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Dedication to William O. Foye

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PART I

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CHAPTER 1

Introduction to Medicinal Chemistry

Andrew Coop

Abbreviations

P-gp P-glycoprotein

GPCRs G protein-coupled receptors

SAR structure-activity relationships

CNS central nervous system

Introduction

Medicinal chemistry has as many definitions as those doing the defining, and is complicated by the concurrent use of terms such as pharmaceutical chemistry, drug chemistry, bioorganic chemistry, chemical biology, and the list goes on. The question of “What is medicinal chemistry?” has been thoroughly discussed,^{1,2} and this author views medicinal chemistry as “the application of chemistry to the continuous improvement of health.” With such a general definition, it is clear that medicinal chemistry forms a critical component of the world’s health care systems, but it is an area that many practitioners do not have a deep understanding of and one that can be initially overwhelming for learners. This short chapter explains the key components of medicinal chemistry and the skills required to fully comprehend the topic and is designed to remove the apprehension often observed by those new to medicinal chemistry.

Medicinal Chemistry is not Just Synthesis and Pushing Electrons

Traditional undergraduate teaching of chemistry is often overwhelming for students; it is a seemingly endless list of named reactions, along with “pushing electrons,” filling an alphabet soup of electron shells, and mathematical equations describing statistical analysis of where electrons are while admitting we have no idea where they are!³ It is therefore of no surprise to this author that some students fear any topic that includes the name chemistry. As an introduction to medicinal chemistry, it needs to be remembered that the whole area originates from the use of natural products to treat conditions, where traditional remedies were handed down through the generations. As scientific understanding of the specific active components increased, the area evolved to one where the active ingredient was extracted, followed by making chemical modifications resulting in new drugs. It is therefore not unexpected that medicinal chemistry education in pharmacy schools was focused on extraction and chemical synthesis, areas close to undergraduate chemistry. However,

medicinal chemistry has continued to evolve, and the health care professional needs to bring a new range of chemical skills to the table.

The Components of Medicinal Chemistry

A medicinal chemist can seem a magician to one not trained in the area. Simply by looking at patterns of hexagons and pentagons, with a few letters (e.g., C, N, O, S), along with charges (+ and -), the medicinal chemist can predict

- the effects of that drug on the human body, including magnitude of effect and duration
- the potential for interactions with other drugs
- possible adverse effects of the drug
- optimal methods of administration of the drug
- how to change the drug to eliminate or attenuate unwanted effects
- how to store the drug to ensure prolonged effectiveness
- how a drug may be misused (abused)

Obviously to be able to predict all such actions, the medicinal chemist must be skilled in many avenues of science, including organic chemistry, inorganic chemistry, physical chemistry, computational chemistry, pharmacology, metabolism, biology, biochemistry, mathematics, and, yes, psychology. That a medicinal chemist is required to apply and integrate all these differing scientific disciplines in order to make drug design decisions is what causes the apprehension for learners, but also what places it as a central discipline in health care. For that reason alone, the discipline of medicinal chemistry should excite learners relative to how they might bring a professionally unique and critically needed skill set to the interprofessional care team and the patient care environment.

So, Where to Start?

If we consider the first bullet above, the effects of the drug depend on the chemical constituents of the molecule, including how they interact with proteins, nucleic acids, or other macromolecules in the human body, what specific macromolecules they interact with, how they are degraded by

enzymes in the body, how they facilitate transport of the drug across membranes, and how they facilitate solubility in aqueous media, among others. It is important to remember that all atoms in all molecules follow simple predictable rules and that all drugs are molecules and will behave as their chemistry dictates they must. Many molecules are small (e.g., aspirin [Chapter 15]); several are large (paclitaxel) (Chapter 33) (Fig. 1.1); and biologics are simply very large molecules or mixtures of large molecules. However, they are all molecules that follow the same rules.

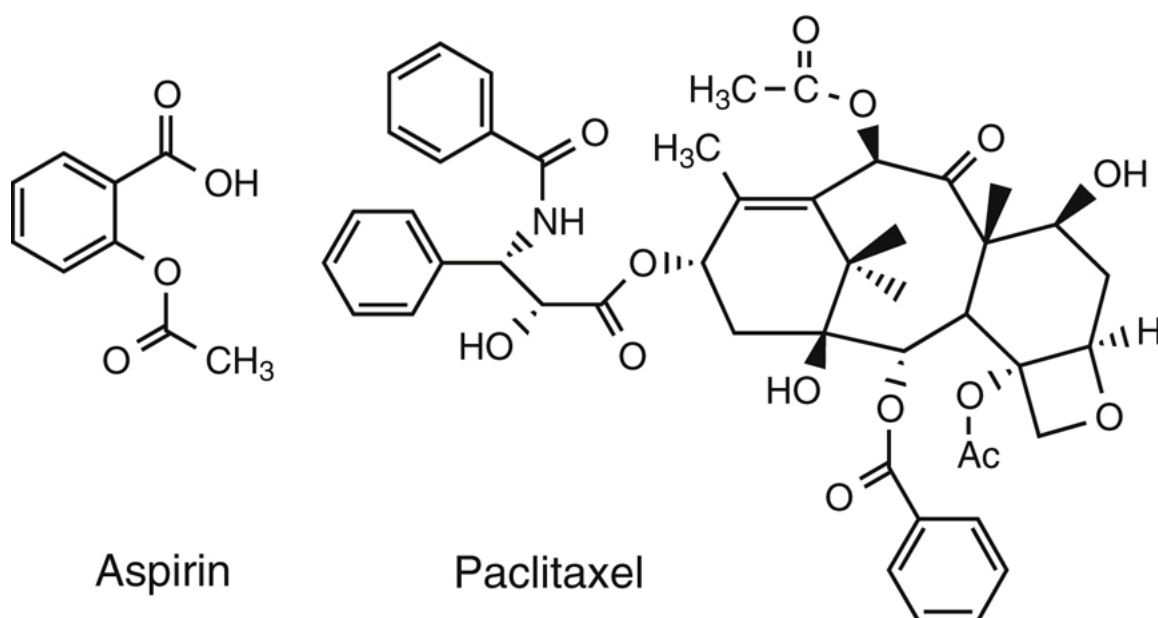


FIGURE 1.1 Example of a small and large drug molecule.

Solubility

Let us consider aqueous (water-based) solubility as an area to apply this metric. Solubility under aqueous conditions is a critical component of the action of a drug, as the human body is mostly water and all life occur in aqueous systems. Whether we consider aspirin or paclitaxel in terms of solubility, we are considering the same principles: do the polar groups overcome the lipophilic groups to allow solubility in the polar solvent of water? If, like aspirin and paclitaxel, the answer is no, the second question is “Are there acidic and/or basic groups that could be utilized to yield an ionized species?” as the introduction of ionization increases polarity. In

the case of aspirin, the answer is “yes,” but in the case of paclitaxel, it is “no,” making aspirin the more water soluble of the two drugs. The theory and application of acid/base principles is involved, but the fundamental concept remains the same for all drugs—is it polar enough to be water soluble?

Crossing Membranes

Biological membranes exist at the surface of cells but also form barriers that drugs are required to cross to reach their target biological system (see below). Membranes are lipids, and this lipophilic character is in contrast to the aqueous environment outside the membrane. One confounding factor that many learners confront is the fact that if a drug is soluble in the aqueous environment, how can it enter the membrane to cross passively? One must remember that there are extremes, such as the opioid antagonist methylnaltrexone that is always fully ionized and designed not to cross the blood-brain barrier to limit actions centrally (Chapter 14) and others that are extreme in terms of lipophilicity, such as short-acting barbiturates (Chapter 12), fentanyl analogues (Chapter 14), or the triglyceride-lowering drug fenofibrate (Chapter 19). However, most drugs are designed at a “happy medium” with a sufficient balance of aqueous and lipid solubility that allows them to traverse the disparate barriers they will encounter in distributing from the site of administration to the site of action and ultimately to the site of elimination.

Interaction With Biological Systems

The majority of drugs exert their effects through direct action on biological systems. These interactions are again consistent for all drugs and all biological systems—a simple effect of thermodynamics. How energetically favorable are the dipole-dipole interactions between biological system (the target receptor or enzyme) and drug compared to the same interaction between drug and the aqueous environment, which includes considering the entropy of both drug-target and drug-water complexes? Again, the science behind the multiple effects is involved, but the fundamental principle is constant. Indeed, it is also the same principle that determines whether the drug interacts with (or binds to) other unwanted biological targets, which leads to adverse effects that can limit therapeutic utility.

The interactions can be modeled using computational chemistry, or a pattern of chemical substituents that lead to stronger interactions can be determined through the analysis of a large set of differing drug analogues. The development of an activity pattern from a large set of drugs is what is called structure-activity relationships (SAR). SAR is a powerful tool, allowing the prediction of the activity of a new drug, but this author's experience is that learners often fall into the trap of thinking that SAR is a hard and fast "law" and are frustrated by compounds that are active but do not satisfy SAR rules. This, again, is where a grasp of the principles behind the terms is required. SAR is a pattern that chemists have developed to help explain the action of drugs and is therefore a model based on known knowledge. All models have limitations, and anything based on current knowledge is one new data point away from no longer being accurate. Thus, this author considers SAR as more of an art than a science, a useful guide that helps predict activity, but not a "set in stone" law. Indeed, even the author has proposed SAR for a class of compounds that was shown not to be accurate for all compounds in that class!

Functional Activity at Receptors

The interaction with biological systems mentioned above is simply recognition or binding between drug and macromolecular receptors (Fig. 1.2). This correlates very well with enzyme inhibitory function, but medicinal chemists also need to understand how receptors function. G protein-coupled receptors (GPCRs) are one main type of receptor which spans cell membranes and functions to translate the message from the drug to the inside of the cell (readers are directed to Chapters 6-9 of this text for more in-depth information on drug receptors). The pharmacology of such receptors and intracellular pathways are complicated, but again, the learner should not lose sight that a drug can bind and activate the receptor (an agonist) or bind and not activate the receptor (an antagonist). Within the pages of this text, you will encounter a range of other activities associated with drugs (ligands), such as inverse agonists, partial agonists, and allosteric binders, but all interact and either activate a receptor macromolecule in some way or inactivate it.

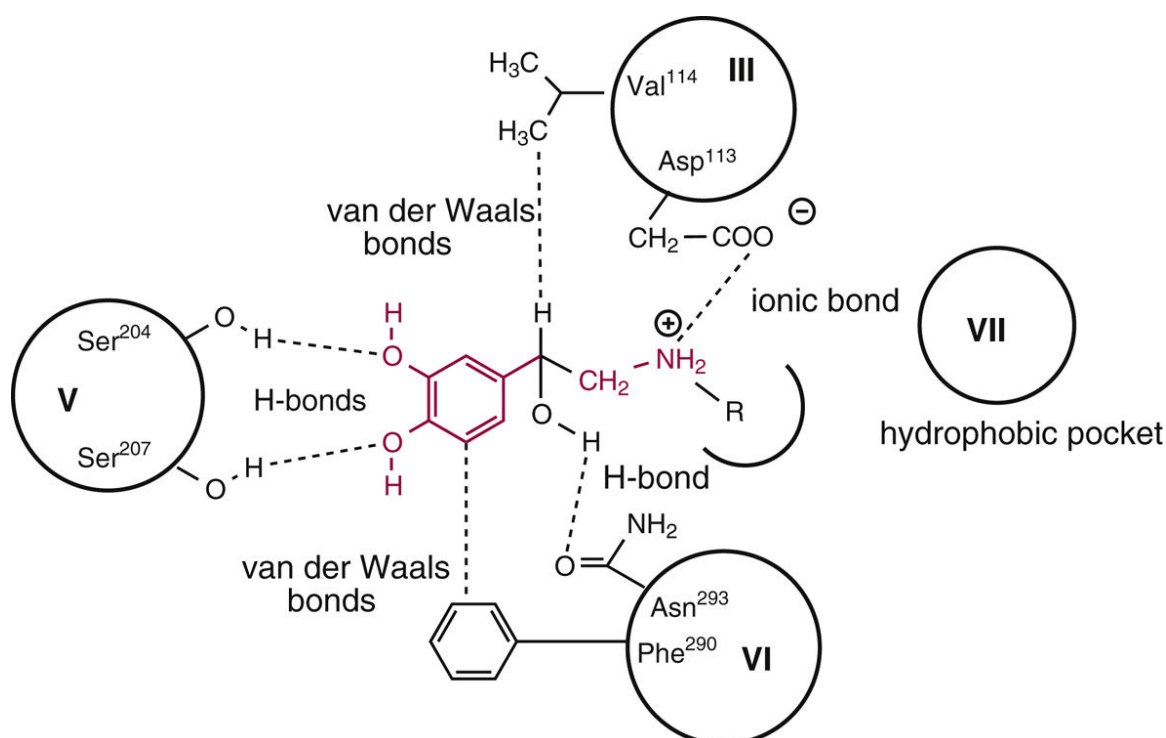
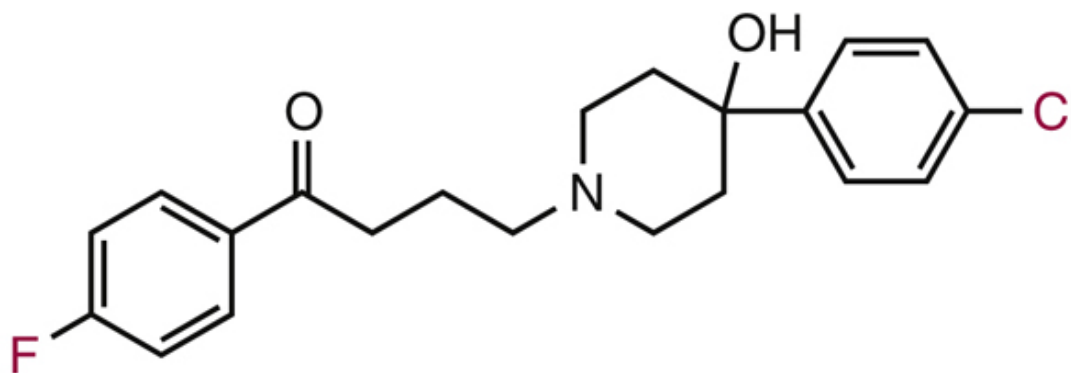
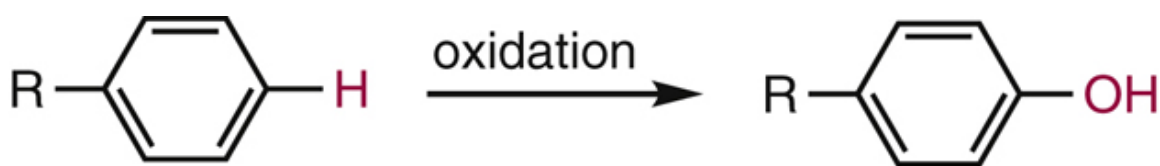


FIGURE 1.2 Norepinephrine binding to key residues in the adrenergic receptor.

Metabolism

One key component of the action of a drug is length of duration. As with the topics above, duration is complicated, but many of the factors that limit duration are due to metabolism. Metabolism is the natural enzymatic modification of molecules by the body, with the goal of increasing polarity for excretion through, for instance, the urine (see Chapter 3). Understanding that metabolism is aimed at increasing polarity and having a grasp of the functional groups that are both prone and resistant to metabolism allow one to predict the rate of metabolism of each group and to design a new molecule that is not prone to metabolism but still satisfies the SAR. The metabolic pathways by all the metabolic enzymes are numerous but can be broken down into Phase 1 (hydrolysis, oxidation, and reduction), Phase 2 (conjugation), and efflux (see below), which is sometimes referred to as Phase 3. Thus, a grasp of the chemistry of the functional groups in a molecule allows an analysis of the rate of metabolism and methods to prevent the actions of the metabolizing enzyme or, in the case of prodrugs that require metabolic activation, to

harness them. There are many approaches you will encounter in this textbook; however, many are simply the replacement of a “hydrogen” that is prone to oxidation through hydroxylation with a metabolism-resistant substituent. A common metabolically vulnerable hydrogen is the *p*-hydrogen on an aromatic ring (Fig. 1.3). Thus, when looking through structures of therapeutics, please take note of the large number that contains *p*-halogens (such as haloperidol [Chapter 11] shown below, with *p*-halogens in red). It may well not be noted that the halogens eliminate such metabolism, but that is a major reason why such halogens are a common feature in drugs.



Haloperidol

FIGURE 1.3 Example of hydrogen replacement with halogens to block oxidative metabolism to extend drug duration of action.

Active Transporters

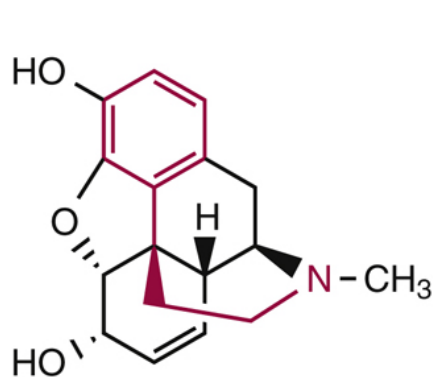
The passive crossing of membranes was discussed above in terms of lipophilicity, but active transporters also exist—biological molecules that span membranes and actively transport the drug both into (influx) and out

of (efflux) the cell. Amino acid transporters are a well-described class of transporters that facilitate influx of chemical species that would not passively cross membranes. P-glycoprotein (P-gp) is a well-described example of an efflux transporter (Chapter 4). P-gp is again a complicated protein, and how it truly functions remains open to debate, but the fundamental principle is that it removes exogenous chemical species from the body. This was obviously beneficial to remove toxins from eating poisonous berries during human evolution but works against us with drugs. P-gp recognizes a broad range of molecular species (its SAR contains many different functional groups that lead to transport) and is actually upregulated in many cancer cells. Thus, low activity as a substrate for P-gp is critical for many central nervous system (CNS) agents and anticancer drugs. By way of example, cancer cells initially responsive to the antimitotic agents paclitaxel and docetaxel can develop resistance to these drugs by upregulating (generating more molecules of) P-gp. However, the newest taxane antimitotic, cabazitaxel, has a lower affinity for this efflux protein and retains efficacy in malignant cells that no longer respond to other taxanes (Chapter 33). On the downside, because cabazitaxel is not rapidly ejected from cells by P-gp, it can accumulate in enterocytes, causing significant diarrhea (an adverse effect of therapy).

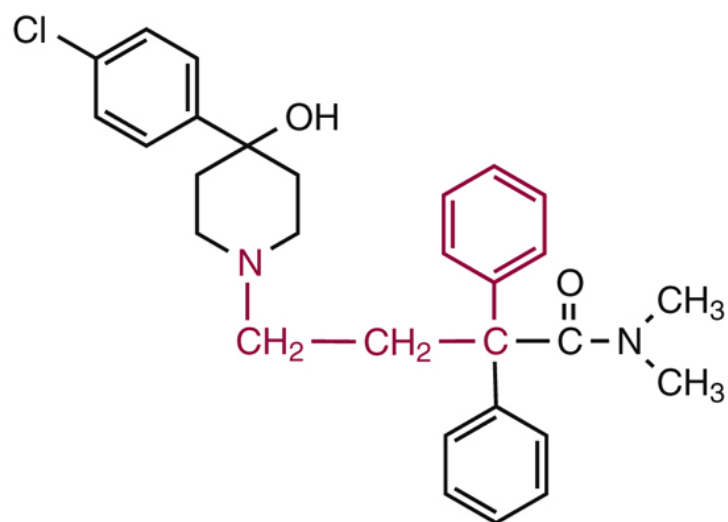
Abuse Liability

What is generally true in life can also be true in medicinal chemistry: a problem is often an opportunity. Drugs of abuse tend to function by acting on targets in the CNS. Thus, the fact that high P-gp substrate activity limits access to the CNS is one reason why the antidiarrheal loperamide has the actions of an opioid (such as morphine) when tested in cells but has very limited central opioid activity (and thus addiction potential with accompanying abuse liability) when taken by patients at recommended doses (Chapter 14). [Figure 1.4](#) shows the relationship between morphine and loperamide; at first, they appear to be very different structures. However, as highlighted in red, they both share a phenylpropyl pattern of atoms; such a common feature between molecules with the same biological actions is termed a pharmacophore, essentially a special form of SAR. This does not mean that all molecules that contain a phenylpropyl

are opioids (remember SAR is not a “law”), but common pharmacophores are a useful guide when determining the biological activity of a molecule.



Morphine



Loperamide

FIGURE 1.4 Comparison of morphine and loperamide showing the common phenylpropyl moiety.

Summary

The short paragraphs above are an introduction to the various aspects of medicinal chemistry, and all will be discussed in greater detail in the various chapters within this text. The goal of this introductory chapter is to bring clarity to many of the concepts and terms and is intended as a starting point from which to progress. Enjoy the journey!

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CHAPTER 2

Foundational Principles and Functional Group Impact on Activity

James J. Knittel and Robin M. Zavod

Abbreviations

BCRP	breast cancer resistance protein
HCl	hydrochloric acid
HPT-1	human peptide transporter 1
IV	intravenous
MRP2, MRP3	multiresistance ^[1] _{SEP} associated proteins 2 & 3
MW	molecular weight
NaOH	sodium hydroxide
OAT	organic anion transporter
OCT	organic cation transporter
PABA	<i>p</i> -aminobenzoic acid
PepT1	peptide transporter 1
PHTs	polyhistidine triad
P-gp	<i>P</i> -glycoprotein
QSAR	quantitative structure-activity relationship
SAR	structure-activity relationship
USP	U.S. Pharmacopeia

Medicinal chemistry is an interdisciplinary science at the intersection of organic chemistry, biochemistry (bio-organic chemistry), computational chemistry, pharmacology, pharmacognosy, molecular biology, and physical chemistry. This branch of chemistry is

involved with the identification, design, synthesis, and development of new drugs that are safe and suitable for therapeutic use in humans and pets. It includes the study of marketed drugs, their biologic properties, and their quantitative structure-activity relationships (QSARs).

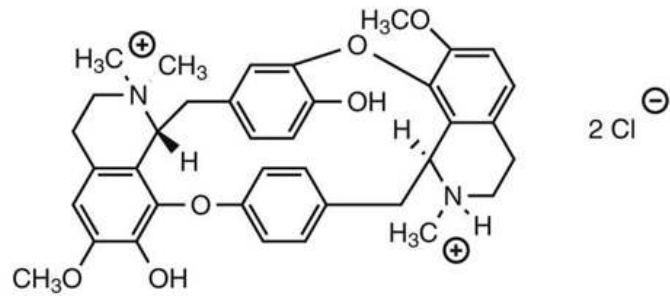
Medicinal chemists study how chemical structure influences biologic activity. As such, it is necessary to understand not only the mechanism by which a drug exerts its effect but also how the molecular and physicochemical properties of the molecule influence the drug's pharmacokinetics (absorption, distribution, metabolism, toxicity, and elimination) and pharmacodynamics (what the drug does to the body). The term "physicochemical properties" refers to how the functional groups present within a molecule influence its acid-base properties, water solubility, partition coefficient, crystal structure, stereochemistry, and ability to interact with biologic systems, such as enzyme active sites and receptor binding sites. To design better medicinal agents, the relative contribution that each functional group (i.e., pharmacophore) makes to the overall physicochemical properties of the molecule must be evaluated. Studies of this type involve modification of the molecule in a systematic fashion followed by a determination of how these changes affect biologic activity. Such studies are referred to as structure-activity relationships (SARs)—that is, the relationship of how structural features of the molecule contribute to, or take away from, the desired biologic activity. Because of the foundational nature of the content of this chapter, there are numerous case studies presented throughout the chapter (as boxes).

Introduction

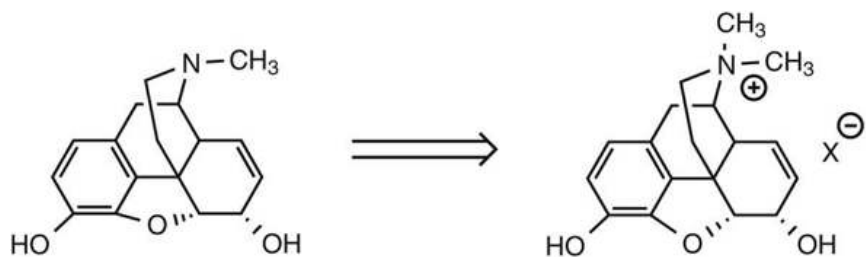
Chemical compounds, usually derived from plants and other natural sources, have been used by humans for thousands of years to alleviate pain, diarrhea, infection, and various other maladies. Until the 19th century, these “remedies” were primarily crude preparations of plant material of unknown constitution. The revolution in synthetic organic chemistry during the 19th century produced a concerted effort toward identification of the structures of the active constituents of these naturally derived medicinals and synthesis of what were hoped to be more efficacious agents. By determining the molecular structures of the active components of these complex mixtures, it was thought that a better understanding of how these components worked could be elucidated.

Relationship Between Molecular Structure and Biologic Activity

Early studies of the relationship between chemical structure and biologic activity were conducted by Crum-Brown and Fraser¹ in 1869. They demonstrated that many compounds containing tertiary amine groups exhibited activity as muscle relaxants when converted to quaternary ammonium compounds. Molecules with widely differing pharmacologic properties, such as strychnine (a convulsant), morphine (an analgesic), nicotine (a deterrent, insecticide), and atropine (an anticholinergic), could be converted to muscle relaxants with properties similar to those of tubocurarine when methylated (Fig. 2.1). Crum-Brown and Fraser therefore concluded that muscle relaxant activity required the presence of a quaternary ammonium group within the structure. This initial hypothesis was later disproven by the discovery of the natural neurotransmitter and activator of muscle contraction, acetylcholine (Fig. 2.2). Even though Crum-Brown and Fraser's initial hypothesis that related chemical structure with action as a muscle relaxant was incorrect, it demonstrated the concept that molecular structure influences the biologic activity of chemical entities and that alterations in structure produce changes in biologic action.



Tubocurarine
(muscle relaxant)



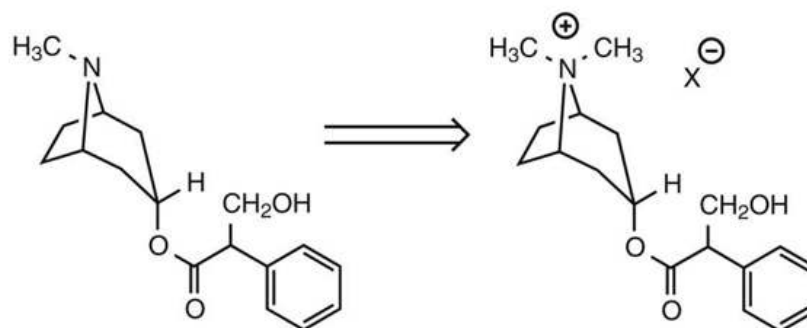
Morphine
(analgesic)

N-Methylmorphine
(muscle relaxant)



Nicotine
(insecticide)

N-Methylnicotine
(muscle relaxant)



Atropine
(mydriatic)

N-Methylatropine
(muscle relaxant)

FIGURE 2.1 Effects of methylation on biologic activity.

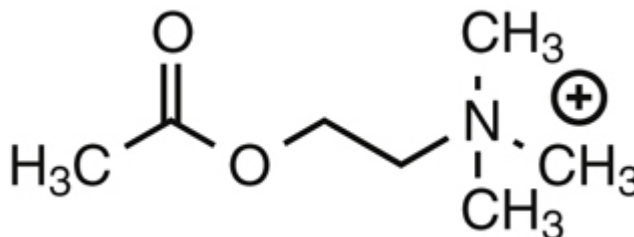


FIGURE 2.2 Acetylcholine, a neurotransmitter and muscle relaxant.

With the discovery by Crum-Brown and Fraser that quaternary ammonium groups could produce molecules with muscle relaxant properties, scientists began to look for other functional groups that produce specific biologic responses. At the time, it was thought that specific chemical groups, or nuclei (rings), were responsible for specific biologic effects. This led to the postulate, which took some time to disprove, that “one chemical group gives one biological action.”² Even after the discovery of acetylcholine by Loewi and Navrati,³ which effectively dispensed with Crum-Brown and Fraser’s concept of all quaternary ammonium compounds being muscle relaxants, this was still considered to be dogma and took a long time to refute.

Selectivity of Drug Action and Drug Receptors

Although the structures of many drugs or xenobiotics, or at least their functional group composition, were known at the start of the 20th century, how these compounds exerted their effects was still a mystery. Using his observations with regard to the staining behavior of microorganisms, Ehrlich⁴ developed the concept of drug receptors. He postulated that certain “side chains” on the surfaces of cells were “complementary” to the dyes (or drug) and suggested that the two could therefore interact with one another. In the case of antimicrobial compounds, interaction of the chemical with the cell surface “side chains” produced a toxic effect. This concept was the first description of what later became known as the receptor hypothesis for explaining the biologic action of chemical entities. Ehrlich also discussed selectivity of drug action via the concept of a “magic bullet.” He suggested that this selectivity permitted eradication of disease states without significant harm coming to the organism being treated (i.e., the patient). This was later modified by Albert⁵ and today is referred to as “selective toxicity.” An example of poor selectivity was demonstrated when Ehrlich developed organic arsenicals that were toxic to trypanosomes as a result of their irreversible reaction with thiol groups (-SH) on vital proteins. The formation of As-S bonds resulted in death of the target organism. Unfortunately, these compounds were toxic not only to the target organism but also to the host once certain blood levels of arsenic were achieved.

The “paradox” that resulted after the discovery of acetylcholine, how one chemical group can produce two different biologic effects (i.e., muscle relaxation and muscle contraction), was explained by Ing⁶ using the actions of acetylcholine and tubocurarine as his examples. Ing hypothesized that both acetylcholine and tubocurarine act at the same receptor, but that one molecule fits the receptor in a more complementary manner and “activates” it, causing muscle contraction. (Ing did not elaborate just how this activation occurred.) The blocking effect of the larger molecule, tubocurarine, could be explained by its occupation of part of the receptor, thereby preventing acetylcholine, the smaller molecule,

from interacting with the receptor. With both molecules, the quaternary ammonium functional group is a common structural feature and interacts with the same region of the receptor. If one closely examines the structures of other molecules with opposing effects on the same pharmacologic system, this appears to be a common theme: Molecules that block the effects of natural neurotransmitters, such as norepinephrine, histamine, dopamine, or serotonin, for example, are called antagonists and are usually larger in size than the native compound, which is not the case for antagonists of peptide neurotransmitters and hormones such as cholecystokinin, melanocortin, or substance P. Antagonists to these peptide molecules are usually smaller in size. However, regardless of the type of neurotransmitter (biogenic amine or peptide), both agonists and antagonists share common structural features with the neurotransmitter that they influence. This provides support to the concept that the structure of a molecule, its composition and arrangement of functional groups, determines the type of pharmacologic effect that it possesses (i.e., SAR). For example, compounds that are muscle relaxants acting via the cholinergic nervous system possess a quaternary ammonium or protonated tertiary ammonium group and are larger than acetylcholine (compare acetylcholine in [Fig. 2.2](#) with tubocurarine in [Fig. 2.1](#)).

SARs are the underlying principle of medicinal chemistry. Similar molecules exert similar biologic actions in a qualitative sense. A corollary to this is that structural elements (functional groups) within a molecule most often contribute in an additive manner to the physicochemical properties of a molecule and, therefore, to its biologic action. One need only peruse the structures of drug molecules in a particular pharmacologic class to become convinced (e.g., histamine H_1 antagonists, histamine H_2 antagonists, β -adrenergic antagonists). In the quest for better medicinal agents (drugs), it must be determined which functional groups within a specific structure are important for its pharmacologic activity and how these groups can be modified to produce more potent, more selective, and safer compounds.

An example of how different functional groups can yield chemical entities with similar physicochemical properties is demonstrated by the

sulfanilamide antibiotics. In Figure 2.3, the structures of sulfanilamide and *p*-aminobenzoic acid (PABA) are shown. In 1940, Woods⁷ demonstrated that PABA reverses the antibacterial action of sulfanilamide (and other sulfonamide-based antibacterials) and both PABA and sulfanilamide have similar steric and electronic properties. Both molecules contain acidic functional groups, with PABA containing an aromatic carboxylic acid (Ar-COOH) and sulfanilamide an aromatic sulfonamide (Ar-SO₂NH₂). When ionized at physiologic pH, both compounds have a similar electronic configuration, and the distance between the ionized acid and the weakly basic amino group is also very similar. It should be no surprise that sulfanilamide acts as an antagonist to PABA metabolism in bacteria.

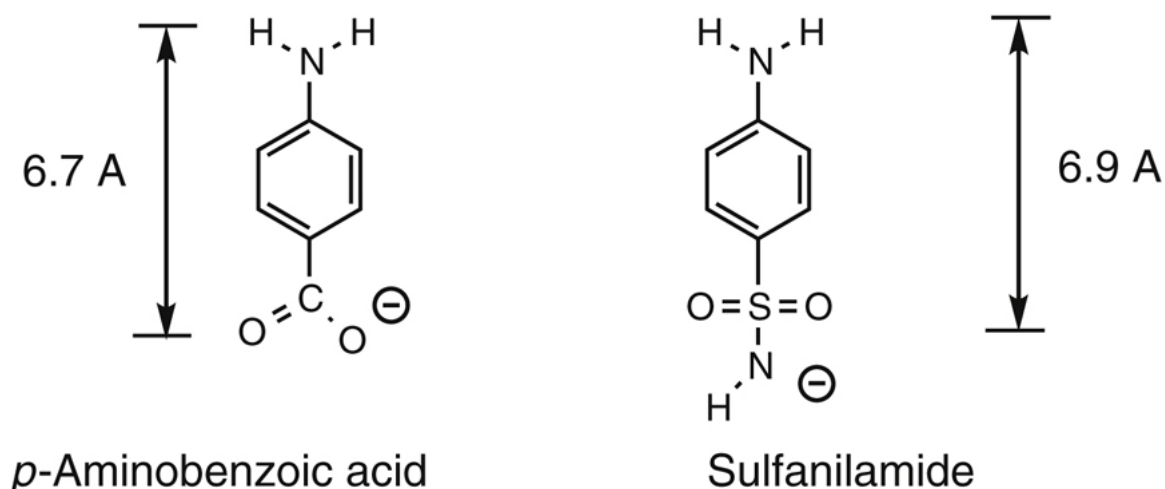


FIGURE 2.3 Ionized forms of *p*-aminobenzoic acid (PABA) and sulfanilamide, with comparison of the distance between amine and ionized acids of each compound.

Note how closely sulfanilamide resembles PABA.

Biologic Targets for Drug Action

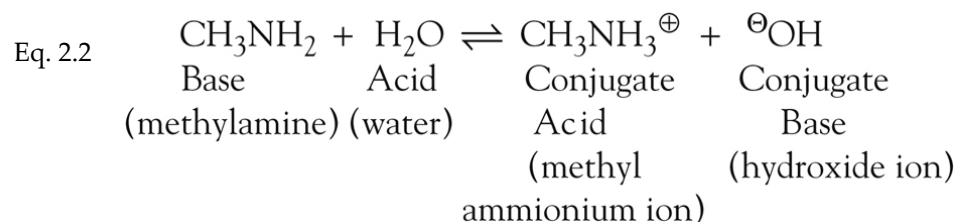
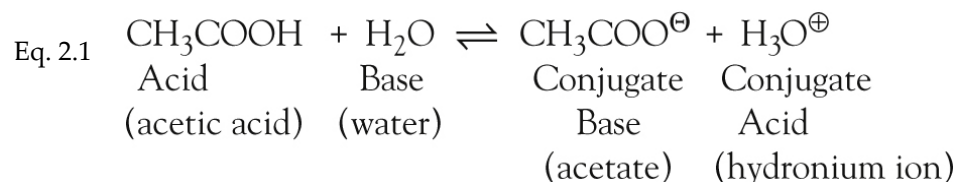
In order for drug molecules to exhibit their pharmacologic activity, they must interact with a biologic target, typically a receptor, enzyme, nucleic acid, excitable membrane or other biopolymer. These interactions occur between the functional groups found in the drug molecule and those found within each biologic target. The relative fit of each drug molecule with its target is a function of a number of physicochemical properties including acid-base chemistry and related ionization, functional group

shape and size, and three-dimensional spatial orientation. The quality of this “fit” has a direct impact on the biologic response produced. In this chapter, functional group characteristics are discussed as a means to better understand overall drug molecule absorption, distribution, metabolism, and excretion, as well as potential interaction with a biologic target.

Physicochemical Properties of Drugs

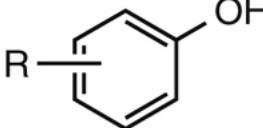
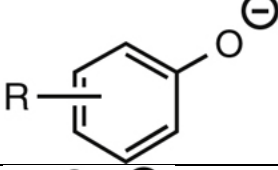
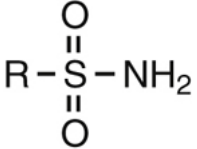
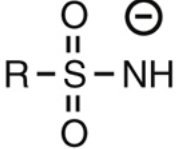
Acid-Base Properties

The human body is 70%-75% water, which amounts to approximately 51-55 L of water for a 160-lb (73-kg) individual. For an average drug molecule with a molecular weight of 200 g/mol and a dose of 20 mg, this leads to a solution concentration of approximately 2×10^{-6} M (2 μ M). When considering the solution behavior of a drug within the body, we are dealing with a dilute solution, for which the Brønsted-Lowry⁸ acid-base theory is most appropriate to explain and predict acid-base behavior. This is a very important concept in medicinal chemistry, because the acid-base properties of drug molecules determine the ionization state of the molecule at a given pH therefore directly affecting absorption, excretion, and compatibility with other drugs in solution. According to the Brønsted-Lowry theory, an “acid” is any substance capable of yielding a proton (H^+), and a “base” is any substance capable of accepting a proton. When an acid gives up a proton to a base, it is converted to its “conjugate base.” Similarly, when a base accepts a proton, it is converted to its “conjugate acid” (Equations (2.1) and (2.2)):



Note that when an acidic functional group loses its proton (often referred to as having undergone “dissociation”), it is left with an extra electron and becomes negatively charged. This is the “ionized” form of the acid. The ability of the ionized functional group to participate in an ion-dipole interaction with water (see the Water Solubility of Drugs section) enhances its water solubility. Many functional groups behave as acids (Table 2.1). The ability to recognize these functional groups and their relative acid strengths helps to predict absorption, distribution, excretion, and potential incompatibilities between drugs.

TABLE 2.1
Common Acidic Organic Functional Groups and Their Ionized (Conjugate Base) Forms

Acids (pKa)			Conjugate Base
Phenol (9-11)			Phenolate
Sulfonamide (9-10)			Sulfonamide

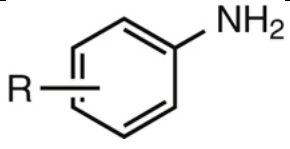
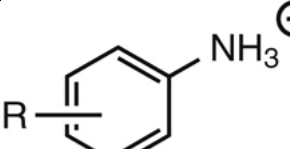
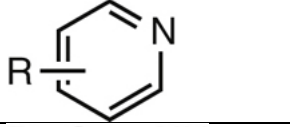
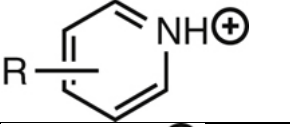
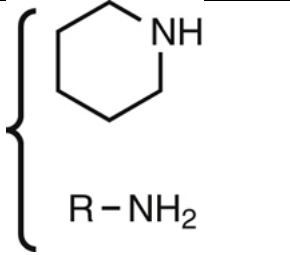
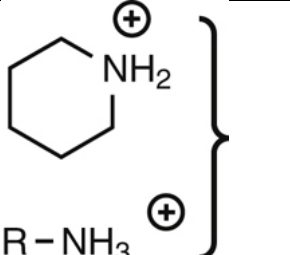
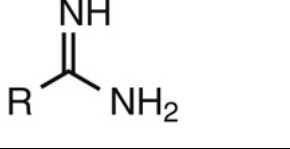
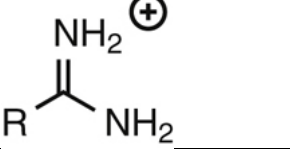
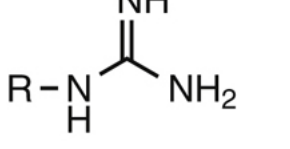
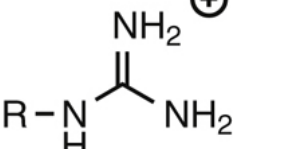
Acids (pKa)			Conjugate
Imide (9-10)			Imide
Alkylthiol (10-11)	$R-SH$	$R-S^-$	Thiolate
Thiophenol (9-10)			Thiophenolate
N-Arylsulfonamide (6-7)			N-Arylsulfonamide
Sulfonimide (5-6)			Sulfonimide
Alkylcarboxylic acid (5-6)	$R-C(=O)-OH$	$R-C(=O)-O^-$	Alkylcarboxylate
Arylcarboxylic acid (4-5)			Arylcarboxylate
Sulfonic acid (0-1)			Sulfonate

Acid strength increases as one moves down the table.

When a basic functional group is converted to the corresponding conjugate acid, it too becomes ionized. In this instance, however, the functional group becomes positively charged due to the extra proton. Most drugs that contain basic functional groups contain primary, secondary, and tertiary amines or imino amines, such as guanidines and amidines. Other functional groups that are basic are shown in [Table 2.2](#). As with the acidic groups, it is important to become familiar with these functional groups and their relative strengths.

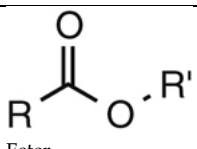
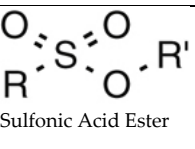
TABLE 2.2
Common Basic Organic Functional Groups and Their Ionized (Conjugate Acid) Forms

Base		Conjugate Acid (pKaHB ⁺)

Base			Conjugate Acid (pKaHB ⁺)
Arylamine			Arylammonium (9-11)
Aromatic amine			Aromatic ammonium (5-6)
Imine	$R-\overset{\text{H}}{\underset{\text{H}}{\text{C}}}=\text{NH}$	$R-\overset{\text{H}}{\underset{\text{H}}{\text{C}}}=\text{NH}^+$	Iminium (3-4)
Alkylamines			Alkylammonium (2° – 10-11) (1° – 9-10)
Amidine			Amidinium (10-11)
Guanidine			Guanidinium (12-13)

Functional groups that cannot give up or accept a proton are considered to be “neutral” (or “nonelectrolytes”) with respect to their acid-base properties. Common neutral functional groups are shown in Table 2.3. Quaternary ammonium compounds are neither acidic nor basic and are not electrically neutral. Additional information about the acid-base properties of the functional groups listed in Tables 2.1-2.3 can be found in Lemke.⁹

TABLE 2.3
Common Organic Functional Groups That are Considered Neutral Under Physiologic Conditions

$R-\text{CH}_2-\text{OH}$ Alkyl Alcohol	$R'-\text{O}-R'$ Ether	 Ester	 Sulfonic Acid Ester

$\text{R}-\text{CH}_2-\text{OH}$ Alkyl Alcohol	$\text{R}-\text{O}-\text{R}'$ Ether	$\text{R}-\text{C}(=\text{O})-\text{O}-\text{R}'$ Ester	$\text{R}-\text{SO}_3-\text{R}'$ Sulfonic Acid Ester
$\text{R}-\text{C}(=\text{O})-\text{NH}_2$ Amide	 Diarylamine	$\text{R}-\text{C}\equiv\text{N}$ Nitrile	$\text{R}-\text{N}^+(\text{R}')(\text{R}'')(\text{R}''')$ Quaternary ammonium
$\text{R}-\text{N}(\text{R}')(\text{R}'')\rightarrow\text{O}$ Amine oxide	$\text{R}-\text{C}(=\text{O})-\text{R}'$ Ketone and Aldehyde	$\text{R}-\text{S}-\text{R}'$ Thioether	$\text{R}-\text{S}(=\text{O})-\text{R}'$ $\text{R}-\text{SO}_2-\text{R}'$ Sulfoxide sulfone

A molecule can contain multiple functional groups with acid-base properties and, therefore, can possess both acidic and basic character. For example, ciprofloxacin (Fig. 2.4), a fluoroquinolone antibacterial agent, contains a secondary alkylamine, two tertiary arylamines (aniline-like amines), and a carboxylic acid. The two arylamines are weakly basic and, therefore, do not contribute significantly to the acid-base properties of ciprofloxacin under physiologic conditions. Depending on the pH of the physiologic environment, this molecule will either accept a proton (secondary alkylamine), donate a proton (carboxylic acid), or both. Thus, it is described as amphoteric (both acidic and basic) in nature. Figure 2.5 shows the acid-base behavior of ciprofloxacin in two different environments. Note that at a given pH (e.g., pH 1.0-3.5), only one of the functional groups (the alkylamine) is significantly ionized. To be able to make this prediction, an appreciation for the relative acid-base strength of both the acidic and basic functional groups is required. Thus, one needs to know which acidic or basic functional group within a molecule containing multiple functional groups is the strongest and which acidic or basic functional group is the weakest. The concept of pK_a not only describes relative acid-base strength of functional groups but also allows one to calculate, for a given pH, the relative percentages of the ionized and unionized forms of the drug. As stated earlier, this helps to predict relative water solubility, absorption, and excretion for a given compound.

