] This enzyme biotransforms S-warfarin, the more active isomer of warfarin. There is an association between slow metabolism and an increased risk of bleeding in patients on warfarin.[78]

CYP2D6 Twenty-five percent of all drugs used today, including 50% of the commonly used antipsychotics, are substrates for CYP2D6. It is sometimes called debrisoquine hydrolase as it was first identified with studying the metabolism of the antihypertensive agent debrisoquine.[12,41,63]

CYP2E1 This enzyme comprises 7% of the total CYP enzyme content in the human liver.[62] It metabolizes small organic compounds including alcohol, carbon tetrachloride, and halogenated anaesthetic agents.[84] It also biotransforms low-molecular-weight xenobiotics including benzene, acetone, and N-nitrosamines.[84] Some of these substrates are procarcinogens which are bioactivated by CYP2E1. Besides CYP1A2, this is the only other CYP enzyme linked to cancer.[63] The assessment for a relationship to cancer is particularly intense because many of its substrates are environmental xenobiotics. The induction of CYP2E1 is associated with increased liver injury by reactive metabolites of carbon tetrachloride and of bromobenzene[37] (Chap. 26). During the metabolism of substrates that include carbon tetrachloride, ethanol, acetaminophen, aniline, and N-nitrosomethylamine, CYP2E1 actively produces free radicals and other reactive metabolites associated with adduct formation and lipid peroxidation[15] (Chaps. 8 and 34). CYP2E1 is inhibited by acute elevations of ethanol, an effect illustrated by the capacity of acute administration of ethanol to inhibit the metabolism of acetaminophen.[10] The chronic ingestion of ethanol hastens its own metabolism through enzyme induction.

CYP3A4 CYP3A4 is the most abundant CYP in the human liver, comprising 40% to 55% of the mass of hepatic CYP enzymes.[12,25] The CYP3A4 enzyme is the most common one found in the intestinal mucosa and is responsible for much first-pass drug metabolism.[12] Numerous xenobiotics are metabolized by CYP3A4. It is involved in the biotransformation of 50% to 60% of all pharmaceuticals.[62,98] It has such broad substrate specificity because it accommodates especially large lipophilic substrates and can adopt multiple conformations. It can even simultaneously fit two relatively large compounds (ketoconazole, erythromycin) in its active site.[77]

An example of an adverse drug interaction related to this enzyme is the QT interval prolongation and torsades de pointes that occurred in patients taking terfenadine or astemizole in combination with ketoconazole or erythromycin.[68,75] Ketoconazole inhibits CYP3A4, causing a 15-fold to 72-fold increase in serum concentrations of terfenadine.[63] Bioflavonoids in grapefruit juice decrease metabolism of some substrates by 5-fold to 12-fold.[12,63] The CYP3A4 enzyme does not exhibit genetic polymorphism; however, there are large interindividual variations in enzyme concentrations.[98]

PHASE II BIOTRANSFORMATION REACTIONS

Phase II biotransformation reactions are synthetic, catalyzing conjugation of the products of phase I reactions or molecules with sites amenable to conjugation. Conjugation usually terminates the pharmacologic activity of the xenobiotic and greatly increases their water solubility and excretability.[56,87,96] Conjugation occurs most commonly with glucuronic acid, sulfates, and glutathione. Less common phase II reactions include amino acid conjugation, such as glycine, glutamic acid, and taurine; acetylation; and methylation.

Glucuronidation is the most common phase II synthesis reaction.[56] It occurs only within microsomal membranes. Glucuronyl transferase has relatively low substrate affinity but it has high capacity at higher substrate concentrations.[96] The glucuronic acid, donated by uridine diphosphate glucuronic acid (UDPG), is conjugated with the nitrogen, sulfhydryl, hydroxyl, or carboxyl groups of substrates. Smaller conjugates usually undergo renal elimination, whereas larger ones undergo biliary elimination.[51]

Sulfation complements glucuronidation because it is a high affinity but low capacity reaction that occurs primarily in the cytosol. For example, the affinity of sulfate for phenol is very high (the K_m is low), so that when low doses of phenol are administered, the predominant excretion product is the sulfate ester. Because the capacity of this reaction is readily saturated, glucuronidation becomes the main method of detoxification when high doses of phenol are administered.[56,96] Sulfate conjugates are highly ionized and very water soluble. Of note, sulfation is reversible by the action of sulfatases within the liver. The resultant metabolites may be resulfated and the cycle may repeat itself further.[96]

Glutathione *S*-transferases are important because they catalyze the conjugation of the tripeptide glutathione (glycine-glutamate-cysteine, or GSH) with a diverse group of reactive, electrophilic metabolites of phase I CYP enzymes. The reactive compounds initiate an attack on the sulfur group of cysteine, resulting in conjugation with GSH that detoxifies the reactive metabolite. Of the three phase II reactions addressed, hepatic concentrations of glutathione by far account for the greatest amount of cofactors used. While intracellular glutathione is difficult to deplete, when it does occur, severe hepatotoxicity often follows.[96] Some GSH conjugates are directly excreted. More commonly, the glycine and glutamate residues are cleaved and the remaining cysteine is acetylated to form an *N*-acetylcysteine (mercapturic acid) conjugate that is readily excreted in the urine. A familiar example of this detoxification is the avid binding of *N*-acetyl-*p*-benzoquinoneimine (NAPQI), the toxic metabolite of acetaminophen, by glutathione.[5,11]

As with the CYP enzymes, many phase II enzymes are inducible. For example, UDP-glucuronosyltransferase which executes glucuronidation is inducible via PXR, CAR, and AhR nuclear receptors after binding with rifampin, phenobarbital, and PAHs, respectively. Its activity varies 6-fold to 15-fold in liver microsomes.[90]

MEMBRANE TRANSPORTERS

While the focus on drug disposition has traditionally been on biotransformation, membrane transporters also impact drug disposition.[38] Because they usually occur after phase I and II biotransformation, their actions are sometimes called phase III metabolism.[42] Their physiologic role is to transport sugars, lipids, amino acids, and hormones so as to regulate cellular solute and fluid balance. However, they affect drug disposition just as do biotransformation processes by facilitating or preventing the passage of xenobiotics through membranes.[46] Uptake transporters translocate drugs into cells while efflux transporters export xenobiotics, often against concentration gradients, out of cells. Most transporters are in the adenosine triphosphate binding cassette (ABC) family of transmembrane proteins that use energy from ATP hydrolysis.[13,38] This family includes the P-glycoprotein family. Some transporters move substrates both into and out of cells. Organs important for drug disposition have multiple transporters that have overlapping substrate capabilities, a redundancy that enhances protection. In the small intestine, P-glycoprotein is important because it can actively extrude xenobiotics back into the intestinal lumen.[13] The degree of phenotypic expression of P-glycoprotein affects the bioavailability of many xenobiotics including paclitaxel, digoxin, and protease inhibitors. Hepatocyte efflux transporters move biotransformed xenobiotics into bile. Transporters in endothelial cells of the blood–brain barrier prevent

CNS entry of substrate xenobiotics.[13,38] As with biotransformation enzymes and nuclear receptors, membrane transporters may be inhibited or induced. Digoxin, a high affinity substrate for P-glycoprotein, has increased bioavailability when administered with P-glycoprotein inhibitors such as clarithromycin or atorvastatin.[38] Loperamide is a substrate for P-glycoprotein that limits its intestinal absorption or CNS entry. Coadministration with quinidine, a P-glycoprotein inhibitor, results in increased opioid CNS effects of loperamide.[38] As with the biotransformation enzymes, polymorphisms exist for membrane transporters. However, the clinical significance of these is not clear.[17]

MECHANISMS OF CELLULAR INJURY

Ideally and commonly, potentially toxic metabolites produced by phase I reactions are detoxified during phase II reactions. However, detoxification does not always occur. This section reviews mechanisms of cellular injury related to xenobiotic biotransformation.

SYNTHESIS OF TOXINS

Sometimes a xenobiotic is mistaken for a natural substrate by synthetic enzymes that biotransform it into an injurious compound. The incorporation of the rodenticide fluoroacetate into the tricarboxylic acid cycle is an example of this mechanism of toxic injury (Fig. 12–3).[70] Another example is illustrated by analogs of purine or pyrimidine bases that are phosphorylated and inserted into growing DNA or RNA chains, resulting in mutations and disruption of cell division. This mechanism is used therapeutically with 5-fluorouracil (5-FU), an antitumor, pyrimidine base analog. When phosphorylated to 5-fluoro-dUTP and incorporated into growing DNA chains, it causes structural instability of the cellular DNA and inhibits tumor growth.[75]

INJURY BY METABOLITES OF BIOTRANSFORMATION

Many toxic products result from metabolic activation (Table 12–2).[43] The CYP enzymes most associated with bioactivation are 1A1, 1B1, 2A6, and 2E1 while 2C9 and 2D6 yield little toxic activation.[34]

Highly reactive metabolites exert damage at the site where they are synthesized; reacting too quickly with local molecules to be transported elsewhere. This commonly occurs in the liver, the major site of biotransformation of xenobiotics[33,82] (Chap. 26). However, the lungs, skin, kidneys, gastrointestinal tract, and nasal mucosa can also create toxic metabolites that cause local injury.[11,49] Overdoses of acetaminophen lead to excessive hepatic production of the highly reactive electrophile NAPQI, which initiates a damaging covalent bond with hepatocytes[5,10] (Chap. 34). Acute renal tubular necrosis also occurs in patients with overdose of acetaminophen, attributed to its biotransformation by

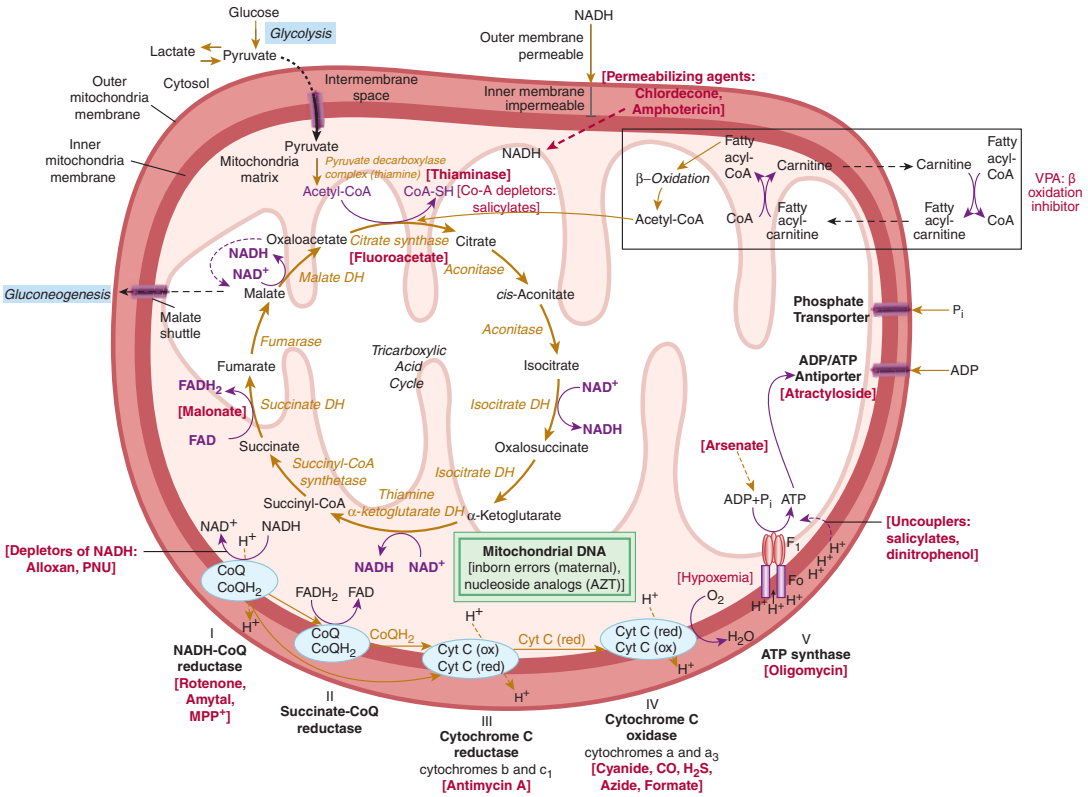

FIGURE 12–3. Pyruvate is converted to acetylcoenzyme A (acetyl-CoA), which enters the Krebs cycle as shown. Reducing equivalents, in the form of NADH, and FADH, donate electrons to a chain of cytochromes beginning with NADH dehydrogenase. These reactions "couple" the energy released during electron transport to the production of ATP. Ultimately, electrons combine with oxygen to form water. The sites of action of xenobiotics that inhibit oxidative metabolism are shown. The sites where thiamine functions as a coenzyme are also illustrated. DH = dehydrogenase.

TABLE 12–2. Examples of Xenobiotics Activated to Toxins by Human Cytochrome P450 Enzymes

CYP Enzyme	Substrate	Toxicity
1A1	Benzo[*a*]pyrene (PAH)	IARC Group 1
1A2	Acetaminophen	Hepatotoxicity
	Aflatoxin B (*Aspergillus* mycotoxin)	IARC Group 1
	2-Naphthylamine (azo dye production)	IARC Group 1
	NNK (nitrosamine in tobacco)	IARC Group 1
	NNK (nitrosamine in tobacco)	IARC Group 1
	N-Nitrosodiethylamine (gas and lubricant additive, copolymer softener)	IARC Group 2A
2B6	Chrysene (PAH)	IARC Group 2B
	Cyclophosphamide	IARC Group 1
2C 8,9	Phenytoin	IARC Group 2B
	Tienilic acid (old diuretic; off market)	Hepatotoxicity
	Valproic acid	Hepatotoxicity
2D6	NNK (nitrosamine in tobacco)	IARC Group 1
2F1	Acetaminophen	Hepatotoxicity
	3-Methylindole (in perfumes, cigarettes for flavor)	Pneumotoxicity
	Valproic acid	Hepatotoxicity
2E1	Acetaminophen	Hepatotoxicity
	Acrylonitrile	IARC Group 2B
	Benzene	IARC Group 1
2E1	Carbon tetrachloride	IARC Group 2B
	Chloroform	IARC Group 2B
	Chloroform	IARC Group 2B
	Ethylene dibromide (former gas additive and fumigant; still indust. intermediate)	IARC Group 2A
	Ethyl carbamate (former antineoplastic)	IARC 2A
	Halothane	Hepatotoxicity
	Methylene Chloride	IARC Group 2B
	N-Nitrosodimethylamine (formerly in rocket fuel)	IARC Group 2A
	Styrene	IARC Group 2A
	Trichloroethylene	IARC Group 2A
	Vinyl chloride	IARC Group 1
3A4	Acetaminophen	Hepatotoxicity
	Aflatoxin B_1 (*Aspergillus* mycotoxin)	IARC Group 1
	Chrysene (PAH)	IARC Group 2B
	Cyclophosphamide	IARC Group 1
	1-Nitropyrene (PAH)	IARC Group 2B
	Senecionine (pyrrolizidine alkaloid)	Hepatotoxicity
	Sterigmatocystin (*Aspergillus* mycotoxin)	IARC Group 2B

IARC – International Agency for Research on Cancer of the World Health Organization. Group 1 – known carcinogen; Group 2A – probable carcinogen; Group 2B – possible carcinogen. PAH – polycyclic aromatic hydrocarbon

prostaglandin H synthase within renal tubular cells to a highly reactive semiquinoneimine.[23,48]

Monoamine oxidases (MAOs) are mitochondrial enzymes present in many tissues. They oxidize many amines, including dopamine, epinephrine, and serotonin, and xenobiotics such as primaquine and haloperidol. The metabolic activity of MAOs was responsible for the outbreak of parkinsonism associated with the use of methylphenyltetrahydropyridine (MPTP), an unintended by-product of attempts to synthesize a "designer" analog of meperidine, methylphenylpropionoxypiperidine (MPPP). After crossing the blood–brain barrier, MPTP is biotransformed by MAO in glial cells to methylphenyldihydropyridine ($MPDP^+$), which is nonenzymatically converted to MPP^+. The MPP^+ is subsequently taken up by specific dopamine transport systems into dopaminergic neurons in the substantia nigra, resulting in inhibition of oxidative phosphorylation and subsequent neuronal death.[31]

FREE RADICAL FORMATION

Within an atom, it is energetically favorable for electrons to exist in lone pairs or as a part of a chemical bond. An element or compound with an unpaired electron, called a *radical* or *free radical*, is very reactive and it generally does not exist for long. It rapidly seeks other species in order to obtain another electron. Radicals include the superoxide anion $O_2^{\bullet -}$, which is produced by adding an electron to O_2, and the highly reactive hydroxyl radical $HO^{\bullet}$, which is produced by splitting the H_2O_2 molecule into two. The H_2O_2 molecule itself is reactive and is also associated with injury. The superoxide and hydroxyl radicals react with other molecules in order to stabilize; however, by taking an electron in order to do so, they generate new free radical species, potentially initiating a chain reaction. The production of radicals is a normal occurrence and the human body has defense mechanisms. Some xenobiotics promote the formation of reactive oxidizing species to the extent that defensive mechanisms against oxidants are overwhelmed, a condition called *oxidative stress*. Oxidizing species are called such because, by reducing themselves by taking away electrons, they oxidize the species from which they took the electrons.[7]

While oxidative stress may result in oxidative damage to nucleic acids and proteins, other classic targets are polyunsaturated fatty acids (PUFA) in cellular membranes, resulting in lipid peroxidation (the oxidative destruction of lipids). This attack removes the particularly reactive hydrogen atom, with its lone electron, from a methylene carbon of a PUFA; leaving an unpaired electron and causing the formation of a lipid radical. This lipid radical attacks other PUFA, causing a chain reaction that destroys the cellular membrane. Membrane degradation products initiate inflammatory reactions in the cells, resulting in further damage.[82,7]

Molecular oxygen (O_2) has a lone pair of electrons in its orbit. Because oxygen is a relatively weak univalent electron acceptor (and most organic molecules are weak univalent electron donors), oxygen cannot efficiently oxidize amino acids and nucleic acids. However, the unpaired electrons of O_2 readily interact with the unpaired electrons of transition metals and organic radicals. Metals frequently catalyze

the creation of oxygen free radicals. The following is an example of hydroxyl radical formation: (1) A first step is the addition of an electron to O_2 to create the superoxide ion. (2) The very reactive superoxide combines with hydrogen and another electron to produce hydrogen peroxide. (3) In the presence of a metal ion catalyst such as iron, hydrogen peroxide undergoes various reactions to produce the hydroxyl radical. The dot in these formulas represents an unpaired electron, the hallmark of a free radical.[40,35,56]

$$(1)\ O_2 + e^- \rightarrow O_2^{\bullet -}$$

$$(2)\ O_2^{\bullet -} + 2H^+ + e^- \rightarrow H_2O_2$$

$$(3)\ H_2O_2 \xrightarrow[Fe^{2+}]{} OH^- + OH\bullet \quad \text{(Fenton)}$$

$$H_2O_2 + O_2^{\bullet -} \xrightarrow[Fe^{2+}]{} O_2 + OH^- + OH\bullet \quad \text{(Haber-Weiss)}$$

The damaging effects of the free radicals are decreased by reaction with antioxidants such as ascorbate, tocopherols, and glutathione.[56] Deficiencies of antioxidants, especially glutathione, are associated with increased oxidative damage. Free radicals are also neutralized by several enzymes, including peroxidase, superoxide dismutase, and catalase.