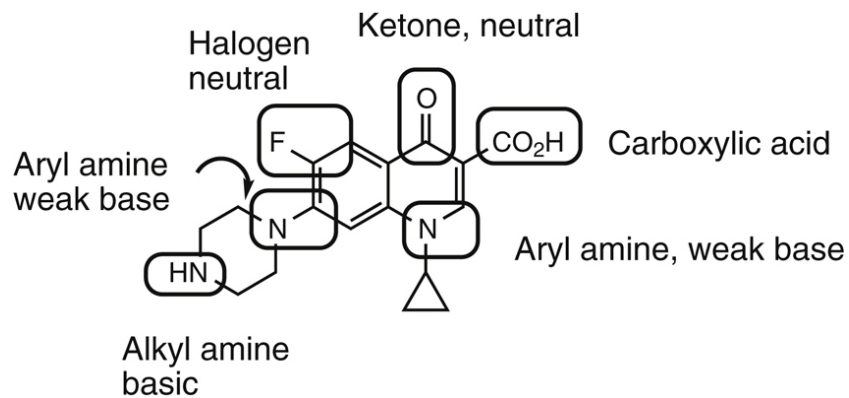
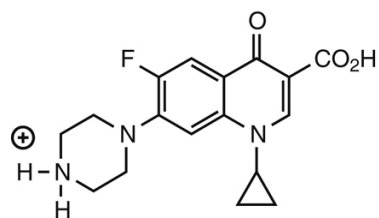
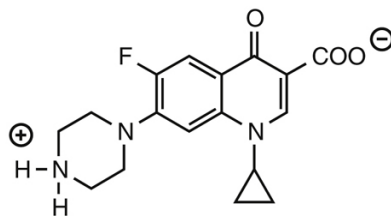


FIGURE 2.4 Chemical structure of ciprofloxacin showing the various organic functional groups.



Stomach (pH 1.0 - 3.5)

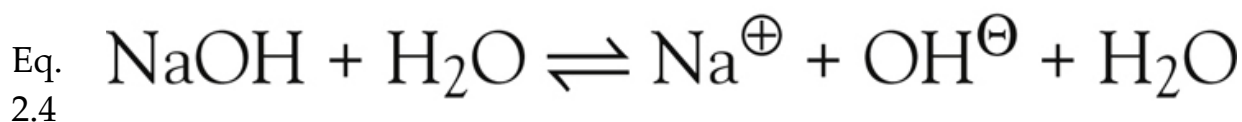


Colon (pH 5.6-7)

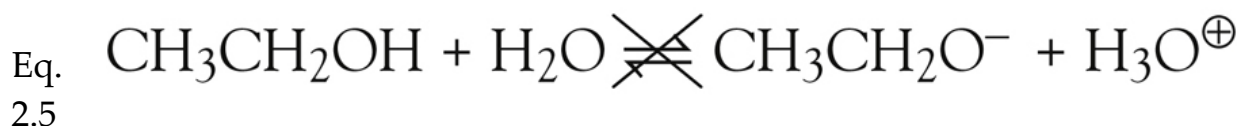
FIGURE 2.5 Predominate forms of ciprofloxacin at two different locations within the gastrointestinal tract.

Relative Acid Strength (pK_a)

Strong acids and bases completely donate (dissociate) or accept a proton in aqueous solution to produce their respective conjugate bases and acids. For example, mineral acids, such as hydrochloric acid (HCl), or bases, such as sodium hydroxide (NaOH), undergo 100% dissociation in water, with the equilibrium between the ionized and un-ionized forms shifted completely to the right (ionized), as shown in Equations (2.3) and (2.4):



Acids and bases of intermediate or weak strength, however, incompletely donate (dissociate) or accept a proton, and the equilibrium between the ionized and un-ionized forms lies somewhere in the middle, such that all possible species can exist at any given time. Note that in Equations (2.3) and (2.4), water acts as a base in one instance and as an acid in the other. Water is therefore *amphoteric*—that is, it can act as an acid or a base, depending on the prevailing pH of the solution. From a physiologic perspective, drug molecules are always present as a dilute aqueous solution. The strongest base that is present is OH^- , and the strongest acid is H_3O^+ . This is known as the “leveling effect” of water. Thus, some functional groups that have acidic or basic character do not behave as such under physiologic conditions in aqueous solution. For example, alkyl alcohols, such as ethyl alcohol, are not sufficiently acidic to become significantly ionized in an aqueous solution at a physiological pH. Water is not sufficiently basic to remove the proton from ethyl alcohol to form the ethoxide ion (Equation (2.5)). Therefore, under physiologic conditions, alcohols are neutral with respect to acid-base properties:

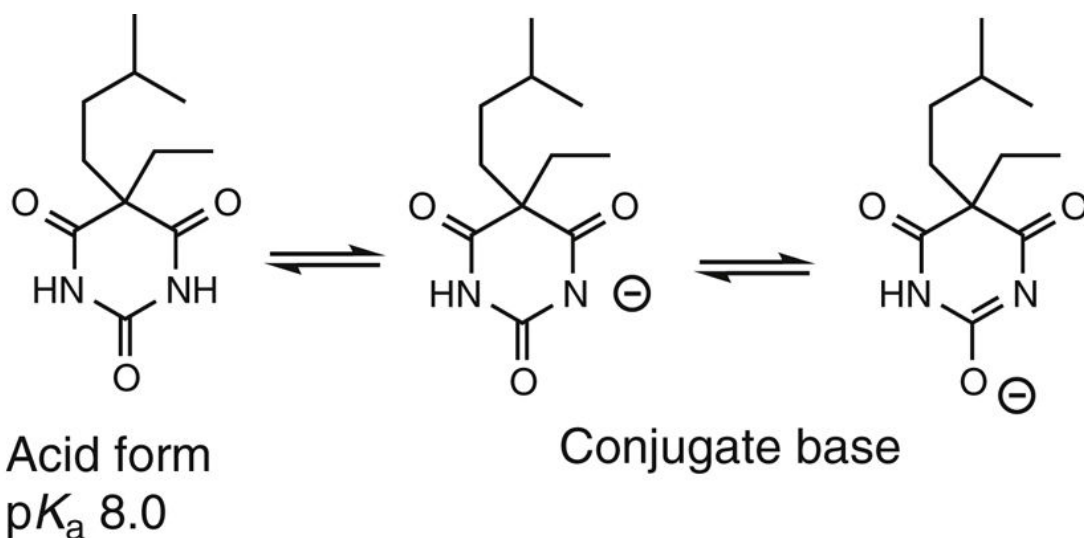


Predicting the Degree of Ionization of a Molecule

By knowing if there are acidic and/or basic functional groups present in a molecule, one can predict whether a molecule is going to be predominantly ionized or un-ionized at a given pH. To be able to quantitatively predict the degree of ionization of a molecule, the pK_a values of each of the acidic and basic functional groups present and the pH of the environment in which the molecule will be located must be known. The magnitude of the pK_a value is a measure of relative acid or base strength, and the Henderson-Hasselbalch equation (Equation (2.6)) can be used to calculate the percent ionization of a compound at a given pH (this equation was used to calculate the major forms of ciprofloxacin in Fig. 2.5):

$$\text{Eq. 2.6} \quad pK_a = \text{pH} + \log \frac{[\text{acid form}]}{[\text{base form}]}$$

The key to understanding the use of the Henderson-Hasselbalch equation for calculating percent ionization is to realize that this equation relates a constant, pK_a , to the ratio of the acidic form of a functional group to its conjugate base form (and conversely, the conjugate acid form to its base). Because pK_a is a constant for any given functional group, the ratio of acid to conjugate base (or conjugate acid to base) will determine the pH of the solution. A sample calculation is shown in Figure 2.6 for the sedative hypnotic amobarbital.



Question: At a pH of 7.4, what is the percent ionization of amobarbital?

Answer: $8.0 = 7.4 + \log \frac{[\text{acid}]}{[\text{base}]}$

$$0.6 = \log \frac{[\text{acid}]}{[\text{base}]}$$

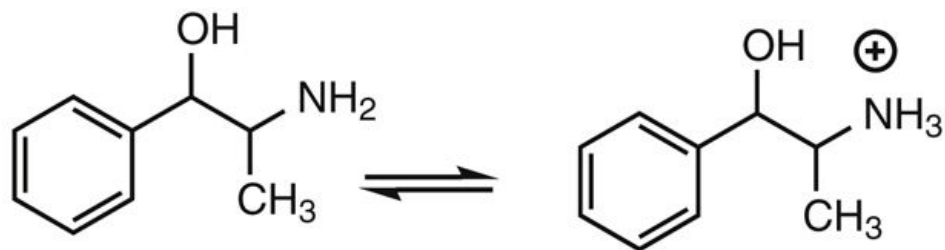
$$10^{0.6} = \frac{[\text{acid}]}{[\text{base}]} = \frac{3.98}{1}$$

$$\% \text{ acid form} = \frac{3.98 \times 100}{4.98} = 79.9\%$$

FIGURE 2.6 Calculation of percent ionization of amobarbital. Calculation indicates that 80% of the molecules are in the acid (or protonated) form, leaving 20% in the conjugate base (ionized) form.

When dealing with a basic functional group, one must recognize the conjugate acid represents the ionized form of the functional group. [Figure 2.7](#) shows the calculated percent ionization for the decongestant phenylpropanolamine. It is very important to understand that for a base, the pK_a refers to the conjugate acid or ionized form of the compound. To

thoroughly comprehend this relationship, calculate the percent ionization of an acidic functional group and a basic functional group at different pH values and carefully observe the trend.



Base form

Conjugate acid form

pK_a 9.4

Question: What is the % ionization (acid form) of phenylpropanolamine at pH 7.4?

Answer: $7.4 = 9.4 + \log \frac{[\text{acid}]}{[\text{base}]}$

$$2.0 = \log \frac{[\text{acid}]}{[\text{base}]}$$

$$10^2 = \frac{[\text{acid}]}{[\text{base}]} = \frac{100}{1}$$

$$\% \text{ ionization} = \frac{100 \times 100}{101} = 99\%$$

FIGURE 2.7 Calculation of percent ionization of phenylpropanolamine. Calculation indicates that 99% of the molecules are in the conjugate acid form, which is the same as the percent ionization.

Water Solubility of Drugs

The solubility of a drug molecule in water greatly affects the routes of administration that are available, as well as its absorption, distribution, and elimination. Two key concepts to keep in mind when considering the water (or fat) solubility of a molecule are the potential for hydrogen bond formation and ionization of one or more functional groups within the molecule.

Hydrogen Bonds

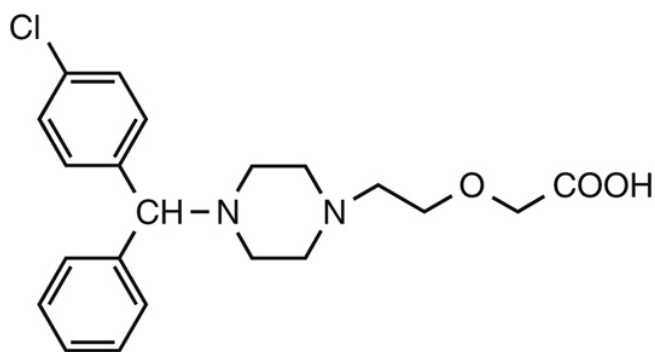
Each functional group capable of donating or accepting a hydrogen bond contributes to the overall water solubility of the compound and increases the hydrophilic (water-loving) nature of the molecule. Conversely, functional groups that cannot form hydrogen bonds do not enhance hydrophilicity and will contribute to the hydrophobic (water-fearing) nature of the molecule. Hydrogen bonds are a special case of what are usually referred to as dipole-dipole interactions. A permanent dipole occurs as a result of an unequal sharing of electrons between two atoms within a covalent bond. This unequal sharing of electrons only occurs when these two atoms have significantly different electronegativities. When a permanent dipole is present, a partial charge is associated with each of these atoms along a single bond (one atom has a partial negative charge, and one atom has a partial positive charge). The atom with a partial negative charge has higher electron density than the other atom. When two functional groups containing one or more permanent dipoles approach one another, they align such that the negative end of one dipole is electrostatically attracted to the positive end of the other. When the positive end of the dipole is a hydrogen atom, this interaction is referred to as a “hydrogen bond” (or H-bond). Thus, for a hydrogen bonding interaction to occur, at least one functional group must contain a dipole with an electropositive hydrogen. The hydrogen atom must be covalently bound to an electronegative atom, such as oxygen (O), nitrogen (N), sulfur (S), or selenium (Se). Of these four elements, only oxygen and nitrogen atoms contribute significantly to the dipole, and we will therefore concern ourselves only with the hydrogen-bonding capability (specifically as hydrogen bond donors) of functional groups that contain a bond between oxygen and hydrogen atoms (e.g., alcohols) and functional groups that

contain a bond between nitrogen and hydrogen atoms (e.g., primary and secondary amines and amides) (e.g., NH and CONH groups).

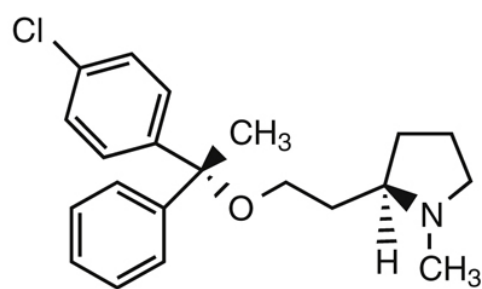
Case Study

Absorption/Acid-Base Case

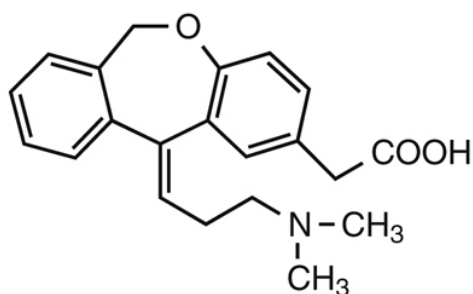
A long-distance truck driver comes into the pharmacy complaining of seasonal allergies. He asks you to recommend an agent that will act as an antihistamine but will not cause drowsiness. He regularly takes TUMS for indigestion due to the bad food he eats while on the road.



Cetirizine (Zyrtec)



Clemastine (Tavist)



Olopatadine (Patanol)

1. Identify the functional groups present in Zyrtec and Tavist, and evaluate the effect of each functional group on the ability of the drug to cross lipophilic membranes (e.g., blood-brain barrier). Based on your assessment of each agent's ability to cross the blood-brain barrier (and, therefore, potentially cause drowsiness), provide a rationale for whether the truck driver should be taking Zyrtec or Tavist.

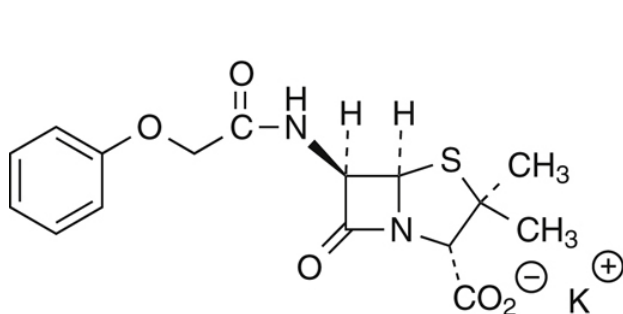
2. Patanol is sold as an aqueous solution of the hydrochloride salt. Modify the structure present in the box to show the appropriate salt form of this agent. This agent is applied to the eye to relieve itching associated with allergies. Describe why this agent is soluble in water  and what properties make it able to be absorbed into membranes that surround the eye.
3. Consider the structural features of Zyrtec and Tavist. In which compartment (stomach [pH 1] or intestine [pH 6-7]) will each of these two drugs be best absorbed?
4. TUMS neutralizes stomach acid to pH 3.5. Based on your answer to question 3, determine whether the truck driver will get the full antihistaminergic effect if he takes his antihistamine at the same time he takes his TUMS. Provide a rationale for your answer.

Case Solution found immediately after References.

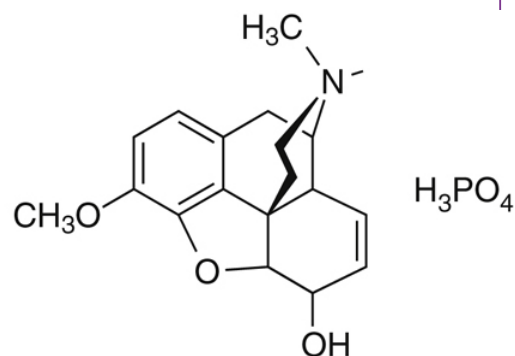
Case Study

Acid-Base Chemistry/Compatibility Case

The intravenous (IV) technician in the hospital pharmacy gets an order for a patient that includes the two drugs drawn below. She is unsure if she can mix the two drugs together in the same IV bag and is not certain how water soluble the agents are.



Penicillin V potassium



Codeine

1. Penicillin V potassium is drawn in its salt form, whereas codeine phosphate is not. Modify the given structure above to show the salt form of codeine phosphate. Determine the acid-base character of the functional groups in the two molecules drawn above as well as the salt form of codeine phosphate.
2. As originally drawn above, which of these two agents is more water soluble? Provide a rationale for your selection that includes appropriate structural properties. Is the salt form of codeine phosphate more or less water soluble than the free base form of the drug? Provide a rationale for your answer based on the structural properties of the salt form of codeine phosphate.
3. What is the chemical consequence of mixing aqueous solutions of each drug in the same IV bag? Provide a rationale that includes an acid-base assessment.

Case Solution found immediately after References.

Even though the energy associated with each hydrogen bond is small (1-10 kcal/mol/bond), it is the additive nature of multiple hydrogen bonds that contributes to the overall water solubility of a given drug molecule. This type of interaction is also important in the interaction between a drug and its biologic target (e.g., receptor). [Figure 2.8](#) shows several types of hydrogen bonding interactions that can occur with a couple of functional groups and water. As a general rule, the more hydrogen bonds that are possible between a drug molecule and water, the greater the water solubility of the molecule. [Table 2.4](#) lists several common functional groups and the number of hydrogen bonds in which they can potentially participate. Note that this table does not take into account the possibility of intramolecular hydrogen bond formation. Each intramolecular hydrogen bond decreases water solubility (and increases lipid solubility) because there is one less interaction possible with water.

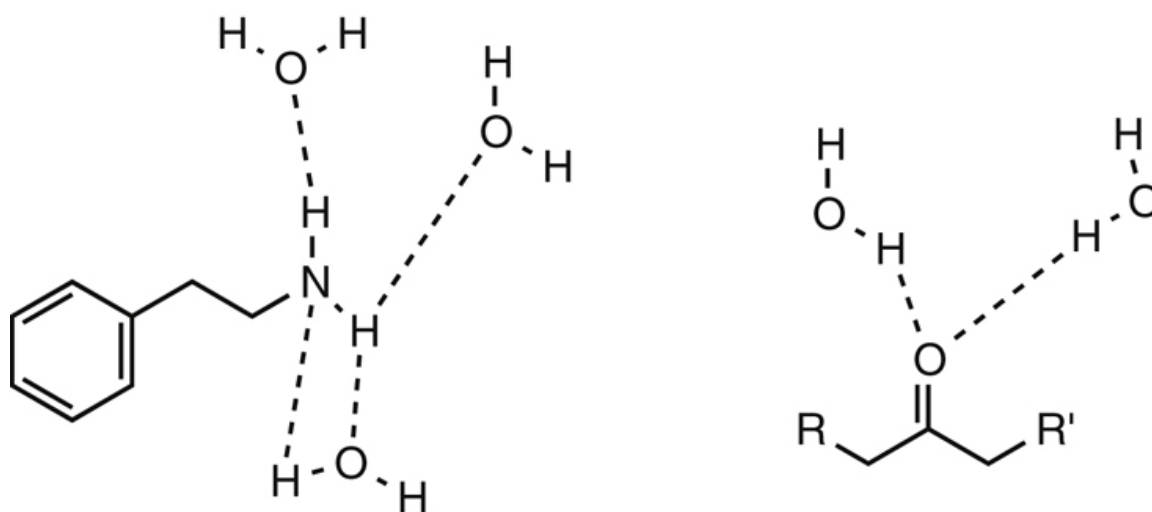


FIGURE 2.8 Examples of hydrogen bonding between water and hypothetical drug molecules.

TABLE 2.4

Common Organic Functional Groups and Their Hydrogen-Bonding Potential

Functional Groups	Number of Potential H-Bonds
R-OH	3

Functional Groups	Number of Potential H-Bonds
$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{R}' \end{array}$	2
$\text{R}-\text{NH}_2$	3
$\begin{array}{c} \text{R}-\text{NH} \\ \\ \text{R}' \end{array}$	2
$\begin{array}{c} \text{R}-\text{N}-\text{R}'' \\ \\ \text{R}' \end{array}$	1
$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{O}-\text{R}' \end{array}$	2

Ionization

In addition to the hydrogen-bonding capacity of a molecule, another type of interaction plays an important role in determining water solubility: the ion-dipole interaction. This type of interaction can occur with organic salts. Ion-dipole interactions occur between either a cation and the partially negatively charged atom found in a permanent dipole (e.g., the oxygen atom in water) or an anion and the partially positively charged atom found in a permanent dipole (e.g., the hydrogen atoms in water) (Fig. 2.9).

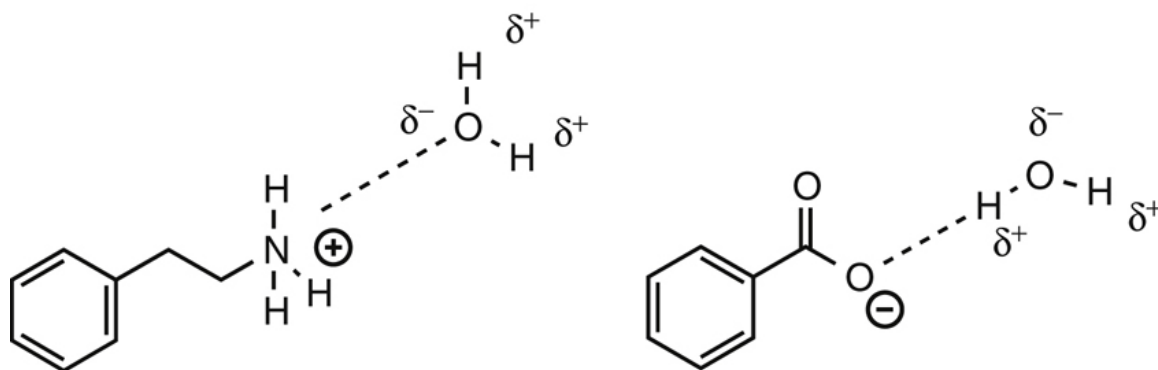
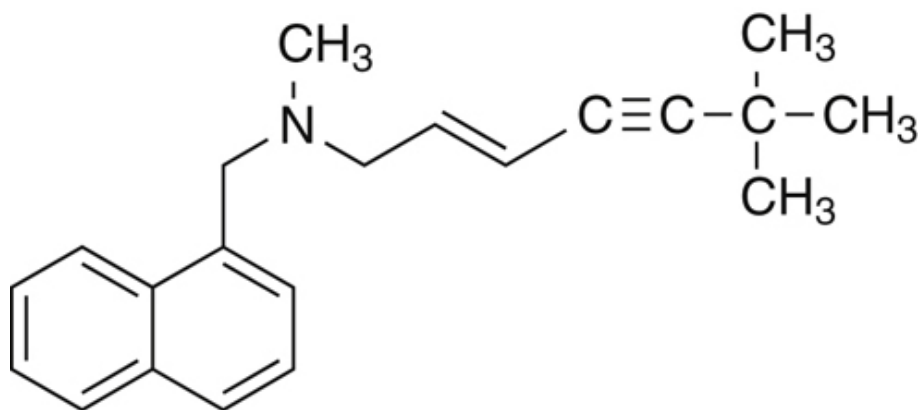


FIGURE 2.9 Examples of ion-dipole interactions.

Case Study

Absorption/Binding Interactions Case

A 24-year-old man comes into the pharmacy and asks you to recommend a treatment for the itching and burning he has recently noticed on both feet. He indicates that he would prefer a cream rather than a spray or a powder. Your recommendation to this patient is to use Lamisil (terbinafine), a very effective topical antifungal agent that is sold over the counter.



Terbinafine (Lamisil)

1. Identify the structural characteristics and the corresponding properties that make terbinafine an agent that can be used topically.
2. The biologic target of drug action for terbinafine is squalene epoxidase. Consider each of the structural features of this antifungal agent and describe the type of interactions that the drug will have with the target for drug action. Which amino acids are likely to be present in the active site of this enzyme?

Case Solution found immediately after References.

Organic salts are composed of a drug molecule in its ionized form and an oppositely charged counterion. For example, the salt of a carboxylic acid is composed of the carboxylate anion (ionized form of the functional group) and a positively charged ion (e.g., Na^+), and the salt of a secondary amine is composed of the ammonium cation (ionized form of the functional group) and a negatively charged ion; e.g., Cl^-). Not all organic salts are very water soluble. To associate with enough water molecules to become soluble, the salt must be highly dissociable; in other words, the cation and anion must be able to separate and interact independently with water molecules. Highly dissociable salts are those formed from strong acids with strong bases (e.g., sodium chloride), weak acids with strong bases (e.g., sodium phenobarbital), or strong acids with weak bases (e.g., atropine sulfate). Examples of strong acids (strong acids are 100% ionized in water [i.e., no ionization constants or pK_a - values >1]) include the hydrohalic (hydrochloric, hydrobromic, and hydrofluoric), sulfuric, nitric, and perchloric acids. All other acids (e.g., phosphoric, tartaric, acetic, and other organic acids and phenols) are partially ionized with pK_a values from 1 to 14 and are, therefore, considered to be moderate or weak acids. Hydroxides of sodium, potassium, and calcium are strong bases because they are 100% ionized, whereas other bases, such as amines, are of moderate or weak strength. The salt formed by a carboxylic acid with an alkylamine is the salt of a weak acid and weak base, respectively. This salt does not dissociate appreciably and cannot significantly contribute to the overall water solubility of a given drug molecule. In general, low-molecular-weight salts are water soluble, and high-molecular-weight salts are water insoluble. Examples of common organic salts used in pharmaceutical preparations are provided in [Figure 2.10](#).

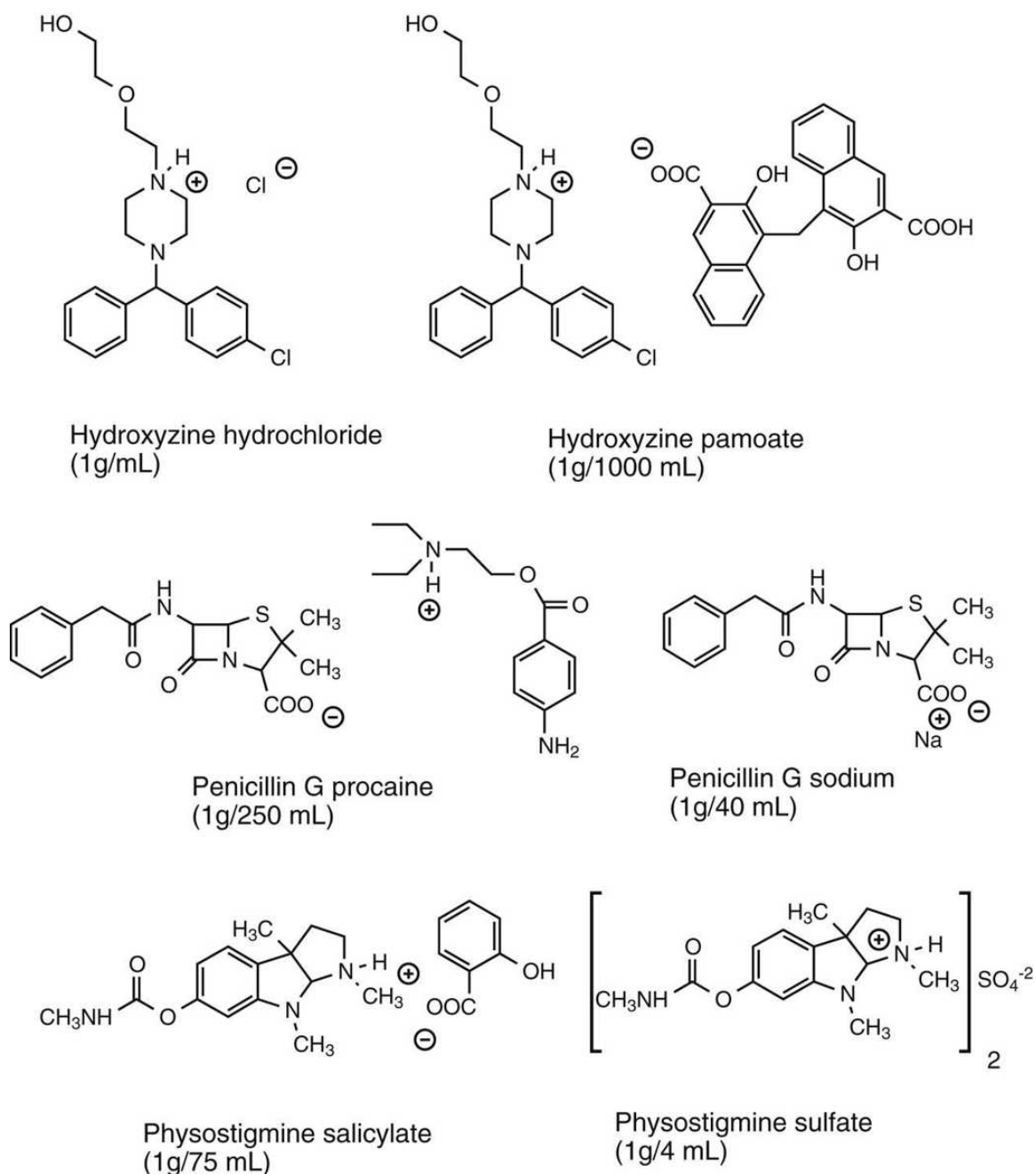


FIGURE 2.10 Water solubilities of different salt forms of selective drugs.

The extent to which ionized molecules are soluble in water is also dependent on the presence of intramolecular ionic interactions. Molecules with ionizable functional groups of opposite charge have the potential to interact with each other rather than with water molecules. When this occurs, these molecules often become water insoluble. A classic example is the amino acid tyrosine (Fig. 2.11). Tyrosine contains three very polar

functional groups, two of which are ionizable (the alkylamine and carboxylic acid) depending on the pH of the environment.

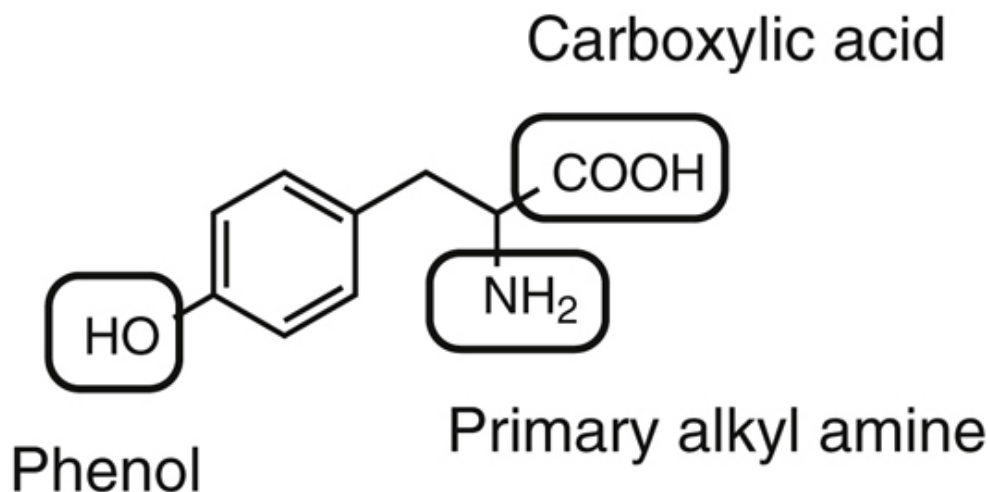


FIGURE 2.11 Functional groups present in tyrosine (see text for pK_a values).

The phenolic hydroxyl group is also ionizable (pK_a 9-10); however, it does not contribute significantly to the ionization of tyrosine under pharmaceutically or physiologically relevant conditions (<1% ionized at pH 7). Because of the presence of three very polar functional groups (two of them being ionizable), one would expect tyrosine to be very soluble in water, yet its solubility is only 0.45 g/1000 mL. The basic alkylamine (pK_a (HB⁺) 9.1 for the conjugate acid) and the carboxylic acid (pK_a 2.2) are both ionized at physiologic pH, and a zwitterionic molecule results. These two charged groups are sufficiently close that a strong ion-ion interaction occurs, thereby keeping each group from participating in ion-dipole interactions with surrounding water molecules. This lack of interaction between the ions and the dipoles found in water results in a molecule that is very water insoluble ([Fig. 2.12](#)). Not all zwitterions or multiply charged molecules demonstrate this behavior; only those that contain ionized functional groups close enough for an ionic interaction to occur will be poorly soluble. Generally, the greater the separation between charges, the more highly water soluble one anticipates the molecule will be. This is only true, however, up to a certain number of carbon atoms. This will be discussed in more detail later.

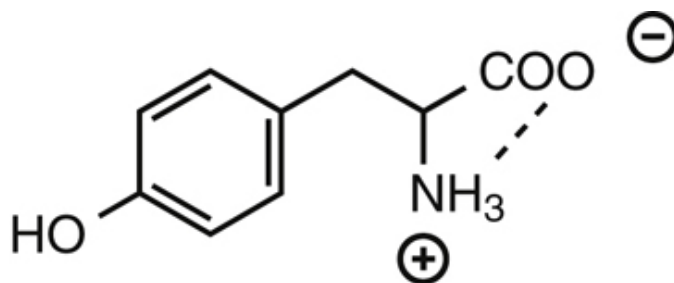


FIGURE 2.12 Zwitterionic form of tyrosine showing ion-ion bond.

Predicting Water Solubility: The Empirical Approach

Lemke⁹ developed an empiric approach to predicting the water solubility of molecules based on the carbon-solubilizing potential of several functional groups. In his approach, if the solubilizing potential of the functional groups exceeds the total number of carbon atoms present, then the molecule is considered to be water soluble. Otherwise, it is considered to be water insoluble. Participation in intramolecular hydrogen bonding or ionic interactions decreases the solubilizing potential of a given functional group. It is difficult to quantitate how much such interactions will decrease a molecule's overall water solubility.

Table 2.5 shows the water-solubilizing potential for several functional groups common to many drugs. Because most drug molecules contain more than one functional group (i.e., are polyfunctional), the second column in the table will be of more utility. To demonstrate Lemke's method, consider the structure of anileridine. Anileridine (Fig. 2.13) is an opioid analgesic containing three functional groups that contribute to water solubility: an aromatic amine (very weak base), a tertiary alkylamine (weak base), and an ester (neutral). There are a total of 22 carbon atoms in the molecule and a solubilizing potential from the three functional groups of 9 carbon atoms. Since the solubilizing potential of the functional groups is less than the total number of carbons present, it is predicted that anileridine is insoluble in water. This is, indeed, the case: The solubility of anileridine is reported in the U.S. Pharmacopeia (USP) as 1 g/10,000 mL, or 0.01%. Now consider the hydrochloride salt of anileridine. Not only do the three functional groups contribute a solubilizing potential of 9 carbon atoms, the positive charge of the alkylammonium also contributes to its

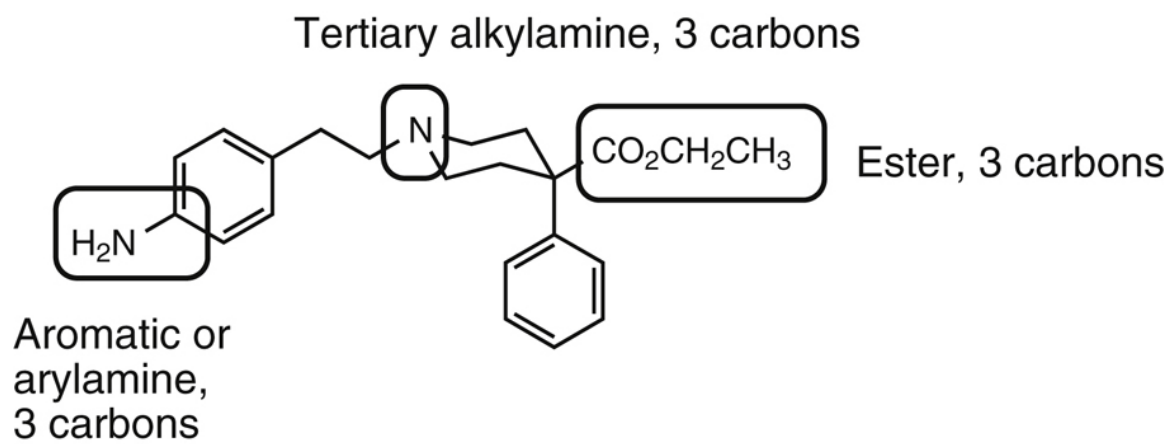
water solubility. Lemke⁹ estimates that each ionized functional group (cationic or anionic) found within a drug molecule contributes a solubilizing potential of 20-30 carbon atoms. Thus, the solubilizing potential for all of the functional groups in anileridine hydrochloride is 29-39 carbon atoms, which is more than the total number of carbon atoms in the molecule. This salt should therefore be soluble in water, and it is to the extent of 0.2 g/mL, or 20%. Solubility data for drug molecules can be found in the USP. In most instances, discrepancies between approximate and actual water solubilities can be rationalized by careful inspection of the chemical structure.

TABLE 2.5

Water-Solubilizing Potential of Organic Functional Groups in a Mono- or Polyfunctional Molecule

Functional Group	Monofunction Molecule	Polyfunctional Molecule
Alcohol	5-6 carbons	3-4 carbons
Phenol	6-7 carbons	3-4 carbons
Ether	4-5 carbons	2 carbons
Aldehyde	4-5 carbons	2 carbons
Ketone	5-6 carbons	2 carbons
Amine	6-7 carbons	3 carbons
Carboxylic acid	5-6 carbons	3 carbons
Ester	6 carbons	3 carbons
Amide	6 carbons	2-3 carbons
Urea, carbonate, carbamate		2 carbons

Water solubility is defined as greater than 1% solubility.⁹



Anileridine

FIGURE 2.13 Identification of functional groups in anileridine.

Case Study

Binding Interactions Case

Each of these drug molecules interacts with a different biologic target and elicits a unique pharmacologic response. For each of the three molecules, list the types of interactions that are possible with a biologic target. For each type of interaction, provide one example of an amino acid that could participate in that interaction.

Case Solution found immediately after References.

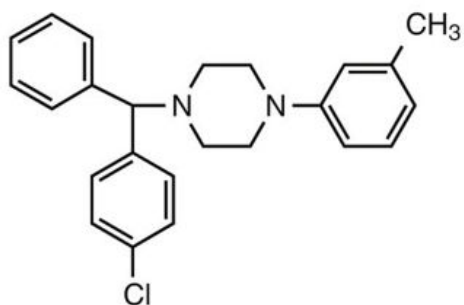
Case Study

Water/Lipid Solubility Case

When you look at any drug molecule, there are a number of functional groups present that contribute to the properties of that drug molecule. Identify the types of functional groups in each molecule and to which physical properties (water/lipid solubility) each contributes.

1. Structural feature

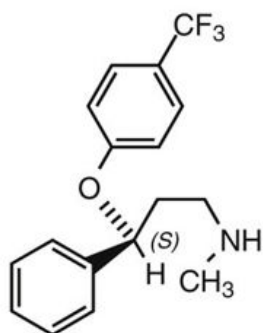
Physical property



Mecclizine (Antivert)

2. Structural feature

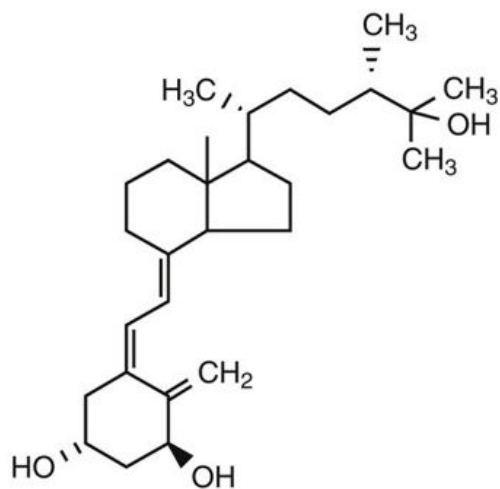
Physical property



Fluoxetine (Prozac)

3. Structural feature

Physical property



1,25-Dihydroxy Vit D₂

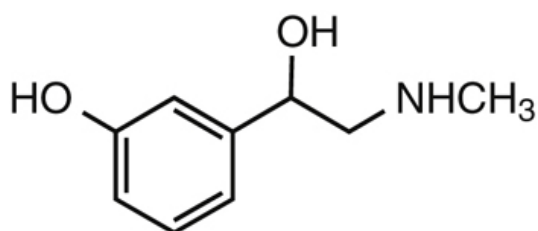
Case Solution found immediately after References.

Case Study

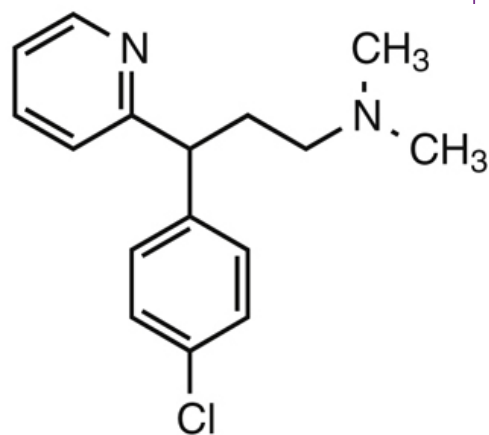
Interactions/Solubility Case Study

J.K. presents a prescription for her 6-month-old daughter for Donatussin Drops. She wants to know if this medication will have an effect on her daughter's alertness.

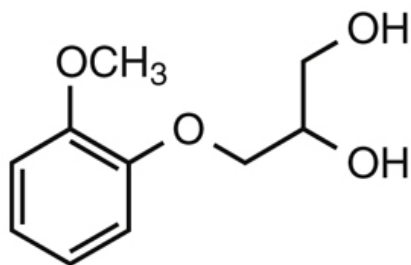
Components of Donatussin:



Phenylephrine
(decongestant)



Chlorpheniramine
(antihistamine)



Guaifenesin
(expectorant)

1. Identify the structural features/functional groups of phenylephrine and guaifenesin that contribute to improved water solubility (medication given as drops). List the type(s) of interactions that these groups have with water, and draw an example of these interactions (with appropriate labels).
2. Evaluate each of the three molecules, and determine if each molecule contains any functional groups that will allow the drug

- to cross the blood-brain barrier and have an effect on this child's alertness (create a list of relevant functional groups for each molecule). Based on your evaluation, which agent is likely to have the most significant effect? Identify what property is necessary for these agents to cross this biologic membrane.
3. Identify the binding interactions that chlorpheniramine and guaifenesin could have with their respective targets for drug action. Be sure to identify which functional groups will participate in each of these binding interactions.

Case Solution found immediately after References.

Predicting Water Solubility: Analytical/Quantitative Approach

Another method for predicting water solubility involves calculation of an approximate log P, or log of the partition coefficient for a molecule. This approach is based on an approximation method developed by Cates¹⁰ and discussed in Lemke.⁹ In this approach, one sums the hydrophobic or hydrophilic properties of each functional group present in the molecule. Before we can calculate log P values, a brief explanation of the concept of partition coefficient is necessary.