The ethanol-inducible CYP2E1 enzyme produces significant amounts of superoxide and peroxide free radicals, and, in the presence of iron, hydroxyl free radicals that readily initiate lipid peroxidation. This has been studied extensively in models of the metabolism of carbon tetrachloride, ethanol, and acetaminophen.[20] The formation of free radicals is implicated in the pulmonary injury caused by paraquat, the myocardial injury caused by doxorubicin, and the liver injury caused by carbon tetrachloride.[60,72] Paraquat reacts with NADPH to form a pyridinyl free radical, which, in turn, reacts with oxygen to generate the superoxide anion radical. Doxorubicin is metabolized to a semiquinone free radical in the cardiac mitochondria, which, in the presence of oxygen, forms a superoxide anion radical that initiates myocardial lipid peroxidation.[60] Carbon tetrachloride (CCl_4) is metabolized to the trichloromethyl radical ($\bullet CCl_3$) that binds covalently to cellular macromolecules. In the presence of oxygen, this is converted to the trichloromethylperoxyl radical ($\bullet CCl_3O_2$) that can initiate lipid peroxidation (Fig. 12–4).[73] See Chap. 106 for a more extensive discussion.

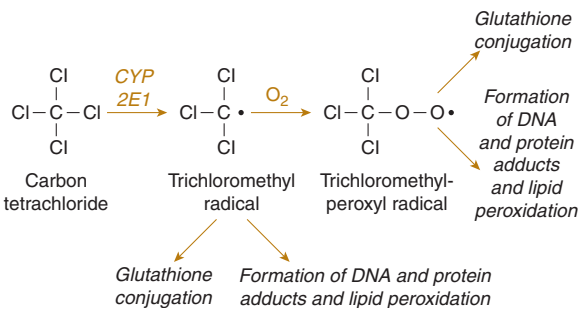

FIGURE 12–4. Carbon tetrachloride metabolism by the hepatocyte. Under hypoxic conditions, the CCl_3 radical is the predominant species formed. At higher oxygen tensions, CCl_3 radical is oxidized to the CCl_3OO radical, which is more readily detoxified by glutathione. Both free radicals bind to hepatocytes and cause cellular injury.

CRITICAL BIOCHEMICAL PATHWAYS AND XENOBIOTICS THAT AFFECT THEM

Energy metabolism is the foundation of cellular function. It provides high-energy fuel, predominantly in the form of ATP, for all energy-dependent cellular processes such as synthesis, active transport, and maintenance of electrolyte balance and membrane integrity. Numerous pathways interconnect glycogen, fat, and protein reserves in many tissues that store and retrieve ATP and glucose. The brain and red blood cells are entirely dependent on glucose for energy production, while other tissues can also use ketone bodies and fatty acids to synthesize ATP. Rapid cell death occurs if the production or use of ATP is inhibited, thus the goal of many metabolic processes is the production and mobilization of cellular energy.

Catabolic pathways that produce cellular energy include glycolysis, the tricarboxylic acid (citric acid, or Krebs) cycle, and oxidative phosphorylation via the electron transport chain. Citric acid occurs in the cytosol while the citric acid cycle and the electron transport chain are located within the mitochondria. Glycolysis produces small amounts of ATP through the anaerobic metabolism of glucose. Pyruvate, the end product of glycolysis, yields far more ATP when it is converted to acetylcoenzyme A (acetyl-CoA) and "processed" in the citric acid cycle (Fig. 12–3). Fat and protein yield their energy through their conversion to acetyl-CoA and other intermediates of the citric acid cycle. The citric acid cycle and oxidative phosphorylation, via the electron transport chain, result in most ATP synthesis. Oxidative phosphorylation disposes of electrons or "reducing equivalents" and converts their energy to ATP. A lack of oxygen stops the electron transport chain and ATP production. Oxidative metabolism is highly energy efficient, producing 36 moles of ATP for each mole of glucose metabolized, compared to the 2 moles of ATP produced by glycolysis. The following sections review the basics of cellular energy metabolism and several important xenobiotics that affect these critical metabolic functions (Table 12–3). [47,25]

GLYCOLYSIS

Glycolysis is the first biochemical pathway in the metabolism of glucose. Other sugars enter the glycolytic pathway after conversion to glycolytic intermediates (Fig. 12–5). The glycolytic process converts 1 molecule of glucose to 2 pyruvate molecules + 2 ATP + 2 NADH. Pyruvate may follow many paths. Under anaerobic conditions, the 2 pyruvates produced from 1 glucose molecule are reduced by lactate dehydrogenase to 2 lactate molecules in an NADH-requiring step that regenerates NAD^+. Thus, anaerobic glycolysis yields 2 molecules of lactate + 2 ATP. When NAD^+ and oxygen are available, pyruvate is converted by pyruvate decarboxylase to acetyl-CoA, which is transported from the cytosol into the mitochondria and condenses with oxaloacetate within the citric acid cycle to form citrate (Fig. 12–3).[47,25] In energy rich conditions, pyruvate is used for fatty acid synthesis.

Arsenate has a toxic effect at the glycolytic step where 3-phosphoglyceraldehyde dehydrogenase (3-PGA) catalyzes the oxidation of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate; a reaction that preserves a high-energy phosphate bond used to synthesize ATP in the next step of glycolysis (Fig. 12–5).[39] Arsenate acts as an analog of phosphate at this step. While glycolysis continues, the resultant unstable arsenate intermediate is rapidly hydrolyzed, preventing the subsequent synthesis of ATP.[39]

CITRIC ACID CYCLE

The citric acid cycle uses acetyl-CoA derived from glycolysis, fat, or protein to regenerate NADH from NAD^+. The cycle is a major source of

TABLE 12–3. Inhibitors of Glucose Metabolism and ATP Synthesis

Step/Location	Action	Examples
Glycolysis	Inhibits NADH production	Iodoacetate (at GAPDH) NO^+ (at GAPDH)
	Bypasses ATP producing step	Arsenate, As^{5+}
Gluconeogenesis	Inhibits NADH production	4-(Dimethylamino)phenol *p*-benzoquinone Hypoglycin A
Fatty acid metabolism	Inhibits NADH production	Aflatoxin Amiodarone Hypoglycin Perhexiline Protease inhibitors Salicylates Tetracycline Valproic acid
TCA Cycle	Inhibits NADH production	Arsenite, As^{3+} *p*-Benzoquinone Fluoroacetate
Electron-transport chain at complex I	Inhibits electron transport	MPP^+ Paraquat Rotenone
Electron-transport chain at complex III	Inhibits electron transport	Antimycin-A Funiculosin Di- and trivalent metal cations (Zn^{2+}, Hg^{2+}, Cu^{2+}, and Cd^{2+}) Substituted phenols* (are also uncouplers)
Electron-transport chain at complex IV	Inhibits electron transport	Azide Carbon monoxide Cyanide Formate Hydrogen sulfide Nitric oxide Phosphine Protamine
Electron-transport chain at ATP synthase	Inhibits ATP production	Arsenate, As^{5+} Mycotoxins (numerous, including oligomycin) Organic chlorines (DDT and chlordecone) Organotins (cyhexatin) Paraquat
Mitochondria ADP/ATP antiporter	Disrupts the movement of ADP into and ATP out of the mitochondria at the ADP/ATP antiporter	Atractyloside DDT Free fatty acids
Mitochondria inner membrane	Uncouples oxidative phosphorylation by disrupting the proton gradient → stops proton flow at ATP synthase → stops ATP synthesis	Substituted phenols (pentachlorophenol and dinitrophenol) Lipophilic amines (amiodarone, perhexiline, buprenorphine) Benzonitrile Thiadiazole herbicides NSAIDs with ionizable groups (salicylates, diclofenac, indomethacin, piroxicam) Valinomycin Chlordecone
Mitochondria inner membrane	Diverts electrons to alternate pathways (vs. to the electron-transport chain)	Doxorubicin MPP^+ Naphthoquinones (menadione) *N*-nitrosoamines Paraquat

GAPDH = glyceraldehyde 3-phosphate dehydrogenase; MPP^+ = 1-methyl-4-phenylpyridinium; TCA – tricarboxylic acid cycle.

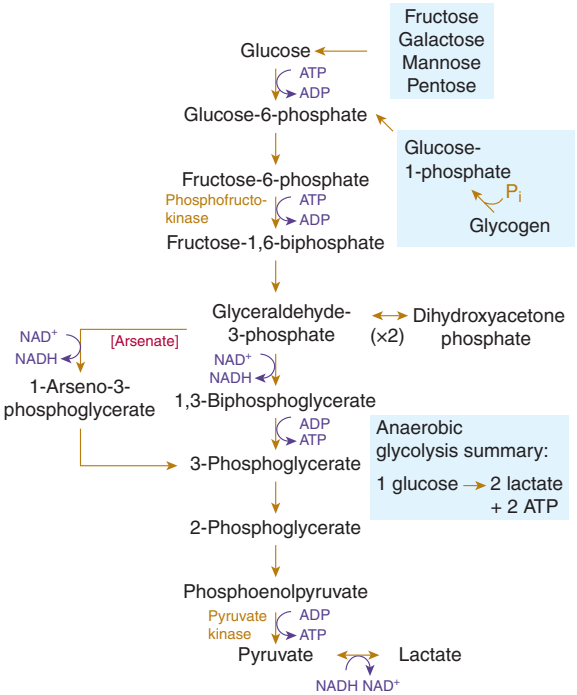

FIGURE 12–5. During glycolysis, the anaerobic metabolism of 1 mole of glucose to 2 moles of pyruvate results in the net production of 2 moles of ATP. Arsenic inhibits 3-phosphoglycerate dehydrogenase, which catalyzes the oxidation of glyceraldehyde-3-phosphate to 1,3-biphosphoglycerate.

electrons (in the form of NADH) and is critical to the aerobic production of ATP (Fig. 12–3). Each acetyl-CoA molecule that is oxidized within the citric acid cycle ultimately forms one molecule each of CO_2 and guanosine triphosphate (GTP), and more importantly, 3 molecules of NADH and one molecule of flavin adenine dinucleotide ($FADH_2$) (reduced form), which enter the electron transport chain, producing a total of 15 molecules of ATP. In addition, the citric acid cycle provides important intermediates for amino acid synthesis and for gluconeogenesis.[47]

Various xenobiotics inhibit the citric acid cycle. The rodenticides, sodium fluoroacetate and fluoroacetamide, are combined with coenzyme A, CoASH, to create fluoroacetyl CoA (FAcCoA). The FAcCoA substitutes for acetyl CoA, entering the TCA cycle by condensation with oxaloacetate to form fluorocitrate, which inhibits citrate metabolism, resulting in inhibition of the cycle and termination of oxidative metabolism (Fig. 12–3) (Chap. 108).[70]

Thiamine is an important cofactor for 2 citric acid cycle enzymes: the conversion of pyruvate to acetyl-CoA by pyruvate decarboxylase and for the conversion of α-ketoglutarate to succinyl-CoA by α-ketoglutarate dehydrogenase (Fig. 12–3).[25] The life-threatening effects of thiamine deficiency are likely related to impairment of these enzyme functions (see Antidotes in Depth: Thiamine Hydrochloride). Arsenite inhibits these thiamine-dependent enzymes within the citric acid cycle.

THE ELECTRON TRANSPORT CHAIN

The electron transport chain is the location where the "phosphorylation" of oxidative phosphorylation occurs. Oxidative phosphorylation is the creation of high energy bonds by phosphorylation of ADP to ATP, "coupled" to the transfer of electrons from reduced coenzymes to molecular oxygen via the electron transport chain. The success of aerobic metabolism requires the disposal of electrons within NADH and FADH, generated by oxidative metabolism within the citric acid cycle. The electron transport chain consists of a series of cytochrome–enzyme complexes within the inner mitochondrial membrane (Fig. 12–3). Within these complexes, NADH is split into $NAD^+ + H^+ +$ 2 electrons at complex I at the beginning of the chain while $FADH_2$ is split into $FADH + H^+ +$ 2 electrons at complex II. These splits have 2 results. First, the regenerated NAD^+ and FADH are recycled back to the citric acid cycle, enabling oxidative metabolism to continue. Second, these actions provide the energy required to pump protons (H^+) from the mitochondrial matrix into the intermembrane space. This action causes the matrix to become relatively alkaline compared to the now acidified intermembrane space, resulting in a proton gradient across the inner mitochondrial membrane. This gradient provides the energy needed to create the high-energy bonds of ATP at complex V. The final step in oxidative phosphorylation is the reduction of molecular oxygen to water by cytochrome a-a_3 (Fig. 12–3).[47,25]

Mitochondria oxidize substrates, consume oxygen, and make ATP. Xenobiotics that interrupt oxidative phosphorylation impair ATP production by either inhibiting specific electron chain complexes or by acting as "uncouplers." Both of these mechanisms result in rapid depletion of cellular energy stores, followed by failure of ATP-dependent active transport pumps, loss of essential electrolyte gradients, and increases in cell volume.[27]

Inhibitors of specific cytochromes block electron transport and cause an accumulation of reduced intermediates proximal to the site of inhibition. This stops the regeneration of oxidized substrates for the citric acid cycle, particularly NAD^+ and FAD, further impairing oxidative metabolism. Cyanide, carbon monoxide, and hydrogen sulfide block the cytochrome a-a_3–mediated reduction of O_2 to H_2O. The very dramatic clinical effects of a significant cyanide exposure illustrate the importance of aerobic metabolism (Chaps. 125, 126). Other xenobiotics are less commonly associated with inhibition of the electron transport chain (Table 12–3).[91]

Severe metabolic acidosis is a clinical manifestation of xenobiotics that inhibits aerobic respiration. This metabolic acidosis is primarily caused by the accumulation of protons in the mitochondrial matrix that are not used in the production of ATP. While lactic acid accumulates, it is only a marker for metabolic acidosis associated with the impairment of oxidative metabolism.[76]

Xenobiotics that uncouple oxidative phosphorylation, like inhibitors of the electron transport chain, stop ATP synthesis. However, protons continue to be pumped into the intermembrane space, electrons continue to flow down the chain to reduce oxygen, and substrate consumption continues. Uncoupling xenobiotics destroy the proton gradient across the mitochondrial inner membrane. They allow the protons to cross back into the mitochondrial matrix, causing the loss of the proton gradient across the inner mitochondria membrane. Since it is the proton gradient that drives the production of ATP at complex V, ATP production is stopped. Thus, oxygen consumption is "uncoupled" from ATP production. The redox energy created by electron transport that cannot be coupled to ATP synthesis is released as heat. Various xenobiotics uncouple ATP synthesis (Table 12–3). A classic one is dinitrophenol, used in the past as an herbicide and as a weight-loss product (Chap. 39). Xenobiotics that are capable of carrying hydrogen ions across membranes are generally lipophilic weak acids. These xenobiotics must have an acid-dissociable group to carry the proton and a bulky lipophilic group to cross a membrane.[91] Dinitrophenol is able to carry its proton from

the cytosol into the more alkaline mitochondrial matrix where it dissociates, acidifying the matrix and destroying the proton gradient across the inner mitochondrial membrane. Interestingly, the phenolate anion of dinitrophenol is relatively lipophilic and can cross back out to the cytosol where it gains a new proton and starts the process over again. Long-chain fatty acids uncouple oxidative phosphorylation by a similar mechanism.[91] Fatal exposures to dinitrophenol and to pentachlorophenol, a wood preservative, are associated with severe hyperthermia attributed to heat generation by uncoupled oxidative phosphorylation.[58] Rats develop fatal hyperthermia following oral ingestion of dinitrophenol.[83] The hyperthermia and acidosis associated with severe salicylate poisoning are attributed to its uncoupling of oxidative phosphorylation.[81]

HEXOSE MONOPHOSPHATE SHUNT

The hexose monophosphate (HMP) shunt provides the only source of cellular NADPH. NADPH is used in biosynthetic reactions, particularly fatty acid synthesis, and is an important source of reducing power for the maintenance of sulfhydryl groups that protect the cell from free radical injury.[8,40] As noted earlier, G6PD is a key enzyme in the pathway (Fig. 12–6). Reduced glutathione, which is quantitatively the most important antioxidant in cells, depends on the availability of NADPH. Red blood cells (RBCs) are especially vulnerable to deficiency of NADPH, which results in hemolysis during oxidative stress.

Another manifestation of oxidative stress in RBCs is the oxidation of the iron in hemoglobin from Fe^{2+} to Fe^{3+}, producing methemoglobin that occurs both spontaneously and as a response to xenobiotics such as nitrites and aminophenols. Because most reduction of methemoglobin is done by NADH-dependent methemoglobin reductase, which is not deficient in persons who lack G6PD, such persons do not develop methemoglobinemia under normal circumstances. However, when oxidative stress is severe and methemoglobinemia develops, people who have G6PD deficiency have limited ability to use the alternative NADPH-dependent methemoglobin reductase (Chap. 127).[89]

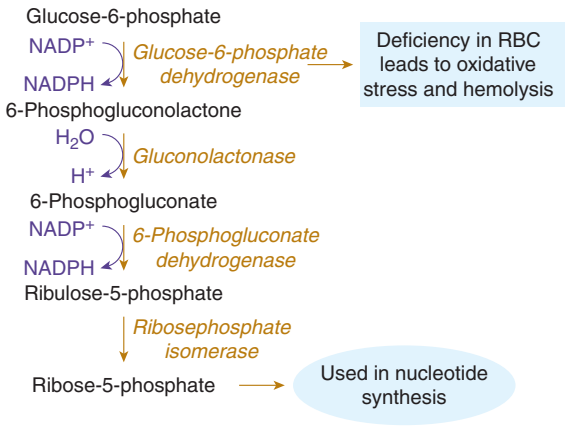

FIGURE 12–6. The oxidation reactions of the hexose monophosphate shunt are an important source of NADPH for reductive biosynthesis and for protection of cells against oxidative stress. Deficiency of G6PD, the first enzyme in the pathway, may result in RBC hemolysis during oxidative stress.

GLUCONEOGENESIS

Gluconeogenesis facilitates the conversion of amino acids and intermediates of the citric acid cycle to glucose. It occurs primarily in the liver but also in the kidney. It is an important source of glucose during fasting and enables maintenance of glycogen stores. Most of the steps in the synthesis of glucose from pyruvate are simply the reverse of glycolysis, with three irreversible exceptions: (1) the conversion of glucose-6-phosphate to glucose; (2) the conversion of fructose-1,6-diphosphate to fructose-6-phosphate; and (3) the synthesis of phosphoenolpyruvate from pyruvate. The synthesis of phosphoenolpyruvate from pyruvate is especially complex. Pyruvate is first converted to oxaloacetate within the mitochondria, then to malate, which is transported out of the mitochondria and converted in the cytosol back to oxaloacetate, and then to phosphoenolpyruvate (Fig. 12–7). Certain amino acids—notably alanine, glutamate, and aspartate—are readily converted to citric acid cycle intermediates and can be used in the synthesis of glucose through this cycle.[47] Glycerol, produced by the breakdown of triglycerides in adipose tissue, is another substrate for gluconeogenesis.

The regulation of gluconeogenesis is opposite to that of glycolysis, stimulated by glucagon and catecholamines but inhibited by insulin. Gluconeogenesis requires the cytosolic NAD^+ and mitochondrial NADH. It is impaired by processes that increase the cytosol-reducing potential as measured by the cytosol NADH/ NAD^+ ratio (see discussion below).