In its simplest form, the partition coefficient, P, refers to the ratio of drug concentration in octanol (C_{oct}) to that in water (C_{water}) (Equation (2.7)). Octanol is used to mimic the amphiphilic nature of lipid, because it has a polar head group (primary alcohol) and a long hydrocarbon chain, or tail, similar to the fatty acid tail that makes up part of a lipid membrane. Because P is logarithmically related to free energy,¹¹ P is generally expressed as log P and is, therefore, the sum of the hydrophobic and hydrophilic characteristics of the functional groups that make up the structure of a molecule. Thus, log P is a measure of the lipid/water solubility characteristics of the entire molecule. Because each functional group contained within the molecule contributes to its overall hydrophilic/hydrophobic character, a hydrophilic/hydrophobic value (the hydrophobic substituent constant, π) can be assigned to each functional group. Equation (2.8) defines this relationship:

$$\text{Eq. 2.7} \quad P = C_{\text{oct}} / C_{\text{water}}$$

$$\text{Eq. 2.8} \quad \text{Log}P = \sum \pi (\text{fragments})$$

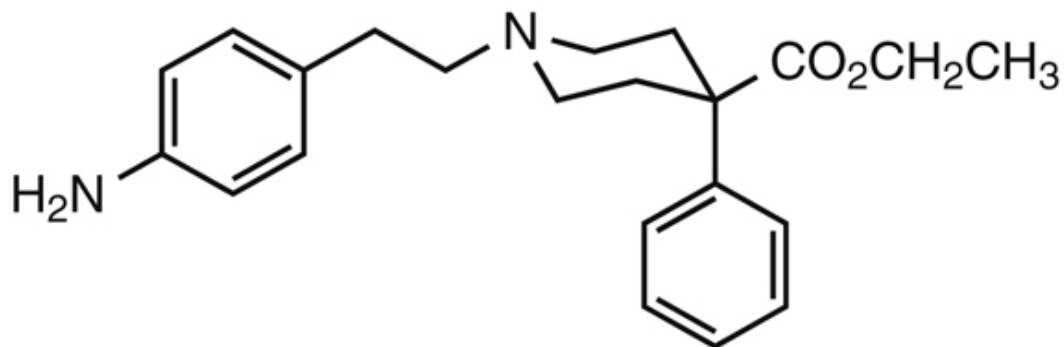
When calculating log P from hydrophobic substituent constants, the sum is usually referred to as log P_{calc} or Clog P (for software sources to calculate Clog P, see Ref. 15) to distinguish it from an experimentally determined value (Mlog P or log P_{meas}). Over the years, extensive tables of π values have been compiled for organic functional groups and molecular fragments¹¹⁻¹⁴ Table 2.6 is a highly abbreviated summary of π values from Lemke,⁹ based largely on the manuscript by Cates.¹⁰ Using the values in this table, a fairly reasonable estimate for the water solubility of many organic compounds (shown as log P) can be determined.

TABLE 2.6
Hydrophilic-Lipophilic Values (π V) for Organic Fragments

Functional Group	π Value (Aliphatic)	π Value (Aromatic)
H		0.00
Alkane	0.50	0.56 (CH ₃); 1.02 (CH ₂ CH ₃)
Alkene		0.82
C ₆ H ₅ (phenyl)	2.15	1.96
Br, Cl, F, I	0.60; 0.39; -0.17; 1.00	0.86; 0.71; 0.14; 1.12
NO ₂	-0.85	-0.28
NH ₂ (primary amine)	-1.19	-1.23
NHR (secondary amine)	-0.67	0.47
NR ₂ (tertiary amine)	-0.30	0.18
-NHC = OR (amide)	-0.97	
SC ₆ H ₅	2.32	
OH	-1.12	-0.67
OCH ₃		-0.02
-OC = OR (ester)	-0.27	-0.64
CHO (aldehyde)		-0.65
C = OCH ₃ (ketone)		-0.55
CO ₂ H		-0.32
SO ₂ NH ₂ (sulfonamide)		-1.82

Based on Cates LA. Calculation of drug solubilities by pharmacy students. *Am J Pharm Ed.* 1981;45:11-13.

Again, we will consider the structure of the opioid analgesic anileridine to demonstrate the calculation of log P (Fig. 2.14). This compound has a total of 22 carbon atoms, some aliphatic and some aromatic. We need to distinguish between the aliphatic and aromatic carbon atoms because the delocalized π orbitals for the sp^2 -hybridized aromatic carbon atoms make them more polar than aliphatic carbons. The compound also contains one tertiary alkylamine, one aromatic or arylamine, and one ester. Evaluation of esters and amides requires that the oxygen, nitrogen, and ester/amide carbon atoms are counted in this π value. The remaining aliphatic carbons are then counted. Figure 2.14 summarizes the log P calculation for anileridine. The calculation gives a Clog P value for anileridine of +7.00. Water solubility as defined by the USP is solubility greater than 3.3%, which equates to an approximate log P of +0.5. Log P values less than +0.5 are therefore considered to be water soluble, and those greater than +0.5 are considered to be water insoluble. According to our calculation, anileridine would be predicted to be insoluble in water. This calculation agrees with the empiric procedure discussed earlier.



Fragments	$\neq$
1 primary arylamine	-1.23
1 tertiary alkylamine	-0.30
9 aliphatic carbons	+4.5
2 phenyl rings	+4.30
1 ester	-0.27
logP	+7.0

FIGURE 2.14 Calculation of log P for anileridine.

Other sample calculations are shown in [Figure 2.15](#). In [Figure 2.15](#), Mlog P values (when available) and Clog P values¹⁵ are included for comparison purposes (see Appendix A for additional Clog P values). Even though the π values from [Table 2.6](#) are not as extensive as those in the computer program, there is good general agreement with most of these compounds with respect to their solubility (or insolubility) in water. In addition, other programs besides Clog P are available to predict log P values; some of these programs are available on the Internet. One must keep in mind that due to the assumptions made in these programs, they cannot produce

results that are in total agreement with measured values or other prediction programs. Clog P values calculated from ACDLog P¹⁵ are generally considered to be more accurate. Other programs for calculating log P values, such as Molinspiration,¹⁶ use different methods and assumptions and, and therefore, do not always agree with Clog P predictions or experimentally determined values. This is not to say that other programs do not give accurate results. Often, one or all of the available programs will have reasonable agreement with measured values, but greater disagreement tends to occur as the number of functional groups in the molecule that participate as hydrogen-bond acceptor and/or hydrogen-bond donor groups increases. This increases the likelihood that intramolecular interactions will occur—something that is not always taken into account with these programs.

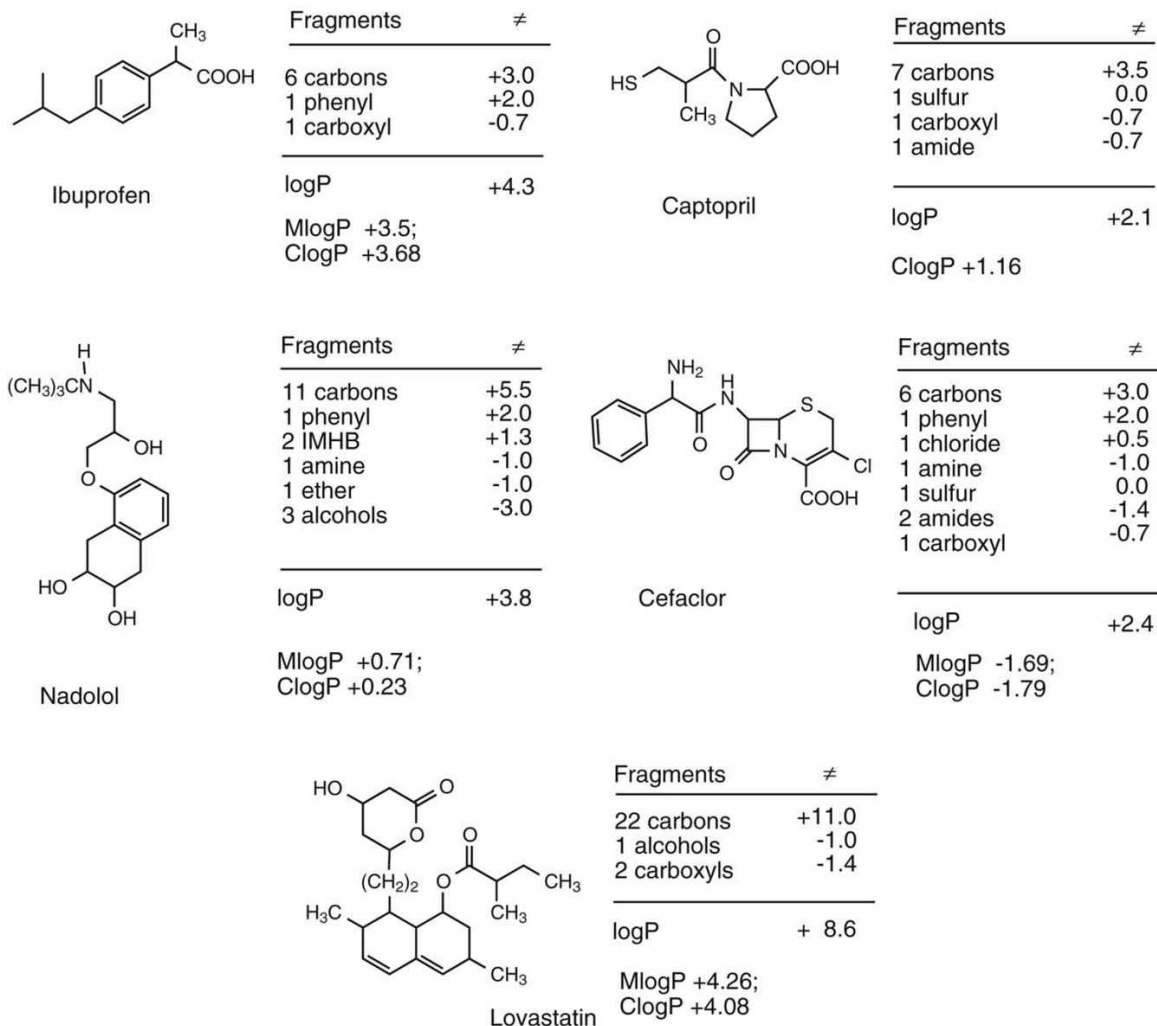


FIGURE 2.15 Clog P calculations for selected compounds.

The ability to predict the percent ionization or water solubility of a molecule should not be viewed as an exercise in arithmetic, but rather as a way to understand the solution behavior of molecules, especially as it relates to admixtures and the pharmacokinetic differences among molecules. The ionization state of a molecule not only influences its water solubility but also its ability to traverse membranes and, therefore, its ability to be absorbed. The distribution of the drug and its ability to bind to proteins other than its target are also greatly influenced by the ionization state and the hydrophilic/hydrophobic nature of the molecule.

Drug Absorption

A variety of routes for administration of drugs exist varying from intravenous (IV), subcutaneous (SC), and intramuscular (IM) injection. Nasal, buccal, inhalation, and rectal administration have also been used. By far the most common, and desirable from a compliance point of view, is that of oral administration: swallowing a tablet, capsule, liquid, or syrup. Each of these approaches to delivery of medications has its advantages and disadvantages, but from a patient perspective the oral route is preferred. As a result, the physical chemical properties of the medication greatly influence the absorption properties of the compound as well as its stability as it traverses the gastrointestinal tract. The type of formulation is also influenced by the physical chemical properties of the compound and will enhance absorption, protect from degradation, or affect the rate of absorption. Because the oral route of drug administration is so important and desirable for drug delivery, a discussion of gastrointestinal physiology is necessary in order to understand the complexities of the conditions encountered by the medication as it passes through this region.

Gastrointestinal Physiology

When administered orally, a drug molecule encounters many physical and chemical barriers to its absorption into the systemic circulation. [Figure 2.16](#) is a diagram of these barriers to absorption and the complexities involved. The stomach is divided into two major anatomical regions: the body of the stomach and the pylorus. The former region secretes pepsin and hydrochloric acid (HCl) and is also muscular, and contractions of these muscles allow for mechanical breakdown of ingested food. By breaking food particles into smaller pieces a larger surface area is obtained allowing for additional digestion to occur with pepsin and HCl. The pyloric region is the mucus-secreting area. This mucus serves as a protective layer preventing damage to the stomach lining by pepsin and HCl. That is, it helps prevent the stomach from digesting itself. It also lubricates the gastrointestinal tract allowing freer movement of solid material. Within

the stomach the dosage form begins to disaggregate and some dissolution of the drug may begin to occur, except in the case of enteric-coated formulations which do not breakdown under acidic conditions and pass through the stomach. These types of formulations will be discussed in later chapters with specific compounds. Depending upon the physical chemical properties of the compound, some dissolution may occur within the stomach. Significant absorption generally does not occur within the stomach due to the low surface area, acidity, and possible association with other contents of the stomach such as fiber, metal ions, etc.

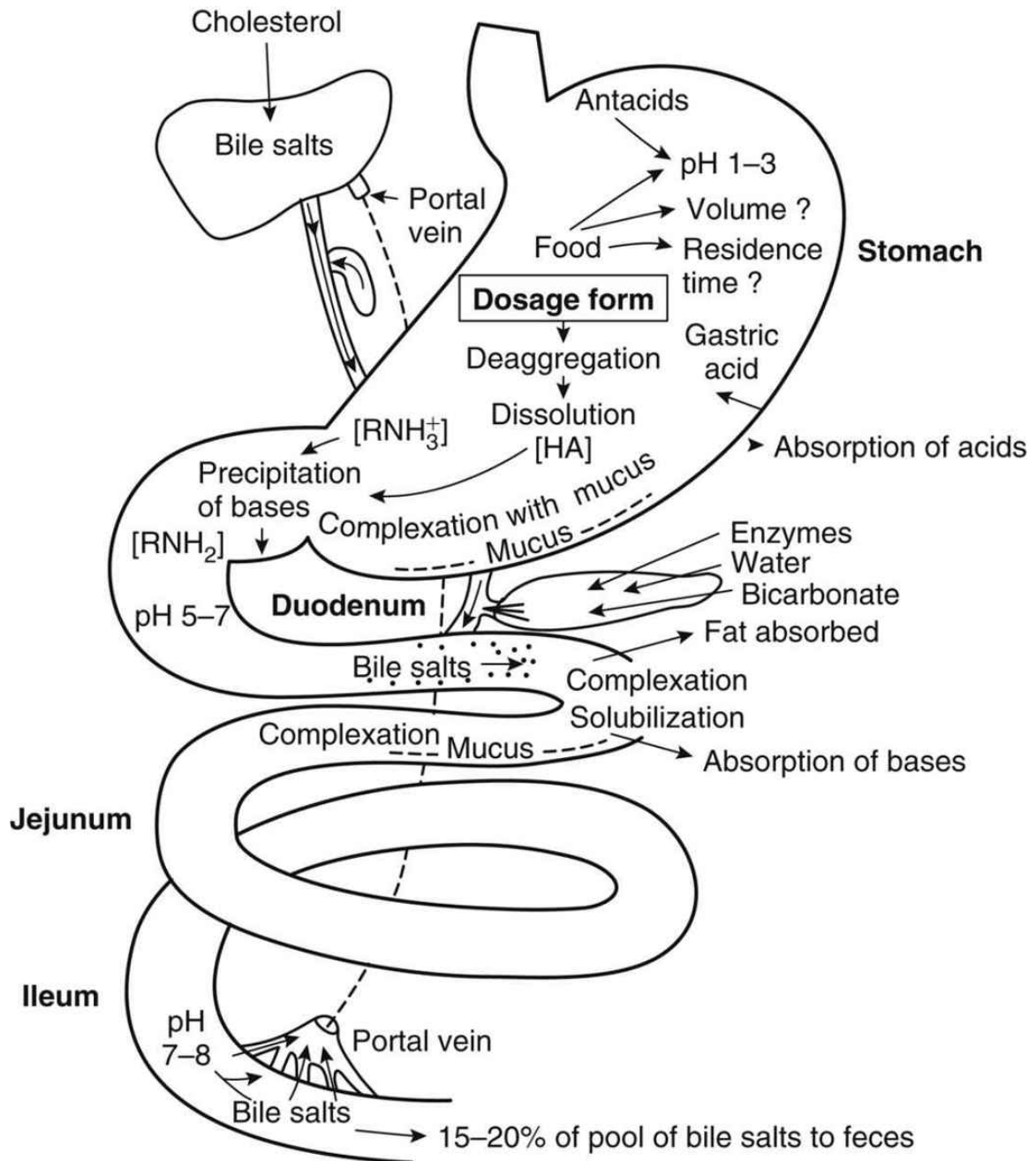


FIGURE 2.16 Processes occurring along with drug absorption when drug molecules travel down the gastrointestinal tract and the factors that affect drug absorption.

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Passage into the small intestine is accompanied by a significant change in pH and in the anatomical structure of the region. The small intestine comprises three distinct regions: duodenum, jejunum, and ileum. Within this region the pH changes from approximately 5 (duodenum) to 7-8

(ileum) and significantly affects the solubility of drugs. Acidic molecules now become ionized and more water soluble, while basic molecules become less ionized and may even begin to precipitate. Because the epithelium of the small intestine is highly convoluted, the nutrients (and drugs) here are efficiently absorbed due to the large surface area that results. Because bases are less ionized within this region, they are more lipophilic and therefore able to cross the cell membranes more efficiently. Basic molecules therefore undergo more rapid absorption in the small intestine. All is not perfect, however. The presence of bile acids, mucus, and food particles can prevent absorption by forming complexes or adsorbing the drug. Such an action results in some drugs passing through the gastrointestinal tract and being excreted in the feces.

In order to get to the systemic circulation, the drug must traverse biomembranes of the epithelial cells lining the ^{[[SEP]]}lumen of the intestine, pass through the cells and through another membrane to reach the blood. This requires the drug to be hydrophilic enough to be soluble in water (the major constituent within the gastrointestinal tract) to be able to come into contact with the membrane and hydrophobic enough to be able to cross the membrane. Thus there is a delicate balance between being too hydrophilic or too hydrophobic to be able to be absorbed into the systemic circulation. To complicate matters even more, the presence on the drug of functional groups that are acidic, basic, or both will influence solubility and therefore absorption. Obviously, these barriers can be overcome; otherwise we would not have orally administered medications in our formularies.

The processes of disaggregation and dissolution of drug formulations are beyond the scope of this chapter, and the reader will become acquainted with this area of physical pharmacy in other parts of the curriculum. For now we will concentrate on what occurs once the drug is in solution within the gastrointestinal tract and how even ionized molecules are able to cross biological membranes. We will mainly concentrate on the process of passive diffusion of drugs across membranes. Other processes will also be mentioned, but will be discussed in more detail in other chapters within this text.

Passive Diffusion

Nonionized Drugs

The simplest case to consider is that of a drug that is neither basic nor acidic with sufficient water solubility to have contact with the membranes of epithelial cells lining the intestine. With this process the molecule is simply undergoing diffusion across the membrane from a region of high drug concentration to that of low concentration. Such diffusion may occur through the membrane lipid layer ([Fig. 2.17A](#)) or via a channel protein ([Fig. 2.17B](#)). In either case no energy expenditure is involved and this is referred to as passive diffusion ([Fig. 2.17](#)).

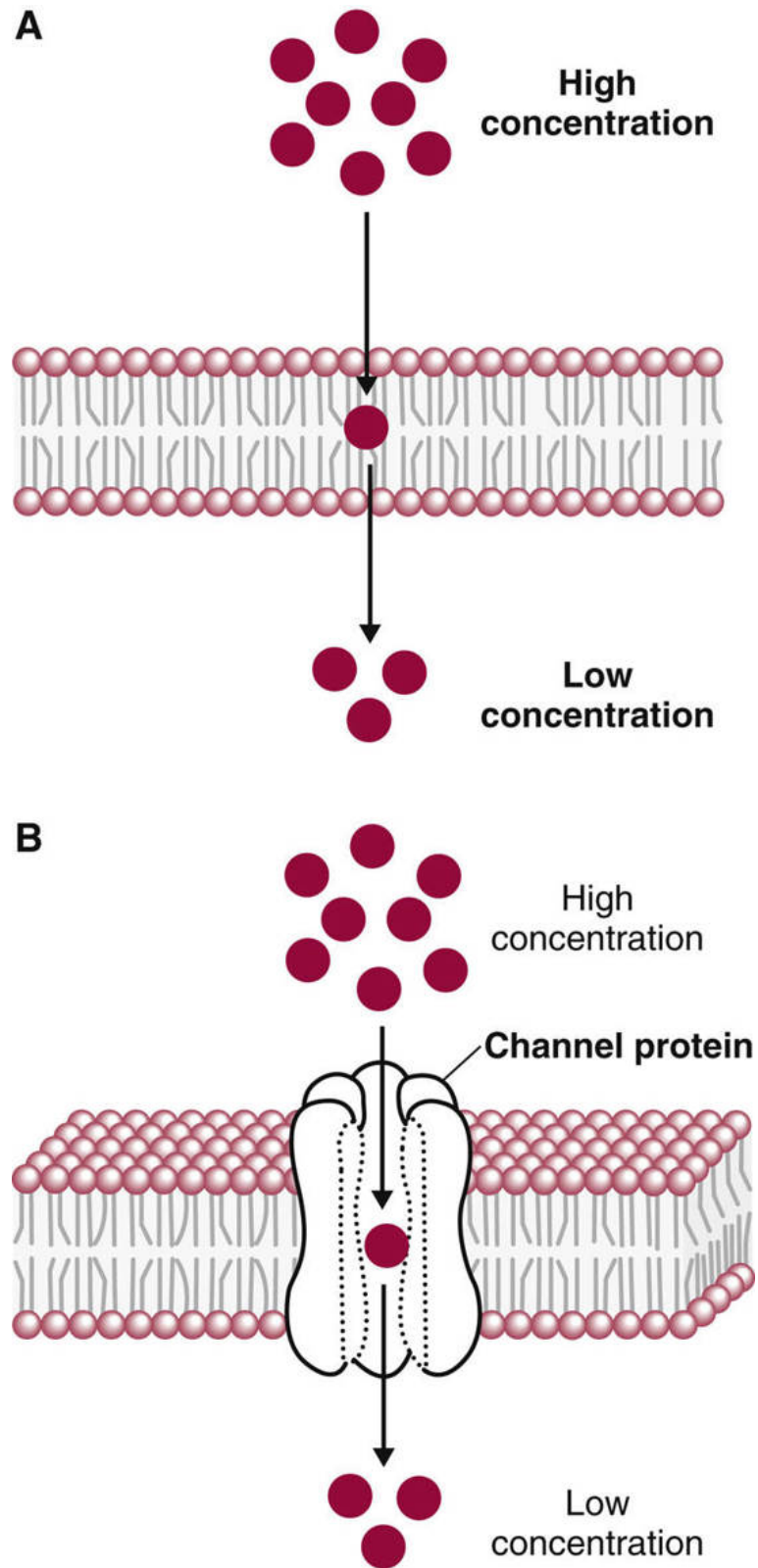


FIGURE 2.17 A, Simple diffusion. B, Membrane channels.

Adapted with permission from Smith C, Marks A, Lieberman M, eds. *Basic Medical Biochemistry*. 2nd ed. Baltimore: Lippincott Williams & Wilkins; 2004.

Acidic Drugs

Acidic drugs encounter an increasingly basic environment as they pass through the gastrointestinal tract that results in an increase in the percentage of ionized drug. Absorption of the acidic drug occurs within the stomach primarily due to the protonated un-ionized species predominating there. However, the greatest absorption occurs just below the stomach, in the duodenum, due to the greater surface area of the microvilli of the brush border. Efficient absorption can still occur even though a significant amount of drug may be ionized due to the equilibria shown in  Figure 2.18, with HA representing the protonated, un-ionized, drug and A^- the deprotonated ionized species.

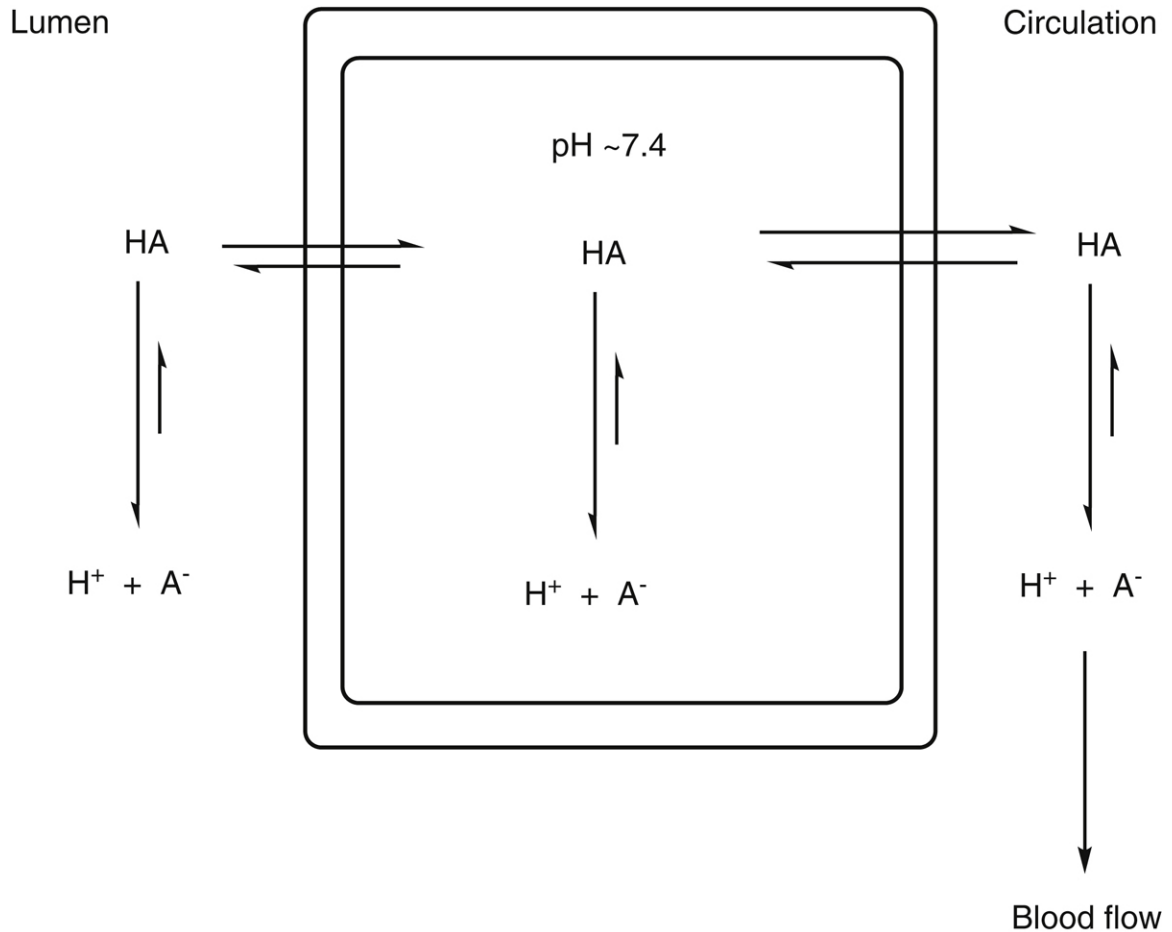


FIGURE 2.18 Equilibria involved in absorption of an acidic drug from the gastrointestinal tract.

In the duodenum the compound is ionized with the equilibrium shifted toward A^- . However, there is always some HA present which can partition through the membrane into the epithelial cell of the brush border. Once through the membrane, another equilibrium is established within the cytoplasm of the cell due to the pH (~ 7.4). As HA partitions through the membrane and into the cell, the equilibrium in the intestinal lumen must shift to form more HA. This is an example of Le Chatelier's principle from your first year chemistry course. The equilibrium within the lumen of the duodenum must be maintained. More HA forms which can now cross the membrane into the cytoplasm of the epithelial cell. But something else must be occurring to keep HA partitioning across the membrane. For this we now need to consider what is happening within the epithelial cell of the brush border.

Within the cell the pH causes deprotonation of the drug resulting in a second equilibrium which is shifted toward A^- . This decreases the amount of HA within the cell causing a shift in the equilibrium across the cell membrane. The HA within the cytoplasm can pass through the membrane of the epithelial cell into the circulation where two things occur. First, due to the pH of the blood, a fourth equilibrium occurs between HA and A^- and is shifted toward A^- . Second, the circulation carries both HA and A^- away which causes the equilibrium across the membrane to shift allowing more HA to cross into the circulation. In summary, Le Chatelier's principle is at play here. As the equilibria shift in response to changes in pH and relative concentrations of un-ionized and ionized drug, absorption of the drug into the systemic circulation occurs.

Basic Drugs

With the increasing basicity in the jejunum and ileum, absorption of basic drugs becomes more efficient. As shown in [Figure 2.19](#), as the pH increases the equilibrium between the conjugate acid (BH^+) and free base (B) form shifts to favor the latter. Following the same line of reasoning as above with acidic drugs, the free base form crosses the membranes and reestablishes equilibria with the conjugate acid form within the cytoplasm and subsequently within the circulation. As the equilibria shift to form more B, and as BH^+ and B are removed by the circulation, diffusion of the drug from high to low concentration occurs.

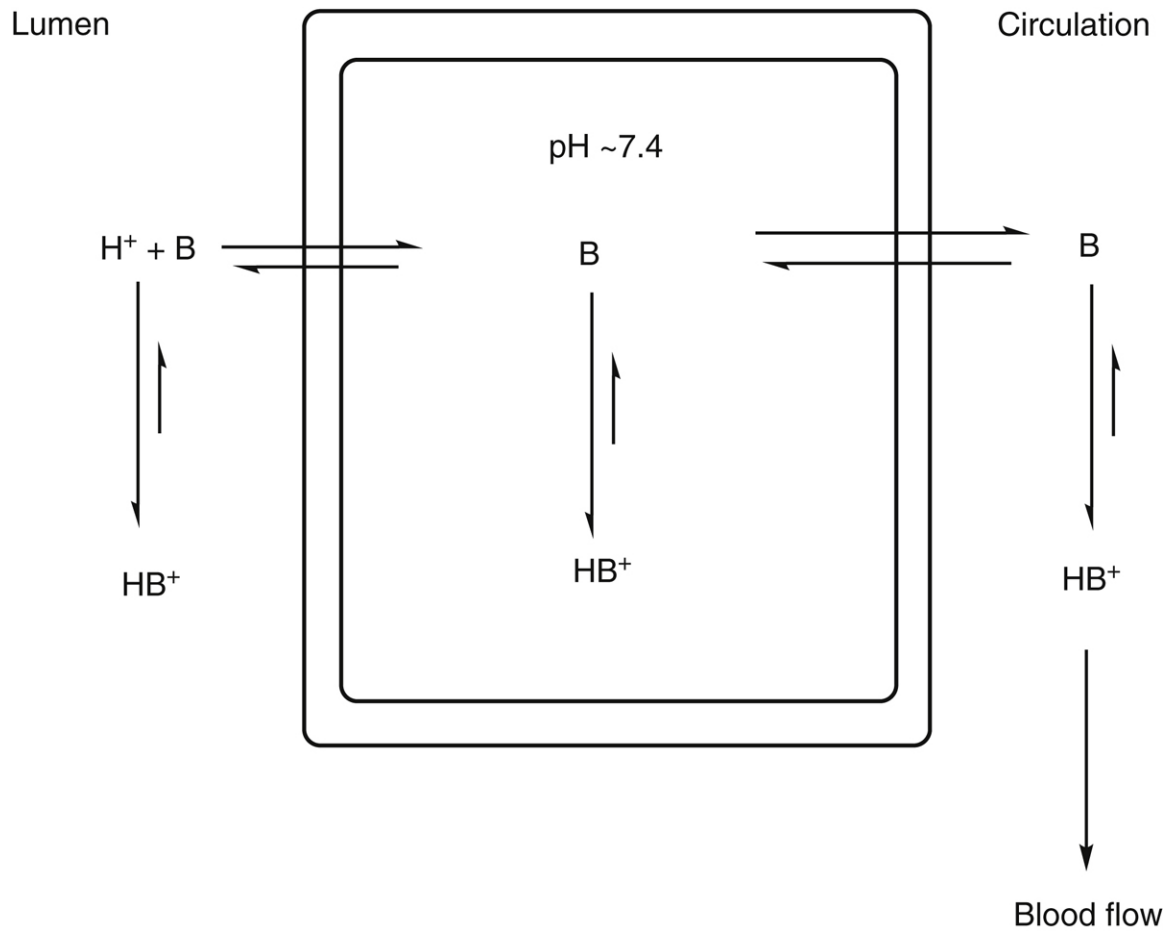


FIGURE 2.19 Equilibria involved in absorption of a basic drug from the gastrointestinal tract.

Active Transport (Influx and Efflux Transporters)

As discussed above, passive diffusion is the passage of a drug from a region of high concentration to that of low concentration and is an energy neutral process. Active transport requires energy and is generally a process of transporting the drug against a concentration gradient. Because active transport requires the drug to be “recognized” by a transport protein that normally mediates movement of a molecule of similar structure across the membrane, it is also a saturable process. That is, at high drug concentrations the ability to transport the drug plateaus due to the limited number of transport proteins available. Another difference is that the structural specificity required to be recognized and transported can result in competition with other compounds of similar structure. These could be natural substances that normally are transported by these proteins, or other drugs.¹⁹ These will be discussed in more detail below (Fig. 2.20).

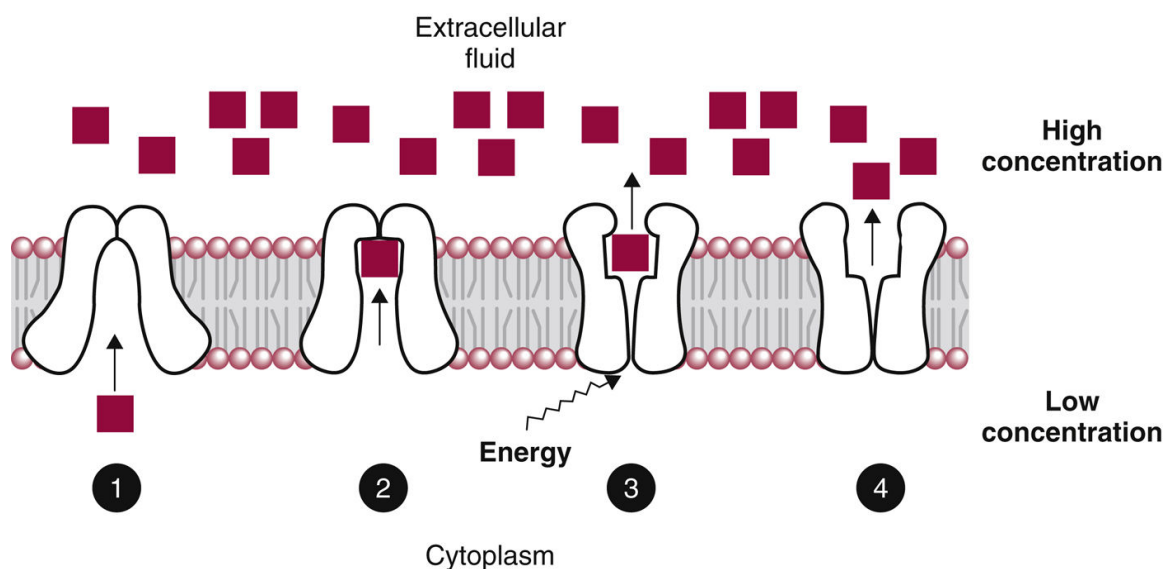


FIGURE 2.20 Active transport.

Adapted with permission from Smith C, Marks A, Lieberman M, eds. *Basic Medical Biochemistry*. 2nd ed. Baltimore: Lippincott Williams & Wilkins; 2004.

Some membrane transporters enhance the passage of drugs across membranes (influx), while others reverse absorption (efflux transporters).

All transporters (influx and efflux) are membrane associated proteins that have multiple transmembrane regions and require energy for the process. Some transporters recognized a broad range of structures, while others are very specific.

Influx Transporters

Several types of influx transporters with differing substrate selectivity exist within the luminal surface of the intestinal mucosa and can influence the rate of oral absorption ^[1](see [Chapter 4](#) for more discussion). Peptide transporters such as the peptide transporter 1 (PepT-1) and human peptide transporter 1 (HPT-1) are responsible for the transport of many diverse peptide and peptide mimetic substrates such as β -lactam antibiotics, ACE inhibitors, and amino acid conjugated drugs (e.g., valacyclovir). Other transporters within the intestinal tract include the bile acid, ^[2]nucleoside, organic cation/anion (OCTP and OATP, respectively) and fatty acid transporters. Just as with PepT-1 and HPT-1, drugs that structurally resemble the natural substrates for these may have enhanced absorption in addition to passive diffusion. Throughout this text, the influence of influx transporters on intestinal absorption of specific drugs will be discussed when relevant.

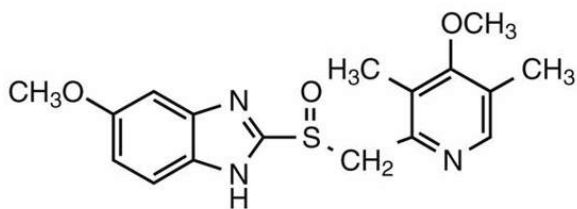
Efflux Transporters

Drug efflux into the intestinal lumen is influenced by P-glycoprotein (P-gp), multiresistance associated proteins 1 and 2 (MRP1 and MRP2), and breast cancer resistance protein (BCRP) (see [Chapter 4](#)). These transporters prevent the influx and accumulation of many drugs into the general circulation as well as specific organs: e.g., transport into bile, preventing blood-brain barrier (BBB) penetration, and tumor tissue. P-gp appears to be the most ubiquitous of these efflux proteins returning a portion of drug entering the intestinal mucosa back to the lumen in a concentration-dependent manner. Two types of P-gp have been shown to exist in mammals: drug-transporting and phospholipid transporting P-gp. Localization of P-gp in the intestine, kidney, and liver suggest that it is a leading player in excreting xenobiotics into the intestinal lumen, urine,

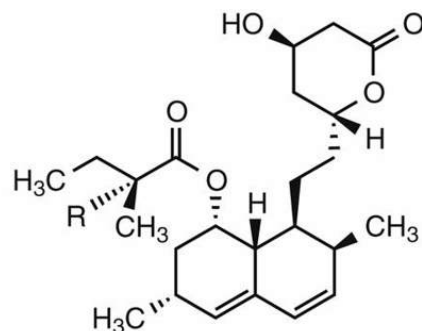
and bile. The number of drugs shown to be substrates increases each year and includes HIV protease inhibitors and verapamil. The latter also acts as an inhibitor of P-gp increasing intestinal permeabilities.

Prodrugs

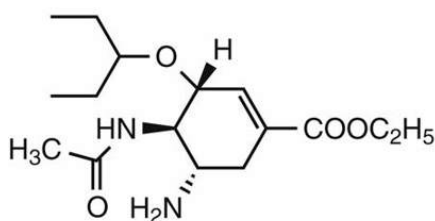
By definition prodrugs are compounds that undergo metabolic or chemical conversion to produce a biologically active substance. That is, the ingested compound is not pharmacologically active and must first be converted via action of an enzyme or chemical degradation to produce the active species. Some prodrugs have been developed because the pharmacologically active form has poor oral bioavailability, usually due to poor absorption in the gastrointestinal tract. Other prodrugs are highly water-soluble derivatives of compounds that are too hydrophobic to be dissolved in aqueous to be given intravenously. Examples of well-known prodrugs include omeprazole, simvastatin, levastatin, enalapril, clopidogel, valacyclovir, acyclovir, and oseltamivir ([Fig. 2.21](#)).



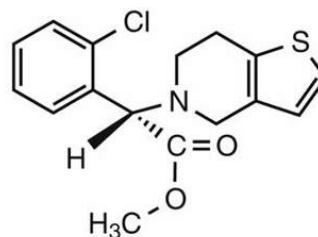
Omeprazole (Prilosec)



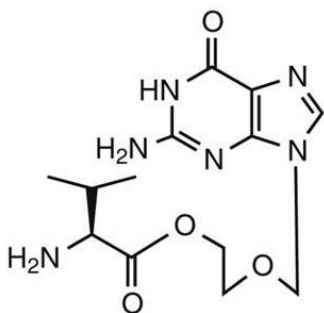
Lovastatin (Mevacor, R = H)
Simvastatin (Zecor, R = CH₃)



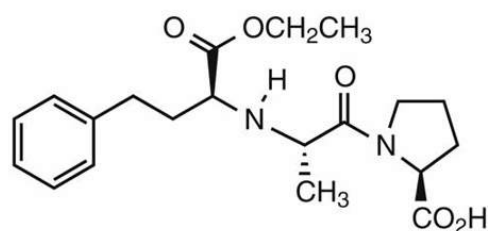
Oseltamivir (Tamiflu)



Clopidogrel (Plavix)



Valacyclovir (Valtrex)



Enalapril (Vasotec)

FIGURE 2.21 Commercially available blockbuster prodrugs and their structures.

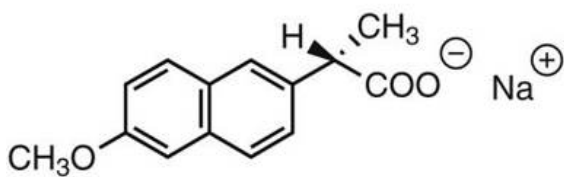
Other reasons for developing prodrugs may be chemical instability, unacceptable odor or taste, gastric irritation, inadequate BBB permeability, presystemic metabolism, or toxicity. The most common approach to prodrug design is to use an ester linkage to the drug (e.g., lovastatin, simvastatin, oseltamivir, clopidogrel, valacyclovir and enalapril in Fig. 2.21). As you will learn in Chapter 3, esters are readily hydrolyzed by ubiquitous esterases within the systemic circulation. The rates at which

these esters are hydrolyzed can be influenced via steric or electronic effects of substituents on either side of the ester bond following basic organic chemical principles. The ester may be hydrophobic (i.e., alkyl esters) to improve diffusion across membranes; ionizable (e.g., succinates or amino acids) to improve aqueous solubility; or sterically hindered to slow hydrolysis providing an acceptable rate for “release” of the active drug.

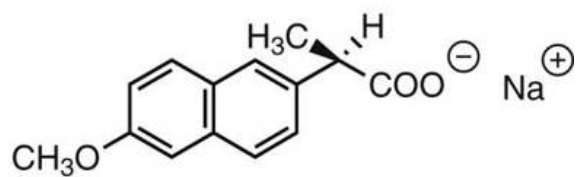
Stereochemistry and Drug Action

Stereoisomers are molecules that contain the same number and kinds of atoms, the same arrangement of bonds, but different three-dimensional structures; in other words, they only differ in the three-dimensional arrangement of atoms in space. There are two types of stereoisomers: enantiomers and diastereoisomers. Enantiomers are pairs of molecules for which the three-dimensional arrangement of atoms represents nonsuperimposable mirror images. Diastereoisomers represent all of the other stereoisomeric compounds that are not enantiomers. Thus, the term “diastereoisomer” includes compounds that contain double bonds (geometric isomers) and ring systems. Unlike enantiomers, diastereoisomers exhibit different physicochemical properties, including, but not limited to, melting point, boiling point, solubility, and chromatographic behavior. These differences in physicochemical properties allow the separation of individual diastereoisomers from mixtures with the use of standard chemical separation techniques, such as column chromatography or crystallization. Enantiomers cannot be separated using such techniques unless a chiral environment is provided or if they are first converted to diastereoisomers (e.g., salt formation with another enantiomer). Examples of enantiomers and diastereoisomers are provided in [Figure 2.22](#).

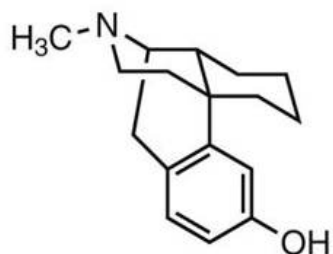
Enantiomers



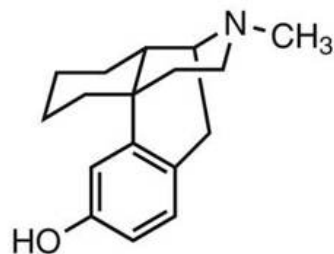
S-(+)-naproxen sodium



R-(-)-naproxen sodium

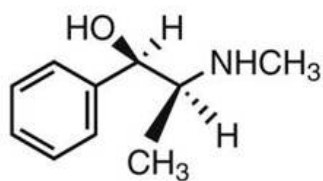


Levorphanol (analgesic)

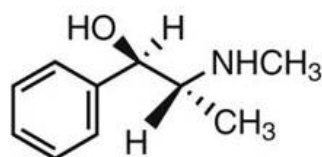


Dextrophan (antitussive)

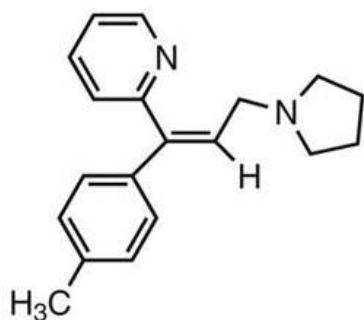
Diastereoisomers



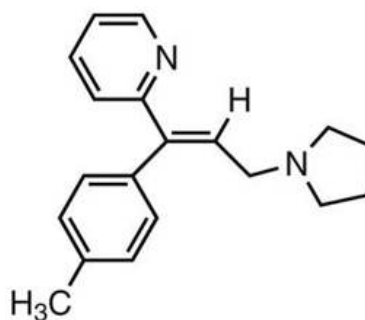
1*R*, 2*S*-(-)-ephedrine



1*R*, 2*R*-(-)-pseudoephedrine



Z-triprolidine (inactive)



E-triprolidine (active)



FIGURE 2.22 Examples of stereoisomers.

The physicochemical properties of a drug molecule are dependent not only on what functional groups are present in the molecule but also on the spatial arrangement of these groups. This becomes an especially important factor when the environment that a molecule is in is asymmetric, such as the human body. Proteins and other biologic targets are asymmetric in nature. How a particular drug molecule interacts with these macromolecules is determined by the three-dimensional orientation of the functional groups present. If critical functional groups in the drug molecule do not occupy the proper spatial region, then productive interactions with the biologic target will not be possible. As a result, it is possible that the desired pharmacologic activity will not be achieved. If, however, the functional groups within a drug molecule are located in the proper three-dimensional orientation, then the drug can participate in multiple key interactions with its biologic target. It is important to understand not only which functional groups contribute to the pharmacologic activity of a drug, but also the importance of the three-dimensional nature of these functional groups in predicting drug potency and potential side effects.

Approximately one in every four drugs currently on the market is some type of isomeric mixture. For many of these drugs, the biologic activity may reside in one isomer (or at least predominate in one isomer). The majority of these isomeric mixtures are termed “racemic mixtures” (or “racemates”). A racemic mixture is comprised of equal amounts of both possible drug enantiomers. As mentioned earlier in this chapter, when enantiomers are introduced into an asymmetric, or chiral, environment, such as the human body, they display different physicochemical properties. This can lead to significant differences in their pharmacokinetic and pharmacodynamic behavior, resulting in adverse side effects or

toxicity. For example, individual isomers in a racemic mixture can exhibit significant differences in absorption (especially active transport), serum protein binding, and metabolism. As it relates to drug metabolism, it is certainly possible that only one of the isomers can be converted into a toxic substance or can influence the metabolism of another drug ([Chapter 3](#)). Since stereochemistry can have a profound effect on both the pharmacokinetic and pharmacodynamic properties of a drug, it is important to review the foundational concepts.

Stereochemical Definitions

Designation of Absolute Configuration

At first, enantiomers were distinguished by their ability to rotate the plane of polarized light. Isomers that rotate the plane of polarized light to the right, or in a clockwise direction, were designated as dextrorotatory, indicated by a (+)- sign before the chemical name (e.g., (+)-amphetamine or dextroamphetamine). The opposite designation, levorotatory or (-)-, was assigned to molecules that rotate the plane of polarized light to the left, or in a counterclockwise direction. The letters *d*- and *l*- are also used to indicate (+)- and (-)-, respectively. A racemate (racemic mixture)—that is, a 1:1 mixture of enantiomers—is indicated by placement of a (±)- before the compound name. This nomenclature is based on a physical property of the molecule and does not describe the absolute configuration or three-dimensional arrangement of atoms around the chiral center.

Questions We Can Now Answer About Any Drug Molecule

Based on your knowledge of acid-base chemistry, from where will this drug primarily be absorbed?

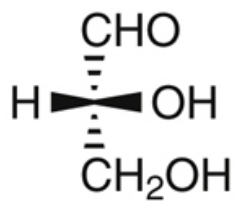
What is the solubility of the drug in the stomach, plasma, or an aqueous IV?

What are the possible interactions that the drug could have with its respective target for drug action?

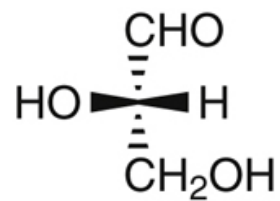
What is the compatibility of the drug if mixed with other drugs?

How should this drug be delivered? Is it stable in stomach acid?

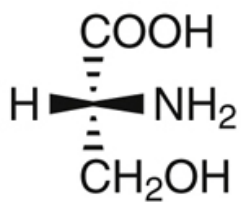
In the late 19th century, Fisher and Rosanoff developed a system of nomenclature based on the structure of glyceraldehyde ([Fig. 2.23](#)). Since there were no methods at that time to determine the absolute three-dimensional arrangement of atoms in space, the two isomers of glyceraldehyde were arbitrarily assigned the designation of d-(+)- and l-(-). It was not until the 1950s that the absolute configurations of these molecules were determined (Fisher had fortuitously guessed correctly). The configurations of other molecules were then assigned based on their relationship to d- or l-glyceraldehyde via synthetic methods or chemical degradation. Thus, via chemical degradation, it was possible to determine that (+)-glucose, (-)-2-deoxyribose, and (-)-fructose had the same terminal configuration as d-(+)-glyceraldehyde and, therefore, were assigned the d-absolute configuration. Amino acid configurations were assigned based on their relationship to d-(+)- and l-(-)-serine ([Fig. 2.23](#)). Unfortunately, this system becomes very cumbersome with molecules that contain more than one chiral center.



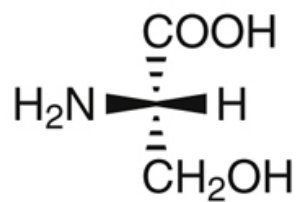
D-(+)-Glyceraldehyde



L-(−)-Glyceraldehyde



D-(+)-Serine



L-(−)-Serine

FIGURE 2.23 Relationship of optical isomers of serine to d- and l-glyceraldehyde.

Learning the Lingo: Drug Molecule Evaluation

Analysis of individual functional groups:

Name of functional group

Shape of functional group

Hydrophobic vs. hydrophilic character

Polar vs. nonpolar character

Acidic vs. basic (pK_a) character

Binding interactions

Chemical/enzymatic stability

Analysis of the whole drug molecule:

Looking for functional group balance: water solubility and absorption

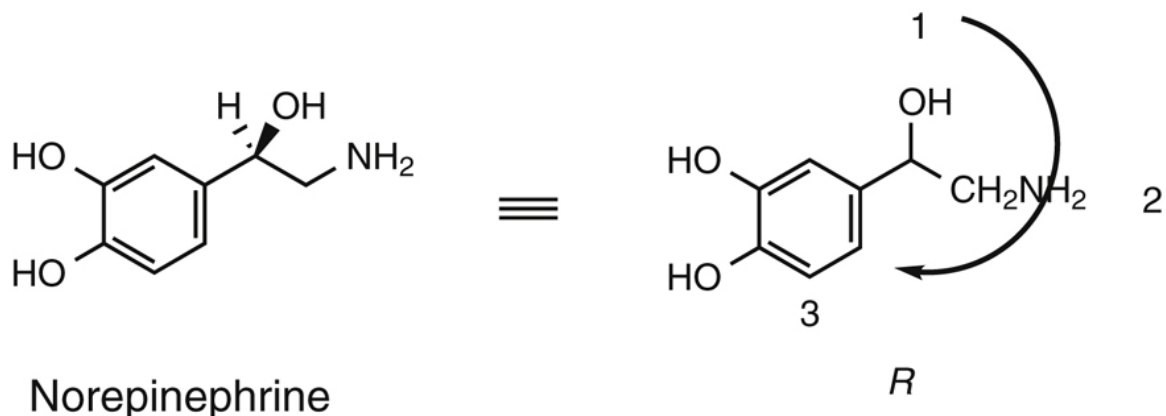
Ionization issues: effect on solubility and absorption

Drug combinations: acid-base interactions

Drug interactions with biologic target: good fit or not?

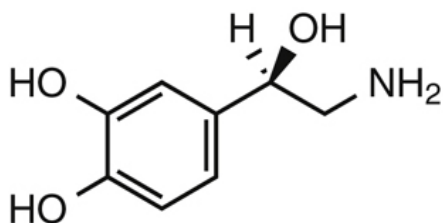
Stability and bioavailability: route of administration

In 1956, a new system of stereochemical nomenclature was introduced by Cahn, Ingold, and Prelog²⁰ and is known as the Sequence Rule or CIP system. With this system, atoms attached to a chiral center are ranked based on their atomic number. Highest priority is given to the atom with the highest atomic number, and subsequent atoms are ranked accordingly, from highest to lowest. When a decision cannot be made in the assignment of priority—for example, two atoms with the same atomic number attached to the chiral center—this evaluation extends to the next atom until a priority can be established. When the molecule is then viewed from the side opposite to the lowest-priority atom, the priority sequence from highest to lowest can then be determined. If the priority sequence proceeds to the right, or in a clockwise direction, the chiral center is designated with an *R*-absolute configuration. The designation is *S* when the priority sequence proceeds to the left, or in a counterclockwise direction. An example of this is seen in the neurotransmitter norepinephrine.

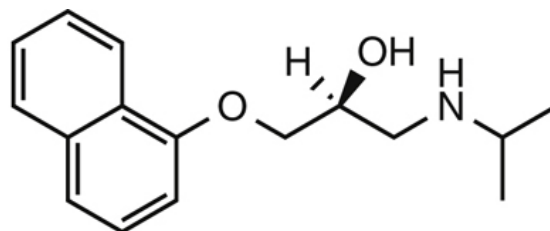


Degradation studies demonstrate that (–)-norepinephrine is related to α-(–)-mandelic acid; therefore, it was given the α-designation using the Fisher system. With the CIP system, norepinephrine is assigned the *R*-absolute configuration.

It should be noted that the CIP nomenclature system uses a set of arbitrary rules and, therefore, should be viewed as a system that tracks absolute configuration only. In many instances, two molecules can have different absolute configurations as designated by the CIP system, but the same relative orientation of the functional groups relevant for biologic activity. An example of this is demonstrated when the absolute configuration of the nonselective β-adrenergic antagonist propranolol is compared to norepinephrine. Because of the presence of the ether oxygen atom, the priority sequence of the functional groups about the chiral center results in the assignment of the *S*-absolute configuration for the more active enantiomer of propranolol. Close inspection of both *R*-norepinephrine and *S*-propranolol, however, shows that the hydroxy group, basic amine, and aromatic rings of both compounds occupy the same regions in three-dimensional space.



(*R*)-Norepinephrine



(*S*)-Propranolol

Stereochemistry and Biologic Activity

Easson-Stedman Hypothesis

In 1886, Piutti²¹ reported different physiologic actions for the enantiomers of asparagine, with (+)-asparagine having a sweet taste and (–)-asparagine a bland one. This was one of the earliest observations that enantiomers can exhibit differences in biologic action. In 1933, Easson and Stedman²² reasoned that differences in biologic activity between enantiomers resulted from selective reactivity of one enantiomer with its receptor. They postulated that such interactions require a minimum of a three-point fit to the receptor. This is demonstrated in Figure 2.24 for two hypothetical enantiomers. In Figure 2.24, the letters A, B, and C represent hypothetical functional groups that can interact with complementary sites on the hypothetical receptor surface, represented by A', B', and C'. Only one enantiomer is capable of attaining the correct special orientation to enable all three functional groups to interact with their respective sites on the receptor surface. The inability of the other enantiomer to achieve the same number of interactions with the hypothetical receptor surface explains its reduced biologic activity. The Easson-Stedman hypothesis states that the more potent enantiomer must be involved in a minimum of three intermolecular interactions with the surface of the biologic target and that the less potent enantiomer only interacts with two sites. This can be illustrated by looking at the differences in vasopressor activity of *R*-(–)-epinephrine, *S*-(+)-epinephrine, and the achiral *N*-methyldopamine (Fig. 2.25). With *R*-(–)-epinephrine, the three points of interaction with the receptor site are the substituted aromatic ring, β-hydroxyl group, and the

protonated secondary ammonium group. All three functional groups interact with their complementary sites on the receptor surface, resulting in receptor stimulation (in this case). With *S*-(+)-epinephrine, only two interactions are possible (the protonated secondary ammonium and the substituted aromatic ring). The β -hydroxyl group is located in the wrong place in space and, therefore, cannot interact properly with the receptor. *N*-methyldopamine can achieve the same interactions with the receptor as *S*-(+)-epinephrine; therefore, it is not surprising that its vasopressor response is the same as that of *S*-(+)-epinephrine and less than that of *R*-(-)-epinephrine.

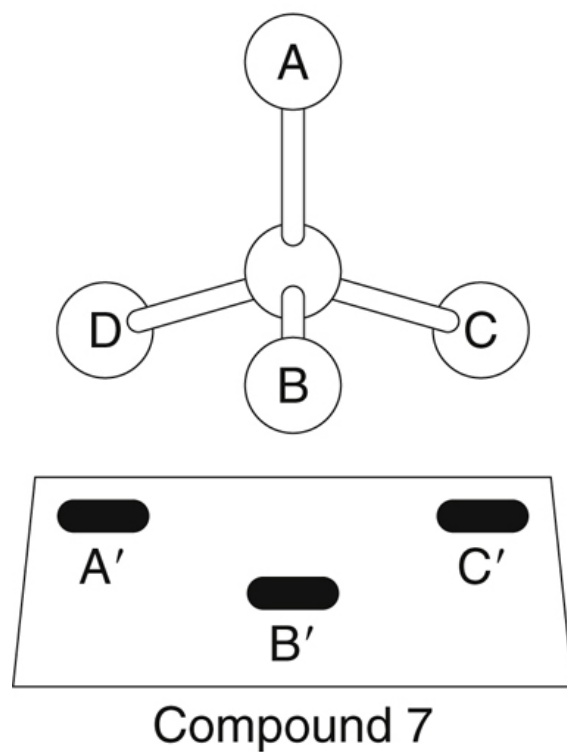
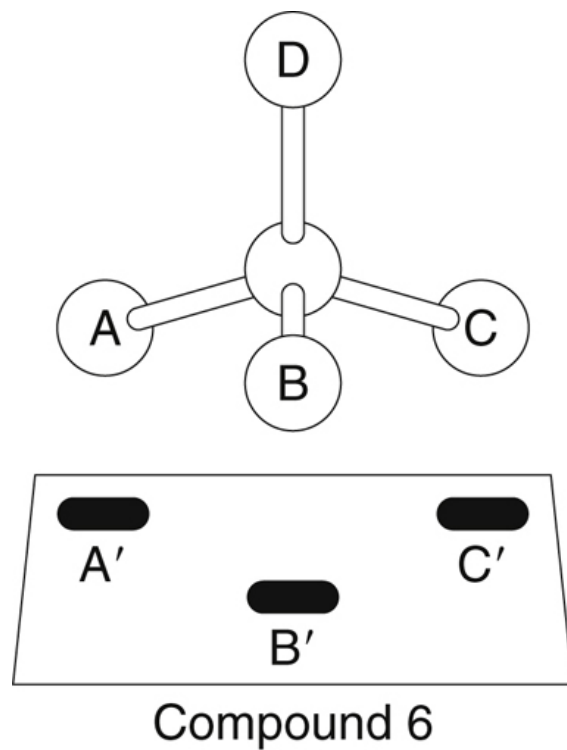
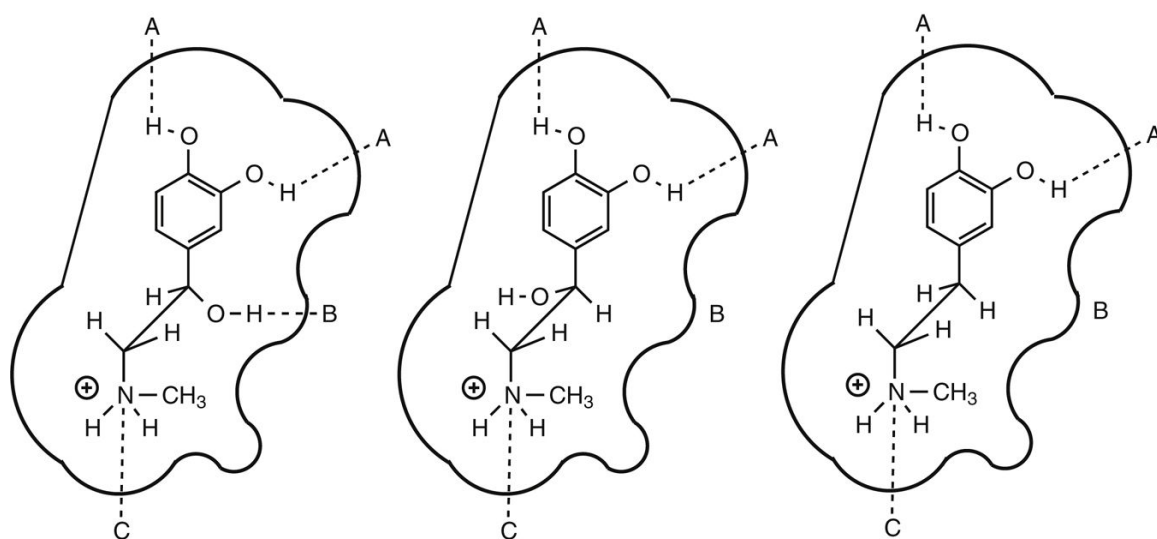


FIGURE 2.24 Optical isomers. Only in compound 6 do the functional groups A, B, and C align with the corresponding sites of binding on the asymmetric surface.



(*R*)-(-)-Epinephrine

(*S*)-(+)-Epinephrine

N-Methyldopamine

FIGURE 2.25 Drug receptor interaction of *R*-(-)-epinephrine, *S*-(+)-epinephrine, and *N*-methyldopamine.

Not all stereoselectivity seen with enantiomers can be attributed to differences in the ability of the drug molecule to interact with its biologic target. Differences in biologic activity can also result from differences in the ability of each enantiomer to reach the biologic target. Because the biologic system encountered by the drug is asymmetric, each enantiomer can experience selective penetration into membranes, metabolism, absorption at sites of loss (e.g., adipose tissue), and/or excretion. [Figure 2.26](#) shows various phases that enantiomers can encounter before reaching the biologic target. An enantiomer may not encounter stereoselective environments at each of these points; however, enantioselectivity at any point can provide enough of an influence to cause one enantiomer to produce a significantly better pharmacologic effect than the other. Conversely, such processes can also contribute to untoward effects of a particular enantiomer. Differences in pharmacologic action among stereoisomers provide an excellent example of how not all pharmacologic effects of a drug are necessarily beneficial to the patient. Although there is no regulatory prohibition on the development of racemic agents, it is reasonable that single enantiomer drugs will become the overwhelming therapeutic choice in the future.

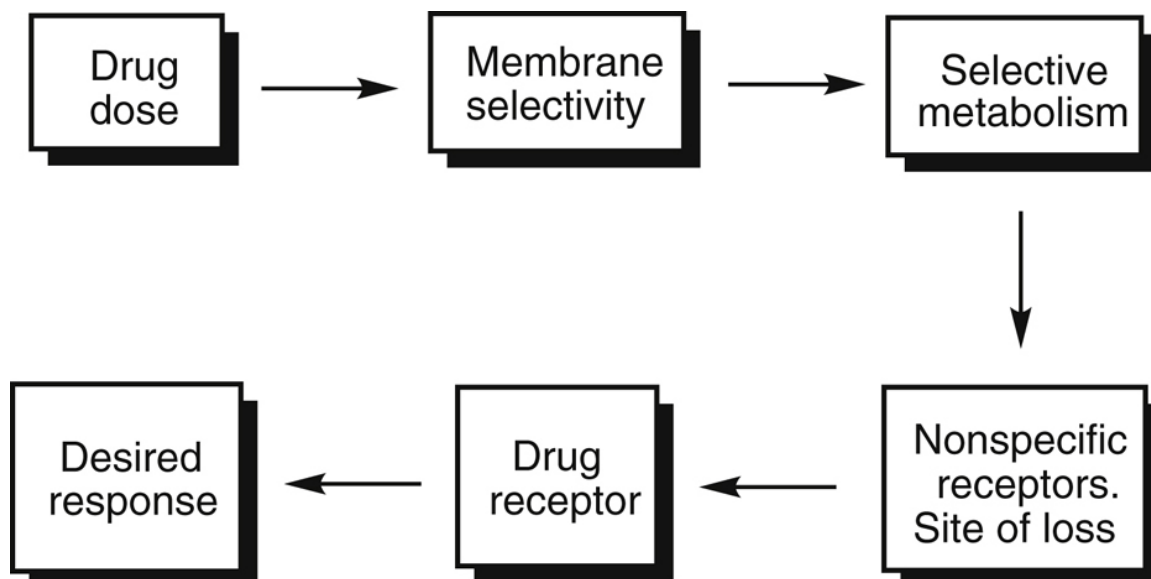


FIGURE 2.26 Selective phases to which optical isomers can be subjected before biologic response.

Diastereomers

As mentioned earlier, diastereoisomers are molecules that are nonsuperimposable, nonmirror images. This type of isomer can result from the presence of more than one chiral center in the molecule, double bonds, or ring systems. These isomers have different physicochemical properties, and as a result, it is possible that they can have differences in biologic activity.

Molecules that contain more than one chiral center probably are the most common type of drug-based diastereoisomers. Classic examples are the diastereoisomers ephedrine and pseudoephedrine (Fig. 2.27). When a molecule contains two chiral centers, there can be as many as four possible stereoisomers consisting of two sets of enantiomeric pairs. When considering an enantiomeric pair of molecules, there is inversion of all chiral centers. In diastereomers, there is inversion of one or more, but not all chiral centers. Figure 2.28 shows several examples that contain two or more chiral centers and, therefore, are diastereoisomeric.

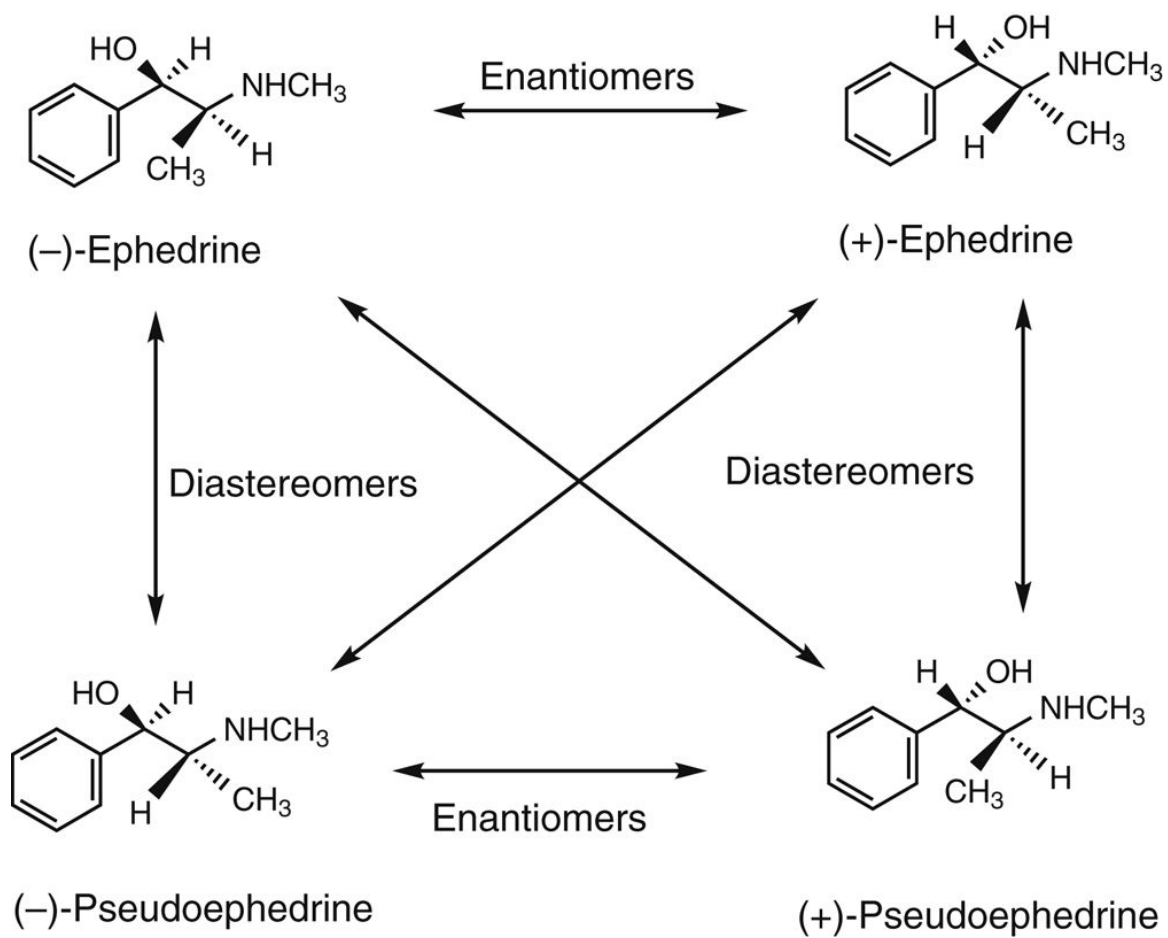
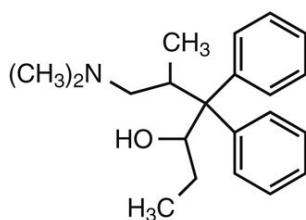
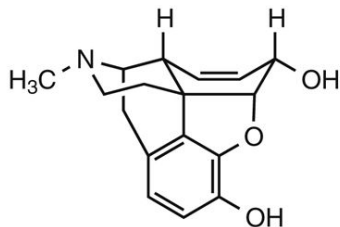


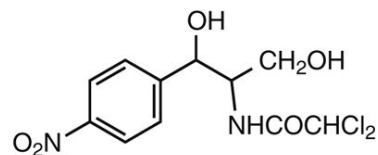
FIGURE 2.27 Relationship between the diastereomers of ephedrine and pseudoephedrine.



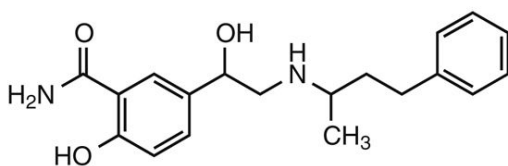
Isomethadol



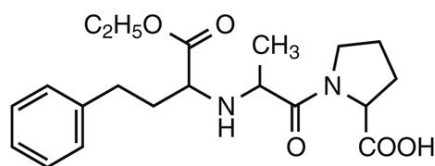
Morphine



Chloramphenicol



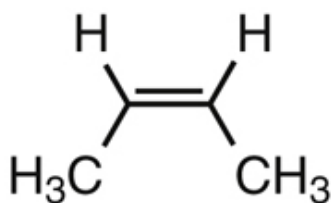
Labetalol



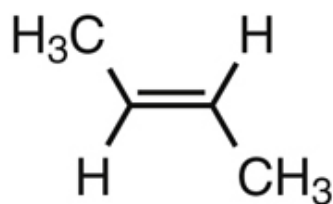
Enalapril

FIGURE 2.28 Examples of chiral drugs with two or more asymmetric centers.

Restricted bond rotation caused by carbon-carbon double bonds (alkenes or olefins) and similar systems, such as imines ($C = N$), can produce stereoisomers. These are also referred to as geometric isomers, although they more properly are classified as diastereoisomers. In this situation, substituents can be oriented on the same side or on opposite sides of the double bond. The alkene 2-butene is a simple example.



cis or *Z* isomer



trans or *E* isomer

With 2-butene, it is readily apparent that the methyl groups can be on the same or on opposite sides of the double bond. When they are on the same side, the molecule is defined as the *cis*- or *Z*-isomer (from the German

zusammen, meaning “together”); when they are on opposite sides, the designation is *trans*- or *E*- (from the German *entgegen*, meaning “opposite”). With simple compounds, such as 2-butene, it is easy to determine which groups in the molecule are *cis* or *trans* to one another. This becomes more difficult to determine, however, with more complex structures, where it is less obvious which substituents should be referred to when naming the compound. In 1968, Blackwood et al.²³ proposed a system for the assignment of “absolute” configuration with respect to double bonds. Using the CIP sequence rules, each of the two substituents attached to the carbon atoms comprising the double bond is assigned a priority of 1 or 2, depending on the atomic number of the atom attached to the double bond. When two substituents of higher priority are on the same side of the double bond, this isomer is given the designation of *cis* or *Z*. When the substituents are on opposite sides, the designation is *trans* or *E*. The histamine H₁-receptor antagonist triprolidine (Fig. 2.29) is a good example for demonstrating how this nomenclature system works. The *E*-isomer of triprolidine is more active both in vitro and in vivo, indicating that the distance between the pyridine and pyrrolidine rings is critical for binding to the receptor.

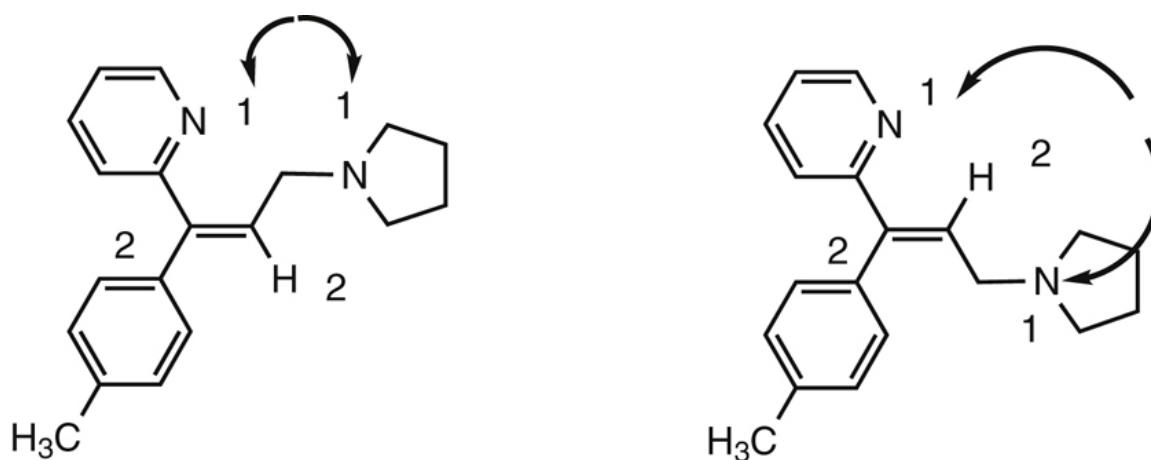


FIGURE 2.29 Geometric isomers of triprolidine.

Diastereoisomers (as well as enantiomers) can also be found in cyclic molecules. For example, the cyclic alkane 1,2-dimethylcyclohexane can exist as *cis/trans*-diastereoisomers, and the *trans* isomer can also exist as an

enantiomeric pair. In [Figure 2.30](#), each of the *trans*-enantiomorphs is depicted in the two possible chair conformations for the cyclohexane ring. Since cyclohexane rings can exhibit significant conformational freedom, this allows for the possibility of conformational isomers. Isomers of this type will be discussed in the next section. When two or more rings share a common bond (e.g., decalin), rotation around the bonds is even more restricted. This prevents ring “flipping” from occurring, and as a result, diastereoisomers and enantiomers are generated.

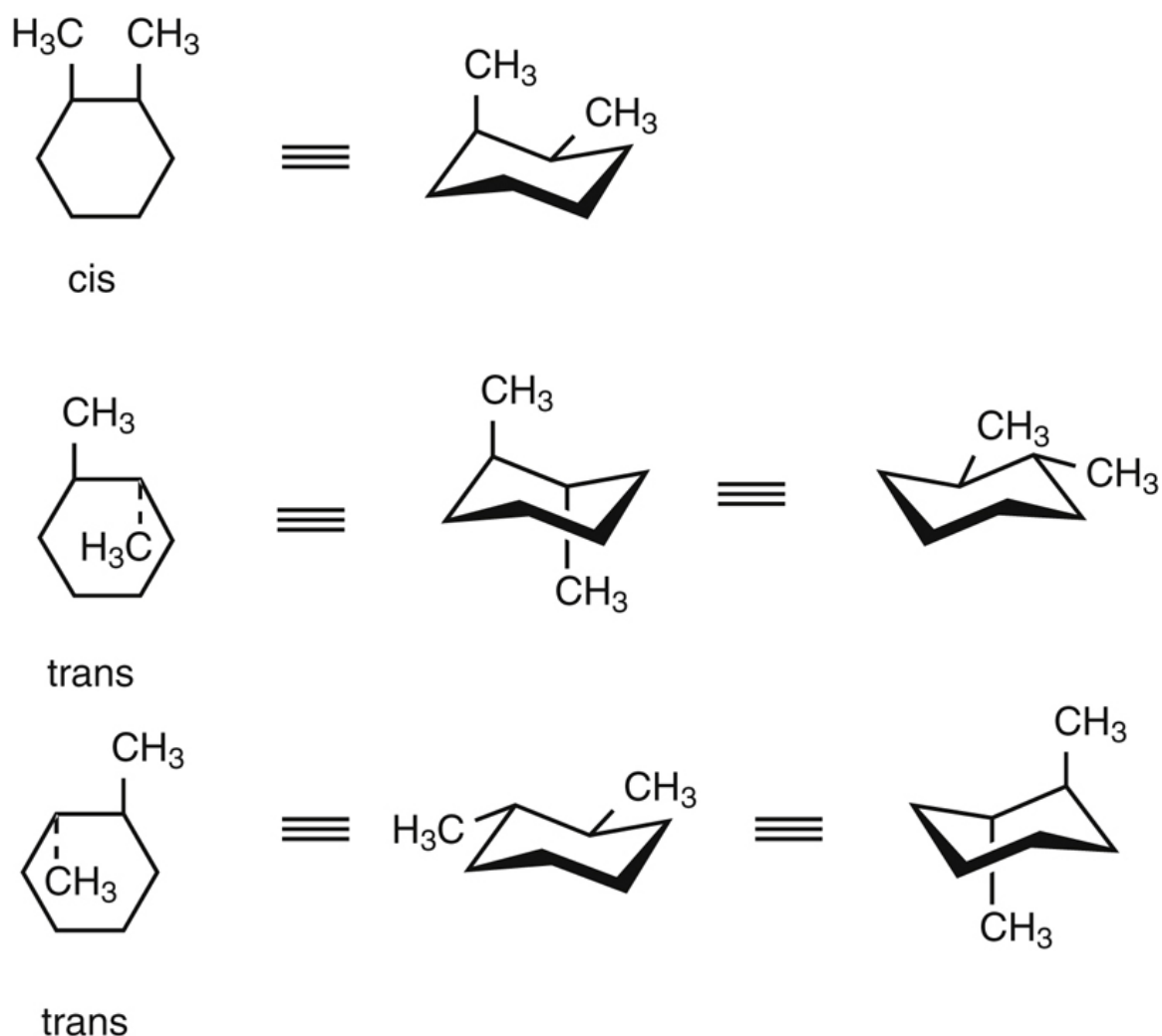
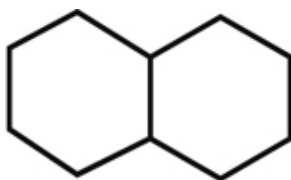
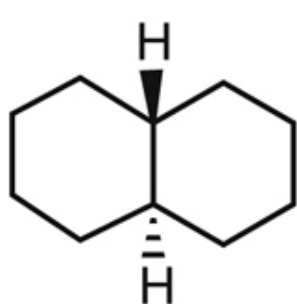


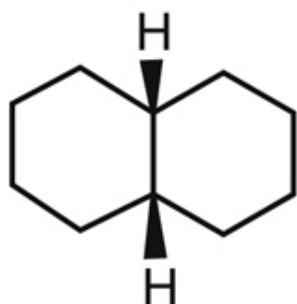
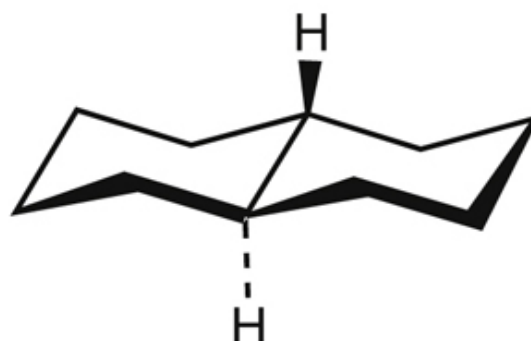
FIGURE 2.30 Diastereomers of 1,2-dimethylcyclohexane.



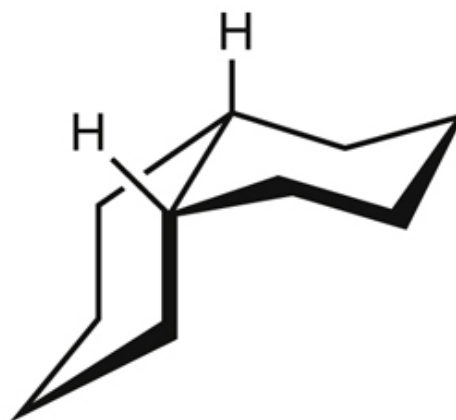
Decalin



trans-decalin



cis-decalin



In the case of decalin, a two-ring system, the rings are fused together via a common bond in either the *trans* or *cis* configuration as shown. Steroids, a class of medically important compounds that consist of four fused rings (three cyclohexanes and one cyclopentane) exhibit significantly different biologic activity when the first two cyclohexane rings are fused into different configurations, referred to as the 5α - or 5β -isomers (Fig. 2.31). The α -designation indicates that the hydrogen atom in the 5-position is

below the “plane” of the ring system; the β -designation refers to the hydrogen atom being above this plane. What appears to be a very minor change in orientation for the substituent results in a very drastic change in the three-dimensional shape of the molecule and in its biologic activity. [Figure 2.31](#) shows the diastereoisomers 5α -cholestane and 5β -cholestane as examples. The chemistry and pharmacology of steroids will be discussed in more detail in later chapters.

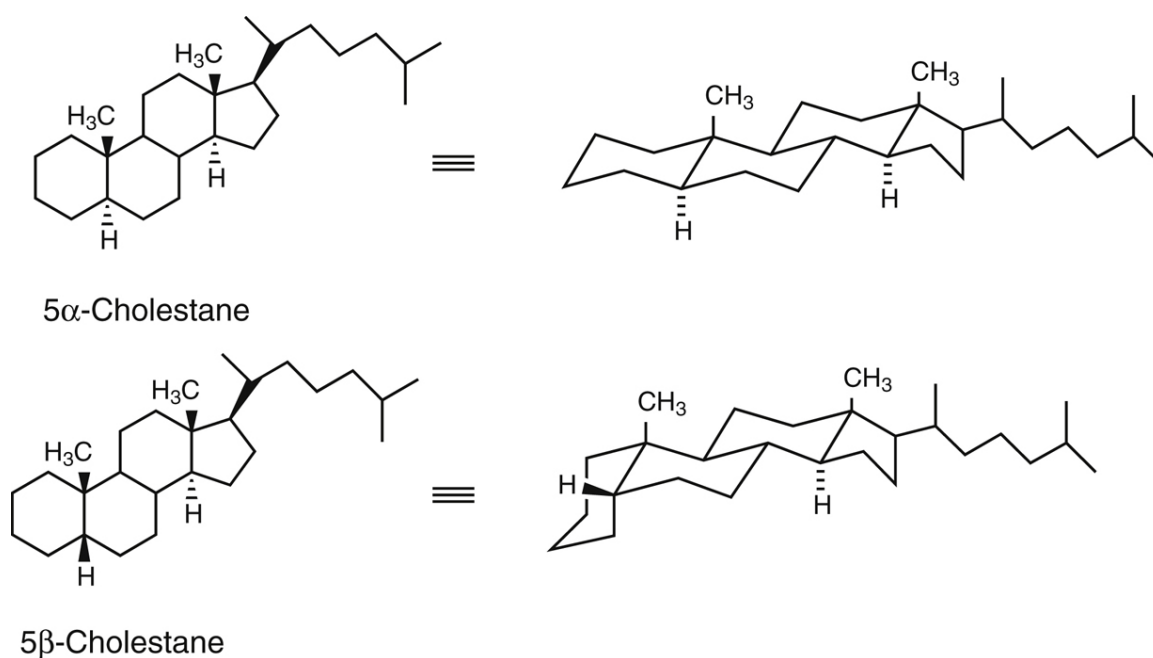
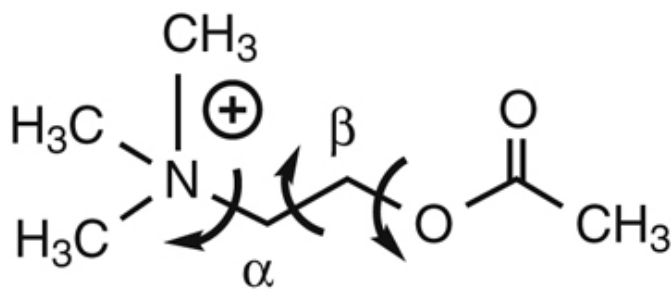


FIGURE 2.31 The 5α and 5β conformations of the steroid nucleus cholestane.

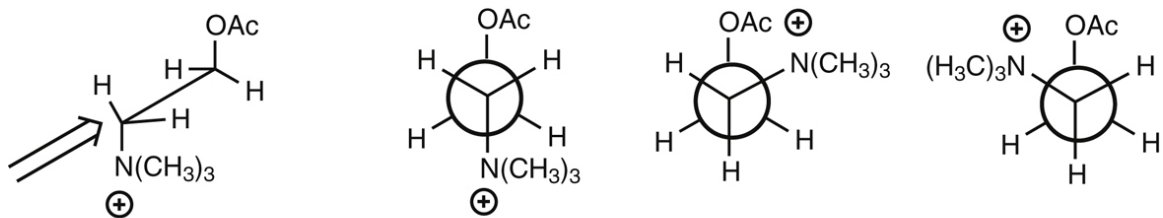
Conformational Isomerism

Conformational isomerism takes place via rotation about one or more single bonds. Such bond rotation results in nonidentical spatial arrangement of atoms in a molecule. This type of isomerism does not require much energy because no bonds are broken. In the conversion of one enantiomer into another (or diastereoisomer), bonds are broken, which requires significantly more energy. The neurotransmitter acetylcholine can be ^[1]used to demonstrate the concept of conformational isomers.



Acetylcholine

Each single bond within the acetylcholine molecule is capable of undergoing rotation, and at room temperature, such rotations readily occur. Rotation around single bonds $\text{C}\alpha\text{-C}\beta$ bond of acetylcholine was shown by Kemp and Pitzer²⁴ in 1936 not to be free but, rather, to have an energy barrier, which is sufficiently low that at room temperature acetylcholine exists in many interconvertible conformations. Rotation around the central $\text{C}\alpha\text{-C}\beta$ bond produces the greatest spatial rearrangement of atoms compared to rotation around any other bond. Since the atoms at the end of some of the bonds within acetylcholine are identical, rotation about several of these bonds produces redundant structures when viewed along the $\text{C}\alpha\text{-C}\beta$ bond and can be depicted in the sawhorse or Newman projections, as shown in [Figure 2.32](#). When the ester and trimethylammonium groups are 180° apart, the molecule is said to be in the anti-, or staggered, conformation (or conformer or rotamer). This conformation allows maximum separation of the functional groups and is the most stable conformation energetically. It is possible that other conformations are more stable if factors other than steric interactions are considered (e.g., intramolecular hydrogen bonds). Rotation of one end of the $\text{C}\alpha\text{-C}\beta$ bond by 120° or 240° results in the two gauche, or skew, conformations shown in [Figure 2.32](#). These are less stable than the anticonformer, although some studies suggest that an electrostatic attraction between the electron-poor trimethylammonium and electron-rich ester oxygen atom stabilizes this conformation. Rotation by 60° , 180° , and 240° produces the least stable conformations in which all of the atoms overlap, what are referred to as eclipsed conformations.



anti- or staggered
conformer

Gauche or skew conformers

FIGURE 2.32 Anti- and gauche conformations of acetylcholine.

Drug Design: Discovery and Structural Modification of Lead Compounds

Process of Drug Discovery

The process of drug discovery begins with the identification of new, previously undiscovered, biologically active compounds, often called “hits,” which are typically found by screening many compounds for the desired biologic properties. We will next explore the various approaches used to identify “hits” and how these are converted into “lead” compounds and, subsequently, into drug candidates suitable for clinical trials. Sources of “hits” can originate from natural sources, such as plants, animals, or fungi; from synthetic chemical libraries, such as those created through combinatorial chemistry or historic chemical compound collections; from chemical and biologic intuition from years of chemical-biologic training; from targeted/rational drug design; or from computational modeling of a target site such as an enzyme. Chemical or functional group modifications are then performed in order to improve the pharmacologic, toxicologic, physiochemical, and pharmacokinetic properties of a “hit” compound to ultimately obtain a “lead” compound. The lead compound to be optimized should be of a known chemical structure and possess a known mechanism of action, including knowledge of its functional groups (pharmacophoric groups) that are recognized by the receptor/active site and are responsible for that molecule’s affinity at the targeted receptor site. “Lead optimization” is the process whereby modifications of the functional groups of the lead compound are carried out in order to improve its recognition, affinity, and binding geometries of the pharmacophoric groups for the targeted site (a receptor or enzyme); its pharmacokinetics; or its reactivity and stability toward metabolic degradation. The final step of the drug discovery process involves rendering the lead compound into a drug candidate that is safe and suitable for use in human clinical trials, including the preparation of a suitable drug formulation.

Natural Product Screening

Perhaps the most difficult aspect of drug discovery is that of lead discovery. Until the late 19th century, the development of new chemical entities for medicinal purposes was achieved primarily through the use of natural products, generally derived from plant sources. As the colonial powers of Europe discovered new lands in the Western Hemisphere and colonized Asia, the Europeans learned from the indigenous peoples of the newly discovered lands of remedies for many ailments derived from plants and animals. Salicylic acid was isolated from the bark of willow trees after learning that Native Americans brewed the bark to treat inflammatory ailments. Structural optimization of this lead compound (salicylic acid) by the Bayer Corporation of Germany resulted in acetylsalicylic acid, or aspirin, the first nonsteroidal anti-inflammatory agent. South American natives used a tea obtained by brewing Cinchona bark to treat chills and fever. Further study in Europe led to the isolation of quinine and quinidine, which subsequently were used to treat malaria and cardiac arrhythmias, respectively. Following “leads” from folklore medicine, chemists of the late 19th and early 20th centuries began to seek new medicinals from plant sources and to assay them for many types of pharmacologic actions. This approach to drug discovery is often referred to as “natural product

screening.” Before the mid-1970s, this was one of the major approaches to obtaining new chemical entities as “leads” for new drugs. Unfortunately, this approach fell out of favor and was replaced with the rational approaches to drug design developed during that period (see the next section). Heightened awareness of the fragility of ecosystems, especially the rainforests, has fueled a resurgence of screening products from plants before they become extinct. A new field of pharmacology, called “ethnopharmacology,” which is the discipline of identifying potential natural product sources with medicinal properties based on native lore, has emerged as a result. Compounds isolated from natural sources are usually tested in one or more bioassays for the ailment(s) that the plant material has been purported to treat. Interestingly, the treatment of different ailments can require different methods of preparation (e.g., brewing, chewing, or direct application to wounds) or different parts of the same plant (e.g., roots, stem, leaves, flowers, or sap). As it turns out, each method of administration or part of the plant used can produce one or more different chemical compounds that are necessary to generate the desired outcome.