A number of xenobiotics impair gluconeogenesis, resulting in hypoglycemia when glycogen stores are depleted (Table 12–3). Hypoglycin A, an unusual amino acid found in unripe ackee fruit that is the cause of Jamaican vomiting sickness, produces profound hypoglycemia.[26,81,85] Its metabolite methylenecyclopropylacetic acid (MCPA) indirectly inhibits gluconeogenesis by blocking the oxidation of long-chain fatty acids, an important source of NADH in mitochondria. It also inhibits the metabolism of several glycogenic amino acids including leucine, isoleucine, and tryptophan; and blocks their entrance into the citric acid cycle. MCPA may also prevent the transport of malate out of the mitochondria.[74,85,86] Hypoglycemia also occurs in fasting patients with elevated ethanol concentrations.[3,29,49] This is likely a result of the impairment of gluconeogenesis by the increased cytosolic NADH:NAD^+ ratio associated with the metabolism of ethanol. This inhibits the two cytosolic steps that require NAD^+—the conversions of lactate to pyruvate and of malate to oxaloacetate.[2,49,73]

FATTY ACID METABOLISM

Fatty acid metabolism occurs primarily in hepatocytes. Fatty acids mobilized in adipose tissue enter hepatocytes by passive diffusion. Fatty acid synthesis is stimulated by insulin and inhibited by glucagon and epinephrine. Acetyl-CoA is the primary building block of free fatty acids (FFAs). In energy-repleted cells, fatty acids are combined with glycerol phosphate to form triacylglycerol (triglycerides), the first step in the synthesis of fat for storage. Hepatic triglycerides are bound to lipoprotein to form very-low-density lipoprotein (VLDL), then transported and stored in adipocytes. When hepatocytes are energy depleted, triglycerides are broken down to FFA and glycerol. This process is suppressed by insulin but supported by glucagon or epinephrine. FFAs undergo β-oxidation in the mitochondria, a process that breaks the FFA into acetyl-CoA molecules that can then enter the citric acid cycle. FFAs require activation before transport into the mitochondria. This is accomplished by acylcoenzyme A (acyl-CoA) synthetase, which adds a CoA group to the FFA in an energy-dependent synthetic reaction. These are transported into the

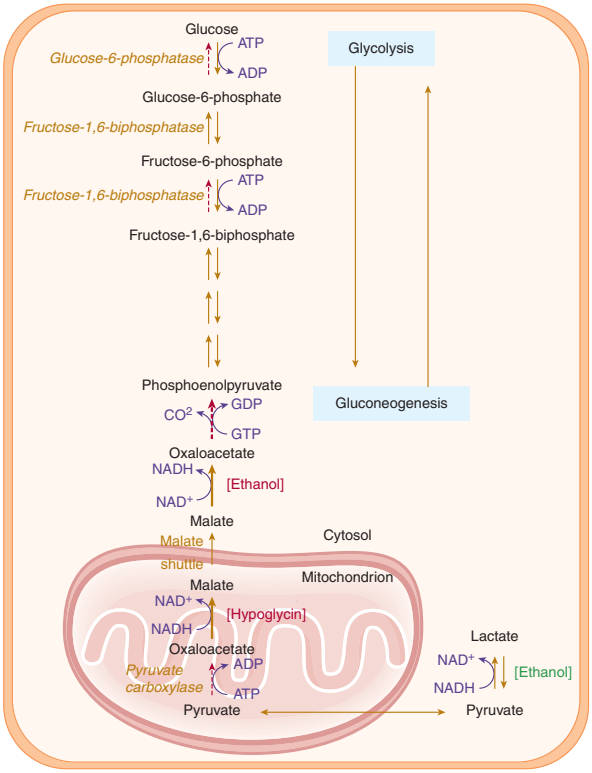

FIGURE 12–7. Gluconeogenesis reverses the steps of glycolysis, with the exception of the bypass of the three irreversible steps shown. The step from pyruvate to phosphoenolpyruvate involves both cytosolic and mitochondrial reactions that use ATP. Hypoglycin A inhibits the intramitochondrial conversion of oxaloacetate to malate by depleting NADH through interference with β-oxidation of fatty acids. Ethanol decreases cytosolic supplies of NAD^+. Pyruvate kinase (PK) and phosphofructokinase (PFK), the enzymes whose activities are regulated by glucagon via cyclic adenosine monophosphate (cAMP)-dependent phosphokinase, are shown.

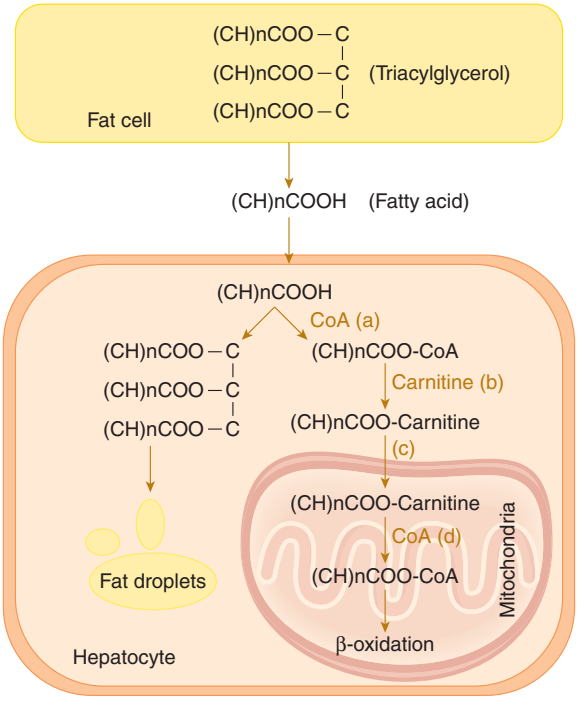

FIGURE 12–8. Steatosis, an accumulation of fat, results when xenobiotics interfere with the oxidation of fatty acids. Other processes that may be associated with intracellular accumulation of fat include: impaired lipoprotein synthesis; impaired lipoprotein release; increased mobilization of free fatty acids; increased uptake of circulating lipids; and increased production of triglycerides. β-Oxidation takes place in the mitochondria after transport of fatty acids from the cellular cytosol across the mitochondrial membrane. The enzymes involved are (a) acyl-CoA synthetase; (b) carnitine palmitoyltransferase I; (c) carnitine acylcarnitine translocase; and (d) carnitine palmitoyltransferase II. Acyl-CoA is the intramitochondrial substrate for β-oxidation. Potential mechanisms of inhibition of β-oxidation include induction of carnitine deficiency, inhibition of the transferase or translocase, and increased NADH:NAD^+ ratio via increased use of NAD^+ or by inhibition of NADH use. The specific site of action is not defined for many toxins that cause steatosis.

mitochondria by a process that utilizes cyclical binding to carnitine, a "carnitine shuttle" (Fig. 12–3). Once inside the mitochondria, FFAs are converted to acetyl-CoA by β-oxidation, involving the sequential removal of 2-carbon fragments, each time acting at the second carbon (the β carbon) position of the fatty acid. Each 2-carbon molecule removed from the FFA produces one NADH and one $FADH_2$, which are oxidized in the electron transport chain, and one mole of acetyl-CoA, which enters the TCA cycle. This process produces 1.3 times more ATP per molecule of carbon metabolized than does the oxidative metabolism of glucose or other carbohydrates.[47]

Many xenobiotics interrupt fatty acid metabolism at various steps, resulting in accumulation of triglycerides in the liver (Table 12–3; Fig. 12–8). The mechanisms of disruption of fatty acid metabolism are poorly defined.[16] Some xenobiotics, including ethanol, hypoglycin, and nucleoside analogs (Chap. 56); inhibit β-oxidation, at least indirectly, through effects on NADH concentrations. Protease inhibitors are associated with a syndrome of peripheral fat wasting, central adiposity, hyperlipidemia, and insulin resistance.

The condition of alcoholic ketoacidosis is related in part to inhibition of gluconeogenesis in the alcoholic patient and in part to an exuberant response to nutritional needs by the fatty acid machinery. Vomiting in the alcoholic patient leads to decreased intake of carbohydrate, which stimulates a starvation response with increases in serum glucagon, cortisol, growth hormone, and epinephrine concentrations, and decreases in serum insulin. When the need for carbohydrate is not met by gluconeogenesis, lipolysis, which is normally inhibited by insulin, is intensified and fatty acid mobilization progresses. Glucagon stimulates mitochondrial carnitine acyltransferase, and β-oxidation of fatty acids is increased. The increased mitochondrial NADH:NAD^+ ratio favors the production of β-hydroxybutyrate over acetoacetate, its oxidized form. The administration of fluids, dextrose, and thiamine to the alcoholic patient leads to correction of this process.[57]

SUMMARY

Humans are exposed to a wide variety of xenobiotics. Some, including therapeutic drugs, are harmless at low doses and toxic only at high doses. The toxicity of those xenobiotics that interrupt important

biologic functions or cause cellular injury is dose related and often rapidly evident. The diverse mechanisms of toxic injury have been discussed in general terms. The capacity of xenobiotics to cause injury is clearly a function of many factors specific to the xenobiotic, the tissue injured, and the individual.