Drug Discovery via Random Screening of Synthetic Organic Compounds

The random screening of synthetic organic compounds approach to the discovery of new chemical entities for a particular biologic action began in the 1930s, after the discovery of the sulfonamide class of antibacterials. All compounds available to the investigator (natural products, synthetic molecules), regardless of structure, were tested in the pharmacologic assays available at the time. This random screening approach was also applied in the 1960s and 1970s in an effort to find agents that were effective against cancer. Some groups did not limit their assays to identify a particular type of biologic activity but, rather, tested compounds in a wide variety of assays. This large-scale screening approach of drug “leads” is referred to as high-throughput screening, which involves the simultaneous bioassay of thousands of compounds in hundreds to thousands of bioassays. These types of bioassays became possible with the advent of computer-controlled robotic systems for the assays and combinatorial chemistry techniques for the synthesis of large numbers of compounds in small (milligram) quantities. This type of random screening eventually gave way to targeted dedicated screening and rational design techniques.

Drug Discovery From Targeted Dedicated Screening and Rational Drug Design

Rational drug design is a more focused approach that uses greater knowledge (structural information) about the drug receptor (targets) or one of its natural ligands as a basis to design, identify, or create drug “leads.” Testing is usually done with one or two models (e.g., specific receptor systems or enzymes) based on the therapeutic target. The drug design component often involves molecular modeling and the use of quantitative structure-activity relationships (QSARs) to better define the physicochemical properties and the pharmacophoric groups that are essential for biologic activity. The development of QSARs relies on the ability to examine multiple relationships between physical properties and biologic activities. In classic QSAR (e.g., Hansch-type analysis), an equation defines biologic activity as a linear free-energy relationship between physicochemical and/or structural properties. It permits evaluation of the nature of interaction forces between a drug and its biological target, as well as the ability to predict

activity in molecules. These approaches are better for the development of a lead compound into a drug candidate than for the discovery of a lead compound.

Drug Discovery via Drug Metabolism Studies

New drug entities have been “discovered” as drug leads through investigation of the metabolism of drug molecules that already are clinical candidates or, in some instances, are already on the market. In this method, metabolites of known drug entities are isolated and assayed for biologic activity using either the same target system or broader screen target systems. The broader screening systems are more useful if the metabolite under evaluation is a chemical structure that was radically altered from the parent molecule through some unusual metabolic rearrangement reaction. In most cases, the metabolite is not radically different from the parent molecule and, therefore, would be expected to exhibit similar pharmacologic effects. One advantage of evaluating this type of drug candidate is that a metabolite can possess better pharmacokinetic properties, such as a longer duration of action, better oral absorption, or less toxicity with fewer side effects (e.g., terfenadine and its antihistaminic hydroxylated metabolite, fexofenadine). As it turns out, the sulfonamide antibacterial agents were discovered in this way. The azo dye prontosil was found to have only antibacterial action in vivo. It was soon discovered that this compound required metabolic activation via reduction of the diazo group to produce the active metabolite 4-aminobenzenesulfonamide (Fig. 2.33). The sulfonamide mimics the physicochemical properties of PABA, a crucial component in microbial metabolism. It is no surprise that the sulfonamide acts as a competitive inhibitor of the enzyme for which PABA is a substrate.

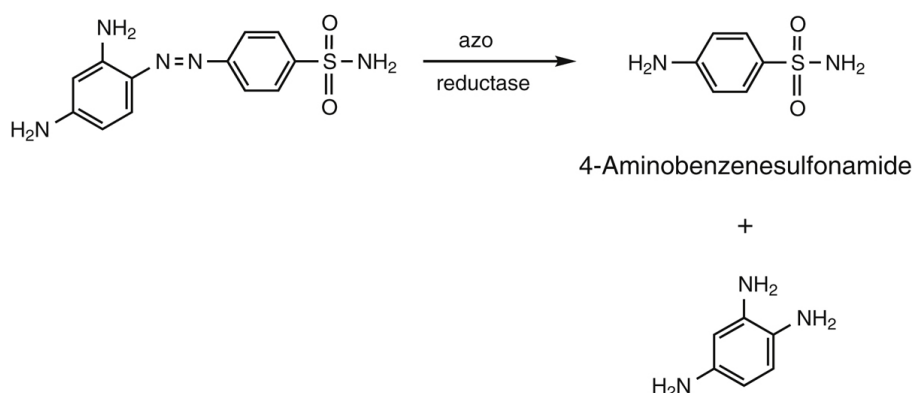


FIGURE 2.33 Metabolic conversion of prontosil to 4-aminobenzenesulfonamide.

Drug Discovery From the Observation of Side Effects

An astute clinician or pharmacologist can detect a side effect in a patient or animal model that could lead, on further development, to a new therapeutic use for a particular chemical entity. Discovery of new lead compounds via exploitation of side-effect profiles of existing agents is discussed below.

One of the more interesting drug development scenarios is that of the phenothiazine antipsychotics (see Chapter 11). Molecules with this type of biologic activity can be traced back

to the first histamine H₁-receptor antagonists developed in the 1930s. In 1937, Bovet and Staub²⁵ were the first to recognize that it should be possible to antagonize the effects of histamine and, thereby, treat allergic reactions. They tested compounds that were known to act on the autonomic nervous system and, eventually, discovered that benzodioxanes (Fig. 2.34) significantly antagonized the effects of histamine. During an attempt to improve the antihistaminergic action of the benzodioxanes, it was discovered that phenyl substituted ethanolamines also demonstrated significant antihistaminergic activity. Further development of this class generated two different classes of antihistamines: the diphenhydramine class of antihistamines represented by diphenhydramine and the ethylenediamine class, represented by tripeleennamine (Fig. 2.34) (see also Chapter 25).

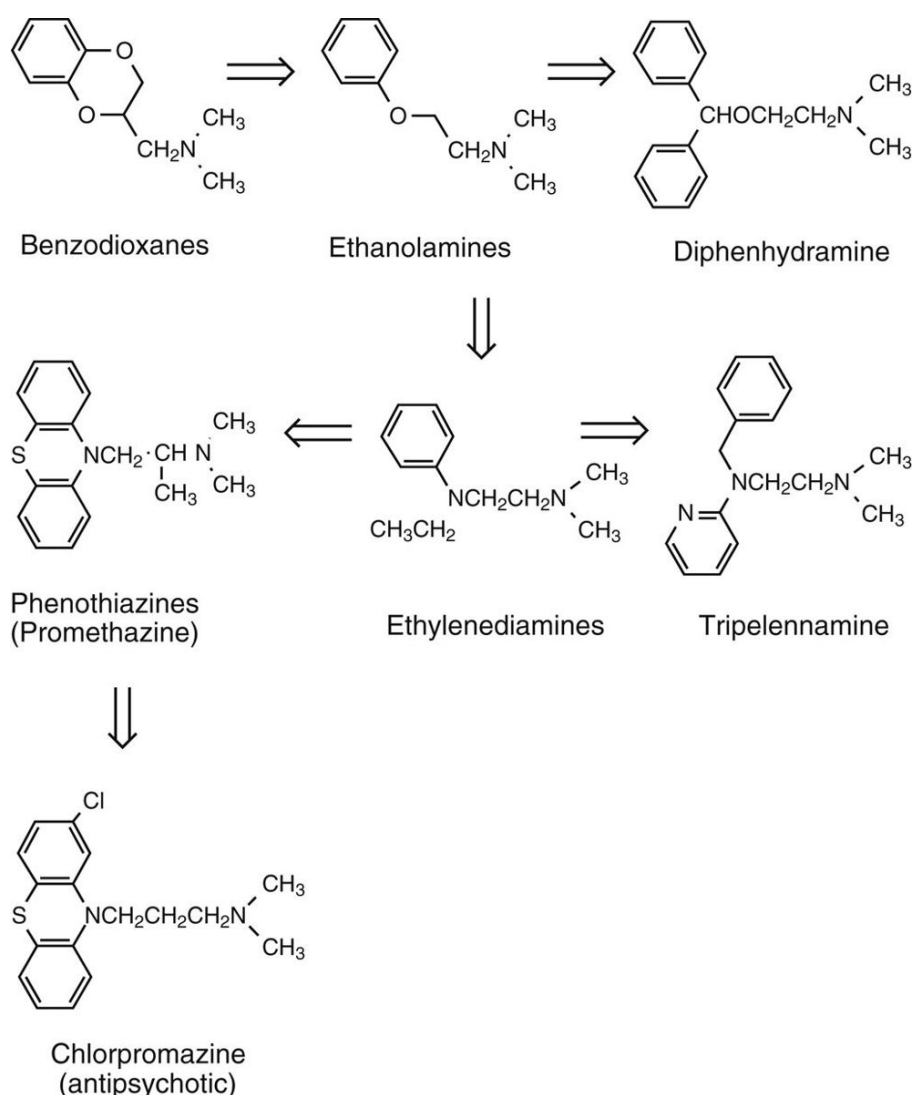


FIGURE 2.34 Development of phenothiazine-type antipsychotic drugs.

Incorporation of the aromatic rings of the ethylenediamines into the rigid and planar tricyclic phenothiazine structure produced molecules (e.g., promethazine) with good antihistaminergic action and relatively strong sedative properties (see also Chapters 11 and 12). At first, these

compounds were found to be useful as antihistamines, but their very strong sedative properties led to their use as potentiating agents for anesthesia.²⁶ Further development to increase the sedative properties of the phenothiazines resulted in the development of chlorpromazine in 1950.²⁷

Chlorpromazine was found to produce a tendency for sleep, but unlike the antihistamine phenothiazines, it also produced a disinterest in patients with regard to their surroundings (i.e., tranquilizing effects). In patients with psychiatric disorders, an ameliorative effect on the psychosis and a relief of anxiety and agitation were noted. These observations suggested that chlorpromazine had potential for the treatment of psychiatric disorders. Thus, what started out as an attempt to improve antihistaminergic activity ultimately resulted in an entirely new class of chemical entities useful in the treatment of an unrelated disorder.²⁸

Another example of how new chemical entities can be derived from biologically unrelated molecules is illustrated by the development of the potassium channel agonist diazoxide (Fig. 2.35). This molecule was developed as a result of the observation that the thiazide diuretics, such as chlorothiazide, not only exhibited diuretic activity, due to inhibition of sodium absorption in the distal convoluted tubule, but also demonstrated a direct effect on the renal vasculature. Structural modification to enhance this direct effect led to the development of diazoxide and related potassium channel agonists for the treatment of hypertension (see Chapter 17).

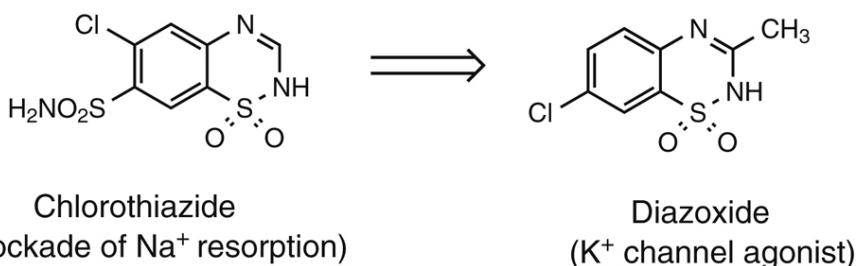


FIGURE 2.35 Structural similarity of chlorothiazide (a diuretic) and diazoxide (an antihypertensive that acts via opening of K⁺ channels).

Refinement of the Lead Structure

Determination of the Pharmacophore

Once a “hit” compound has been discovered for a particular therapeutic use, the next step is to identify the pharmacophoric groups. The pharmacophore of a drug molecule is that portion of the molecule that contains the essential functional group(s) that directly bind with the active site of the biologic target to produce the desired biologic activity. Because drug-target interactions can be very specific (think of a lock [receptor] and key [drug] relationship), the pharmacophore can constitute a small portion of the molecule. In many cases, a very structurally complex molecule can be “stripped down” to a simpler structure with retention of the pharmacophoric groups while maintaining the desired biologic action. An example of this is the opioid analgesic morphine, a tetracyclic compound with five chiral centers. Not only would structure simplification possibly provide molecules with fewer side effects, but a reduction in the number of chiral centers would greatly simplify the synthesis of morphine derivatives. Figure 2.36

shows how the morphine structure has been simplified in the search for molecules with fewer deleterious side effects, such as respiratory depression and addiction potential. Within each class are analogues that are less potent, equipotent, and many times more potent than morphine. As shown in the figure, the pharmacophore of morphine consists of a tertiary alkylamine that is at least four atoms away from an aromatic ring. A more detailed discussion of the chemistry and pharmacology of morphine can be found in [Chapter 14](#).

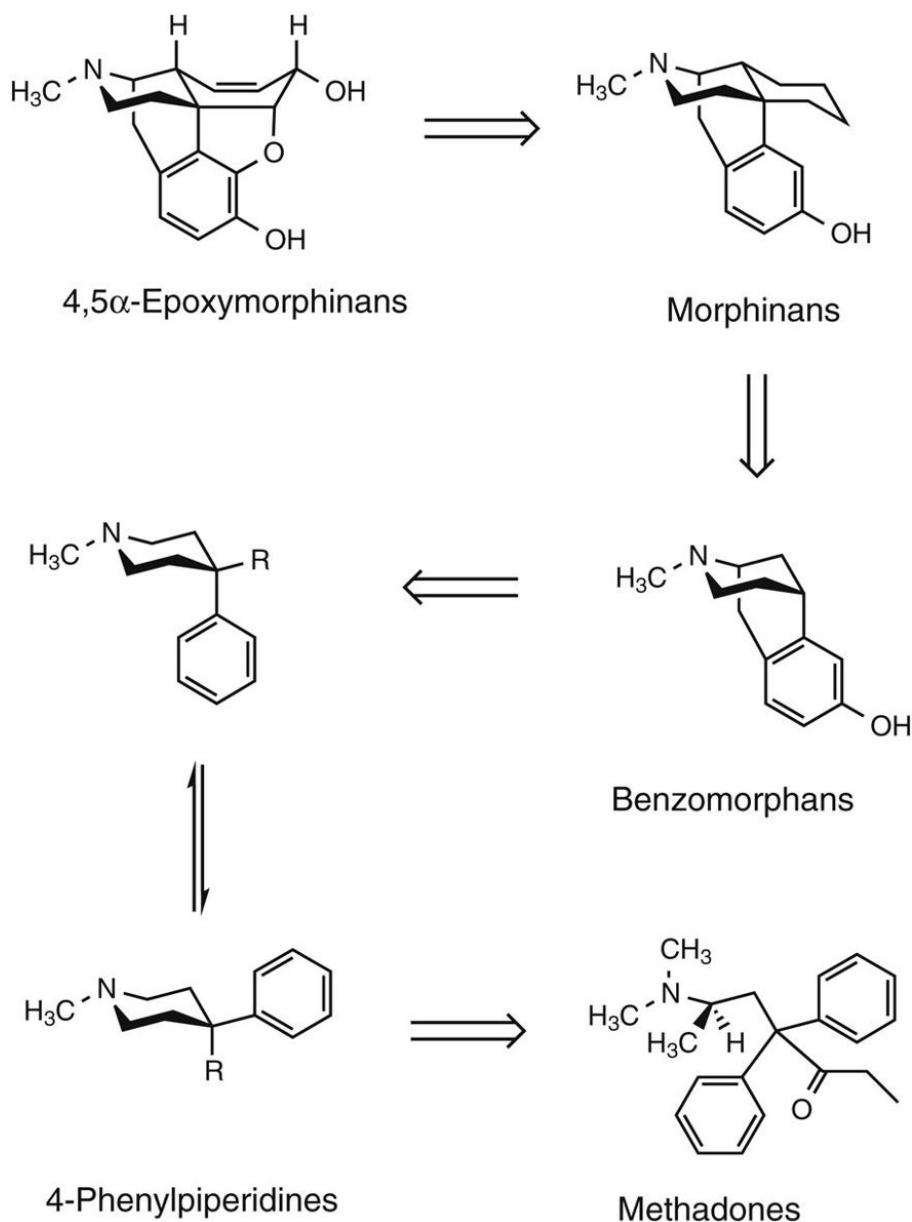


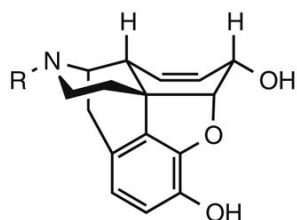
FIGURE 2.36 Morphine pharmacophore and its relationship to analgesic derivatives.

Alterations in Alkyl Chains: Chain Length, Branching, and Rings

An increase or decrease in the length of an alkyl chain (homologation), branching, and alteration of ring size can have a profound effect on the potency and pharmacologic activity of

the molecule. A change in the length of an alkyl chain by one CH_2 unit or branch alters the lipophilic character of the molecule and, therefore, its properties of absorption, distribution, and excretion. If the alkyl chain is directly involved in an interaction with the biologic target, then this type of alteration can influence the quality of those interactions. Molecules that are conformationally flexible can become less flexible if branching is introduced at a key position of an alkyl chain or the alkyl chain is incorporated into a ring equivalent. Changes in conformation can alter the spatial relationship between the pharmacophoric (functional) groups in the molecule and thereby influence interactions with the biologic target. Small structural changes are important to consider in the design of structural analogues.

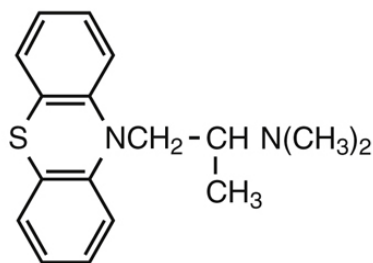
An example that demonstrates how an increase in hydrocarbon chain length has significant effects not only on potency but also on drug action (agonist vs. antagonist) is provided by a series of *N*-alkyl morphine analogues (Fig. 2.37). In this series, homologation of $\text{R} = \text{CH}_3$ (morphine) to $\text{R} = \text{CH}_2\text{CH}_2\text{CH}_3$ (*N*-propylnormorphine) produces a pronounced decrease in agonist activity and an increase in antagonist activity. When further homologated by one methylene unit $\text{R} = \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ (*N*-butylnormorphine), the resulting analogue is totally devoid of agonist or antagonist activity (i.e., the compound is inactive). Additional increases in chain length ($\text{R} = \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ and $\text{R} = \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$) produce compounds with increasing potency as antagonists. When R is β -phenylethyl, the compound is a full agonist, with a potency approximately 14-fold that of morphine.^{29,30}



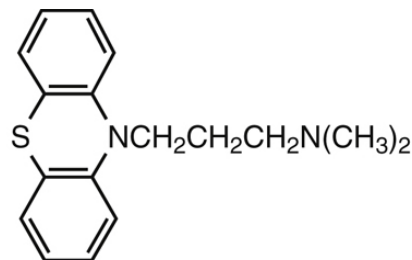
R	Pharmacological activity
$-\text{CH}_3$	Analgesic (morphine)
$-\text{CH}_2\text{CH}_3$	Opioid agonist activity decreased
$-\text{CH}_2\text{CH}_2\text{CH}_3$	Opioid antagonist activity increased
$-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$	Inactive as opioid agonist or antagonist
$-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$	} Opioid antagonist activity increased
$-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$	
$-\text{CH}_2\text{CH}_2$ - 	14X potency of morphine

FIGURE 2.37 Effect of alkyl chain length on bioactivity of morphine.

Branching of alkyl chains can also produce drastic changes in potency and pharmacologic activity. If the mechanism of action is closely related to the lipophilicity of the molecule, then hydrocarbon chain branching will result in a less lipophilic compound and a significant alteration in biologic effect. This decrease in lipophilicity is a result of the alkyl chain becoming more compact and causes less disruption of the hydrogen-bonding network of water. If the hydrocarbon chain is directly involved in interactions with its biologic target, then branching can produce major changes in pharmacologic activity. For example, consider the phenothiazines promethazine and promazine.



Promethazine



Promazine

The primary pharmacologic activity of promethazine is that of an antihistamine, whereas promazine is an antipsychotic. The only difference between the two molecules is the alkylamine side chain. In the case of promethazine, there is an isopropylamine side chain, whereas promazine contains an *n*-propylamine. In this case, simple modification of one carbon atom from a branched to a linear hydrocarbon radically alters the pharmacologic activity.

Positional isomers of aromatic ring substituents can also possess different pharmacologic properties. Substituents on aromatic rings can alter the electron distribution throughout the ring, which, in turn, can influence how the ring interacts with the biologic target. Aromatic ring substituents can also influence the conformation of the flexible portion of a molecule, especially if the substituents are located ortho to the same carbon attached to the flexible side chain. Ring substituents influence the conformations of adjacent substituents via steric interactions and can significantly alter interactions with the biologic target.

Functional Group Modification: Bioisosterism

Bioisosterism

When a lead compound is first discovered, it often lacks the required potency and pharmacokinetic properties suitable for making it a viable clinical candidate. These can include undesirable side effects, physicochemical properties, other factors that affect oral bioavailability, and adverse metabolic or excretion properties. These undesirable properties are often the result of the presence of specific pharmacophoric (functional) groups in the molecule. Successful modification of the compound to reduce or eliminate these undesirable features without losing the desired biologic activity is the goal. Replacement or modification of specific pharmacophoric (functional) groups with other groups having similar properties is known as “isosteric replacement” or “bioisosteric replacement.”

In 1919, Langmuir^{31,32} first developed the concept of chemical isosterism to describe the similarities in physical properties among atoms, functional groups, radicals, and molecules. The similarities among atoms described by Langmuir resulted primarily from the fact that these atoms contained the same number of valence electrons and came from the same columns within the periodic table. This concept of isosterism was limited to elements in adjacent rows and columns, inorganic molecules, ions, and small organic molecules, such as diazomethane and ketene. Table 2.7 shows a comparison of the physical properties of N₂O and CO₂ to illustrate Langmuir's concept.

Table 2.7
Comparison of Physical Properties of N₂O and CO₂

Property	N ₂ O	CO ₂
Viscosity at 20°C	148 × 10 ⁶	148 × 10 ⁶
Density of liquid at 10°C	0.856	0.858
Refractive index of liquid, n_D^{20} D line 16°C	1.193	1.190
Dielectric constant of liquid at 0°C	1.593	1.582
Solubility in alcohol at 15°C	3.250	3.130

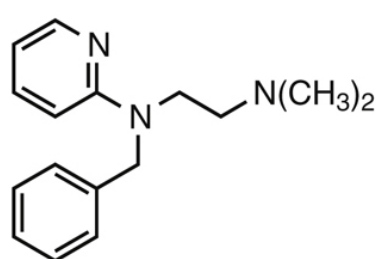
To account for similarities between functional groups with the same number of valence electrons but different numbers of atoms, Grimm³³ developed his hydride displacement law. This is not a "law" in the strict sense but, rather, more an illustration of similar physical properties among closely related functional groups. Table 2.8 presents an example of Grimm's hydride displacement. Descending diagonally from left to right in the table, hydrogen atoms are progressively added to maintain the same number of valence electrons for each group of atoms within a column (thus the term "hydride"). Within each column, the groups are considered to be "pseudoatoms" with respect to each another. Thus, NH₂ is considered to be isosteric to OH, and so on. This early view of isosterism did not consider the actual location, motion, and resonance of electrons within the orbitals of these functional group replacements. Careful observation of this table reveals that some groups do share similar physicochemical properties, but others have very different properties, despite having the same number of valence electrons. For example, OH and NH₂ share similar hydrogen-bonding properties and, therefore, should be interchangeable if that is the only important criterion. The NH₂ group is basic, whereas the OH is neutral. Hence, at physiologic pH, the NH₂ group exists in its protonated or conjugate acid form and the molecule becomes positively charged. If OH is being replaced by NH₂, the additional positive charge could have a significant effect on the overall physicochemical properties of the molecule in which it is being introduced. The difference in physicochemical properties of the CH₃ group relative to the OH and NH₂ groups is even greater. In addition to acid-base character, this "law" fails to take into account other important physicochemical parameters, such as electronegativity, polarizability, bond angles, size, shape of molecular orbitals, electron density, and partition coefficients, all of which contribute significantly to the overall physicochemical properties of a molecule.

TABLE 2.8
Grimm's Hydride Displacement "Law"

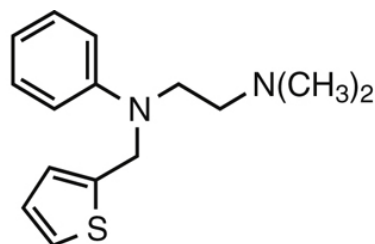
C	N	O	F	Ne

C	N	O	F	Ne
	CH	NH	OH	FH
		CH ₂	NH ₂	OH ₂
			CH ₃	NH ₃

Instead of considering only partial structures, Hinsberg³⁴ applied the concept of isosterism to entire molecules. He developed the concept of “ring equivalents”—that is, functional groups that can be exchanged for one another in aromatic ring systems without drastic changes in physicochemical properties relative to the parent structure. Benzene, thiophene, and pyridine illustrate this concept (Fig. 2.38). A $-\text{CH}=\text{CH}-$ group in benzene is replaced by the divalent sulfur, $-\text{S}-$, in thiophene, and a $-\text{CH}=\text{N}-$ is replaced by the trivalent $-\text{N}=\text{C}-$ to give pyridine. The physical properties of benzene and thiophene are very similar. For example, the boiling point of benzene is 81.1°C, and that of thiophene is 84.4°C (at 760 mm Hg). Pyridine, however, deviates, with a boiling point of 115–116°C. Hinsberg therefore concluded that divalent sulfur ($-\text{S}-$ or thioether) must resemble $-\text{C}=\text{C}-$ in shape, and these groups were considered to be isosteric. Note that hydrogen atoms are ignored in this comparison. Today, this isosteric relationship is seen in many drugs e.g., H₁-receptor antagonists (Fig. 2.38).



Tripelennamine



Methaphenilene

FIGURE 2.38 Isosteric substitution of thiophene for benzene and benzene for pyridine.

It is difficult to relate biologic properties to physicochemical properties of individual atoms, functional groups, or entire molecules, because many physicochemical parameters are involved simultaneously and, therefore, are difficult to quantitate. Simple relationships as described earlier often do not hold up across the many types of biologic systems seen with medicinal agents. That is, what can work as an isosteric replacement in one biologic system cannot work in another. Because of this, it was necessary to introduce the term “bioisosterism” to describe functional groups related in structure that have similar biologic effects. Friedman³⁵ introduced the term bioisosterism and defined it as follows: “Bioisosteres are (functional) groups or molecules that have chemical and physical similarities producing broadly similar biological properties.” Burger³⁶ expanded this definition to take into account biochemical views of biological activity: “Bioisosteres are compounds or groups that possess near equal molecular shapes and volumes, approximately the same distribution of electrons, and which exhibit similar physical properties such as hydrophobicity. Bioisosteric compounds affect the same biochemically associated systems as agonist or antagonists and thereby produce biological properties that are related to each other.”

Classical and Nonclassical Bioisosteres

Bioisosteric groups can be subdivided into two categories: classical and nonclassical bioisosteres. Functional groups that satisfy the original conditions of Langmuir and Grimm are referred to as classical bioisosteres. Nonclassical bioisosteres do not obey steric and electronic definitions of classical bioisosteres and do not necessarily have the same number of atoms as the functional group that they replace. A wider set of compounds and functional groups are encompassed by nonclassical bioisosteres that produce, at the molecular level, qualitatively similar agonist or antagonist action. In animals, many hormones and neurotransmitters with very similar structures and biologic actions can be classified as bioisosteres. An example is the insulins isolated from various mammalian species. Even though these insulins can differ by several amino acid residues, they still produce the same biologic effects. (If this did not occur, the use of insulin to treat diabetes would have had to wait another 60 years for recombinant DNA technology to allow production of human insulin.)

What can be a successful bioisosteric replacement for a given molecule that interacts with a particular biologic target quite has often no effect or abolishes biologic activity in another. Thus, the use of bioisosteric replacement (classical or nonclassical) in the design of new chemical entities (drug discovery) is highly dependent on the biologic system under investigation. No hard-and-fast rules exist to determine which bioisosteric replacement is going to work with a given molecule, although as the following tables and examples demonstrate, some generalizations are possible. Each category of bioisostere can be further subdivided as shown below, and examples are provided in [Tables 2.9](#) and [2.10](#):

1. Classical bioisosteres
 - a. Monovalent atoms and groups
 - b. Divalent atoms and groups
 - c. Trivalent atoms and groups
 - d. Tetrasubstituted atoms
 - e. Ring equivalents
2. Nonclassical bioisosteres
 - a. Exchangeable groups
 - b. Rings versus noncyclic structure

TABLE 2.9
Classical Bioisosteres (Groups Within the Row Can Replace Each Other)

Monovalent Bioisosteres F, H OH, NH F, OH, NH, or CH ₃ for H SH, OH Cl, Br, CF ₃
Divalent bioisosteres $\text{—C=S, —C=O, —C=NH, —C=C—}$
Trivalent atoms or groups:

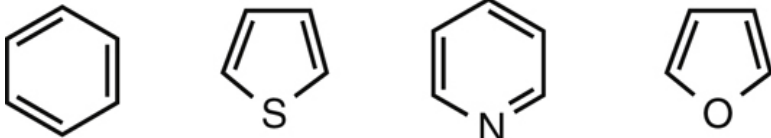
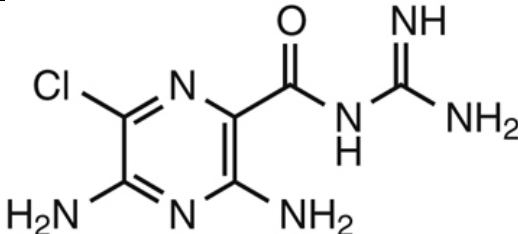
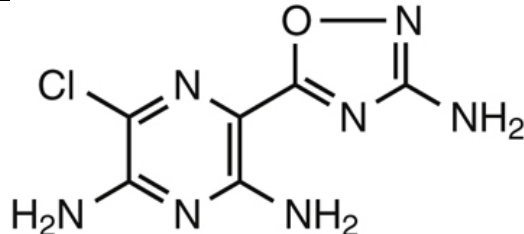
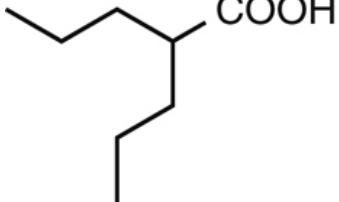
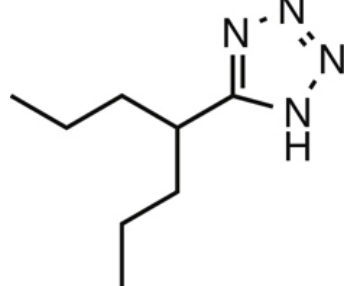
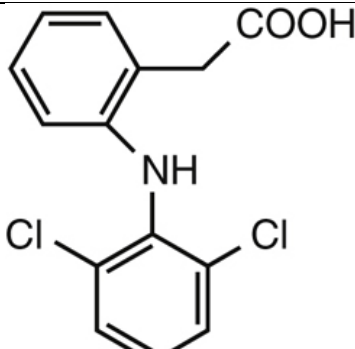
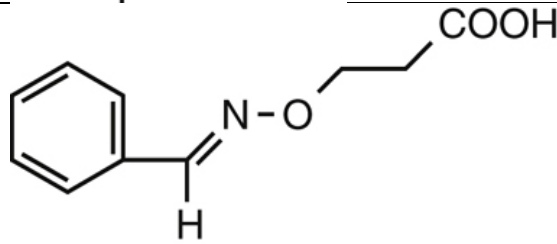
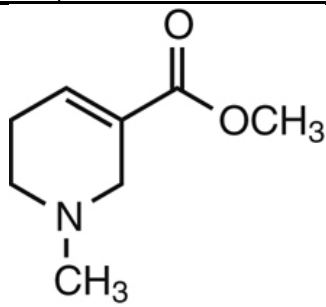
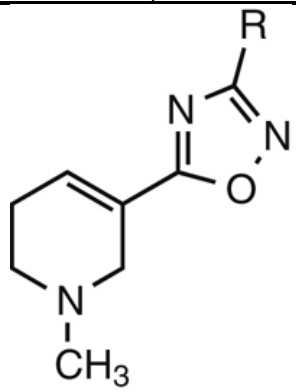
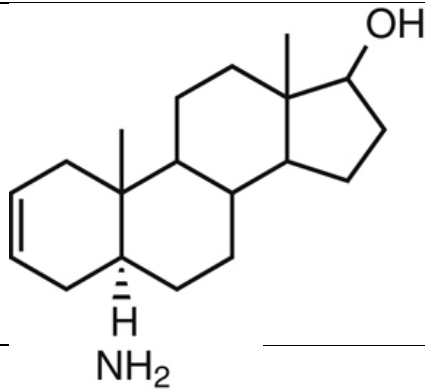
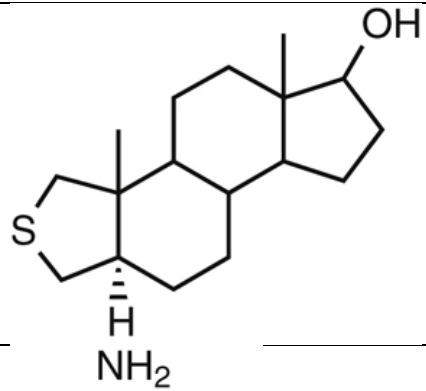
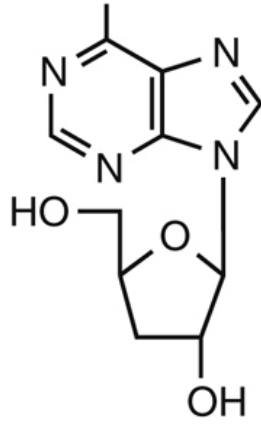
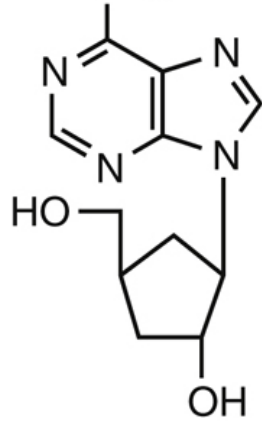
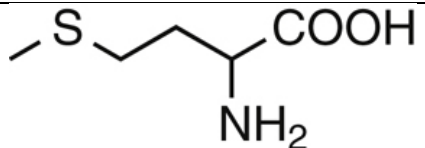
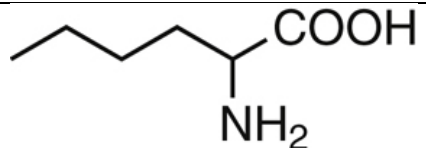
$\text{—}\overset{\text{H}}{\underset{\text{H}}{\text{C}}}=\text{ , } \text{—}\text{N}=\text{}$
$\text{—}\text{P}=\text{ , } \text{—}\text{As}=\text{}$
Tetrasubstituted atoms:
$\text{—}\overset{\oplus}{\underset{\text{H}}{\text{N}}}\text{—} \quad \text{—}\overset{\oplus}{\text{C}}\text{—} \quad \text{—}\overset{\oplus}{\text{P}}\text{—} \quad \text{—}\overset{\oplus}{\text{As}}\text{—}$
Ring equivalents:


TABLE 2.10
Nonclassical Bioisosteric Replacements

Compounds	Bioisosteric Replacement	References
		40
		41
		42

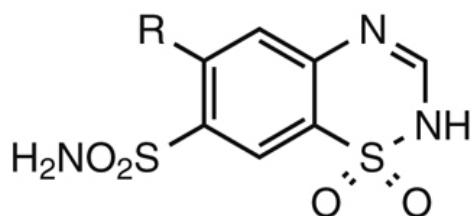
Compounds	Bioisosteric Replacement	References
		43
		44
		45
		46

Classical Bioisosteres

Substitution of hydrogen with fluorine is a common monovalent isosteric replacement. Sterically, hydrogen and fluorine are quite similar, with their van der Waals radii measuring 1.2 and 1.35 Å, respectively. Because fluorine is the most electronegative element in the periodic table, any differences in biologic activity resulting from replacement of hydrogen with fluorine can be attributed to this property.

A classic example of hydrogen replacement by fluorine is development of the antineoplastic agent 5-fluorouracil from uracil. Another example is shown in [Figure 2.39](#), in which the chlorine

of chlorothiazide has been replaced with bromine or a trifluoromethyl group. For each of the substitutions, the electronic (σ , where σ^+ is electron withdrawing and σ^- is electron donating) and hydrophobic (π) properties of each group are maintained relatively constant, but the size of each group varies significantly, as indicated by the Taft steric parameter (E_s).



R =	Cl	Br	CF ₃
σ	+0.23	+0.23	+0.54
π	+0.71	+0.86	+0.88
E_s	-0.97	-1.16	-2.40

FIGURE 2.39 Isosteric replacement of chlorine in thiazide diuretics. Comparison of physicochemical properties of the substituents.

Figure 2.40 shows an example of classical isosteric substitution of an amino group for a hydroxyl group in folic acid. The amino group is capable of mimicking the tautomeric forms of folic acid and providing the appropriate hydrogen bonds to the enzyme active site.

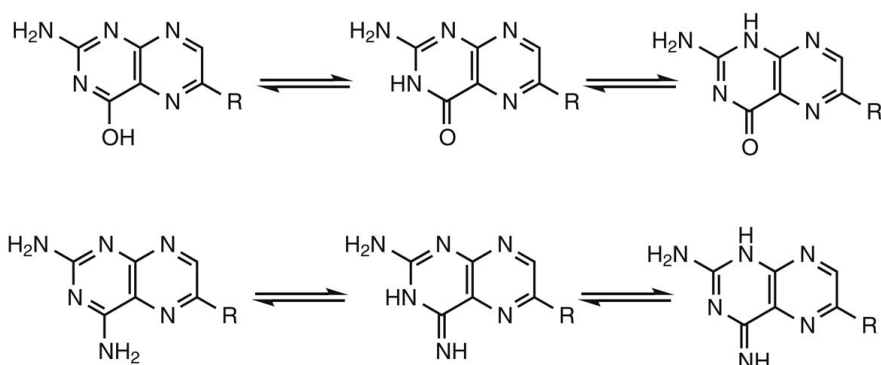
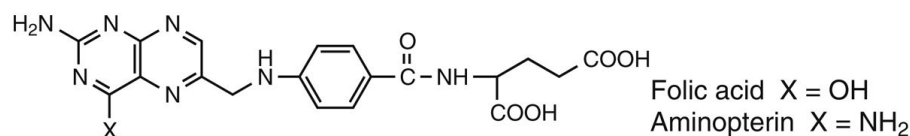
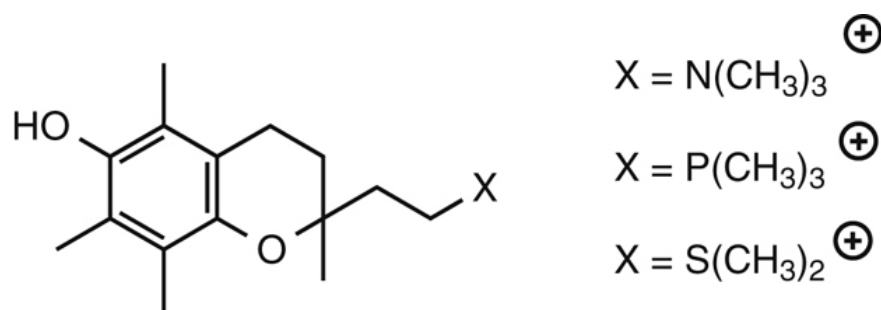


FIGURE 2.40 Isosteric replacement of OH by NH₂ in folic acid and possible tautomers of folic acid and aminopterin.

A tetravalent bioisosteric replacement study was conducted by Grisar et al.³⁷ with a series of α -tocopherol analogues (Fig. 2.41). α -Tocopherol has been shown to scavenge lipoperoxyl and superoxide radicals that accumulate in heart tissue. This is thought to be part of its mechanism of action to reduce cardiac damage resulting from myocardial infarction. All of the bioisosteric analogues were found to produce similar biologic activity.



α -Tocopherol X = C₁₄H₂₉

FIGURE 2.41 Tetravalent bioisosteres of α -tocopherol.

Nonclassical Bioisosteres

As mentioned earlier, nonclassical bioisosteres are replacements of functional groups not defined by classical definitions. Some of these groups, however, mimic spatial arrangements, electronic properties, or some other physicochemical property of the molecule or functional group critical for biologic activity. One example is the use of a double bond to position essential functional groups into a particular spatial configuration critical for activity. This is shown in

Figure 2.42 with the naturally occurring hormone estradiol and the synthetic analogue diethylstilbestrol. The *trans* isomer of diethylstilbestrol has approximately the same potency as estradiol, whereas the *cis* isomer is only one-fourteenth as active. In the *trans* configuration, the phenolic hydroxy groups mimic the correct orientation of the phenol and alcohol in estradiol.^{38,39} This is not possible with the *cis* isomer, and more flexible analogues (Fig. 2.42) have little or no activity.^{40,41}

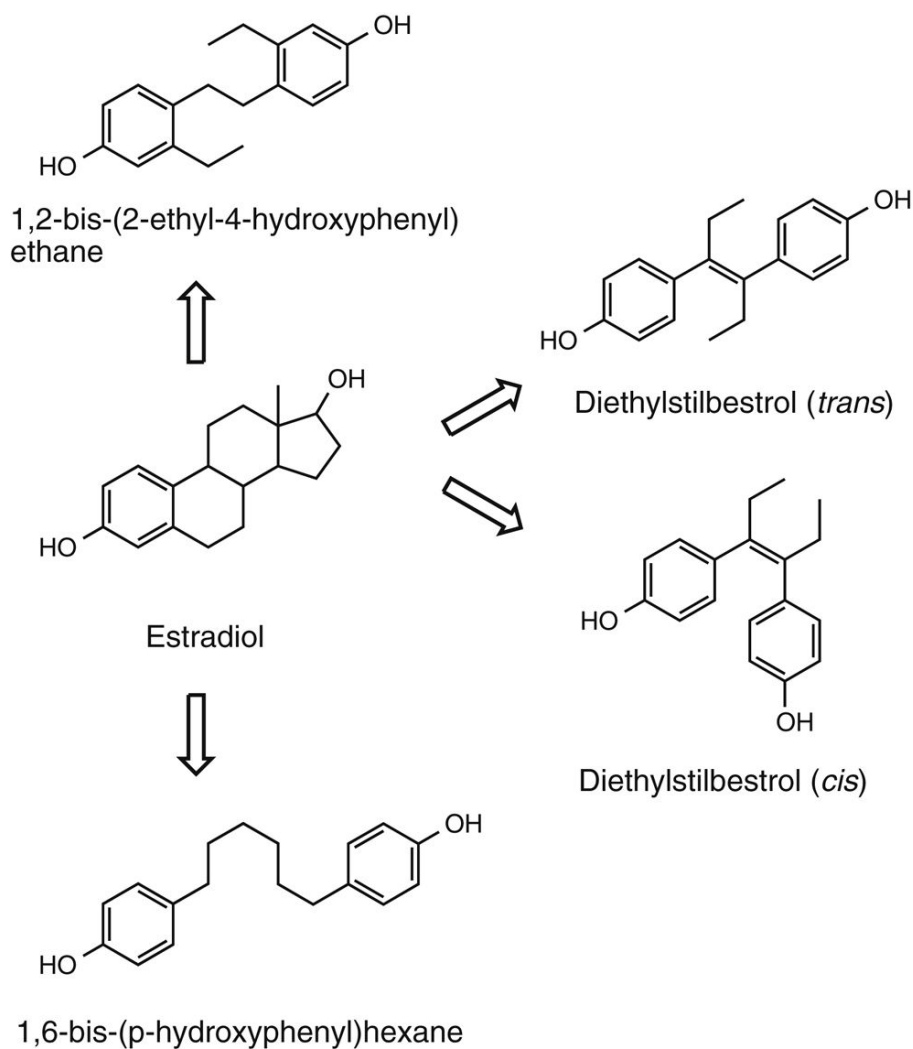


FIGURE 2.42 Noncyclic analogues of estradiol.

Another example of a nonclassical replacement is that of a sulfonamide group for a phenol in catecholamines (Fig. 2.43). With this example, steric factors appear to have less influence on receptor binding than acidity and hydrogen-bonding potential of the functional group on the aromatic ring. Both the phenolic hydroxyl of isoproterenol and the acidic proton of the arylsulfonamide have nearly the same pK_a (~ 10).⁴² Both groups are weakly acidic and capable of losing a proton and interacting with the biologic target as anions (Fig. 2.43). Because the replacement is not susceptible to metabolism by catechol O-methyltransferase, it has also the added advantage of increasing the duration of action and making the compound orally active.

Other examples of successful bioisosteric replacements are shown in Table 2.10, and a more detailed description of the role of bioisosterism can be found in the review by Patani and LaVoie.⁴³

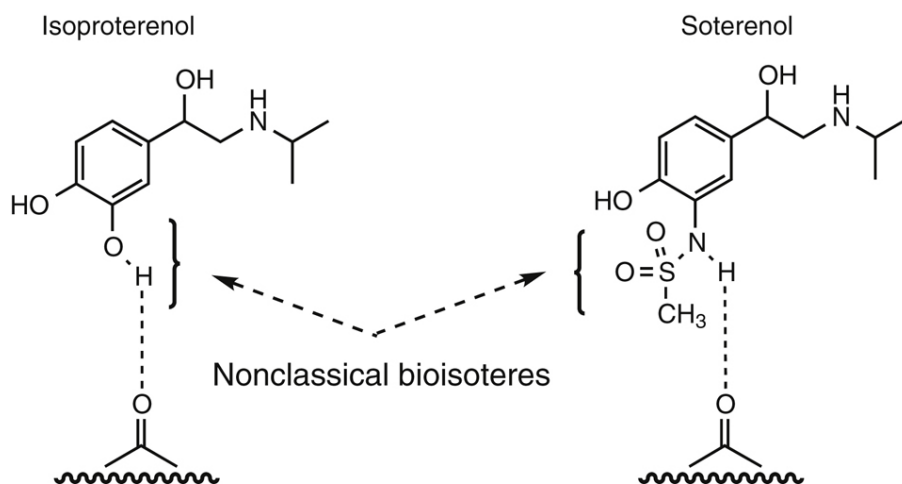


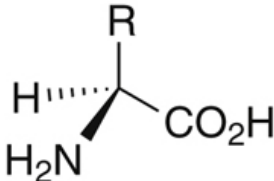
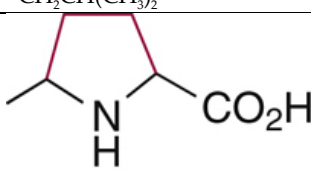
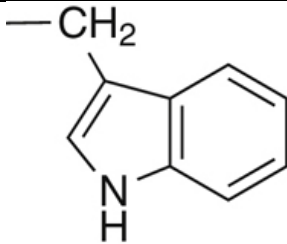
FIGURE 2.43 Bioisosteric replacement of *m*-OH of isoproterenol with a sulfonamido group and similar hydrogen-bonding capacity to a possible drug receptor.

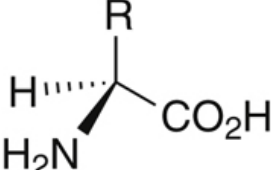
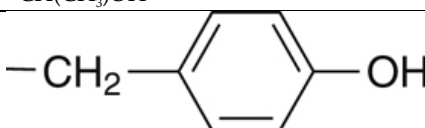
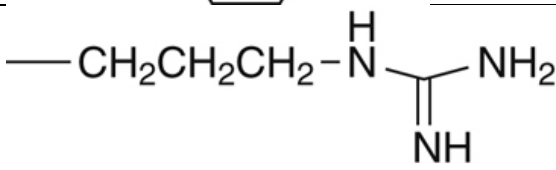
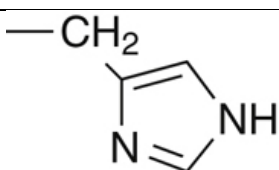
Peptide and Protein Drugs

Not all drugs are small molecules as described thus far. Some very important therapeutic agents are peptidic in nature (e.g., insulin, calcitonin) and, due to their physical chemical properties, generally cannot be delivered orally and must be administered parenterally. Peptides and proteins are very similar in that they are made up of units, or residues, of amino acids that are linked by amide bonds, also referred to as peptide bonds. There is no definitive number of amino acid residues that delineates a peptide from a protein. However, the term peptide refers generally to molecules that contain 15-50 amino acids. Molecules composed of more than 50 residues are generally referred to as proteins.

There are 20 naturally occurring amino acids that serve as the building blocks for both peptide and protein drugs (Table 2.11). Each amino acid contains a common functional group “backbone” that includes a basic amine attached to the α -carbon of an acidic carboxylic acid. The α -carbon for each amino acid is substituted with a unique side chain. The amino acid side chains contribute significantly to the physical chemical properties of the peptide that is formed from a unique sequence of amino acids.

TABLE 2.11
The Twenty Natural Occurring Amino Acids

Name	General Structure			
		Three-Letter Code	Single-Letter Code	Structure R =
Glycine	Gly	G	-H	None
Alanine	Ala	A	-CH ₃	None
Valine	Val	V	-CH(CH ₃) ₂	None
Isoleucine	Ile	I	-CH(CH ₃)CH ₂ CH ₃	None
Leucine	Leu	L	-CH ₂ CH(CH ₃) ₂	None
Proline	Pro	P		(Acidic) ~4
Phenylalanine	Phe	F	-CH ₂ -C ₆ H ₅	None
Tryptophan	Trp	W		None

Name			General Structure	
	Three-Letter Code	Single-Letter Code	Structure R =	pK _a of Side Chain
Methionine	Met	M	$-\text{CH}_2\text{CH}_2\text{SCH}_3$	None
Cysteine	Cys	C	$-\text{CH}_2\text{SH}$	(Acidic) ~8
Serine	Ser	S	$-\text{CH}_2\text{OH}$	None
Threonine	Thr	T	$-\text{CH}(\text{CH}_3)\text{OH}$	None
Tyrosine	Tyr	Y		(Acidic) ~10
Arginine	Arg	R		(Basic) ~12.5
Lysine	Lys	K	$-\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$	(Basic) ~10.5
Histidine	His	H		(Basic) ~6
Asparagine	Asn	N	$-\text{CH}_2\text{CONH}_2$	None
Glutamine	Gln	Q	$-\text{CH}_2\text{CH}_2\text{CONH}_2$	None
Aspartic acid	Asp	D	$-\text{CH}_2\text{COOH}$	(Acidic) ~3.8
Glutamic acid	Glu	E	$-\text{CH}_2\text{CH}_2\text{COOH}$	(Acidic) ~4

As previously mentioned, the amino acid residues are linked by amide bonds, as shown in [Figure 2.44](#). Each carboxylic acid forms an amide bond with the amine group of the next amino acid in the sequence. As with other amide bonds, conjugation between the lone pair of electrons on the nitrogen atom and the adjacent carbonyl group results in the amide bond having partial double-bond character due to a resonance structure as shown in [Figure 2.44](#). This property has two major consequences: (1) the amide bond is therefore co-planar; and (2) there is restricted rotation around the C-N bond ([Fig. 2.44](#)). Because of this restricted rotation, there are two conformations possible, *cis* and *trans* ([Fig 2.45](#)). The *trans*-conformation is lower in energy due to fewer steric interactions (similar to that found with a carbon-carbon double bond) and is favored. When proline is one of the amino acid residues, the *cis*-conformation can be favored as a result of the amine group being part of a

pyrrolidine ring. For this reason, the presence of proline in peptides and proteins is associated with a “kink” or bend in the overall conformation of the peptide chain.

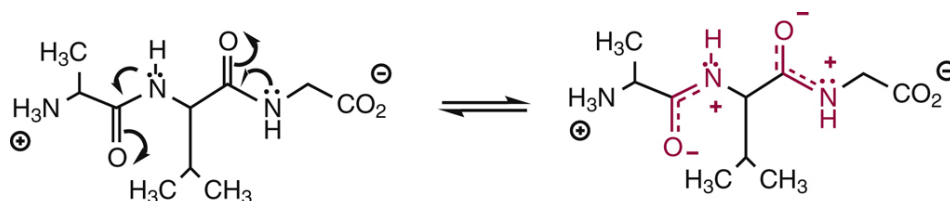
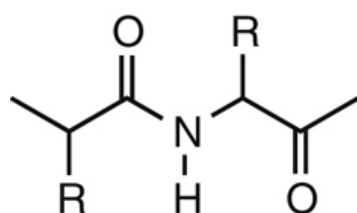
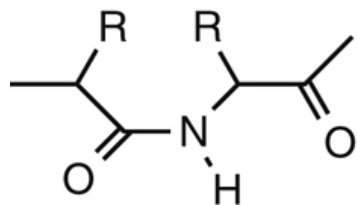


FIGURE 2.44 A tripeptide, Ala-Val-Gly, indicating the planarity of the peptide bonds caused by the restricted rotation around the amide bond.



Trans peptide bond



Cis peptide bond

FIGURE 2.45 *Cis/trans* peptide bond configuration.

Physical Chemical Properties of Peptides

Since the α -amine and α -carboxylic acid of each amino acid are involved in the peptide backbone (except at each terminus of the chain), the basic and acidic nature of these functional groups does not contribute to the overall physical chemical properties of the molecule. The functional groups found within the amino acid side chains are what are integral to the physicochemical properties of the peptide or protein and represent important points of interaction with the corresponding biologic target. Examination of [Table 2.11](#) shows that the functional groups found within the amino acid side chains can be basic (e.g., amine, guanidine, imidazole), acidic (e.g., carboxylic acid, phenol, thiol), neutral (e.g., thioether, amide), or hydrocarbon (e.g., alkyl, aromatic rings) in nature. All of the functional groups found in amino acid side chains were discussed previously as components of small-molecule drugs. The primary differences in physical chemical properties between peptides and small molecules are due to their large size (molecular

weight [MW]) and, as a result, the sheer number of different side chains (i.e., functional groups) present in a given structure. As might be expected, the types and number of functional groups present in the side chains dictate how much more or less polar the peptide is compared to a small-molecule drug. It is certainly plausible that peptide-based drugs will not have optimal log P values for passive absorption across membranes and, given their large MW, will not readily cross membranes.

Metabolism/Degradation of Peptide and Protein Drugs

Peptides and proteins are metabolized extensively by enzymes in the gastrointestinal tract, blood, interstitial fluid, vascular bed, and cell membranes, which results in very poor oral absorption and a short half-life for these molecules. The primary route of metabolism of peptides and proteins involves hydrolysis of the peptide bonds that link the amino acids by enzymes called peptidases. Some of these peptidases exhibit specificity for certain amino acid sequences or have specificity for either the amino or carboxyl terminus of the peptide (exopeptidase). For example, carboxypeptidases cleave off one C-terminal residue, dipeptidyl carboxypeptidases cleave dipeptides from the C terminus, aminopeptidases cleave off one N-terminal residue, and amidases (endopeptidases) cleave internal peptide bonds. There are also peptidase subclasses that exhibit specificity for certain amino acid sequences within the peptide or at either of the termini. Some peptidases (e.g., dipeptidyl peptidase IV) have been found to catalyze the degradation of naturally occurring peptides (e.g., GLP-1) (Fig. 2.46) (see also Chapters 5 and 20).

Subject to proteolytic degradation
catalyzed by dipeptidyl peptidase



His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu—

Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg

GLP-1

FIGURE 2.46 GLP-1 degradation by dipeptidyl peptidase IV.

Summary

Medicinal chemistry involves the discovery of new chemical entities and the systematic study of the SARs of these compounds for disease state management. Such studies provide the basis for development of better and therapeutically safer medicinal agents from lead compounds found from natural sources, random screening, systematic screening, and focused rational design. Drug design goals include increasing the potency and duration of action of newly discovered compounds and decreasing adverse side effects.

For the pharmacist, it is also important to understand how the physicochemical properties influence the pharmacokinetic properties of the medicinal agents being dispensed. Such knowledge will help the pharmacist not only to better understand the clinical properties of these compounds but also to anticipate the properties of newly marketed agents. An understanding of the chemical properties of the molecule will allow the pharmacist to anticipate formulation problems (especially IV admixtures), as well as potential adverse interactions with other drugs as the result of serum protein binding and metabolism.

Case Study

Absorption/Acid-Base Case Solution

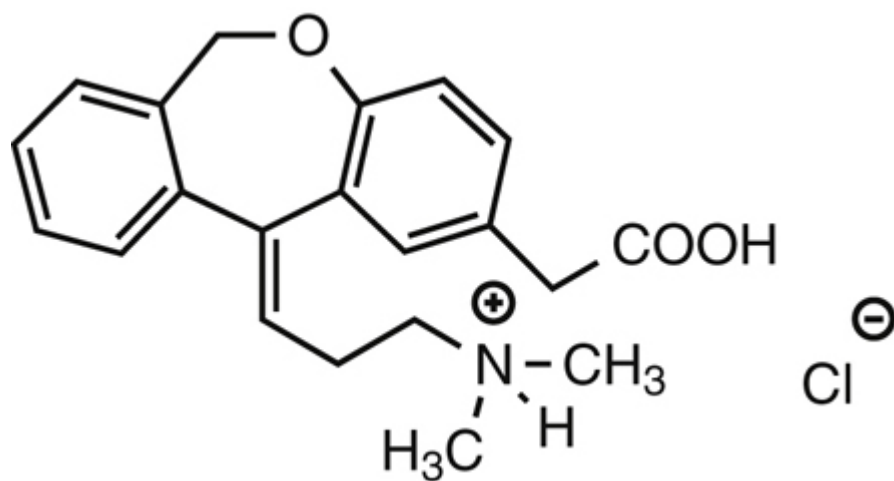
1.

Name of Functional Group	Drug in Which Functional Group Is Present	Character: Hydrophilic or Hydrophobic	Function: $\uparrow$ Aqueous Solubility or Drug Absorption	Character: Acidic, Basic or Neutral	Function: Contributes to Ability of Drug to Cross BBB?
Aromatic hydrocarbon	Zyrtec, Tavist, Patanol	Hydrophobic	$\uparrow$ Absorption	Neutral	Yes
Halogenated aromatic hydrocarbon	Zyrtec	Hydrophobic	$\uparrow$ Absorption	Neutral	Yes
Alkene	Patanol	Hydrophobic	$\uparrow$ Absorption	Neutral	Yes
Tertiary amine	Zyrtec, Tavist, Patanol	Hydrophobic (R) Hydrophilic (N)	$\uparrow$ Absorption $\uparrow$ Solubility	Basic ($pK_a = 9-11$)	Yes (in un-ionized form to a $\frac{1}{10}$ limited extent via R groups)
Ether	Zyrtec, Patanol	Hydrophobic (R) Hydrophilic (O)	$\uparrow$ Absorption $\uparrow$ Solubility (limited)	Neutral	Yes (via R groups)
Carboxylic Acid	Zyrtec, Patanol	Hydrophilic	$\uparrow$ Solubility	Acidic ($pK_a = 2-5$)	No

Cetirizine is a second-generation H_1 antagonist and is purported to be nonsedating. Clemastine is a first-generation H_1 antagonist and is considered to be a sedating antihistamine. Based on the structure evaluation process, both cetirizine and clemastine contain several hydrophobic functional groups that would facilitate their passive diffusion across the blood-brain barrier. Both molecules contain an ionizable amine that will be predominantly ionized in the plasma

(pH = 7.4). A key structural difference between these drug molecules is the presence of a carboxylic acid in cetirizine. This functional group is very hydrophilic and will also be predominantly ionized in the plasma (pH = 7.4) and therefore may limit the extent of absorption across the blood-brain barrier. Though both of these drugs will be predominantly ionized at pH = 7.4, it is important to remember that ionizable functional groups exist in equilibrium with their unionized forms. In the case of clemastine, it is possible that for a small fraction of time there will be some unionized drug available to cross the blood-brain barrier. In the case of cetirizine, with multiple ionizable functional groups, it is highly unlikely that the drug will exist in its un-ionized form. In order to limit the degree of drowsiness, it would be appropriate to recommend cetirizine (Zyrtec®) for this patient.

2.



Olopatadine contains functional groups (e.g., aromatic hydrocarbons, alkene, R groups of ethers and amines) with ^[L]_[SEP] hydrophobic character. Since hydrophobic character contributes to the ability of a drug to cross lipophilic membranes ^[L]_[SEP] (e.g., the

membranes that surround the eye) passively, then it is no surprise based on our evaluation thus far that there is ^[1]_{SEP} the potential for this agent to be absorbed.

The next set of functional groups to evaluate are those that exist predominantly in their ionized form at physiological pH. Olopatadine contains both an ionizable amine ($pK_a = 9-11$) and an ionizable carboxylic acid ($pK_a = 3-5$) that will be predominantly ionized in the ocular fluid. These ionizable functional groups contribute to the drug's ability to be soluble in an aqueous environment, including in the aqueous eye drop formulation. These same functional groups limit the ability of the drug to be absorbed passively into the membranes of the eye.

3.

<u>Assumptions:</u>	pK_a (tertiary amine) = 9.5	
	pK_a (carboxylic acid) = 3.0	
	<u>pH = 1 (stomach)</u>	<u>pH = 10 (intestine)</u>
Tertiary amine (basic)	Predominantly ionized	Close to 50/50% ionized/unionized
Carboxylic acid (acidic)	Unionized	Predominantly ionized

Zyrtec: In both compartments there will be at least one ionized functional group. In the intestine roughly 50% of the time there will be two functional groups ionized that will limit the extent of absorption from this compartment. Zyrtec is probably absorbed from both sites but is probably absorbed from the stomach to a greater extent.

Tavist: Absorbed best in the intestine where it exists in its un-ionized form ~ 50% of the time.

4. If the truck driver takes Zyrtec at the same time that he takes his TUMs, then the pH of the stomach will be elevated to 3.5 from pH = 1. At this pH the carboxylic acid will become

ionized and the extent of absorption from the stomach may be decreased to a limited extent. The truck driver may not receive the full antihistaminergic effect if he takes these two medications at the same time.

<u>Assumptions:</u>	pK_a (tertiary amine) = 9.5	
	pK_a (carboxylic acid) = 3.0	
	<u>pH = 3.5 (stomach + TUMS)</u>	<u>pH = 10 (intestine)</u>
Tertiary amine (basic)	Predominantly ionized	Close to 50/50% ionized/unionized
Carboxylic acid (acidic)	Close to 50/50% ionized/unionized	Predominantly ionized

Case Study

Acid-Base Chemistry/Compatibility Case Solution

1. *Acid/base evaluation of codeine phosphate:*

Ethers—neutral

Secondary alcohol—neutral

Aromatic hydrocarbon—neutral

Cycloalkenes—neutral

Tertiary amine (in salt form)—acidic (conjugate acid of a weak base)

Tertiary amine (as parent drug)—basic (capable of accepting a proton)

2. *Acid/base evaluation of penicillin V potassium:*

Aromatic hydrocarbon—neutral

Thioether—neutral

Amide/lactam—neutral

Ether—neutral

Alkane—neutral

Carboxylic acid (in salt form)—basic (conjugate base of a weak acid)

Carboxylic acid (as parent drug)—acidic (capable of donating a proton)

3. Penicillin V potassium contains several hydrophilic (polar) functional groups (amide, lactam, potassium salt of carboxylic acid) and only a moderate amount of hydrophobic character (aromatic hydrocarbon, thioether, alkane). Codeine phosphate (as parent drug) contains a tertiary amine and an alcohol that are hydrophilic in character. It contains a couple of functional groups that have mixed hydrophobic/hydrophilic character (ethers) and a fair amount of hydrophobic character (aromatic

hydrocarbon, cycloalkane, alkene). In order for a drug to be water soluble it must be able to interact with water *via* hydrogen bonding or an ion/dipole interaction. Hydrophilic functional groups can typically participate in these types of interactions. Considering the structural features of both agents, Penicillin V potassium will be more water soluble due to the presence of several hydrophilic functional groups, including an ionized functional group. The ionized form of codeine is more water soluble than the free base/parent form since the ionized tertiary amine can participate in ion/dipole interactions with water (a strong interaction).

4. If these two salts are mixed in the same IV bag, then it is anticipated that the salts will dissociate in the aqueous environment. In their dissociated forms, the free forms of the drugs (Penicillin V potassium is an acid in its free form and codeine phosphate is a base in its free form) may not be as water soluble as their salt forms. Reduced aqueous solubility could result in one or both of these agents forming a precipitate. Since they are in the same IV bag, it is certainly possible that these drugs (acid + base) could form a complex (ionic). This complex is not likely to be particularly water soluble and may very well form a precipitate in the IV bag.

Case Study

Absorption/Binding Interactions Case Solution

Name of Functional Group	Character: Hydrophilic and/OR Hydrophobic	Function: ↑ Aqueous Solubility or Drug Absorption	Function: Contributes to Ability of Drug to Penetrate Skin	Binding Interactions Possible with Enzyme	Amino Acids that Could Interact with Group
Aromatic hydrocarbon	Hydrophobic	↑ Absorption	Yes	Hydrophobic Stacking interactions	Phenylalanine Tyrosine
Alkene	Hydrophobic	↑ Absorption	Yes	Hydrophobic	Isoleucine Leucine Valine Alanine Methionine
Alkane	Hydrophobic	↑ Absorption	Yes	Hydrophobic (too bulky for Van der Waals)	Isoleucine Leucine Valine Alanine Methionine
Alkyne	Hydrophobic	↑ Absorption	Yes	Hydrophobic	Isoleucine Leucine Valine Alanine Methionine
Tertiary amine	Hydrophobic (R) Hydrophilic (N)	↑ Absorption (R) ↑ Solubility (N)	Yes (to a limited extent via the R groups)	H-bonding Dipole/dipole Ion/dipole Ionic	Serine Threonine Cysteine Tyrosine Glutamic acid, etc.

1. Those functional groups that are hydrophobic in character will facilitate the absorption of this medication into the skin.

2. Selection of the amino acids is based on the types of interactions that are possible with the particular functional group. With the tertiary amine it is essential to consider the ionization of this functional group prior to pairing with an amino ^[L]_{SEP} acid. For the ion/dipole interaction it is important to determine whether the drug is participating as the ion or the dipole when coupling potential amino acids to this type of functional group.

Case Study

Binding Interactions Case Solution

Name of Functional Group	Drugs in Which Functional Group Is Present	Binding Interactions Possible with Enzyme	Amino Acids that Could Interact with Group
Aromatic hydrocarbon	Betaxolol Salmeterol	a. Hydrophobic b. Stacking interactions	a. Phenylalanine b. Tyrosine
Alkane	Betaxolol Misoprostol Salmeterol	Hydrophobic (too bulky for Van der Waals)	Leucine
Ether	Betaxolol Salmeterol	a. H-bonding b. Dipole/dipole c. Hydrophobic	a. Serine b. Threonine c. Valine
Ketone	Misoprostol	a. H-bonding b. Dipole/dipole c. Ion/dipole	a. Cysteine b. Lysine c. Glutamic acid $\begin{matrix} \text{[O}^- \\ \text{SEP} \end{matrix}$ (in ionized form)
Secondary amine	Betaxolol Salmeterol	a. H-bonding b. Dipole/dipole c. Ion/dipole d. Ionic	a. Histidine b. Glutamine c. Cysteine d. Aspartic acid
Ester	Misoprostol	a. H-bonding b. Dipole/dipole c. Ion/dipole	a. Serine b. Threonine c. Arginine

Name of Functional Group	Drugs in Which Functional Group Is Present	Binding Interactions Possible with Enzyme	Amino Acids that Could Interact with Group
Phenol	Salmeterol	a. H-bonding b. Dipole/dipole c. Ion/dipole d. Ionic	a. Tyrosine b. Threonine c. Cysteine d. Lysine
Primary alcohol	Salmeterol	a. H-bonding b. Dipole/dipole c. Ion/dipole	a. Glutamic acid b. Glutamine c. Arginine
Secondary alcohol	Betaxolol Salmeterol Misoprostol	a. H-bonding b. Dipole/dipole c. Ion/dipole	a. Aspartic acid b. Glutamine c. Lysine
Tertiary alcohol	Misoprostol	a. H-bonding b. Dipole/dipole c. Ion/dipole	a. Glutamic acid b. Asparagine c. Arginine

Case Study

Water/Lipid Solubility Case Solution

Name of Functional Group	Drugs in Which Functional Group Is Present	Character: Hydrophilic and/Or Hydrophobic	Function: ↑ Aqueous Solubility or Drug Absorption
Aromatic hydrocarbon	Meclizine Fluoxetine	Hydrophobic	↑ Absorption
Cycloalkane	1,25 di (OH) Vit D ₂	Hydrophobic	↑ Absorption
Halogenated aromatic hydrocarbon	Meclizine	Hydrophobic	↑ Absorption
Halogen (F)	Fluoxetine	Hydrophilic	↑ Solubility
Ether	Fluoxetine	Hydrophobic (R) Hydrophilic (O)	↑ Absorption (R) ↑ Solubility (O)
Alkene	1,25-di(OH) vit D ₂	Hydrophobic	↑ Solubility
Secondary or tertiary amine	Meclizine Fluoxetine	Hydrophobic (R) Hydrophilic (N)	↑ Absorption (R) ↑ Solubility (N)
Alcohol	1,25-di(OH) vit D ₂	Hydrophobic (R) Hydrophilic (OH)	↑ Absorption (R) ↑ Solubility (OH)

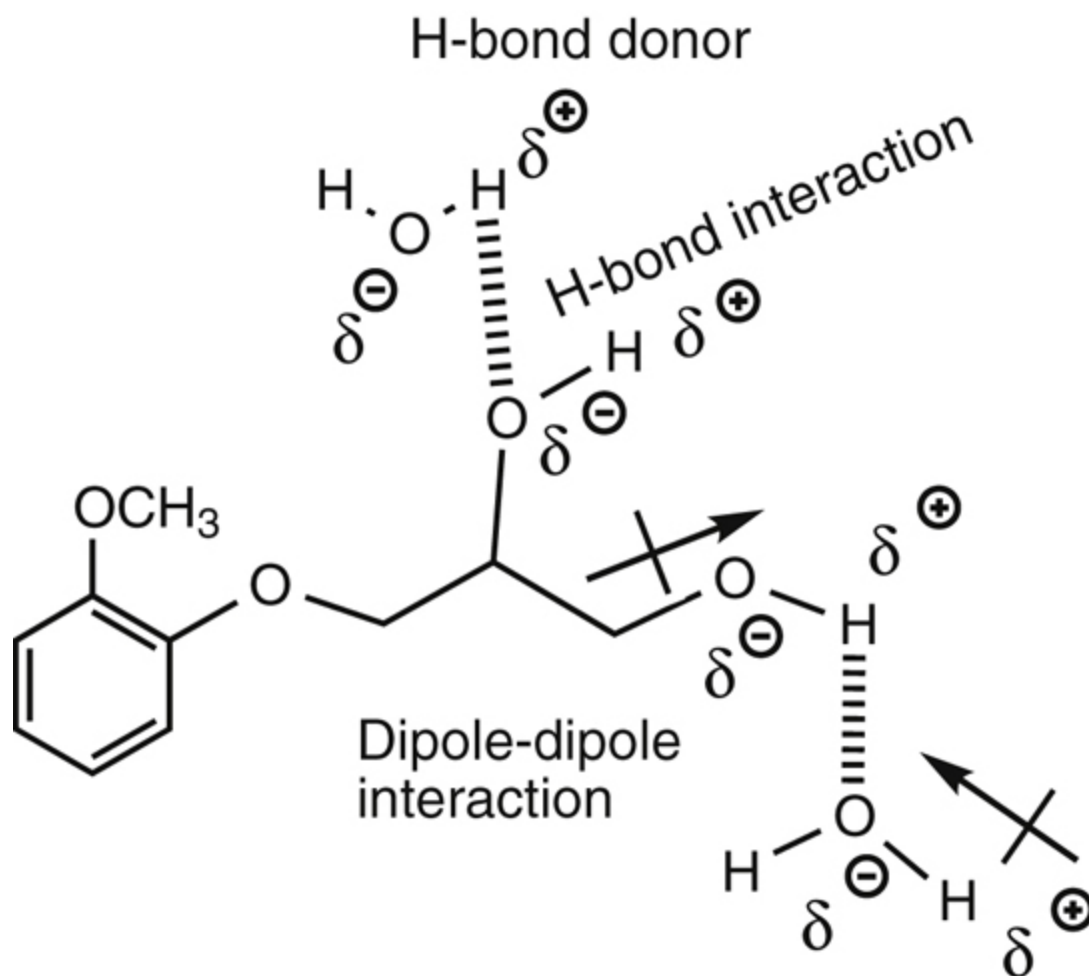
Case Study

Interactions/Solubility Case Solution

1.

Name of Functional Group	Drugs in Which Functional Group Is Present	Character: Hydrophilic and/Or Hydrophobic	Function: ↑ Aqueous Solubility and/Or ↑ Absorption Across BBB	Binding Interactions Possible with Target of Drug Action
Aromatic hydrocarbon	Phenylephrine Chlorpheniramine Guaifenesin	Hydrophobic	↑ Absorption	Hydrophobic stacking interactions
Halogenated aromatic hydrocarbon (Cl)	Chlorpheniramine	Hydrophobic	↑ Absorption	Hydrophobic stacking interactions Dipole-dipole
Ether	Guaifenesin	Hydrophobic (R) Hydrophilic (O)	↑ Absorption (R) ↑ Solubility (O)	H-bonding Dipole-dipole Ion-dipole
Secondary or tertiary amine	Phenylephrine Chlorpheniramine	Hydrophobic (R) Hydrophilic (N)	↑ Absorption (R) ↑ Solubility (N)	H-bonding Dipole-dipole Ion-dipole Ionic
Primary or secondary alcohol	Phenylephrine Guaifenesin	Hydrophobic (R) Hydrophilic (OH)	↑ Absorption (R) ↑ Solubility (OH)	H-bonding Dipole-dipole Ion-dipole
Phenol	Phenylephrine	Hydrophobic Hydrophilic (OH)	↑ Absorption (R) ↑ Solubility (OH)	H-bonding Dipole-dipole Ion-dipole Ionic

Functional groups that contribute to aqueous solubility are hydrophilic in character and are able to interact with water *via* hydrogen bonding or dipole-dipole interactions. If the functional group is ionizable (e.g., carboxylic acids), then the group can participate in an ion-dipole interaction with water.



1. The agent that has the most hydrophobic character is the one that is most likely to cross the lipophilic blood-brain barrier and have an effect on the child's alertness. Of these agents, chlorpheniramine has the most hydrophobic character (see list of structural features above).

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CHAPTER 3

Drug Metabolism

David A. Williams

What is a poison?

All substances are poisons;

There is none that is not a poison.

The right dose differentiates a poison from a drug.

Paracelsus (1493-1541)

Abbreviations

ADH alcohol dehydrogenases

AhR aromatic hormone receptor

ALDH aldehyde dehydrogenases

ATP adenosine triphosphate

CAR constitutive androstane receptor

CNS central nervous system

BBB blood-brain barrier

EHC extrahepatic cycling

EM fast or extensive metabolizer

ER endoplasmic reticulum

FAD flavin dinucleotide

FADH₂ reduced form of flavin dinucleotide

FDA U.S. Food and Drug Administration

FMN flavin mononucleotide

FMNH₂ reduced form of flavin mononucleotide

FMO flavin-containing monooxygenase

GIT gastrointestinal tract

GST glutathione S-transferases

IDR idiosyncratic drug reaction

IM intermediate metabolizer
MAO monoamine oxidase
NAT N-acetyltransferase
NAD⁺ nicotinamide adenine dinucleotide
NADH the reduced form of nicotinamide adenine dinucleotide
NADP⁺ nicotinamide adenine dinucleotide phosphate
NADPH the reduced form of nicotinamide adenine dinucleotide phosphate
P450 cytochrome P450 monooxygenase (P450 genes are in italics)
PAH polycyclic aromatic hydrocarbon
PAPS 3'-phosphoadenosine-5'-phosphosulfate
P-gp P-glycoprotein
PM poor metabolizer
ROS reactive oxygen species
SULT sulfotransferase
UGT UDP-glucuronosyl transferase
UM ultrafast metabolizer

Introduction

Humans are exposed throughout their lives to a large variety of drugs and nonessential exogenous (foreign) compounds (collectively termed “xenobiotics”) that can pose health hazards. Drugs taken for therapeutic purposes, as well as exposures to vapors of volatile chemicals or solvents, pose possible health risks; smoking and drinking lead to the absorption of large amounts of xenobiotics with potentially adverse health effects. Furthermore, ingestion of natural toxins in vegetables and fruits, pesticide residues in food, and carcinogenic pyrolysis products from fats and protein formed during the charbroiling of meat poses additional health risks. Most of these exogenous substances undergo enzymatic biotransformations by xenobiotic-metabolizing enzymes in the liver and extrahepatic tissues and are eliminated by excretion as hydrophilic metabolites. In some cases, especially during oxidative metabolism, numerous chemical procarcinogens form reactive metabolites capable of covalently binding to critical biopolymers, such as proteins or nucleic acids, which can lead to mutagenicity, cytotoxicity, and carcinogenicity. Therefore, insight into the biotransformation and bioactivation of xenobiotics becomes an indisputable prerequisite to assess drug safety and estimate risks associated with chemicals and drugs.

Detoxication and toxic effects of drugs and other xenobiotics have been studied extensively in various mammalian species. Frequently, differences in sensitivity to these toxic effects were observed and can now be attributed to genetic differences between species in the isoenzyme/isoforms of cytochrome P450 monooxygenases (P450). The level of expression of the P450 enzymes is regulated by genetics and by a variety of endogenous factors such as hormones, gender, age, and disease, as well as the presence of environmental factors,

such as inducing agents. Drugs were developed and prescribed under the old paradigm that “one dose fits all,” which ignored largely the fact that humans (both adults and children) are genetically and metabolically different, resulting in variable responses to drugs and risks of drug-drug interactions.

Drugs can no longer be regarded as chemically stable entities that elicit the desired pharmacological responses and then are excreted from the body. Drugs undergo a variety of chemical changes in humans brought about by enzymes of the liver, intestine, kidney, lung, and other tissues, with subsequent alterations of their pharmacological activities, durations of activity, and toxicities. Thus, the pharmacological and toxicological activities of a drug (or xenobiotic) are, in many ways, consequences of its metabolism.

The practice of simultaneous prescriptions of several drugs has become common. Thus, an awareness of possible drug-drug interactions is essential to avoid catastrophic synergistic effects and chemical, enzymic, and pharmacokinetic interactions that can produce toxic adverse effects.

The study of xenobiotic metabolism has developed rapidly during the past few decades.¹⁻¹⁰ These studies have been fundamental in the assessment of drug efficacy and safety and in the design of dosage regimens; in the development of food additives and the assessment of potential hazards of contaminants; in the evaluation of toxic chemicals; and in the safety development of pesticides and herbicides and the assessment of their metabolic fates in insects, other animals, and plants. The metabolism of drugs and other xenobiotics is fundamental to many toxic processes, such as carcinogenesis, teratogenesis, and tissue necrosis. Often the same enzymes involved in drug metabolism also carry out the regulation and metabolism of endogenous substances such as steroids, vitamin D, prostaglandins, and lipids. Consequently, the inhibition and induction of these enzymes by drugs and xenobiotics can have a

profound effect on the normal processes of intermediary metabolism, such as tissue growth and development, hematopoiesis, calcification, and lipid metabolism.

Familiarity with the mechanisms of drug metabolism can often aid the prediction of the consequences of drug-drug interactions, drug-food interactions, and herbal drug-drug interactions and help to explain patients' adverse responses to drug regimens. Incorporating pharmacogenomics into the selection of drug regimens will change the way in which drugs are prescribed. Selection based on a patient's individual genetic makeup could eliminate previously unpredictable responses to drug treatment caused by genetic polymorphisms that affect metabolism, clearance, and tolerance. Pharmacogenomic testing to determine a patient's phenotype (i.e., poor metabolizer) and thus, his or her ability to metabolize drugs, will become common in the future. Such knowledge will assure improved selection of proper drug regimens and doses before therapy begins. The increased knowledge of drug metabolism, fed by the need for better safety evaluations of drugs and chemicals, has resulted in a proliferation of publications (e.g., *Drug Metabolism Reviews*, *Drug Metabolism and Disposition*, and *Xenobiotica*) and a series of monographs that present the current state of knowledge of foreign compound metabolism from biochemical and pharmacological viewpoints.³⁻¹⁰

Pathways of Metabolism

The goals of drug metabolism are to change/terminate the physical and pharmacological properties of a drug/xenobiotic through metabolic biotransformations (and therefore its action and effectiveness), and to effectively remove these apparently chemically stable drugs from the body. The metabolite thus becomes less lipophilic, more water-soluble (hydrophilic), and possibly more easily eliminated (increased body clearance) by multiple routes (e.g., urine, feces).

To accomplish these goals, drug metabolism changes the molecular structure and shape of the drug/xenobiotic by the addition or exposure of a “handle” so that the substance no longer binds to its receptors/enzymes. The “handle” often increases molecular hydrophilicity to ensure increased water solubility by oxidation and conjugation and ensures elimination from the body by one or more routes. Common handles include alcoholic or phenolic hydroxyl groups, ionic carboxyl groups, ionic glucuronides, ionic sulfate esters, and ionic mercapturates.

The term “detoxication” describes the result of these metabolic biotransformations. Although, as a rule, drug metabolism leads to detoxication, the processes of oxidation, reduction, glucuronidation, sulfonation (sulfo-conjugation), and other enzyme-catalyzed reactions can lead to the formation of metabolites having therapeutic or toxic effects from inactive parent drugs. This process is often referred to as “bioactivation.” One of the earliest discoveries of bioactivation to an active metabolite was the reduction of prontosil to the antibacterial agent sulfanilamide. Further examples of drug bioactivation to active metabolites include the hydroxylation of the peripheral analgesic acetanilide to acetaminophen, the N-demethylation of the antidepressant imipramine to form

desipramine, and conversion of the anxiolytic diazepam to desmethyldiazepam. The insecticide parathion is desulfurized by both insects and mammals to form the toxic metabolite paraoxon.

Most drugs and other xenobiotics are metabolized by enzymes normally associated with the metabolism of endogenous constituents (e.g., steroids and biogenic amines). The liver is the primary site of drug metabolism, although other xenobiotic-metabolizing enzymes are found in nervous tissue, kidney, lung, plasma, and the gastrointestinal tract (GIT; digestive secretions, bacterial flora, and the intestinal wall).

Although hepatic metabolism continues to be the most important route of metabolism for xenobiotics and drugs, other biotransformation pathways play a significant role in the metabolism of these substances. Among the more active extrahepatic tissues capable of metabolizing drugs are the intestinal mucosa, kidney, and lung (see section Extrahepatic Metabolism). The ability of the liver and extrahepatic tissues to metabolize substances to either pharmacologically inactive or bioactive metabolites before they achieve systemic blood levels is termed “first-pass metabolism” or the “presystemic first-pass effect.” Other metabolite reactions occurring in the GIT are associated with bacteria and other microflora in the tract. The bacterial flora can affect metabolism through: (1) production of toxic metabolites, (2) formation of carcinogens from inactive precursors, (3) detoxication, (4) exhibition of species differences in drug metabolism, (5) exhibition of individual differences in drug metabolism, (6) production of pharmacologically active metabolites from inactive precursors, and (7) production of metabolites not formed by animal tissues.

Phase 1 Reactions

The pathways of xenobiotic metabolism are divided into three major categories. Phase 1 reactions (biotransformations) include oxidation, hydroxylation, reduction, and hydrolysis. In each of these enzymatic reactions, a new functional group is introduced into the substrate molecule, an existing functional group is modified, or a functional group or acceptor site for Phase 2 transfer reactions is exposed, thus making the xenobiotic more polar and, therefore, more readily excreted.

Phase 2 Reactions

Phase 2 reactions (conjugation) are enzymatic syntheses whereby a functional group, such as an alcohol, a phenol, an amine, or a carboxylic acid, is masked by the addition of a new group, such as acetyl, sulfate, glucuronic acid, or certain amino acids, which usually increases the polarity of the drug or xenobiotic. Most substances undergo both Phase 1 and Phase 2 reactions sequentially.

Those xenobiotics that are resistant to metabolizing enzymes or are already hydrophilic are excreted largely unchanged. This basic pattern of xenobiotic metabolism is common to all animal species, including humans, but species can differ in details of the reaction and enzyme control.

Phase 3 Transporters

Once xenobiotics have been converted into low-toxicity and water-soluble metabolites by the combination of Phase 1 and Phase 2 reactions, these metabolites must be transported against a concentration gradient out of the cell into the interstitial space between cells, and then into the bloodstream for filtration by the kidneys. The biggest hurdle is the transport of these hydrophilic metabolites out of the cell. Charged Phase 2 metabolites will be effectively “ion-trapped” in the cell, as the cell membrane is highly

lipophilic and is an effective barrier to the exit, as well as entry, of most hydrophilic molecules. In addition, failure to remove the hydrophilic products of conjugation reactions can lead to toxicity. Consequently, an array of multipurpose membrane-bound transport carrier systems has evolved which can actively remove hydrophilic metabolites and many other low-molecular-weight drugs and toxins from cells (see [Chapter 4](#)). Thus, the term of Phase 3 metabolism has been applied to the study of this essential arm of the detoxification process. Efflux transporters transport hydrophilic substrates out of cells into interstitial fluid, blood, kidneys, and the GIT. Influx transporters transport hydrophilic substrates into cells from the bloodstream.

Factors Affecting Metabolism

Drug therapy is becoming oriented more toward controlling metabolic, genetic, and environmental illnesses rather than short-term therapy associated with infectious diseases. In most cases, drug therapy lasts for months or even years, and the problems of drug-drug interactions and chronic toxicity from long-term drug therapy have become more serious. Therefore, a greater knowledge of drug metabolism is essential. Several factors influencing xenobiotic metabolism include:

- *Genetic polymorphism.* Genetic polymorphisms of drug-metabolizing enzymes give rise to distinct subgroups in the population that differ in their ability to perform certain drug biotransformation reactions. Polymorphisms are generated by mutations in the genes for these enzymes, which cause decreased, increased, or absent enzyme expression or activity by multiple molecular mechanisms. Individual differences in drug effectiveness (drug sensitivity or drug resistance), drug-drug interactions, and drug toxicity can depend on racial and ethnic characteristics impacting the population frequencies of the many polymorphic genes and the expression of the metabolizing enzymes. Pharmacogenetics focuses primarily on genetic polymorphisms (mutations) responsible for interindividual differences in drug metabolism and disposition. Genotype-phenotype correlation studies have validated that inherited mutations result in two or more distinct phenotypes causing very different responses following drug administration. The genes encoding for CYP2A6, CYP2C9, CYP2C19, and CYP2D6 are functionally polymorphic; therefore, at least 30% of P450-dependent metabolism is performed by polymorphic

enzymes. For example, mutations in the *CYP2D6* gene result in poor (PM), intermediate (IM), or ultrarapid (UM) metabolizers of more than 30 cardiovascular and central nervous system (CNS) drugs. Thus, each of these phenotypic subgroups experiences different responses to drugs extensively metabolized by the *CYP2D6* pathway ranging from severe toxicity to complete lack of efficacy (see section Genetic Polymorphism).

- *Physiologic factors.* Age is a factor, as both very young and old have impaired metabolism. Hormones (including those induced by stress), sex differences, pregnancy, changes in intestinal microflora, diseases (especially those involving the liver), and nutritional status can also influence drug and xenobiotic metabolism.

Because the liver is the principal site for xenobiotic and drug metabolism, liver disease can modify the pharmacokinetics of drugs hepatically metabolized. Liver disease affects the elimination half-life of some drugs but not of others, even if they all undergo hepatic biotransformation. Some studies have shown that the capacity for drug metabolism is impaired in chronic liver disease, which could lead to unintentional drug overdosage. Because of the unpredictability of drug effects in the presence of liver disorders, drug therapy in these circumstances is complex, and more than usual caution is needed.

Protein deficiency leads to reduced hepatic microsomal protein and lipid metabolism, and oxidative metabolism is decreased due to an alteration in endoplasmic reticulum (ER) membrane permeability affecting electron transfer. Protein deficiency would increase the toxicity of drugs and xenobiotics by reducing their oxidative P450 metabolism and clearance from the body.

- *Pharmacodynamic factors.* Dose, frequency, and route of administration, plus tissue distribution and protein binding of a drug, affect its metabolism.
- *Environmental factors.* Competition of ingested environmental substances for metabolizing enzymes and poisoning of enzymes by toxic chemicals such as carbon monoxide can alter drug and other xenobiotic metabolism. Induction of enzyme expression (in which the number of enzyme molecules is increased, while the activity is constant) by other drugs and xenobiotics is another consideration. Environmental factors can change not only the kinetics of an enzyme reaction but also the whole pattern of metabolism, thereby altering bioavailability, pharmacokinetics, pharmacological activity, and/or toxicity of a xenobiotic. Species differences in response to xenobiotics must be considered in the extrapolation of pharmacological and toxicological data from animal experiments to predict effects in humans.