REFERENCES

1. Abernathy D, Flockhart, DA: Molecular basis of cardiovascular drug metabolism implications for predicting clinically important drug interactions. *Circulation*. 2000;101:1749-1753.
2. Albert A: Fundamental aspects of selective toxicity. *Ann N Y Acad Sci*. 1965;123:5-18.
3. Arky RA, Freinkel N: Alcohol hypoglycemia. Effects of ethanol on plasma. 3. Glucose, ketones, and free fatty acids in "juvenile" diabetics: A model for "nonketotic diabetic acidosis"? *Arch Intern Med*. 1964;114:501-507.
4. Audi J, Belson M, Patel M, et al: Ricin poisoning: a comprehensive review. *JAMA*. 2005;294:2342-2351.
5. Badr MZ, Belinsky SA, Kauffman FC, et al: Mechanism of hepatotoxicity to periportal regions of the liver lobule due to allyl alcohol: role of oxygen and lipid peroxidation. *J Pharmacol Exp Ther*. 1986;238:1138-1142.
6. Baud FJ: Cyanide: critical issues in diagnosis and treatment. *Hum Exp Toxicol*. 2007;26:191-201.
7. Bayir H: Reactive oxygen species. *Crit Care Med*. 2005;33:S498-501.
8. Beutler E: Glucose-6-phosphate dehydrogenase deficiency. *N Engl J Med*. 1991;324:169-174.
9. Bock KW, Kohle C: Coordinate regulation of drug metabolism by xenobiotic nuclear receptors: UGTs acting together with CYPs and glucuronide transporters. *Drug Metab Rev*. 2004;36:595-615.
10. Boyland E, Chasseaud LF: The role of glutathione and glutathione S-transferases in mercapturic acid biosynthesis. *Adv Enzymol Relat Areas Mol Biol*. 1969;32:173-219.
11. Brittebo EB: Metabolism of xenobiotics in the nasal olfactory mucosa: implications for local toxicity. *Pharmacol Toxicol*. 1993;72 Suppl 3:50-52.
12. Brown C: Overview of drug interactions modulated by cytochrome P450. *US Pharmacists*. 2001;26:20-35.
13. Callaghan R, Crowley E, Potter S, et al: P-glycoprotein: so many ways to turn it on. *J Clin Pharmacol*. 2008;48:365-378.
14. Cappellini MD, Fiorelli G: Glucose-6-phosphate dehydrogenase deficiency. *Lancet*. 2008;371:64-74.
15. Caro AA, Cederbaum AI: Oxidative stress, toxicology, and pharmacology of CYP2E1. *Annu Rev Pharmacol Toxicol*. 2004;44:27-42.
16. Carr A, Samaras K, Chisholm DJ, et al: Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. *Lancet*. 1998;351:1881-1883.
17. Chinn LW, Kroetz DL: ABCB1 pharmacogenetics: progress, pitfalls, and promise. *Clin Pharmacol Ther*. 2007;81:265-269.
18. Conney AH: Induction of drug-metabolizing enzymes: a path to the discovery of multiple cytochromes P450. *Annu Rev Pharmacol Toxicol*. 2003;43:1-30.
19. Cribb AE, Peyrou M, Muruganandan S, et al: The endoplasmic reticulum in xenobiotic toxicity. *Drug Metab Rev*. 2005;37:405-442.
20. Dai Y, Rashba-Step J, Cederbaum AI: Stable expression of human cytochrome P4502E1 in HepG2 cells: Characterization of catalytic activities and production of reactive oxygen intermediates. *Biochemistry*. 1993;32:6928-6937.
21. Ding X, Kaminsky LS: Human extrahepatic cytochromes P450: Function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annu Rev Pharmacol Toxicol*. 2003;43:149-173.
22. Dutheil F, Beaune P, Loriot MA: Xenobiotic metabolizing enzymes in the central nervous system: Contribution of cytochrome P450 enzymes in normal and pathological human brain. *Biochimie*. 2008;90:426-436.
23. Eling TE, Thompson DC, Foureman GL, et al: Prostaglandin H synthase and xenobiotic oxidation. *Annu Rev Pharmacol Toxicol*. 1990;30:1-45.
24. Evans WE: Pharmacogenomics: Marshalling the human genome to individualise drug therapy. *Gut*. 2003;52 Suppl 2:ii10-18.
25. Farabee M. OnLine Biology Book. Vol. 2009; 2009. http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookTOC.html.
26. Feng PC, Patrick SJ: Studies of the action of hypoglycin-A, an hypoglycaemic substance. *Br J Pharmacol*. 1958;13:125-130.
27. Fenteany G. *BioChemWeb*. Vol. 2009; 2009. http://www.biochemweb.org/.
28. Flockhart D, Tanus-Santos, JE: Implications of cytochrome P450 interactions when prescribing medication of hypertension. *Arch Intern Med*. 2002;162:405-412.
29. Freinkel N, Singer, DL, Arky, RA, et al: Alcohol hypoglycemia. I. Carbohydrate metabolism of patients with clinical alcohol hypoglycemia and the experimental reproduction of the syndrome with pure ethanol. *J Clin Invest*. 1963;42:1112-1113.
30. Gardiner SJ, Begg EJ: Pharmacogenetics, drug-metabolizing enzymes, and clinical practice. *Pharmacol Rev*. 2006;58:521-590.
31. Gerlach M, Riederer P, Przuntek H, et al: MPTP mechanisms of neurotoxicity and their implications for Parkinson's disease. *Eur J Pharmacol*. 1991;208:273-286.
32. Goshman L, Fish J, Roller, K.: Clinically significant cytochrome P450 drug interactions. *J Pharm Soc Wisconsin*. 1999:23-38.
33. Guengerich FP: Catalytic selectivity of human cytochrome P450 enzymes: Relevance to drug metabolism and toxicity. *Toxicol Lett*. 1994;70:133-138.
34. Guengerich FP: Cytochrome p450 and chemical toxicology. *Chem Res Toxicol*. 2008;21:70-83.
35. Halpert JR, Guengerich FP, Bend JR, et al: Selective inhibitors of cytochromes P450. *Toxicol Appl Pharmacol*. 1994;125:163-175.
36. Handschin C, Meyer UA: Induction of drug metabolism: The role of nuclear receptors. *Pharmacol Rev*. 2003;55:649-673.
37. Hetu C, Dumont A, Joly JG: Effect of chronic ethanol administration on bromobenzene liver toxicity in the rat. *Toxicol Appl Pharmacol*. 1983;67:166-177.
38. Ho RH, Kim RB: Transporters and drug therapy: Implications for drug disposition and disease. *Clin Pharmacol Ther*. 2005;78:260-277.
39. Hughes MF: Arsenic toxicity and potential mechanisms of action. *Toxicol Lett*. 2002;133:1-16.
40. Imlay JA: Pathways of oxidative damage. *Annu Rev Microbiol*. 2003;57:395-418.
41. Ingleman-Sundberg M: Genetic polymorphisms of cytochrome P450 2D6 (CY2D6): Clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J*. 2005;5:6-13.
42. Josephy D, Guengerich P, Miners JO: "Phase I and Phase II" drug metabolism: Terminology that we should phase out? *Drug Metab Rev*. 2005;37:575-580.
43. Kalgutkar AS, Gardner I, Obach RS, et al: A comprehensive listing of bioactivation pathways of organic functional groups. *Curr Drug Metab*. 2005;6:161-225.
44. Kato M: Intestinal first-pass metabolism of CYP3A4 substrates. *Drug Metab Pharmacokinet*. 2008;23:87-94.
45. Kim D, Guengerich FP: Cytochrome P450 activation of arylamines and heterocyclic amines. *Annu Rev Pharmacol Toxicol*. 2005;45:27-49.
46. Kim RB: Drugs as P-glycoprotein substrates, inhibitors, and inducers. *Drug Metab Rev*. 2002;34:47-54.
47. King M. The Medical Biochemistry Page. Vol. 2009; 2009. http://themedicalbiochemistrypage.org/.
48. Kleinman JG, Breitenfield RV, Roth DA: Acute renal failure associated with acetaminophen ingestion: Report of a case and review of the literature. *Clin Nephrol*. 1980;14:201-205.
49. Krishna DR, Klotz U: Extrahepatic metabolism of drugs in humans. *Clin Pharmacokinet*. 1994;26:144-160.
50. Kroncke KD, Fricker G, Meier PJ, et al: alpha-Amanitin uptake into hepatocytes. Identification of hepatic membrane transport systems used by amatoxins. *J Biol Chem*. 1986;261:12562-12567.
51. Lewis D: On the recognition of mammalian microsomal cytochrome P450 substrates and their characteristics. *Biochem Pharmacol*. 2000;60:293-306.
52. Lewis MS, Youle RJ: Ricin subunit association. Thermodynamics and the role of the disulfide bond in toxicity. *J Biol Chem*. 1986;261:11571-11577.
53. Lieber CS: Metabolism of alcohol. *Clin Liver Dis*. 2005;9:1-35.
54. Lin JH: CYP induction-mediated drug interactions: in vitro assessment and clinical implications. *Pharm Res*. 2006;23:1089-1116.
55. Lindell TJ, Weinberg F, Morris PW, et al: Specific inhibition of nuclear RNA polymerase II by alpha-amanitin. *Science*. 1970;170:447-449.
56. Manahan S: *Toxicological Chemistry and Biochemistry*. 3rd ed. New York: Lewis Publishers; 2003.
57. McGuire LC, Cruickshank AM, Munro PT: Alcoholic ketoacidosis. *Emerg Med J*. 2006;23:417-420.
58. Menon JA: Tropical hazards associated with the use of pentachlorophenol. *Br Med J*. 1958;14:1156-1158.
59. Miller EC, Miller JA: The presence and significance of bound aminoazo dyes in the livers of rats fed p-demethylaminoazobenzene. *Cancer Res*. 1947;7:468-480.

60. Myers CE, McGuire WP, Liss RH, et al: Adriamycin: The role of lipid peroxidation in cardiac toxicity and tumor response. *Science*. 1977;197:165-167.
61. Nagata K: Genetic polymorphism of human cytochrome P450 involved in drug metabolism. *Drug Metabol. Pharmacokin*. 2002;17:167-189.
62. Nelson D: Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice varients. *Pharmacogenetics*. 2004;14:1-18.
63. Nelson D. Cytochrome P450 homepage. Vol. 2009; 2009. http://drnelson.utmem.edu/CytochromeP450.html.
64. Nolin TD: Altered nonrenal drug clearance in ESRD. *Curr Opin Nephrol Hypertens*. 2008;17:555-559.
65. Obach RS, Walsky RL, Venkatakrishnan K, et al: In vitro cytochrome P450 inhibition data and the prediction of drug-drug interactions: Qualitative relationships, quantitative predictions, and the rank-order approach. *Clin Pharmacol Ther*. 2005;78:582-592.
66. Park B: Cytochrome P450 enzymes in the heart. *Lancet*. 2000;355:945-946.
67. Pavek P, Dvorak Z: Xenobiotic-induced transcriptional regulation of xenobiotic metabolizing enzymes of the cytochrome P450 superfamily in human extrahepatic tissues. *Curr Drug Metab*. 2008;9:129-143.
68. Peck CC, Temple R, Collins JM: Understanding consequences of concurrent therapies. *JAMA*. 1993;269:1550-1552.
69. Pelkonen O, Turpeinen M, Hakkola J, et al: Inhibition and induction of human cytochrome P450 enzymes: Current status. *Arch Toxicol*. 2008;82:667-715.
70. Peters RA, Wakelin RW: The synthesis of fluorocitric acid and its inhibition in acetate. *Biochem J*. 1957;67:280-286.
71. Piscitelli S: Indinavir concentrations and St John's wort. *Lancet*. 2000;355:547-548.
72. Rose MS, Lock EA, Smith LL, et al: Paraquat accumulation: Tissue and species specificity. *Biochem Pharmacol*. 1976;25:419-423.
73. Rosen GM, Rauckman EJ: Carbon tetrachloride-induced lipid peroxidation: A spin trapping study. *Toxicol Lett*. 1982;10:337-344.
74. Ruderman N, Shafrir E, Bressler R: Relation of fatty acid oxidation tgluconeogenesis: Effect of pentenoic acid. *Life Sci*. 1968;7:1083-1089.
75. Santi DV, McHenry CS, Sommer H: Mechanism of interaction of thymidylate synthetase with 5-fluorodeoxyuridylate. *Biochemistry*. 1974;13:471-481.
76. Schafer DF, Sorrell MF: Power failure, liver failure. *N Engl J Med*. 1997;336:1173-1174.
77. Schuster I, Bernhardt R: Inhibition of cytochromes p450: Existing and new promising therapeutic targets. *Drug Metab Rev*. 2007;39:481-499.
78. Schwarz UI, Stein CM: Genetic determinants of dose and clinical outcomes in patients receiving oral anticoagulants. *Clin Pharmacol Ther*. 2006;80:7-12.
79. Si D, Wang Y, Zhou YH, et al: Mechanism of CYP2C9 inhibition by flavones and flavonols. *Drug Metab Dispos*. 2009;37:629-634.
80. Sim E, Lack N, Wang C-J, et al: Arylamine N-acetyltransferases: Structural and functional implications of polymorphisms. *Toxicology*. 2008;254:170-183.
81. Smith MJ, Jeffrey SW: The effects of salicylate on oxygen consumption and carbohydrate metabolism in the isolated rat diaphragm. *Biochem J*. 1956;63:524-528.
82. Southorn PA, Powis G: Free radicals in medicine. I. Chemical nature and biologic reactions. *Mayo Clin Proc*. 1988;63:381-389.
83. Spencer HC RV, Adams EM, Irish DD: Toxicological studies on laboratory animals of certain alkyldinitrophenols used in agriculture. *J Indian Hyg Toxicol*. 1948;30:10-25.
84. Sweeney BP, Bromilow J: Liver enzyme induction and inhibition: Implications for anaesthesia. *Anaesthesia*. 2006;61:159-177.
85. Tanaka K: On the mode of action of hypoglycin A. *J Biol Chem*. 1972;247:7465-7478.
86. Tanaka K, Kean EA, Johnson B: Jamaican vomiting sickness. Biochemical investigation of two cases. *N Engl J Med*. 1976;295:461-467.
87. Timbrell J: *Principles of Biochemical Toxicology*. 3rd ed. Philadelphia: Taylor & Francis; 2000.
88. Timsit YE, Negishi M: CAR and PXR: The xenobiotic-sensing receptors. *Steroids*. 2007;72:231-246.
89. Umbreit J: Methemoglobin—it's not just blue: A concise review. *Am J Hematol*. 2007;82:134-144.
90. Urquhart BL, Tirona RG, Kim RB: Nuclear receptors and the regulation of drug-metabolizing enzymes and drug transporters: Implications for interindividual variability in response to drugs. *J Clin Pharmacol*. 2007;47:566-578.
91. Wallace KB, Starkov AA: Mitochondrial targets of drug toxicity. *Annu Rev Pharmacol Toxicol*. 2000;40:353-388.
92. Waxman D: P450 Gene induction by structurally diverse xenochemicals: Central role of nuclear receptors CAR, P, and PPR. *Arch Biochem Biophys*. 1999;369:11-23.
93. Wienkers LC, Heath TG: Predicting in vivo drug interactions from in vitro drug discovery data. *Nat Rev Drug Discov*. 2005;4:825-833.
94. Williams RT: *Detoxication Mechanisms: The Metabolism of Drugs and Allied Organic Compounds*. 1st ed. London: Chapman and Hall; 1949.
95. Williams RT: *Detoxication Mechanisms: The Metabolism and Detoxication of Drugs, Toxic Substances, and Other Organic Compounds*. 2nd ed. London: Chapman and Hall; 1959.
96. Zamek-Gliszczynski MJ, Hoffmaster KA, Nezasa K, et al: Integration of hepatic drug transporters and phase II metabolizing enzymes: Mechanisms of hepatic excretion of sulfate, glucuronide, and glutathione metabolites. *Eur J Pharm Sci*. 2006;27:447-486.
97. Zhang JY, Wang Y, Prakash C: Xenobiotic-metabolizing enzymes in human lung. *Curr Drug Metab*. 2006;7:939-948.
98. Zhou SF, Xue CC, Yu XQ, et al: Clinically important drug interactions potentially involving mechanism-based inhibition of cytochrome P450 3A4 and the role of therapeutic drug monitoring. *Ther Drug Monit*. 2007;29:687-710.

APPENDIX

Common Cytochrome P450 Substrates, Inhibitors, and Inducers[1]

	SUBSTRATES	INHIBITORS	INDUCERS
1A2	**Analgesics** Acetaminophen Naproxen **Antidepressants** Amitriptyline Clomipramine Duloxetine[2] Fluvoxamine Imipramine Mirtazapine **Antipsychotics** Clozapine Haloperidol Olanzapine Thioridazine **Cardiovascular** Mexiletine Propranolol Verapamil **Hormones** Estradiol Flutamide **Other Medications** Caffeine Cyclobenzaprine Ondansetron Theophylline[3] Tizanidine[3] Warfarin-R Zolmitriptan	**Antibiotics (Fluoroquinolones)** Ciprofloxacin Norfloxacin **Antibiotics (Macrolides)** Clarithromycin Erythromycin Troleandomycin **Antidepressants** Duloxetine Fluvoxamine **Cardiovascular** Amiodarone Mexiletine Mibefradil[4] Verapamil **Other Medications** Acyclovir Cimetidine Famotidine Grapefruit juice	**Anticonvulsants** Carbamazepine Phenobarbital Phenytoin **Proton Pump Inhibitors** Lansoprazole Omeprazole **Other** Nafcillin Polycyclic hydrocarbons (chargrilled meat, cigarette smoke) Rifampicin Rifampin Ritonavir[5]
3A4	**Antibiotics** Clarithromycin Dapsone Erythromycin Rifabutin Telithromycin **Antidepressants** (Minor for most) Amitriptyline Buspirone[2] Citalopram Clomipramine Escitalopram Imipramine Mirtazapine Nefazodone Sertraline Trazodone **Antidysrhythmics** Amiodarone Disopyramide Quinidine[3] **Antifungals** Itraconazole Ketoconazole Voriconazole **Immune Modulators** Cyclosporine[3] Sirolimus[3] Tacrolimus[3] Tamoxifen **Opioids** Alfentanil[3] Buprenorphine Codeine Dextromethorphan Fentanyl[3] Meperidine Methadone Morphine Oxycodone Propoxyphene Sufentanyl Tramadol **Protease Inhibitors** Indinavir Nelfinavir Ritonavir Saquinavir[2]	**Antidepressants** Fluoxetine Fluvoxamine Nefazodone Norfluoxetine Sertraline **Antibiotics (Macrolide)** Clarithromycin Erythromycin Telithromycin **Antibiotics** Chloramphenicol Ciprofloxacin Isoniazid Norfloxacin **Antifungals** Fluconazole Itraconazole Ketoconazole Voriconazole **Calcium Channel Blockers** Diltiazem Mibefradil[4] Nifedipine Verapamil **Protease Inhibitors** Amprenavir Atazanavir Fosamprenavir Indinavir Nelfinavir[1] Ritonavir[5] Saquinavir **Other** Amiodarone Cimetidine Cisapride Cocaine Cyclosporine Ergotamines Felbamate Grapefruit juice[1]	**Antibiotics** Rifabutin Rifampicin Rifampin Rifapentine **Protease Inhibitors** Amprenavir Efavirenz Nelfinavir[1] Nevirapine Ritonavir[5] **Anticonvulsants** Carbamazepine Felbamate Oxcarbazepine Phenobarbital Phenytoin Topiramate **Steroids** Dexamethasone Methylprednisolone Prednisolone **Other** St. John's wort

	SUBSTRATES		INHIBITORS		INDUCERS
	Antihistamines Astemizole[4] Chlorpheniramine Desloratidine Loratidine Terfenadine[4] **Antipsychotics** (Minor for most) Aripiprazole Clozapine Haloperidol Quetiapine Risperidone Thioridazine Ziprasidone **Benzodiazepines** Alprazolam Clonazepam Diazepam Midazolam[2] Triazolam[2] **Calcium Channel Blockers** Amlodipine Diltiazem Felodipine[2] Nicardipine Nifedipine Nimodipine Nisoldipine Verapamil	**Proton Pump Inhibitors** (Minor for most) Esomeprazole Lansoprazole Omeprazole Pantoprazole Rabeprazole **Statins** Atorvastatin Cerivastatin[4] Lovastatin[2] Simvastatin[2] **Steroids and Hormones** Dexamethasone Estradiol Fluticasone[2] Hydrocortisone Methylprednisolone Prednisone Progesterone **Other Medications** Carbamazepine Cisapride Cyclobenzaprine Diclofenac Ergotamines[3] Losartan Ondansetron Pioglitazone Propranolol Salmeterol Sildenafil[2] Vardenafil[2] Warfarin-R Zaleplon Zolpidem			
2C9	**Angiotensin II Blockers** Irbesartan Losartan **Hypoglycemics** Chlorpropamide Glimepiride Glipizide Glyburide Tolbutamide **NSAIDs** Celecoxib Diclofenac Flurbiprofen Ibuprofen Indomethacin Meloxicam Naproxen Piroxicam	**Other Medications** Amitriptyline Fluoxetine Fluvastatin Phenobarbital Phenytoin[3] Rosiglitazone Tamoxifen Sertraline Rosuvastatin Warfarin-S[3]	**Antibiotics (Macrolide)** Clarithromycin Erythromycin Troleandomycin **Antibiotics** Isoniazid Metronidazole Sulfamethoxazole **Antidepressants** Fluoxetine Fluvoxamine Paroxetine Sertraline	**Antifungals (Azoles)** Fluconazole Itraconazole Ketoconazole Voriconazole **Other** Amiodarone Cimetidine Ritonavir[5] Grapefruit juice Valproic acid	**Anticonvulsants** Carbamazepine Phenobarbital Phenytoin **Other** Nelfinavir Rifampicin Rifampin Rifapentine Ritonavir[5] St. John's wort