Drug Biotransformation Pathway (Phase 1)

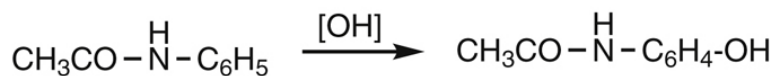
Human Hepatic Cytochrome P450 Enzyme System

Introduction

Oxidation, the most common reaction in xenobiotic metabolism, is catalyzed by a group of membrane-bound monooxygenases found in the smooth ER of the liver and other extrahepatic tissues, termed the “cytochrome P450 monooxygenase enzyme system” (hereafter, abbreviated as P450). P450 has also been called a mixed-function oxidase or microsomal hydroxylase. The tissue homogenate fraction containing the smooth ER is called the microsomal fraction. P450 functions as a multicomponent electron-transport system, responsible for the oxidative metabolism of a variety of endogenous substrates (e.g., steroids, fatty acids, prostaglandins, and bile acids) and xenobiotics including drugs, carcinogens, insecticides, plant toxins, environmental pollutants, and other foreign chemicals. Central to the functioning of this unique superfamily of heme proteins is an iron protoporphyrin. The iron protoporphyrin is coordinated to the sulfur of cysteine and can form a complex with carbon monoxide, resulting in a complex which has its primary absorption maximum at 450 nm (thus the title of these metabolizing P450 enzymes). P450 has an absolute requirement for NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate) and molecular oxygen (dioxygen). The rate at which various compounds are metabolized by this system depends on the animal species, strain, nutritional status, type of tissue, age, and pretreatment of animals. P450 has the ability to catalyze the C-H oxidation of a wide range of structurally dissimilar functional groups. All of these reactions have a common characteristic: H atom abstraction mechanism from C-H, with the resultant formation of a hydroxyl group via oxygen rebound.¹¹ The same active enzyme site binds to structurally dissimilar substrates, resulting in indiscriminate substrate binding. The variety of reactions catalyzed by P450 (Table 3.1) include the oxidation of alkanes and aromatic compounds; the epoxidation of alkenes, polycyclic hydrocarbons, and halogenated benzenes; the dealkylation of secondary and tertiary amines and ethers; the deamination of amines; the conversion of amines to N-oxides, hydroxylamine, and nitroso derivatives; and the dehalogenation of halogenated hydrocarbons. It also catalyzes the oxidative cleavage of organic thiophosphate esters, the sulfoxidation of some thioethers, and the reduction of azo and nitro compounds to primary aromatic amines. A nonheme, microsomal flavoprotein monooxygenase is responsible for the oxidation of certain nitrogen- and sulfur-containing organic compounds. Substrate and inhibitor promiscuity have an impact on drug-drug interactions.

TABLE 3.1
Hydroxylation Mechanisms Catalyzed by Cytochrome P450

Aromatic hydroxylation



While all P450 enzymes share a common tertiary protein structure, the flexibility and functional amino acids around the active site account for the ability of P450 enzymes to accommodate a vast number of structurally dissimilar substrates and support a wide range of selective oxidations. The P450 superfamily has a remarkable adaptability (plasticity) for substrate recognition and regio- and stereoselectivity in the catalytic chemistry. This is possibly due to secondary protein structural features surrounding the P450 active site. Not only do these features differ between enzymes, but they change even between different states of individual enzymes.

The most important function of P450 is to “activate” molecular oxygen (dioxygen) to a ferric iron-oxene intermediate, permitting the incorporation of one atom of oxygen into an organic substrate molecule concomitant with the reduction of the other atom of oxygen to water. The introduction of a hydroxyl group into the hydrophobic substrate provides a site for subsequent conjugation with hydrophilic compounds (Phase 2), thereby increasing the aqueous solubility of the product for transport and excretion from the body. The P450 enzyme system not only catalyzes xenobiotic transformations in ways that lead typically to detoxication but, in some cases, in ways that lead to products having greater cytotoxic, mutagenic, or carcinogenic properties.

Components of P450

P450 consists of at least two protein components: a heme protein called P450 and a flavoprotein called NADPH-P450 reductase, containing both flavin mononucleotide (FMN) and flavin dinucleotide (FAD). P450 is the substrate- and oxygen-binding site of the enzyme system, whereas the reductase serves as an electron carrier, shuttling electrons from NADPH to P450. A third component essential for electron transport from NADPH to P450 is a phospholipid, phosphatidylcholine, which facilitates the transfer of electrons from NADPH-P450 reductase to P450.⁶ Although the phospholipid does not function in the system as an electron carrier, it has a great influence on the P450 monooxygenase system. The phospholipid constitutes approximately one-third of the hepatic ER and contributes to a negatively charged environment at physiological pH.

Of the three components involved in microsomal oxidative xenobiotic metabolism, P450 is important due to its vital role in oxygen activation and substrate binding. P450 is an integral protein embedded in the membrane matrix. The electron components of P450 are located on the cytoplasmic side of the ER membrane, and the hydrophobic active site is oriented toward the lumen of the ER.⁶ The active site of P450 consists of a hydrophobic substrate-binding domain in which is embedded an iron protoporphyrin (heme) prosthetic group. This group is exactly like that of hemoglobin, peroxidase, and the *b*-type cytochromes. The iron in the iron protoporphyrin is coordinated with four nitrogens via a tetradentate link to the porphyrin ring. X-ray studies reveal that in the ferric state, the two nonporphyrin ligands are water and cysteine (Fig. 3.1). The cysteine thiolate ligand (proximal) is absolutely essential for the formation of the reactive ferric oxenoid intermediate. The sixth (distal) coordination position is occupied by an easily exchangeable ligand, most likely water, which is labile and readily displaced by stronger drug or xenobiotic ligands. The ferrous form loses the water ligand completely, leaving the sixth position open for binding ligands such as oxygen and carbon monoxide.

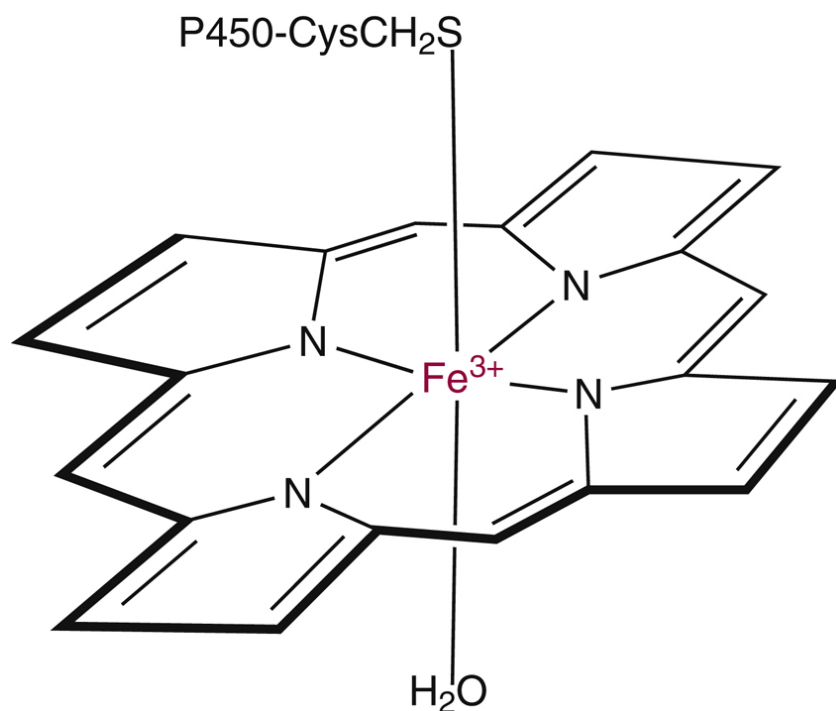


FIGURE 3.1 Ferric heme thiolate catalytic center of CYP450. The porphyrin side chains are deleted for clarity.

The vast array of xenobiotics presents a unique challenge to the human body to metabolize these many lipophilic foreign compounds. This makes it impractical to have one enzyme to metabolize each compound or each class of compounds. Therefore, whereas most cellular functions are normally very specific, oxidation of xenobiotics necessitates P450s with diverse substrate specificities and regioselectivities (i.e., multiple sites of oxidation). Several types of P450 enzymes can be found in a single animal species. Humans have 57 genes and more than 59 pseudogenes divided among 18 families and 43 subfamilies of P450 genes, each coding for a different version of the enzyme (isoform) so that, together, the P450s can metabolize almost any lipophilic compound to which the individual is exposed.

Classification of the P450 Multigene Family

Nebert et al.^{12,13} classified the P450 supergene family on the basis of structural (evolutionary) relationships. P450 monooxygenases resulting from this supergene family have been subdivided into families, which possess greater than 40% amino acid homology, with subfamilies possessing greater than 55% homology.^{12,13} The P450s are named with the root symbol CYP followed by an Arabic numeral designating the family (e.g., CYP1, CYP2, or CYP3), a letter denoting the subfamily (e.g., CYP1A, CYP2C, CYP2D, or CYP2E), and another Arabic numeral representing the individual gene (e.g., CYP3A4). Names of genes are written in italics. The nomenclature system is based solely on sequence similarity among the P450s and does not indicate the properties or functions of individual P450s. Of the more than 17 P450 isoforms that have been identified so far, the primary isoforms responsible for drug metabolism in the liver are presented in [Figure 3.2](#). It is evident that the CYP3A and CYP2C families are the isoforms most involved in the metabolism of clinically relevant drugs, while the CYP1A2 isoform is predominantly involved in the bioactivation of environmental substances.

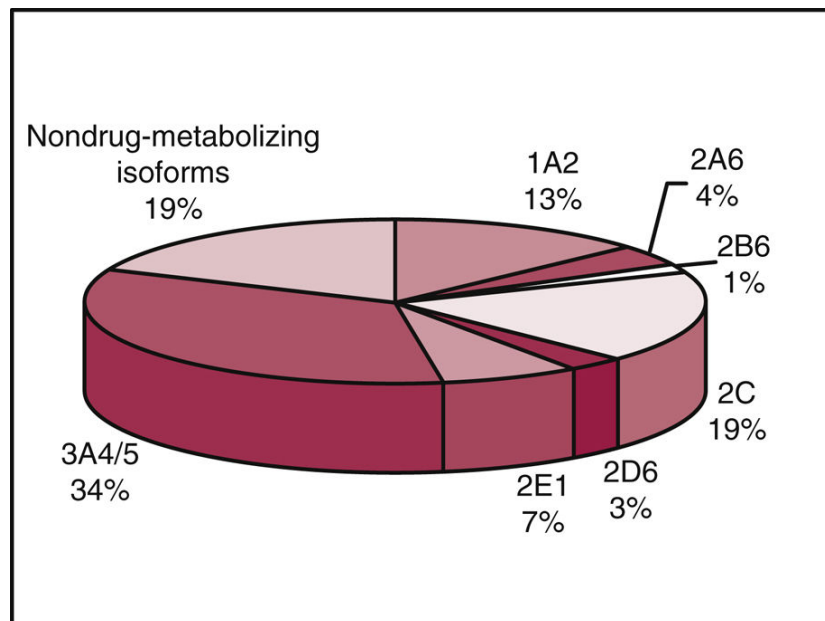


FIGURE 3.2 Total human CYP450 isoforms expressed in the liver that metabolize drugs.

P450s initially evolved for the regulation of endogenous substances, such as the metabolism of cholesterol necessary to maintain membrane integrity, and for steroid biosynthesis and metabolism, rather than for metabolizing toxic xenobiotics. P450s are either located in the inner mitochondrial membrane and involved in highly specific steroid hydroxylations or bound to the ER of the cell and have broad substrate specificity. In evolutionary terms, P450s evolved from a common ancestor, and only more recently (during the last 100 million years) have P450 genes taken on the role of producing enzymes to metabolize a vast array of lipophilic foreign compounds. The xenobiotic P450 genes probably emerged from the steroidogenic P450s to enhance animal survival by synthesizing new P450s to metabolize plant toxins concentrated in the food chain. It is not surprising that animals and humans possess a vast array of P450 enzymes capable of handling a multitude of xenobiotics. Interindividual variation in the expression of xenobiotic P450 genes (genetic polymorphism) or in their inducibility can be associated with differences such as individual susceptibilities to cigarette smoke-induced carcinogenesis. Certain P450 isoforms that clearly exhibit genetic polymorphisms are known to metabolize (and as a rule inactivate) therapeutic agents. The extent of P450 polymorphism in humans is being investigated to determine the potential for protection against cancers. Food mutagens are typically carcinogens in tissues, but they are activated by CYP1A2 and CYP3A in the liver. Specific forms of P450 in hepatic microsomes are regulated by hormones (e.g., the CYP3A subfamily) and are induced or inhibited by drugs, food toxins, or other environmental xenobiotics (see section Induction and Inhibition of Cytochrome P450 Isoforms). Identification of a specific P450 isoform as the primary form responsible for the metabolism of a specific drug in humans permits reconciliation of the drug's toxicity and/or other pharmacological effects.

Substrate Specificity

The substrate specificities, affinities, regioselectivities, and rates of hydrogen abstraction are probably consequences of the conformational changes resulting from ligand binding at the site of hydroxylation. Protein-protein interactions and amino acid side chains play a key role in substrate cooperativity, as well as flexible and dynamic arrangements that change depending upon the presence of substrate, cofactor, and oxidation state.^{14,15} Because a primary function of these enzymes is the metabolism of hydrophobic substrates, it is likely that hydrophobic forces are important in the binding of many

substrates to the apoproteins. Nonspecific binding is consistent with the multiple substrate orientations at the active site necessary for the broad regioselectivities observed. A specific binding requirement would decrease the diversity of substrates. Some P450 isoforms have constrained binding sites, thus they metabolize only small organic molecules (e.g., CYP2E1). CYP1A1/2 isoforms have planar binding sites and metabolize only aromatic planar compounds (i.e., polycyclic aromatic hydrocarbons [PAHs]). CYP2D6 exhibits high affinity for specific apoprotein interactions (hydrogen bonds, ion-pair formation) with specific substrates such as lipophilic amines. CYP3A4 has a broader affinity for a variety of lipophilic substrates with molecular weights of 200-1200 daltons. If P450 isoforms are tightly membrane bound, substrate access to the active sites is limited to compounds that can diffuse through the membranes. P450 isoforms that are less tightly bound will metabolize hydrophilic compounds.

The site of hydroxylation is determined by accessibility to, and topography of, the active site of the protein, the degree of steric hindrance encountered by substrate to the heme ferric-oxenoid species at the site of reaction, the ease of H atom abstraction or electron transfer from the compound (metabolic soft spot), and the stability of the carbon center radical, which follows the carbocation (C⁺) stability; tertiary > secondary > primary > CH₃.^{15,16} Alkyl groups are electron donating so they neutralize the positive charge on the C⁺. The more alkyl substituents attached to the C⁺, the greater the surface area over which to spread the positive charge. The lower charge density translates to greater stability. Since the tertiary carbons are electron donating, this will stabilize a partial negative charge on an electronegative atom.

The P450s were often referred to as having broad and overlapping specificities, but it became apparent that the broad substrate specificity could be attributed to multiple isoenzymic forms of P450. The phenotype of an individual with respect to the forms and amounts of individual P450s expressed in the liver can determine the rate and pathway of the metabolic clearance of a compound (see section Genetic Polymorphism). Significant differences exist between humans and other animal species with respect to the catalytic activities and regulation of expression of hepatic drug-metabolizing P450s. These differences often make it difficult to extrapolate results of P450-mediated metabolism studies performed experimentally in animal species to humans. Caution is warranted in the extrapolation of rodent data to humans, as some isoforms are similar between species (e.g., CYP1A and CYP3A subfamilies) whereas others are different (e.g., CYP2A, CYP2B, CYP2C, and CYP2D subfamilies). The unique and diverse characteristics of the P450 ensure that predicting the metabolism of xenobiotics will be difficult.

Other P450 Isoforms

Other P450 isoforms catalyzing the oxidation of steroids, bile acids, fat-soluble vitamins, and other endogenous substances are shown in [Table 3.2](#).

TABLE 3.2
Other Cytochromes With Indicated Specificities and Actions

CYP450s	Activity	Location	Actions
CYP4	ω -hydroxylases		ω -Hydroxylation of fatty acids, leukotrienes, eicosanic acids
CYP5	Thromboxane A ₂ synthase		Arachidonic acid to thromboxane A ₂ effecting platelet aggregation
CYP7A	7 α -Hydroxylase		Cholesterol to bile acids formation
CYP7B	7 α -Hydroxylase	Brain	Neurosteroids formation: 7 α -hydroxydehydroepiandrosterone and 7 α -hydroxypregnenolone
CYP8A	Prostacyclin synthase		Synthesis of prostaglandin I ₂ regulating hemostasis
CYP8B	12 α -Hydroxylase		Catalyzes bile acid biosynthesis
CYP11A1	Steroid 20 α -/22-hydroxylase	Adrenal mitochondria	Catalyzes mitochondrial steroid biosynthesis via 17-side chain cleavage of cholesterol to pregnenolone—lack of leads to feminization and hypertension
CYP11B1	11 β -Hydroxylase	Adrenal cortex	Catalyzes 11-deoxycortisol to hydrocortisone or 11-deoxycorticosterone to corticosterone

CYP450s	Activity	Location	Actions
CYP11B2	18-Hydroxylase	Adrenal zona glomerulosa	Hydroxylates corticosterone at the 18-position leading to aldosterone
CYP17A1	17 α -Hydroxylase and 17,20-lyase	ER of adrenal cortex	Production of testosterone and estrogen—lack of affects sexual development
CYP19A	Aromatase	ER of gonads, brain, adipose tissue	Aromatization of ring A of testosterone leading to estrogen—lack of causes estrogen deficiency
CYP21A1	C21 steroid hydroxylase	Adrenal cortex	17-hydroxyprogesterone to cortisol
CYP24	24-Hydroxylase	Mitochondria	Catalyzes the degradation/inactivation of vitamin D metabolites
CYP26A1	<i>Trans</i> -retinoic acid hydroxylase		Terminates retinoic acid signal
CYP26B1	Retinoic acid hydroxylase		Catalyzes the hydroxylation of <i>cis</i> -retinoic acids
CYP26C	Retinoic acid hydroxylase		Unknown
CYP27A1	27-hydroxylase		Catalyzes the oxidation of the cholesterol in bile acid biosynthesis
CYP27B1	Vitamin D ₃ 1- α -hydroxylase	Mitochondria	Activates vitamin D3
CYP27C1			Unknown
CYP39			Catalyzes the 7-hydroxylation of 24-hydroxy cholesterol
CYP46	Cholesterol 24-hydroxylase		Unknown
CYP51	Lanosterol 14 α -demethylase		Converts lanosterol into cholesterol

Cytochrome P450 Isoforms Metabolizing Drugs/Xenobiotics

Figure 3.3 shows the % participation of the hepatic P450 isoforms in the metabolism of drugs and xenobiotics. P450s are the most important enzymes responsible for phase 1 drug metabolism.¹⁷ The polymorphic nature of the P450s influences individual drug responses and drug-drug interactions and induces adverse drug reactions. The crystal structures of eight mammalian P450s (CYP2C5, CYP2C8, CYP2C9, CYP3A4, CYP2D6, CYP2B4, CYP2A6, and CYP1A2) have been published. Approximately one-third of all drugs are metabolized by one isoform, CYP3A4, increasing the potential for drug-drug interactions.¹⁷ When two drugs are metabolized by the same isoform, only one drug can serve as a substrate at a time at an individual enzyme active site, increasing the likelihood of a drug-drug interaction, especially if one drug has a low therapeutic threshold.

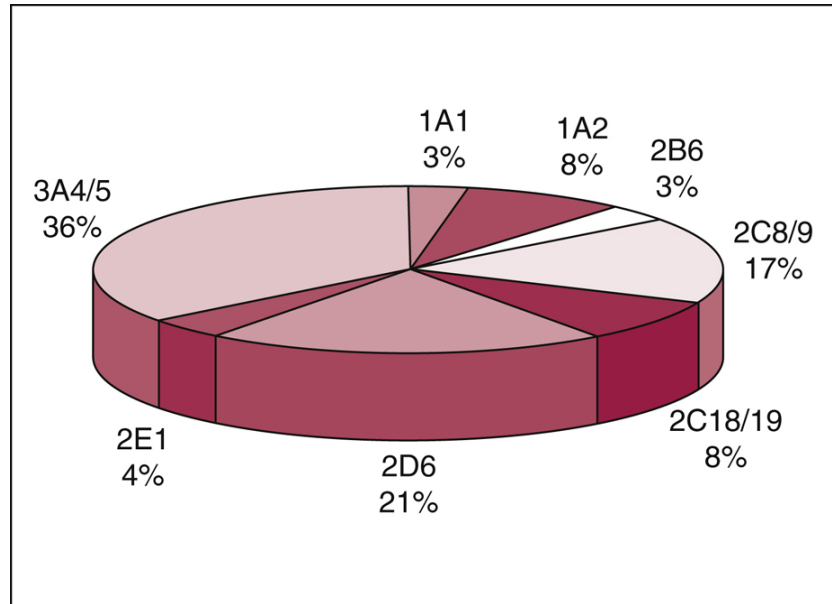


FIGURE 3.3 Percentage of clinically important drugs metabolized by human CYP450 isoforms.

Family 1

CYP 1 consists of three isoforms, CYP1A1, CYP1A2, and CYP1B1, three genes, and one pseudogene. The human CYP1A subfamily has an integral role in the metabolism of estrogens and two important classes of environmental carcinogens, PAHs and arylamines (Tables 3.3 and 3.4). The PAHs are commonly present in the environment through industrial combustion processes and tobacco products. Several potent carcinogenic arylamines result from the pyrolysis of amino acids in cooked meats and cause colon cancer in rats. Environmental and genetic factors can alter the expression of this subfamily of enzymes.

Table 3.3

Some Substrates and Reaction Type for Human Subfamily CYP1A2*

Acetaminophen (Iminoquinone)
<i>Amitriptyline</i> (N-demethylation)
Caffeine (N1- and N3-demethylation)
Chlordiazepoxide
<i>Clomipramine</i> (N-demethylation)
<i>Clopidogrel</i>
<i>Clozapine</i>
Cyclobenzaprine
<i>Desipramine</i> (N-demethylation)
Diazepam
<i>Duloxetine</i>
Erlotinib
Estradiol (2- and 4-hydroxylation)
Flutamide
Fluoroquinolones (3'-hydroxylation of piperazine ring)
<i>Fluvoxamine</i>
<i>Haloperidol</i>
<i>Imipramine</i> (N-demethylation)
Levobupivacaine
Mexiletine
Mirtazapine
Naproxen
Nortriptyline

Olanzapine
Ondansetron
Propafenone
Propranolol
Ramelteon
Riluzole
Ropinirole
Ropivacaine
Tacrine
Theophylline
Tizanidine
Verapamil
R-Warfarin
Zileuton
Zolmitriptan

^aDrugs in bold italic have been reported to cause drug-drug interactions.

TABLE 3.4
Some Procarcinogens and Other Toxins Activated by Human Cytochrome P450s

CYP1A1	CYP1A2	CYP2E1	CYP3A4
Benzo[a]pyrene and other polycyclic aromatic hydrocarbons	4-Aminobiphenyl 2-Naphthylamine 2-Aminofluorene 2-Acetylaminofluorene 2-Aminoanthracene Heteropolycyclic amines (2-aminoquinolines) Aflatoxin B1 Ipomeanol	Benzene Styrene Acrylonitrile Vinyl bromide Trichloroethylene Carbon tetrachloride Chloroform Methylene chloride 1,2-Dichloropropane Ethyl carbamate	Aflatoxin B1 Aflatoxin G1 Estradiol 6-Aminochrysene Polycyclic hydrocarbons Dihydrodiols

CYP1A1

The CYP1A1 isoform (also called aromatic hydrocarbon hydroxylase) is expressed primarily in extrahepatic tissues, the small intestine, placenta, skin, and lung as well as in the liver in response to the presence of CYP1A1 inducers such as PAHs (i.e., in cigarette smoke and the carcinogen 3-methylcholanthrene), α -naphthoflavone (a noncarcinogenic inducer related to dietary flavones), and indole-3-carbinol (found in Brussels sprouts and related vegetables). CYP1A1 metabolizes a range of PAHs, including a large number of procarcinogens and promutagens (Table 3.4). Interindividual variations in the inducible expression of CYP1A1 might be related to genetic differences in aromatic hydrocarbon receptor (AhR) expression, which could explain differences in individual susceptibilities to cigarette smoke-induced lung cancer. Therefore, genetic factors appear to be important in the expression of the *CYP1A1* gene in humans and its involvement in human carcinogenesis. Women who smoke are at greater risk than men to develop lung cancer (adenocarcinoma) and chronic obstructive pulmonary disease.

The mechanism for induction of the *CYP1A1* gene begins with binding of the inducing agents to a cytosolic receptor protein, the AhR, which is then translocated to the nucleus where it binds to the DNA of the *CYP1A1* gene, thus enhancing the rate of transcription. The presence of the Ah receptor in hepatic and intestinal tissues can have implications beyond xenobiotic metabolism and can have a role in the induction of other genes for the control of cellular growth and differentiation. On the other hand, CYP1A1 can metabolize procarcinogens to hydroxylated inactive compounds that are not mutagenic.

CYP1A2

The CYP1A2 isoform (also known as phenacetin O-deethylase, caffeine demethylase, or antipyrine N-demethylase) is one of the major P450s in human liver metabolizing approximately 8% of the clinically important drugs (Fig. 3.3) and catalyzes the oxidation (and in some cases the bioactivation) of arylamines, nitrosamines and aromatic hydrocarbons, the bioactivation of promutagens and procarcinogens, estrogens and other substances (Table 3.4). CYP1A2 is expressed in the liver (13%) (Fig. 3.2), intestine, and stomach and is induced by smoking and polyaromatic hydrocarbons. CYP1A2 is primarily responsible for the activation of the carcinogen aflatoxin B1 found in contaminated peanuts. CYP1A2 is subject to reversible and/or irreversible inhibition by a number of drugs, natural substances, and other compounds. The CYP1A2 gene expresses a 515-residue protein with a molecular mass of 58.3 KDa.

The CYP1A2 structure exhibits a relatively compact and planar active site cavity that is highly adapted for the size and shape of its substrates. A large interindividual variability in the expression and activity of CYP1A2 has been observed, which is largely caused by genetic, epigenetic, and environmental factors (e.g., smoking). CYP1A2 is primarily regulated by the AhR. Induction or inhibition of CYP1A2 may provide a partial explanation for some clinical drug interactions. More than 15 variant alleles of the CYP1A2 gene have been identified and some of them have been associated with altered drug clearance and response and disease susceptibility. Evidence for polymorphism of this isoform has been reported, and it is likely that low CYP1A2 activity will be associated with altered susceptibility to the bioactivation of procarcinogens, promutagens, and other xenobiotic substrates. The expression of the CYP1A2 gene in the stomach becomes an important issue for gastric carcinogenesis, which is induced by smoking and by the metabolic activation of the procarcinogenic arylamines to mutagens.¹⁸

Family 2

The human CYP2 family comprises 16 isoforms, CYP2A6, CYP2A7, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP2R1, CYP2S1, CYP2U1, CYP2W1, 16 genes, and 16 pseudogenes. CYP2A7 is nonfunctional.

CYP2A6

CYP2A6 has a low level of hepatic expression and represents only approximately 4% of the total of hepatic P450 isoforms (Fig. 3.3). It is also expressed at low level in lung and nasal epithelium and exhibits high interindividual variability (polymorphism). Lung CYP2A6 bioactivates many tobacco-smoke-specific carcinogens, procarcinogens, and nitrosoamines. Mutant alleles of this isoenzyme have been associated with an elevated risk for small cell lung cancer. Hepatically, CYP2A6 is the major enzyme catalyzing the 7-hydroxylation of coumarin (coumarin 7-hydroxylase) and the C5 oxidation of nicotine to cotinine (nicotine C-oxidase) (Table 3.5). Other substrates include aflatoxin B1, naproxen, tacrine, clozapine, mexiletine, and cyclobenzaprineas.

TABLE 3.5
Some Substrates for CYP2A6

Acetaminophen	Nicotine
Cisapride	Omeprazole
Clomethiazole	Pilocarpine
Cyclophosphamide	Promazine
Disulfiram	Propofol
Efavirenz	Tamoxifen
EtodolacIfosfamide	Tretinoin
Methoxsalen	Zidovudine

Adapted from Di YM, Chow VD, Yang LP, et al. Structure, function, regulation and polymorphism of human cytochrome CYP2A6. *Curr Drug Metab.* 2009;10:754-780.

In vitro studies with human microsomes and expressed human CYP2A6 have shown that selegiline and its desmethyl metabolite are mechanism-based CYP2A6 inhibitors of nicotine metabolism, and inhibition of nicotine metabolism by selegiline could increase plasma nicotine in vivo. There is overlapping substrate specificity with CYP2A13. CYP2A6 exhibits polymorphism with an incidence of 2% in the Caucasian population, a population characterized as PMs. Smokers with a defective CYP2A6 gene smoke fewer cigarettes, implicating a genetic factor in nicotine dependence.

CYP2A13

CYP2A13 is expressed highest in nasal epithelium, followed by lung and trachea with high interindividual variability. CYP2A13 activates tobacco-smoke-specific carcinogens, procarcinogens, and nitrosoamines into DNA-altering compounds that cause lung cancer. Individuals with a high level of CYP2A13 expression could likely have an increased risk of developing tobacco-smoking-related lung cancers. Although CYP2A13 and CYP2A6 are 93.5% identical in amino acid sequence, CYP2A13 exhibits higher affinity for metabolizing tobacco smoke carcinogens than does CYP2A6. A comparison of the crystal structures of CYP2A13 and CYP2A6 has shown them to be very similar and, like CYP2A6, the CYP2A13 active site cavity is small and highly hydrophobic with a cluster of phenylalanine residues. Amino acid differences between CYP2A6 and CYP2A13 at key positions could be the cause of the significant variations observed in ligand binding and catalysis.

CYP2B6

The CYP2B6 subfamily has not been as fully characterized at the molecular level as other members of the human P450 family. This family represents less than 1% of the total of hepatic P450 isoforms (Fig. 3.2), and its level of expression in the liver is low. This enzyme exhibits polymorphic and ethnic differences in expression and therefore could be important in drug-drug interactions. CYP2B6 metabolizes approximately 3% of the clinically used drugs (Fig. 3.3) (Table 3.6). CYP2B6 also metabolizes arachidonic acid, lauric acid, 17 β -estradiol, estrone, ethinyl estradiol, and testosterone and can also bioactivate several procarcinogens and toxicants. CYP2B6 is closely regulated by the androstane receptor which can activate CYP2B6 expression upon ligand binding. Pregnane X receptor and glucocorticoid receptor also have a role in the regulation of CYP2B6.

TABLE 3.6
Some Substrates for Human Subfamily CYP2B6

Amitriptyline	Diclofenac	Methadone	Sevoflurane
Artemisinin	Diphenhydramine	Methoxyflurane	S-mephenytoin
Bupropion	Disulfiram	Mianserin	S-mephobarbital
Carbamazepine	Efavirenz	Nevirapine	Tamoxifen
Carbaryl	Fluoxetine	Nicotine	Temazepam
Clomethiazole	Haloperidol	Prasugrel	Thiotepa
Clopidogrel	Ifosfamide	Promazine	Tramadol
Cyclophosphamide	Imipramine	Propofol	Tretinoin
Desmethylselegiline	Ketamine	Selegiline	Valproic acid
Diazepam	Meperidine	Sertraline	Verapamil

Adapted from Mo SL, Liu YH, Duan W, et al. Substrate specificity, regulation, and polymorphism of human cytochrome CYP2B6. *Curr Drug Metab.* 2009;10:730-753.

Induction of CYP2B6 may partially explain some clinically relevant drug-drug interactions observed. For example, coadministered carbamazepine decreases the blood levels of bupropion. There is a wide interindividual variability in the expression and activity of CYP2B6. Such a large variability is the result of genetic polymorphisms and exposure to drugs that are inducers or inhibitors of CYP2B6. CYP2B6 substrates are by and large small nonplanar molecules, unionized or weakly basic, highly lipophilic with one or two hydrogen-bond acceptors, and frequently active in the CNS. Phenobarbital appears to induce its formation.

CYP2C

The CYP2C subfamily is the most complex subfamily, consisting of three isoforms, CYP2C8, CYP2C9, and CYP2C19, metabolizing approximately 25% of the clinically important drugs (Fig. 3.3) (Table 3.7). The CYP2C subfamily represents approximately 20% of the total of P450 isoforms in the liver (Fig. 3.2). CYP2C8 is expressed primarily in extrahepatic tissues (kidney, adrenal, brain, uterus, breast, ovary, and intestine) and metabolizes the tricyclic antidepressants, diazepam and verapamil. Its level of expression is less than that of the other CYP2C9 and CYP2C19 isoforms.

TABLE 3.7
Some Substrates and Reaction Type for Human Subfamily CYP2C^a

CYP2C8	CYP2C9	CYP2C9 (cont.)	CYP2C19
Amiodarone	Amitriptyline	Losartan (hydroxymethyl to carboxylic acid)	Amitriptyline
Amodiaquine	Bosentan	Mefenamic acid	Carisoprodol
Benzphetamine	Buprenorphine	Meloxicam	Cilostazol
Carbamazepine	Carvedilol	R-Mephenytoin	Citalopram
Docetaxel	Celecoxib	Mestranol	Clomipramine
Fluvastatin	Chlorpheniramine	Montelukast	Clopidogrel
Isotretinoin	Chloramphenicol	Naproxen	Cyclophosphamide
Paclitaxel	Clomipramine	Nateglinide	Desipramine
Phenytoin	Chlorpropamide	Omeprazole	Diazepam (N-demethylation)
Pioglitazone	Clopidogrel	Phenobarbital	Escitalopram
Repaglinide	Dapsone	Phenytoin (4'-hydroxylation)	Esomeprazole
Retinol	Desogestrel	Phenylbutazone (4-hydroxylation)	Formoterol
Rosiglitazone	Diclofenac (4'-hydroxylation)	Piroxicam	Hexobarbital
Tolbutamide	Diazepam	Prasugrel	Imipramine (N-demethylation)
Torsemide	Diphenhydramine	Ramelteon	Indomethacin
Verapamil	Donepezil	Rosiglitazone	Lansoprazole
Zopiclone	Dorzolamide	Rosuvastatin	Loratadine (N-descarboxylation)
	Dronabinol	Sildenafil	S-Mephenytoin (4'-hydroxylation)
	Eletriptan	Sulfamethoxazole	R-Mephenytoin (N-demethylation)
	Etodolac	Sulfapyrazole (aromatic hydroxylation)	R-Mephobarbital
	Fluoxetine	Suprofen	Moclobemide
	Flurbiprofen (4'-hydroxylation)	Tamoxifen	Nelfinavir
	Fluvastatin	Tenoxicam	Nilutamide
	Formoterol	Terbinafine	Omeprazole (hydroxylation)
	Glibenclamide	Tienilic acid (thiophene ring hydroxylation)	Pantoprazole
	Gliclazide	Tolbutamide (p-methyl-hydroxylation)	Pentamidine
	Glimepiride	Tolterodine	Phenobarbital
	Glipizide	Torsemide	Phenytoin (ring hydroxylation)
	Glyburide	Δ^1 -THC (7-hydroxylation)	Progesterone
	Halothane	Testosterone (16 α -hydroxylation)	Proguanil (cyclization)
	Hexobarbital (3'-hydroxylation)	Trimethadione	Propranolol (side chain hydroxylation)
	Ibuprofen (i-butylmethyl-hydroxylation)	Valdecocix	Rabeprazole
	Imipramine	Vardenafil	Teniposide
	Indomethacin	Valproic acid	Thioridazine
	Irbesartan	Valsartan	Tolbutamide
	Irinotecan	Voriconazole	Voriconazole
	Ketamine	S-warfarin (7'-hydroxylation)	R-Warfarin
	Lornoxicam	Zafirlukast	
		Zileuton	
		Zopiclone	

^a Drugs in bold italic have been reported to cause drug-drug interactions.

CYP2C9 metabolizes approximately 15% of clinically relevant drugs (Table 3.7). CYP2C9, a highly polymorphic isoform, also metabolizes endogenous compounds such as steroids, melatonin, retinoids, and arachidonic acid. Many CYP2C9 substrates are weak acids, but CYP2C9 has also the capacity to

metabolize un-ionized, highly lipophilic compounds. Ligand-based and homology models of CYP2C9 have been reported which have provided insights into how its substrates are bound to the active site of CYP2C9. The resolved crystal structure of CYP2C9 has confirmed the importance of substrate specificity and ligand orientation. CYP2C9 is subject to induction by rifampin, phenobarbital, and dexamethasone, indicating the involvement of pregnane X receptor, the androstane receptor and the glucocorticoid receptor in the regulation of CYP2C9.

A number of compounds inhibit CYP2C9, which could provide an explanation for some clinically important drug-drug interactions. Tienilic acid, suprofen, and silybin are mechanism-based inhibitors of CYP2C9. Given the importance of CYP2C9 in drug metabolism and the presence of polymorphisms, it is important to identify drugs as potential substrates, inducers, or inhibitors of CYP2C9.

CYP2C19 is found primarily in the liver and intestine. The expression of CYP2C19 in the liver is less than that of CYP2C9 and also exhibits polymorphism. Differences in the DNA sequence for the CYP2C gene changes the enzyme's ability to metabolize substrates (i.e., PM phenotype). Because of this genetic difference in expressing CYP2C isoforms, it is important to be aware of a person's race when prescribing and/or dispensing drugs that are commonly metabolized differently by different racial populations (see section Genetic Polymorphism). This allows pharmacists to be proactive in evaluating the potential risk to patients who may be of the PM phenotype by assessing their reaction to previously prescribed CYP2C substrates and encouraging communication if the patient experiences adverse reactions. CYP2C19 (*S*-mephenytoin hydroxylase) is the isoform associated with the 4'-hydroxylation of *S*-mephenytoin. The CYP2C subfamily is apparently not inducible in humans.

CYP2D6

CYP2D6 polymorphism is the most studied P450. This enzyme is responsible for at least 30 different drug oxidations, representing approximately 21% of clinically important drugs (Fig. 3.3). CYP2D6 is only 3% expressed in the liver and minimally expressed in the intestine, and it does not appear to be inducible. Because there might not be any other way to clear drugs metabolized by CYP2D6 from the system, PMs of CYP2D6 substrates can be at severe risk for adverse drug reactions or drug overdoses. This isoform metabolizes a wide variety of lipophilic amines (Table 3.8) and is probably the only P450 for which a charged or ion-pair interaction is important for substrate binding. It appears also to preferentially catalyze the hydroxylation of a single enantiomer (stereoselectivity) in the presence of enantiomeric mixtures.

TABLE 3.8
Some Substrates and Reaction Type for Human CYP2D6 Isoform^a

Alprenolol (4-Hydroxylation)	Dexfenfluramine	Lidocaine (3-hydroxylation)	Pindolol
<i>Amitriptyline</i> (10-hydroxylation)	<i>Dextromethorphan</i> (O-demethylation)	Maprotiline	Promethazine (ring hydroxylation, S-oxidation, N-demethylation)
Amphetamine	Diphenhydramine (N-demethylation, ring hydroxylation, cleavage ether bond)	<i>Meperidine</i>	<i>Propafenone</i> (4-hydroxylation)
Aripiprazole	Dolasetron (hydroxylation of indole ring)	<i>Methadone</i>	<i>Propoxyphene</i>
<i>Atomoxetine</i>	<i>Donepezil</i>	Methamphetamine	Propranolol (4'-hydroxylation)
Bufuralol (1'-hydroxylation)	<i>Doxepin</i>	Methoxyamphetamine (4-hydroxylation, N-demethylation)	Quetiapine
Bisoprolol	<i>Duloxetine</i>	Metoclopramide	Quinidine (hydroxylation)
Captopril	Encainide (N-demethylation, O-demethylation)	Metoprolol (O-demethylation)	Ranolazine
Carvedilol	Fenfluramine	<i>Mexiletine</i> (4-hydroxylation and methyl	Risperidone

		hydroxylation)	
Cevimeline	Fluphenazine	Minaprine	Ritonavir
Chlorpheniramine (N-demethylation, ring hydroxylation, deamination)	Fentanyl	Mirtazapine	Sertraline
Chlorpromazine	Flecainide (O-dealkylation)	Morphine	S-metoprolol
Chlorpropamide	Fluoxetine(N-dealkylation)	Nebivolol	Sparteine (N-oxidation)
Clemastine	Fluvoxamine	Nortriptyline (10-hydroxylation)	Tamoxifen
Clomipramine (hydroxylation)	Formoterol	Olanzapine	Thioridazine (aromatic hydroxylation)
Clozapine (aromatic hydroxylation)	Galantamine	Ondansetron (hydroxylation of indole ring)	Timolol (O-dealkylation)
Codeine (O-demethylation)	Guanoxan (6- and 7-hydroxylation)	Oxycodone (O-demethylation)	Tolterodine (2-hydroxylation)
Cyclobenzaprine	Haloperidol	Paroxetine	Tramadol (O-demethylation)
Darifenacin	Hydrocodone	Perhexiline (4'-hydroxylation)	Trazodone
Debrisoquine (4-hydroxylation)	Hydroxyzine (ring hydroxylation)	Perphenazine (aromatic hydroxylation)	Tripelennamine
Desipramine	Imipramine (2-hydroxylation)		Tropisetron (hydroxylation of indole ring)
	Indoramin (6-hydroxylation)		Venlafaxine

^aDrugs in bold italic have been reported to cause drug-drug interactions.

Quinidine is an inhibitor of CYP2D6 and its concurrent administration with CYP2D6 substrates results in increased blood levels and toxicities for these substrates. If the pharmacological action of the CYP2D6 substrate depends on the formation of active metabolites, 2D6 inhibition results in a lack of a therapeutic response. The competition of two substrates for CYP2D6 can prompt a number of clinical responses. For example, when substrates have unequal affinities for CYP2D6, the first-pass hepatic metabolism of a substrate (drug) with high affinity for CYP2D6 will inhibit binding of a second substrate which has a lower affinity for this enzyme. The result will be a rapid absorption of the second nonmetabolized substrate leading to a higher plasma concentration and to the increased potential for an adverse reaction or toxicity.

CYP2E1

Few drugs are metabolized by CYP2E1; however, this isoform has a major role in the metabolism of numerous halogenated hydrocarbons (including volatile general anesthetics) and of a range of low-molecular-weight organic compounds including dimethylformamide, acetonitrile, acetone, ethanol, and benzene, as well as in the activation of acetaminophen to its reactive metabolite, N-acetyl-p-benzoquinoneimine¹⁹ (Table 3.9). CYP2E1 is of high interest because of the oxidation of ethanol into its reactive products, acetaldehyde and 1-hydroxyethyl radicals, and its ability to activate low-molecular-weight products into electrophilic reactive metabolites which result in toxicity and carcinogenicity. This enzyme then oxidizes acetaldehyde produced from ethanol to acetic acid. This isoform is only 7% expressed in the liver (Fig. 3.2) and expressed also in the kidney, intestine, and lung.

Table 3.9
Some Substrates and Reaction Type for Human CYP2E1 Isoform^a

Acetaminophen (p-benzoquinoneimine)
Styrene (epoxidation)
Theophylline (8-oxidation)
Disulfiram
Halogenated hydrocarbons
Dehalogenation of chloroform, methylene chloride

Volatile anesthetics (fluorinated hydrocarbons)
Enflurane, halothane, methoxyflurane, sevoflurane, desflurane
Miscellaneous organic solvents
Ethanol (to acetaldehyde)
Glycerin
Dimethylformamide (N-demethylation)
Acetone
Diethylether
Benzene (hydroxylation)
Aniline (hydroxylation)
Acetonitrile (hydroxylation to cyanohydrin)
Pyridine (hydroxylation)

^aDrugs in bold italics have been reported to cause drug-drug interactions.

CYP2E1 is inducible under diverse pathophysiological conditions including diabetes, obesity, fasting, cancer, alcoholic liver disease, and nonalcoholic liver disease and by the chemical substances ethanol, isoniazid, and 4-methylpyrazole, among others (Table 3.9). Mitochondrial CYP2E1 induces the formation of reactive oxygen species (ROS) which is associated with oxidative stress, lipid peroxidation, and it augments ethanol-induced hepatocellular injury. CYP2E1 is also known as the microsomal ethanol-oxidizing system, benzene hydroxylase, or aniline hydroxylase. CYP2E1 is induced in alcoholics and there is a polymorphism associated with this isoform which is most common in Chinese people. This isoform appears also to be involved in smoking-induced cancer (c.f., CYP1A2). Most of the compounds that induce CYP2E1 are also substrates for this enzyme.

The induction of this enzyme in humans can cause enhanced susceptibility to the toxicity and carcinogenicity of CYP2E1 substrates. Some evidence shows interindividual variation in the in vitro liver expression of this isoform. The mechanism of induction appears to be a combination of an increase in CYP2E1 transcription, mRNA translation efficiency, and stabilization of CYP2E1 against proteolytic degradation. The induction of CYP2E1 resulting from ketosis (i.e., due to starvation, a high-fat diet, uncontrolled diabetes or obesity) or from exposure to alcoholic beverages or other xenobiotics can be detrimental to individuals simultaneously exposed to halogenated hydrocarbons (increased hepatotoxicity because of exposure to halothane or chloroform).²⁰ Chronic alcohol intake is known to enhance the hepatotoxicity of halogenated hydrocarbons. Testosterone appears to regulate CYP2E1 levels in the kidney, and pituitary growth hormone regulates hepatic levels of CYP2E1.