	SUBSTRATES		INHIBITORS		INDUCERS
2C19	**Antidepressants** Amitriptyline Citalopram Clomipramine Desipramine Doxepin Escitalopram Fluoxetine Imipramine **Anticonvulsants** Diazepam Phenobarbital Phenytoin	**Proton Pump Inhibitors** (Major for most) Esomeprazole Lansoprazole Omeprazole[2] Pantoprazole Rabeprazole **Other Medications** Atomoxetine Carisoprodol Clopidogrel Cyclophosphamide Indomethacin Nelfinavir Olanzapine Methadone Progesterone Propranolol Voriconazole Warfarin-R	**Antibiotics (Macrolide)** Clarithromycin Erythromycin Troleandomycin **Antidepressants (SSRI)** Citalopram Fluoxetine Fluvoxamine Paroxetine Sertraline **Antifungals (Azoles)** Fluconazole Ketoconazole Voriconazole	**Anticonvulsants** Felbamate Oxcarbazepine Topiramate **Other Medications** Chloramphenicol Cimetidine Grapefruit juice Indomethacin Isoniazid Ritonavir[5] Ticlopidine **Proton Pump Inhibitors** Lansoprazole Omeprazole Pantoprazole Rabeprazole	**Anticonvulsants** Carbamazepine Phenobarbital Phenytoin **Other** Prednisone Rifampicin Rifampin Rifapentine Ritonavir[5] St. John's wort
2D6	**Antidepressants (SSRI)** Escitalopram Fluoxetine Fluvoxamine Paroxetine Sertraline **Antidepressants (Other)** (Major for most) Amitriptyline Clomipramine Desipramine[2] Doxepin Duloxetine Escitalopram Imipramine Maprotiline Mirtazapine Nortriptyline Venlafaxine **Antidysrhythmics** Flecainide Mexiletine Quinidine **Antihistamines** Chlorpheniramine Desloratidine Diphenhydramine Loratadine	**Antipsychotics** (Major for most) Aripiprazole Chlorpromazine Fluphenazine Haloperidol Perphenazine Promethazine Risperidone Thioridazine[3] **β-Adrenergic Antagonists** Metoprolol Pindolol Propranolol Timolol **Opioids** Codeine Dextromethorphan Hydrocodone Oxycodone Tramadol **Other Medications** Amphetamine Atomoxetine Cyclobenzaprine Debrisoquine Metoclopramide Ondansetron Tamoxifen	**Antidepressants** Bupropion Citalopram Duloxetine Escitalopram Fluoxetine Paroxetine Sertraline **Antihistamines** Chlorpheniramine Cimetidine Diphenhydramine Hydroxyzine Ranitidine **Antipsychotics** Chlorpromazine Haloperidol Perphenazine Promethazine Thioridazine	**Other Medications** Amiodarone Celecoxib Chloramphenicol Chloroquine Cocaine Doxorubicin Ticlopidine Methadone Ritonavir[5]	Dexamethasone Rifampicin Rifampin Ritonavir[5] Tramadol

	SUBSTRATES		INHIBITORS	INDUCERS
2E1	Acetaminophen Chlorzoxazone Ethanol Theophylline Isoniazid	**Inhaled Anesthetics** Enflurane Halothane Isoflurane Methoxyflurane	Disulfiram Fomepizole	Ethanol Isoniazid St. John's wort

[1] This list is not complete and may reflect some variation in author opinions as to whether a xenobiotic is a substrate, inhibitor, or inducer. Many drugs are metabolized by several isoenzymes with some representing major pathways and others minor pathways.

[2] The area-under-the-curve of this substrate has been shown to increase 5-fold or more when coadministered with a known CYP3A inhibitor.

[3] This substrate has a narrow therapeutic range and safety concerns occur when coadministered with an inhibitor.

[4] These medications were withdrawn from the market due to complications associated with drug–drug interactions.

[5] Ritonavir has paradoxical dose- and time-dependent inhibitory and induction effects.

NSAID – Non-steroidal anti-inflammatory drugs; PPI – proton pump inhibitors.

REFERENCES

1. Anderson GD: Pharmacogenetics and enzyme induction/inhibition properties of antiepileptic drugs. *Neurology*. 2004;63:S3-8.
2. Armstrong SC, Cozza KL: Antihistamines. *Psychosomatics*. 2003;44:430-434.
3. Bartra J, Valero AL, del Cuvillo A, et al: Interactions of the H1 antihistamines. *J Investig Allergol Clin Immunol*. 2006;16 Suppl 1:29-36.
4. Bertilsson L: Metabolism of antidepressant and neuroleptic drugs by cytochrome p450s: Clinical and interethnic aspects. *Clin Pharmacol Ther*. 2007;82:606-609.
5. Bondy B, Spellmann I: Pharmacogenetics of antipsychotics: Useful for the clinician? *Curr Opin Psychiatry*. 2007;20:126-130.
6. Dixit V, Hariparsad N, Li F, et al: Cytochrome P450 enzymes and transporters induced by anti-human immunodeficiency virus protease inhibitors in human hepatocytes: Implications for predicting clinical drug interactions. *Drug Metab Dispos*. 2007;35:1853-1859.
7. Epocrates: Medication formulary. 2009. Available at: http://www.epocrates.com/
8. FDA: Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers. US Department of Health and Human Services. 2009.
9. Flockhart D: P450 Drug Interaction Table 2009. Available at http://medicine.iupui.edu/clinpharm/ddis/
10. Flockhart D, Tanus-Santos, JE: Implications of cytochrome P450 interactions when prescribing medication of hypertension. *Arch Intern Med*. 2002;162:405-412.
11. Foisy MM, Yakiwchuk EM, Hughes CA: Induction effects of ritonavir: Implications for drug interactions. *Ann Pharmacother*. 2008;42:1048-1059.
12. Furuta T, Sugimoto M, Shirai N, et al: CYP2C19 pharmacogenomics associated with therapy of *Helicobacter pylori* infection and gastro-esophageal reflux diseases with a proton pump inhibitor. *Pharmacogenomics*. 2007;8:1199-1210.
13. Ku HY, Ahn HJ, Seo KA, et al: The contributions of cytochromes P450 3A4 and 3A5 to the metabolism of the phosphodiesterase type 5 inhibitors sildenafil, udenafil, and vardenafil. *Drug Metab Dispos*. 2008;36:986-990.
14. Lotsch J, Skarke C, Liefhold J, et al: Genetic predictors of the clinical response to opioid analgesics: Clinical utility and future perspectives. *Clin Pharmacokinet*. 2004;43:983-1013.
15. Martinez C, Albet C, Agundez JA, et al: Comparative in vitro and in vivo inhibition of cytochrome P450 CYP1A2, CYP2D6, and CYP3A by H2-receptor antagonists. *Clin Pharmacol Ther*. 1999;65:369-376.
16. Mega JL, Close SL, Wiviott SD, et al: Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med*. 2009;360:354-362.
17. Neuvonen PJ, Niemi M, Backman JT: Drug interactions with lipid-lowering drugs: Mechanisms and clinical relevance. *Clin Pharmacol Ther*. 2006;80:565-581.
18. Nivoix Y, Leveque D, Herbrecht R, et al: The enzymatic basis of drug-drug interactions with systemic triazole antifungals. *Clin Pharmacokinet*. 2008;47:779-792.
19. Nowack R: Review article: Cytochrome P450 enzyme, and transport protein mediated herb-drug interactions in renal transplant patients: Grapefruit juice, St. John's Wort—and beyond! *Nephrology (Carlton)*. 2008;13:337-347.
20. Pai MP, Momary KM, Rodvold KA: Antibiotic drug interactions. *Med Clin N Am*. 2006;90:1223-1255.
21. Pelkonen O, Turpeinen M, Hakkola J, et al: Inhibition and induction of human cytochrome P450 enzymes: current status. *Arch Toxicol*. 2008;82:667-715.
22. Picard N, Cresteil T, Djebli N, et al: In vitro metabolism study of buprenorphine: evidence for new metabolic pathways. *Drug Metab Dispos*. 2005;33:689-695.
23. Ramirez J, Innocenti F, Schuetz EG, et al: CYP2B6, CYP3A4, and CYP2C19 are responsible for the in vitro N-demethylation of meperidine in human liver microsomes. *Drug Metab Dispos*. 2004;32:930-936.
24. Somogyi AA, Menelaou A, Fullston SV: CYP3A4 mediates dextropropoxyphene N-demethylation to nordextropropoxyphene: human in vitro and in vivo studies and lack of CYP2D6 involvement. *Xenobiotica*. 2004;34:875-887.
25. Sweeney BP, Bromilow J: Liver enzyme induction and inhibition: Implications for anaesthesia. *Anaesthesia*. 2006;61:159-177.
26. Wojcikowski J, Maurel P, Daniel WA: Characterization of human cytochrome p450 enzymes involved in the metabolism of the piperidine-type phenothiazine neuroleptic thioridazine. *Drug Metab Dispos*. 2006;34:471-476.
27. Zanger UM, Turpeinen M, Klein K, et al: Functional pharmacogenetics/genomics of human cytochromes P450 involved in drug biotransformation. *Anal Bioanal Chem*. 2008;392:1093-1108.