Family 3

The human CYP3 family comprises one subfamily, four isoforms, CYP3A4, CYP3A5, CYP3A7, CYP3A43, four genes, and two pseudogenes.

CYP3A4

The CYP3A subfamily includes the most abundantly expressed P450s in the human liver and intestine (extrahepatic metabolism) (Fig. 3.2). Although CYP3A4 is responsible for approximately two-thirds of

CYP3A-mediated drug metabolism, the other minor isoforms also (CYP3A5, CYP3A7, and CYP3A43) contribute.

Approximately one-third of the total CYP450 in the liver and two-thirds in the intestine are CYP3A4. This isoform is responsible for the metabolism of testosterone and more than one-third of clinically important drugs (Table 3.10) and is inhibited by a number of xenobiotics (Table 3.11). CYP3A4 appears also to activate aflatoxin B1 and, possibly, to metabolize benzo[a]pyrene. The interindividual differences reported for metabolism of nifedipine, cyclosporine, triazolam, and midazolam are probably related to changes in induction and not to polymorphism. CYP3A4 is expressed in the intestine, lung, placenta, kidney, uterus, and brain and is glucocorticoid-inducible.

TABLE 3.10
Substrates and Reaction Type for Human CYP3A4 Isoform^a

<i>Alfentanil</i>	Clomipramine	Dronabinol	Isradipine	<i>Odansetron</i>
Alfuzosin	Clonazepam	Dutasteride	(aromatization)	<i>Omeprazole</i>
Almotriptan	<i>Clopidogrel</i>	Efavirenz	<i>Itraconazole</i>	<i>Oral</i>
<i>Alprazolam</i>	Clozapine	<i>Eplerenone</i>	<i>Ketoconazole</i>	<i>contraceptives/progesti</i>
Amitriptyline	Cocaine	<i>Ergotamine</i>	Lansoprazole	Oxybutynin
Amiodarone (N-deethylation)	Codeine (N-demethylation)	<i>Erlotinib</i>	Letrozole	<i>Paclitaxel</i>
<i>Amlodipine</i>	Cyclophosphamide	<i>Erythromycin</i>	<i>Lidocaine</i> (N-deethylation)	Pantoprazole
Amprenavir	<i>Cyclosporine</i>	(N-demethylation)	Lopinavir	Pioglitazone
<i>Aprepitant</i>	Dapsone (N-oxide)	Esomeprazole	Loratadine	Prasugrel
Aripiprazole	<i>Darifenacin</i>	<i>Eszopiclone</i>	<i>Lovastatin</i> (6-hydroxylation)	Propranolol
Astemizole	Delavirdine	<i>Ethinyl estradiol</i>	<i>Methadone</i>	<i>Quetiapine</i>
<i>Atazanavir</i>	Desogestrel	<i>Ethosuximide</i>	<i>Midazolam</i> ^[1,1] _{SEP}	<i>Quinidine</i> (3-hydroxylation)
<i>Atorvastatin</i>	Dextromethorphan (N-demethylation)	Etonogestrel	(methyl hydroxylation)	Quinine
"Azole"	Diazepam (C7 hydroxylation)	<i>Etoposide</i>	<i>Mifepristone</i>	Rabeprazole
antifungals	<i>Dihydroergotamine</i>	Exemestane	<i>Mirtazapine</i>	<i>Ramelteon</i>
Bepidil	<i>Disopyramide</i>	<i>Felodipine</i>	<i>Modafinil</i>	<i>Ranolazine</i>
Bromocriptine	<i>Diltiazem</i> (N-deethylation)	<i>Fentanyl</i>	<i>Mometasone</i>	Repaglinide
Budesonide	Docetaxel	Fexofenadine	<i>Montelukast</i>	Rifampin, <i>rifabutin</i> ^[1,1] _{SEP}
Buprenorphine	Dofetilide	Finasteride	<i>Nateglinide</i>	and related compounds
Buspirone	Dolasetron (N-oxide)	Flutamide	<i>Nelfinavir</i>	<i>Ritonavir</i>
Cafegot	Domperidone	<i>Fluticasone</i>	Nevirapine	Salmeterol
Caffeine	Donepezil	Galantamine	<i>Nicardipine</i>	<i>Saquinavir</i>
Cannabinoids	Doxorubicin	Gleevec	(aromatization)	<i>Sertraline</i>
<i>Carbamazepine</i>		Haloperidol	<i>Nifedipine</i>	Sibutramine
(epoxidation)		Hydrocodone	<i>Nisoldipine</i>	<i>Sildenafil</i>
Cevimeline		Imatinib	Nitrendipine	<i>Simvastatin</i>
Chlorpheniramine		Imipramine (N-demethylation)	Norethindrone	<i>Sirolimus</i>
Cilostazol		<i>Indinavir</i>		
Citopram		Irinotecan		
Clarithromycin				
Clindamycin				

^aDrugs in bold italic have been reported to cause drug-drug interactions.

Table 3.11
Cytochrome P450 Inhibitors^a

CYP1A2	CYP2B6	CYP2C8	CYP2C19	CYP2C9	CYP2D6	CYP2E1	CYP3A4
Amiodarone	Amiodarone	Anastrozole	Cimetidine	<i>Amiodarone</i>	<i>Amiodarone</i>	Diethyl dithiocarbamate	<i>Amiodarone</i>
Atazanavir	Amlodipine	<i>Gemfibrozil</i>	Citalopram	Atazanavir	Bupropion	Disulfiram	<i>Amiodarone</i>
<i>Cimetidine</i>	Azelastine	Glitazones	Delavirdine	<i>Cimetidine</i>	Celecoxib		<i>Apr</i>
<i>Ciprofloxacin</i>	Citalopram	Montelukast	Efavirenz	Clopidogrel	Chloroquine		<i>Ata</i>
Citalopram	Clotrimazole	Nicardipine	Felbamate	Cotrimoxazole	Chlorpheniramine		<i>Cim</i>
<i>Clarithromycin</i>	Desipramine	Sulfapyrazole	Fluconazole	Delavirdine	Chlorpromazine		Cip

CYP1A2	CYP2B6	CYP2C8	CYP2C19	CYP2C9	CYP2D6	CYP2E1	CYP
Diltiazem	Disulfiram	Trimethoprim	Fluoxetine	Disulfiram	Cimetidine		Clai
Enoxacin	Doxorubicin		Fluvastatin	Efavirenz	Cinacalcet		Cyc
Erythromycin	Ethinyl estradiol		Fluvoxamine	Fenofibrate	Citalopram		Dan
Ethinyl Estradiol	Fluoxetine		Indomethacin	Fluconazole	Clemastine		Delu
Fluoroquinolones	Fluvoxamine		Isoniazid	Fluorouracil	Clomipramine		Diet dith
Fluvoxamine	Isoflurane		Ketoconazole	Fluoxetine	Cocaine		Dilt
Interferon	Ketoconazole		Lansoprazole	Fluvastatin	Darifenacin		Efa
Isoniazid	Mestranol		Modafinil	Fluvoxamine	Desipramine		Ery
Ketoconazole	Methimazole		Omeprazole	Gemfibrozil	Diphenhydramine		Ethi
Methoxsalen	Miconazole		Oxcarbazepine	Imatinib	Doxepin		Fluc
Mibefradil	Nelfinavir		Probenecid		Doxorubicin		Fluc
	Orphenadrine		Ticlopidine	Itraconazole	Duloxetine		Flu
	Paroxetine		Topiramate	Ketoconazole	Escitalopram		Ges
	Sertraline			Leflunomide	Fluoxetine		Ima
	Sorafenib			Lovastatin	Fluphenazine		Indi
	Tamoxifen			Methoxsalen	Halofantrine		Ison
	Ticlopidine			Metronidazole	Haloperidol		Itra
	Thiotepa			Mexiletine	Hydroxychloroquine		Keti
	Venlafaxine			Modafinil	Hydroxyzine		Met
				Nalidixic acid	Imatinib		Met
				Norethindrone	Levomopromazine		Mib
				Norfloxacin	Methadone		Mic
				Omeprazole	Metoclopramide		Mifi
				Oral contraceptives	Mibefradil		Neli
				Paroxetine	Midodrine		Nici
				Phenylbutazone	Moclobemide		Nife
				Probenecid	Norfluoxetine		Nor
				Sertraline	Paroxetine		Nor
				Sulfamethoxazole	Perphenazine		Nor
				Sulfaphenazole	Propafenone		Oxi
				Sulfonamides	Propoxyphene		Prec
				Tacrine	Propranolol		Qui
				Teniposide	Quinacrine		Ran
				Ticlopidine	Quinidine		Riti
				Tipranavir	Ranitidine		Rox
				Troleandomycin	Ranolazine		Saq
				Voriconazole	Ritonavir		Sert
				Zafirlukast	Sertraline		Teli
				Zileuton	Terbinafine		Troi
					Thioridazine		Veri
					Ticlopidine		Vor
					Tipranavir		Zafi
					Tripeleminamine		Zile
							Zoli

Data from *Stockley's Drug Interactions: A Source Book of Interactions, Their Mechanisms, Clinical Importance and Management*. 11th ed. London and Chicago: Pharmaceutical Press; 2016.

^aP450 isoform inhibitors presented in bold italics have been associated with drug-drug interactions of clinical relevance or with drug-drug interaction warnings that can require dosage adjustment.

Binding to CYP3A4 is predominantly lipophilic. Drugs known to be substrates for CYP3A4 have low and variable oral bioavailabilities that might be explained by prehepatic metabolism by a combination of intestinal CYP3A4 and P-glycoprotein (P-gp) in the enterocytes of the intestinal wall (see section Presystemic First-Pass Metabolism). Therefore, it is the expression and function of CYP3A4 that governs the rate and extent of metabolism of the substrates for the CYP3A subfamily. The induction of the CYP3A subfamily by phenobarbital in humans could ultimately be responsible for many of the well-documented interactions between barbiturates and other drugs.^{14,15}

CYP3A5 is the best studied of the minor CYP3A isoforms, with 85% sequence identity with CYP3A4. Approximately 20% of human livers express this isoform. The expression of CYP3A5 shows ethnic differences, with the wild-type *CYP3A5*1* allele more common in Africans than in Caucasians or Asians. In individuals who express CYP3A5, 17%-50% of the total hepatic CYP3A is this isoform. Additionally, CYP3A5 is also expressed in a range of extrahepatic tissues (kidney, small intestine, lung, adrenal glands) and is inducible via the pregnane X receptor. Both CYP3A4 and CYP3A5 exhibit significant overlap in substrate specificity but can differ in catalytic activity and regioselectivity. Results from a comparison of CYP3A4 and CYP3A5 enzyme kinetics indicate that CYP3A5 has different enzymatic characteristics than CYP3A4 in some CYP3A-catalyzed reactions. The enzyme kinetics of CYP3A5 suggests faster substrate turnover than that observed with CYP3A4.^{6,7}

CYP3A7 is predominantly expressed in fetal liver (approximately 50% of total fetal CYP450 enzymes) but is also found in some adult livers and extrahepatically. CYP3A7 has a specific role in the hydroxylation of retinoic acid, 16 α -hydroxylation of steroids, and hydroxylation of allylic and benzylic carbons, and, therefore, it is relevant in both normal development and in carcinogenesis.

Family 4

The human CYP450 4 family consists of 6 subfamilies and 13 CYP450 4 proteins, of which the CYP4A, 4B and 4F subfamilies have been most studied. The CYP4A subfamily (fatty acid hydroxylases) catalyzes the hydroxylation of the terminal ω -carbon and, to a lesser extent, the ω -1 position of saturated and unsaturated fatty acids, as well as the ω -hydroxylation of various prostaglandins. The *CYP4A1*, *A2*, and *A3* genes are expressed mostly in the liver and kidney and their expression is induced by a class of chemicals known as peroxisome proliferators. Induction of CYP4A expression by fibrate antihyperlipidemic agents is due to transcriptional activation, mediated possibly via a peroxisome proliferator-activated receptor (PPAR). CYP4A gene expression is hormonally regulated. There is a close association between microsomal CYP4A1 induction, peroxisome proliferation, and induction of the peroxisomal fatty acid metabolizing system.

The CYP4A subfamily is involved in the metabolism of eicosanoic acids (e.g., arachidonic acid) leading to the formation of physiologically important metabolites involved in processes such as blood flow in the kidney, cornea, and brain. CYP4B1 is a major bioactivating factor catalyzing the formation of reactive metabolites, such as the pneumotoxin and 4-ipomeanol. The CYP4F subfamily consists of seven proteins, ω -hydroxylases which function as eicosanoid regulators. This family is responsible for inactivating the vascular effects of leukotrienes, omega-3 fatty acids, and tocopherols (vitamin E analogues).

Clearly, no one animal model or combination of animal models represents the metabolic capabilities of humans. By having a complete understanding of the factors (e.g., inducers, inhibitors, and effects of disease states) that alter the expression and activity of an enzyme responsible for the metabolism of a particular compound, and by a determination of responsible isoforms and patient phenotyping, it might be possible to predict drug-drug interactions and metabolic clearance.

An alphabetical listing of the clinically important drugs and the CYP450 isoforms catalyzing their oxidative metabolism is presented in [Table 3.12](#).

TABLE 3.12
Some Substrates for the P450 Isoforms Catalyzing Their Metabolism

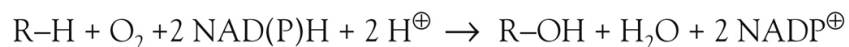
Acetaminophen	1A2, 2E1, 3A4, 2C9	Caffeine	1A2
Albendazole	3A4, 1A2	Cannabinoids	3A4
Alfentanil	3A4	Carbamazepine	2C8, 3A4, 2B6
Alprazolam	3A4	Carisoprodol	2C19
Amiodarone	3A4, 2C9	Carvedilol	2C9, 2D6
Amitriptyline	1A2, 2C9, 2D6, 3A4, 2C19	Celecoxib	2C9
Amlodipine	3A4	Cevimeline	2D6

Amphetamine	2D6	Chlordiazepoxide	1A2
Aripiprazole	2D6, 3A4	Chloroquine	3A4
Anastrozole	3A4	Chlorpromazine	2D6, 3A4
Astemizole	3A4	Chlorzoxazone	2E1
Atomoxetine	2D6	Cimetidine	3A4
Atorvastatin	3A4	Cisapride	3A4
Bepridil	3A4	Citalopram	2C19, 3A4
Bisoprolol	2D6	Clarithromycin	3A4
Bosentan	2C9	Clindamycin	3A4
Bupropion	2C9, 2B6, 2A6, 1A2	Clomipramine	1A2, 2C9, 2C19, 2D6, 3A4
Busulfan	3A4	Clopidogrel	2C19, 2C9, 1A2, 3A4/5
Clonazepam	3A4	Estrogens, oral	3A4
Clozapine	1A2, 2D6, 2C19, 3A4	Ethanol	2E1
Cocaine	3A4	Ethinyl estradiol	3A4
Codeine	2D6, 3A4	Etodolac	2C9
Cyclobenzaprine	1A2, 2A6, 2D6, 3A4	Etoposide	3A4
Cyclophosphamide	2B6, 2C19, 3A4	Felodipine	3A4
Cyclosporine	3A4	Fenfluramine	2D6
Dapsone	2C9, 3A4	Fentanyl	2D6, 3A4
Delavirdine	3A4	Fexofenadine	3A4
Desipramine	1A2, 2C19, 2D6	Finasteride	3A4
Desogestrel	2C9	Flecainide	2D6
Dexamethasone	3A4	Fluconazole	3A4
Dexfenfluramine	2D6	Flurbiprofen	2C9
Dextromethorphan	2D6, 3A4	Fluoxetine	2C9, 2D6, 2B6
Diazepam	1A2, 2C19, 2C9, 3A4	Fluvastatin	2C8, 2C9
Diclofenac	2C8, 2C9	Fluphenazine	2D6
Diltiazem	3A4	Flutamide	1A2, 3A4
Diphenhydramine	2C9	Fluvoxamine	1A2, 2D6
Disopyramide	3A4	Formoterol	2C9, 2C19, 2D6
Divalproex sodium	2C19	Galantamine	2D6
Docetaxel	2C8, 3A4	Glimepiride	2C9
Dolasetron	2D6, 3A4	Glipizide	2C9
Donepezil	2D6, 3A4, 2C9	Glyburide	2C9, 3A4
Dorazolamide	2C9	Granisetron	3A4
Doxepin	2D6	Halofantrine	3A4
Doxorubicin	3A4	Haloperidol	1A2, 2D6
Dronabinol	2C9	Halothane	2E1, 2C9
Efavirenz	2B6	Hydrocodone	2D6, 3A4
Eletriptan	2C9	Hydrocortisone	2D6, 3A4
Enalapril	3A4	Ibuprofen	2C9
Encainide	2D6	Ifosfamide	2B6, 3A4
Enflurane	2E1	Imipramine	1A2, 2C19, 2C9, 2D6, 3A4
Ergot alkaloids	3A4	Indinavir	2D6, 3A4
Erythromycin	3A4	Indomethacin	2C9, 2C19
Esomeprazole	2C19	Irbesartan	2C9
Estradiol	1A2	Isoflurane	2E1, 2B6
Isotretinoin (retinoids)	1A2, 2C8, 3A4	Nelfinavir	2B6, 3A4
Isradipine	3A4	Nevirapine	3A4
Itraconazole	3A4	Nicardipine	3A4
Ketamine	2B6, 2C9	Nicotine	2A6, 2B6, 2A13
Ketoconazole	3A4	Nifedipine	3A4/5
Labetalol	2D6	Nilutamide	2C19
Lansoprazole	2C19, 3A4	Nimodipine	3A4
Lidocaine	2D6, 3A4	Nisoldipine	3A4
Leflunomide	1A2, 2C9, 2C19	Nitrendipine	3A4
Losartan	2C9, 3A4	Nortriptyline	1A2, 2D6
Lovastatin	3A4	Olanzapine	1A2, 2D6, 3A4
Maprotiline	2D6	Omeprazole	2C19, 2C9, 3A4
Moclobemide	2C19	Ondansetron	1A2, 2D6, 2E1, 3A4
Mefenamic acid	2C9	Oral contraceptives	3A4

Mefloquine	3A4	Oxycodone	2D6
Meloxicam	2C9	Paclitaxel	2C8, 3A4
Meperidine	2D6, 2B6	Pantoprazole	2C19
Mephenytoin	2C19	Paroxetine	2D6
Mephobarbital	2C9, 2B6	Perphenazine	2D6
Mestranol	2C9	Phenol	2E1
Methadone	1A2, 2D6, 2B6	Phenobarbital	2B6, 2C9
Methamphetamine	2D6	Phenytoin	2C19, 2C8, 2C9
Metoprolol	2D6	Pimozide	3A4
Mexiletine	1A6, 2D6, 2A6	Pindolol	2D6, 3A4
Mibefradil	3A4	Pioglitazone	2C8, 3A4
Miconazole	3A4	Piroxicam	2C18, 2C9
Midazolam	3A4	Prasugrel	3A4, 2B6, 2C9
Mirtazapine	1A2, 2D6, 3A4	Pravastatin	3A4
Modafinil	3A4	Praziquantel	2B6, 3A4
Montelukast	2C9	Prednisone	3A4
Morphine	2D6	Progesterone	3A4, 2C19
Naproxen	1A2, 2C18, 2C9, 2A6	Proguanil	2C18, 2C19
Nateglinide	2C9	Propafenone	1A2, 2D6, 3A4
Navelbine	3A4	Propofol	2B6, 2C9
Nefazodone	3A4	Propoxyphene	2D6
Propranolol	1A2, 2C18, 2C19, 2D6	Terfenadine	3A4
Quetiapine	3A4	Testosterone	3A4
Quinidine	3A4	Δ^9 -THC	2C9
Quinine	3A4	Thiabendazole	1A2
Rabeprazole	2C19	Timolol	2D6
Rapaglinide	2C8	Tolbutamide	2C8, 2C9, 2C19
Retinoic acid	2C8	Tolterodine	2D6, 2C9
Rifabutin	3A4	Torsemide	2C9
Rifampin	3A4	Tramadol	2D6
Riluzole	1A2	Trazodone	2D6
Risperidone	2D6, 3A4	Tretinoin	2C8, 3A4
Ritonavir	2A6, 2C19, 2C9, 2D6, 2E1, 3A4	Triazolam	3A4
Ropinirole	1A2	Troleandomycin	3A4
Ropivacaine	1A2, 2D6	Tropisetron	2D6
Rosiglitazone	2C8, 2C9	Valsartan	2C9
Rosuvastatin	2C9	Valproic acid	2C9, 2A6, 2B6
Salmeterol	3A4	Valdecocib	2C9
Saquinavir	3A4	Vardenafil	3A4
Selegiline	2D6, 2B6, 2A6	Venlafaxine	2D6
Sertindole	2D6	Verapamil	1A2, 3A4, 2C8
Sertraline	2D6, 2C19, 3A4, 2B6	Vinblastine	3A4
Sevoflurane	2E1	Vincristine	3A4
Sildenafil	2C9, 3A4	Voriconazole	2C9
Simvastatin	3A4	Warfarin	2C18, 2C9
Sufentanil	3A4	R-warfarin	1A2
Suprofen	2C9	S-warfarin	2C9, 2C18
Sulfamethoxazole	2C9	Yohimbine	2D6
Tacrine	1A2, 2A6	Zafirlukast	2C9
Tacrolimus	3A4	Zaleplon	3A4
Tamoxifen	1A2, 2A6, 2B6, 2D6, 2E1, 3A4	Zileuton	1A2, 2C9, 3A4
Temazepam	3A4	Zolpidem	3A4
Teniposide	3A4, 2C19	Zopiclone	2C8, 2C9, 3A4
Terbinafine	2C9		

Oxygen Activation

Elemental oxygen (dioxygen) is a relatively unreactive form of oxygen that exists as an unpaired diradical in the triplet form. Dioxygen activation by P450s requires the sequential addition of two single electrons to the P450 from NAD(P)H through the redox partners.



The function of CYP450 monooxygenases is mostly hydroxylation of a substrate. A reactive radical-like iron oxenoid intermediate is generated that is reactive enough to split aliphatic $\text{C}=\text{C}$ H bonds, add to $\text{C}=\text{C}$ H bonds α to heteroatoms, or remove single electrons from heteroatoms to produce radical-cations. The reactive oxygen intermediate has been spectroscopically observed.^{20,21}

Catalytic Cycle of Cytochrome P450: Steps of the Catalytic Cycle

Many variant CYP450 isoforms that have been isolated show a remarkable uniformity for the catalytic mechanism. The net C-H hydroxylation reaction is an insertion of oxygen with a ferric-oxenoid complex into a $\text{C}=\text{C}$ H bond to produce an alcohol or phenol. The ease with which P450 enzymes perform hydroxylation in unreactive substrates such as hydrocarbons indicates a highly reactive enzyme intermediate capable of mediating such chemistry. A major determinant in the regioselectivity of hydroxylation is the hydrogen atom abstraction and the stability of the radical intermediate being formed.^{10,20,21}

The current view illustrating the cyclic (“wheel”) mechanism for reduction and oxygenation of P450 with stepwise interactions with substrate molecules, electron donors, and oxygen is shown in Figure 3.4 and can be summarized as follows.^{21,22}

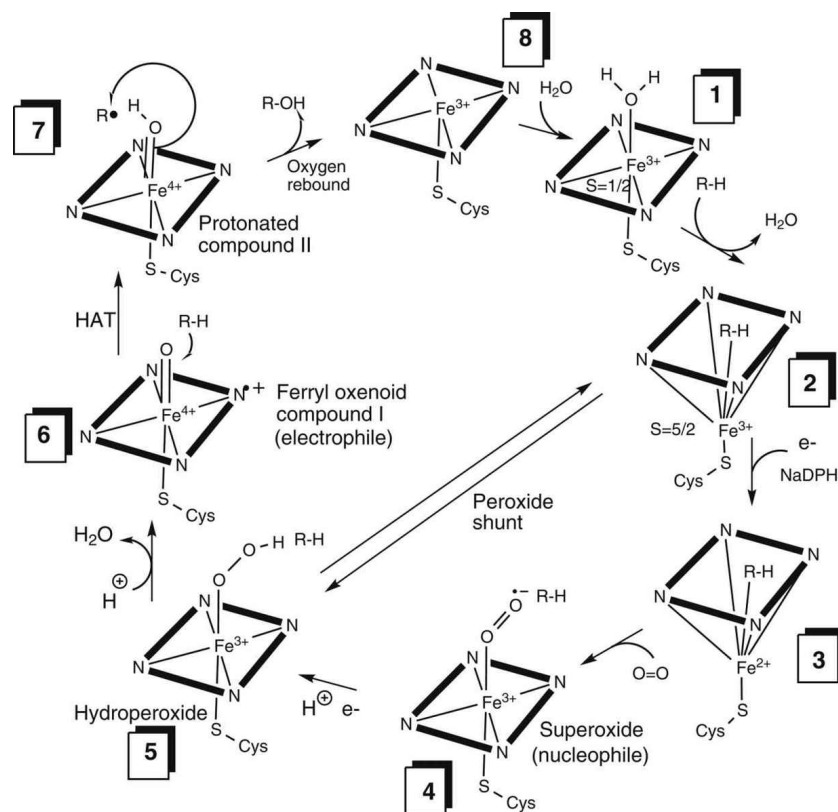


FIGURE 3.4 Generalized cyclic mechanism for CYP450.

The catalytic P450 cycle

Step 1. The resting $[\text{Fe}^{3+}\text{-P450}]$ complex binds reversibly with a molecule of the substrate (RH) displacing the distal water resulting in a complex resembling enzyme-substrate complex $[\text{Fe}^{3+}\text{-P450*RH}]$. The binding of the substrate triggers/facilitates the first one-electron reduction step from NADPH.

Step 2. The substrate complex of $[\text{Fe}^{3+}\text{-P450*RH}]$ undergoes reduction to a $[\text{Fe}^{2+}\text{-P450*RH}]$ substrate complex by an electron originating from its redox partner by the flavoprotein [NADPH-P450 reductase FNMH₂/FADH complex].

Step 3. The reduced $[\text{Fe}^{2+}\text{-P450*RH}]$ substrate complex readily binds dioxygen as the sixth ligand of Fe^{2+} to form a $[\text{dioxy-Fe}^{2+}\text{-P450*RH}]$ substrate complex.

Step 4. The $[\text{dioxy-Fe}^{2+}\text{-P450*RH}]$ complex rearranges by resonance because of the strong electronegativity of O_2 to form the $[\text{Fe}^{3+}\text{-P450*RH-superoxide anion } \text{O}_2^{\cdot-}]_{\text{SEP}}^{\text{L}}$ complex.

Step 5. The $[\text{Fe}^{3+}\text{-P450*RH-superoxide}]$ complex undergoes further reduction by accepting a second electron from NADPH-P450 reductase to form the equivalent of a two-electron reduced $[\text{peroxy-Fe}^{3+}\text{-P450*RH}]$ (hydroperoxide anion) complex (the step where O_2 is split into an oxygen atom). If the electron is not delivered rapidly, this complex dissociates and is aborted (uncoupled) from subsequent substrate hydroxylation at this step by xenobiotics which can cause release of superoxide, which decomposes to hydrogen peroxide and dioxygen with regeneration of the starting point of the cycle, the $\text{Fe}^{3+}\text{-P450}$ complex.

Step 6. The unstable $[\text{Fe}^{3+}\text{-peroxy-P450*RH}]$ complex undergoes heterolytic cleavage of peroxide anion upon protonation to form water and a highly electrophilic porphyrin-radical cation intermediate (ferryl oxenoid species, $\text{Fe}^{4+}=\text{O}$) (Compound I, the more favorable oxygen-cysteine-porphyrin radical cation resonance-stabilized complex). The $\text{Fe}^{4+}=\text{O}$ species represents the catalytically active oxygenation species. One role of the cysteine sulfur ligand is thought to be electron donation (push) that weakens the peroxide O-O bond, causing peroxide bond scission to produce a highly reactive and strong oxidizing intermediate (Compound I).

Step 7. Abstraction of a hydrogen atom (HAT) from the substrate (RH) by the electrophilic $[\text{Fe}^{4+}=\text{O*RH}]_{\text{SEP}}^{\text{L}}$ species to produce either a carbon-centered radical, radical addition to a π -bond, or single electron transfer (SET) from a heteroatom to form a heteroatom-centered radical-cation ferryl intermediate. Subsequent radical recombination with the ferric-bound hydroxyl radical $[\text{Fe}^{4+}\text{*OH}]$ (step 7/8, oxygen rebound) or single electron-transfer (SET, deprotonation) yields the carbon oxidized hydroxylated product (Compound II) and the regeneration of the initial $\text{Fe}^{3+}\text{-P450}$ enzyme complex (Fig. 3.5).^{23,24}

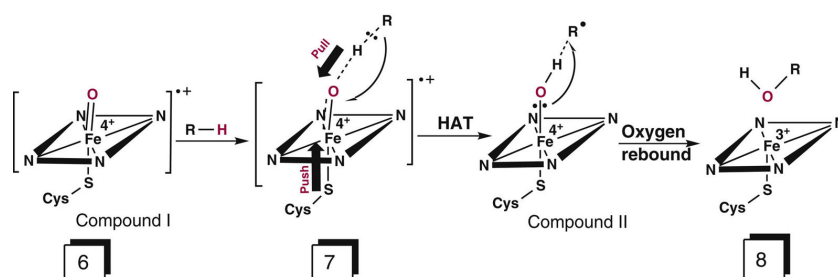


FIGURE 3.5 Oxygen rebound: a two step mechanism: the electrophilic ferryl oxenoid species $[\text{Fe}^{4+}=\text{O}]$ abstracts a hydrogen

(step 7) from the substrate (R-H) to give a carbon radical intermediate ($\text{R} \text{C}=\text{C}$) that recombines with the ferric-bound hydroxyl radical $[\text{Fe}^{4+}\text{*OH}]$ (step 7/8) to give the final hydroxyl product.

Until the final step, the oxidizable substrate has been an inactive spectator in the chemical events of oxygen activation. None of the peroxide or superoxide intermediates in the cycle are sufficiently reactive to abstract hydrogen from the substrate. The ferryl oxenoid complex (Step 7) is a good hydrogen abstractor, even for relatively inert terminal methyl groups on hydrocarbon chains. The ferric-oxenoid complex exhibits regioselectivity in its choice of hydrogen atoms, balancing stability of the resulting carbon radical with stereochemical constraints. The reactivity of C-H abstraction depends on carbon radical stability. Some of the factors that stabilize carbon radicals include neighboring carbon atoms, neighboring carbon-carbon multiple bonds, and neighboring heteroatoms with lone pairs of electrons, e.g., oxygen, nitrogen. Carbon radicals are electron deficient and are seeking electrons and therefore are stabilized by nearby electron-donating groups. Because the inert aliphatic region of the substrate has been converted to a highly reactive radical, the process is described as substrate activation.²⁵

Oxidations Catalyzed by Cytochrome P450 Isoforms

Aliphatic and Alicyclic Hydroxylations

The accepted mechanism of hydroxylation of alkane $\text{[SEP]} \text{C}=\text{C} \text{H}$ bonds is shown in Figure 3.6 and has been reviewed in detail elsewhere.²⁵⁻²⁷ The principal metabolic pathway of the methyl group is oxidation to the hydroxymethyl derivative followed by microsomal oxidation to the carboxylic acid (e.g., tolbutamide) [SEP] (Fig. 3.7). On the other hand, some methyl groups are oxidized only to the hydroxymethyl derivative, without further oxidation to the acid. Where there are several equivalent methyl groups, as a rule only one methyl group is oxidized. In the case of aromatic methyl groups, the para-methyl is the most vulnerable because it is less sterically hindered.

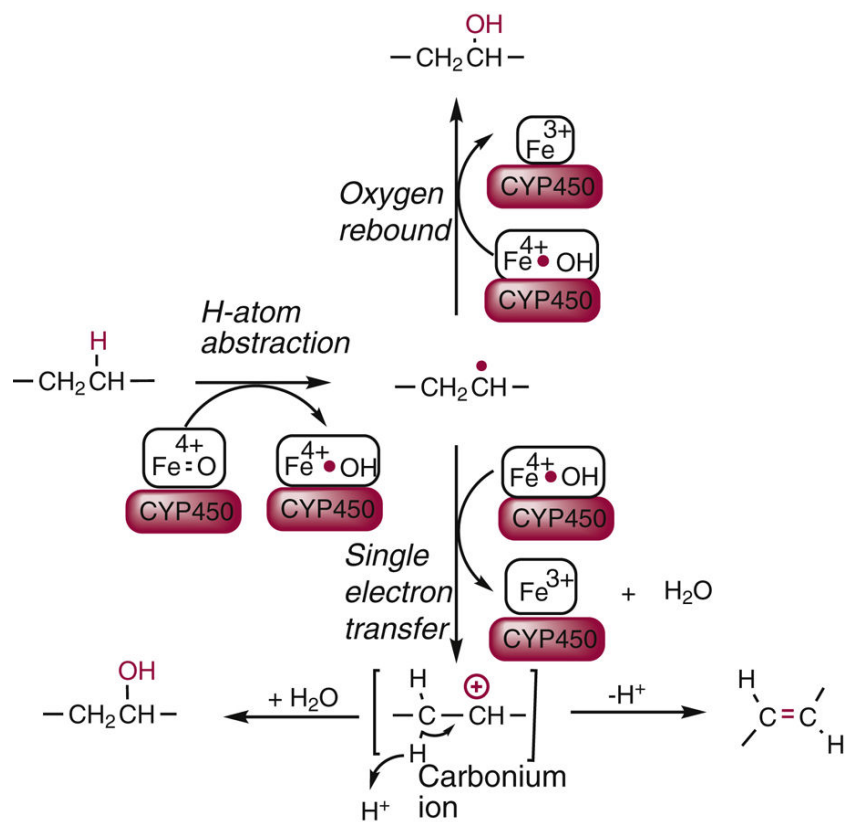


FIGURE 3.6 Proposed mechanisms for the hydroxylation and dehydrogenation of alkanes.

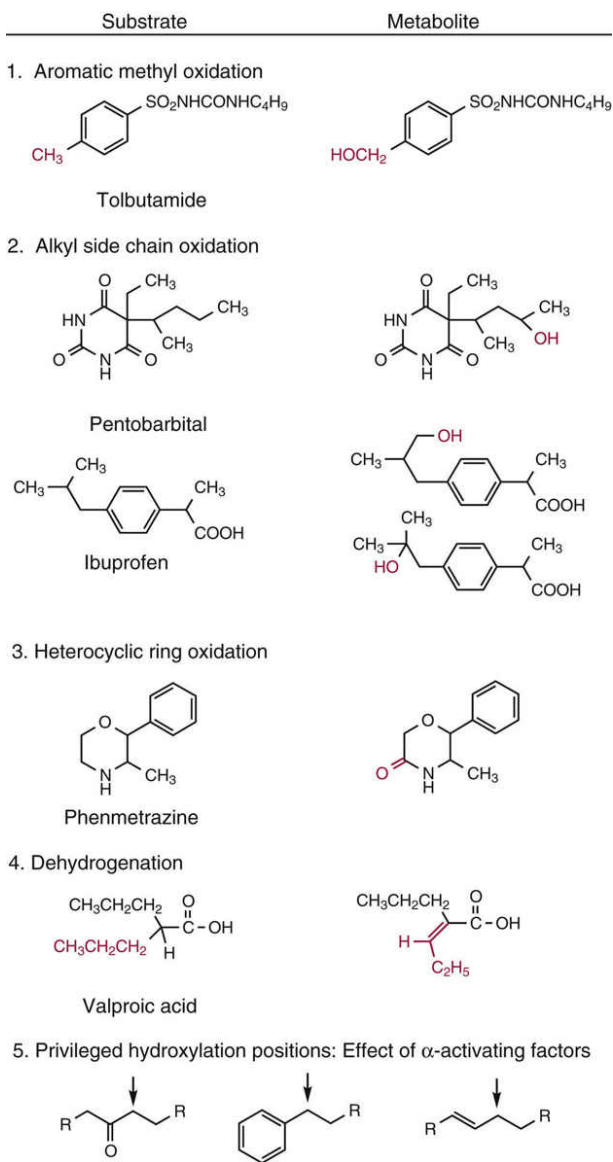


FIGURE 3.7 Examples of oxidative metabolism of aliphatic and alicyclic hydrocarbons catalyzed by CYP450.

Alkyl side chains exhibit regioselectivity and are often hydroxylated on the terminal or penultimate (secondary) carbon atoms (e.g., pentobarbital) (Fig. 3.7). The isopropyl group is an interesting side chain which is hydroxylated at the tertiary carbon and at either of the equivalent methyl groups (e.g., ibuprofen) (Fig. 3.7). Hydroxylation of alkyl side chains attached to an aromatic ring does not follow the general rules for alkyl side chains, because the aromatic ring activates the α -position of hydroxylation (Fig. 3.7). Oxidation occurs preferentially on the benzylic methylene group and, to a lesser extent, at other positions on the side chain.

The methylene groups of an alicycle are readily hydroxylated, usually at the least hindered position, or at an activated position; for example, α to a carbonyl (cyclohexanone), α to a double bond (cyclohexene), or α to a phenyl ring (tetralin). The products of hydroxylation often show regioselectivity and stereoisomerism. Nonaromatic heterocycles normally undergo oxidation at the α -carbon adjacent to the heteroatom (e.g., phenmetrazine) (Fig. 3.7).²⁷

In addition to hydroxylation reactions, P450s can catalyze the dehydrogenation of an alkane to an alkene (olefin). The reaction is thought to involve formation of a carbon radical and electron transfer to

the ferryl complex of CYP450, which produces a carbocation that is deprotonated to a dehydrogenated product alkene (Fig. 3.7).²⁷⁻²⁹ An example of the ability of CYP450 to function as both a dehydrogenase and a monooxygenase has been demonstrated with the antiseizure drug valproic acid. Whereas the major metabolic products in humans are formed by β -oxidation and Phase 2 acyl glucuronidation, several alkenes are also formed, including (*E*)-2-ene isomer (Fig. 3.7).²⁶ Presumably, the CYP3A subfamily catalyzes these reactions. The factors which determine whether CYP450 catalyzes hydroxylation (oxygen rebound/recombination) or dehydrogenation (electron transfer) remain unknown, but hydroxylation is usually favored. In some instances, the product of dehydrogenation can be the primary product (e.g., 6,7-dehydrogenation of testosterone).

Alkene and Alkyne Hydroxylation

The proposed mechanism for the oxidation of π -bonds in alkenes is a stepwise sequence of one-electron transfers between the radical complex and the ferryl oxenoid intermediate [$\text{Fe}^{4+}=\text{O}$], leading to alkene oxidation (Fig. 3.8).²⁷ Following the initial formation of an unsaturated CYP450 π -complex, the one-electron transfer either (1) yields a radical σ -complex which can collapse either to an arene or to an alkene epoxide (step a or d, Fig. 3.8), (2) undergoes a 1,2-group migration to form a carbonyl product (steps a and b, Fig. 3.8), or (3) produces a vinyl hydroxylated product (step c, Fig. 3.8) or a σ -complex which can break down to a phenol (step e, Fig. 3.8). The presence of a hydroxyl radical in the porphyrin ring allows some substrate radicals to covalently bond by N-alkylation of a pyrrole nitrogen rather than recombination with $(\text{Fe}-\text{OH})^{+3}$ radical. This deviation from the normal course of reaction explains the suicide inhibition exhibited by some xenobiotics, such as the oral contraceptives, erythromycin, and paroxetine.^{26,27}

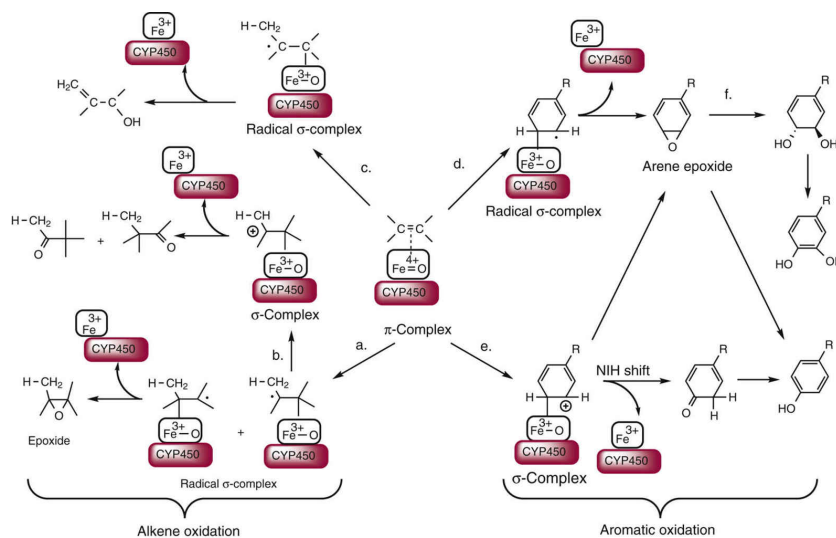


FIGURE 3.8 Proposed mechanisms for the oxidation of alkene and aromatic compounds.

The oxidation of alkenes yields primarily epoxides and a series of products derived from 1,2-migration (Fig. 3.8). The stereochemical configuration of the alkene is retained during epoxidation. The epoxides can differ in reactivity. Those that are highly reactive undergo either pH-catalyzed hydrolysis to excretable vicinal dihydrodiols or covalent reactions (alkylation) with macromolecules such as proteins or nucleic acids, which lead to tissue necrosis or carcinogenicity. Moreover, the ubiquitous epoxide hydrolase can catalyze the rapid hydrolysis of epoxides to nontoxic vicinal dihydrodiols. Several drugs (carbamazepine, cyproheptadine, and protriptyline), however, were found to form stable epoxides at the 10,11-position during biotransformation (Fig. 3.9). The fact that these epoxides could be detected in

the urine indicates that these oxides are not particularly reactive and should not readily react covalently with macromolecules.

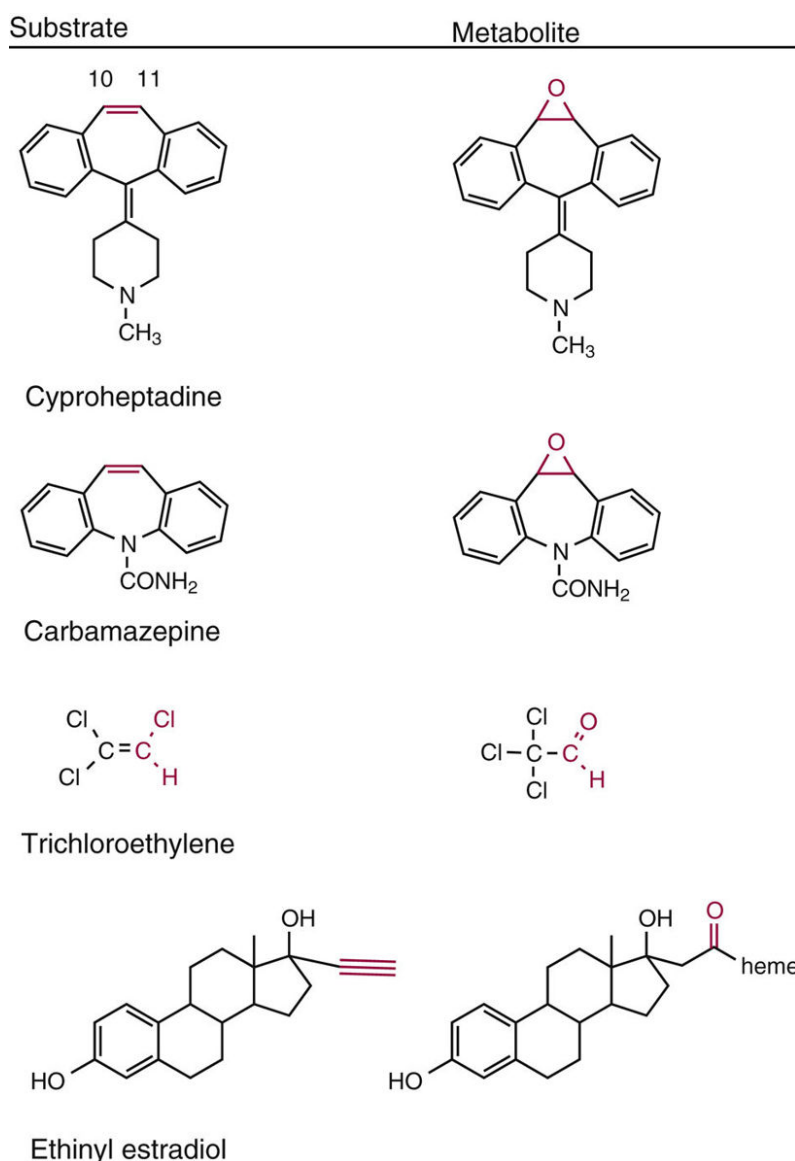


FIGURE 3.9 Examples of oxidative metabolism of alkenes and alkynes catalyzed by CYP450.

The epoxidation of terminal alkenes is accompanied by the mechanism-based (“suicide”) N-alkylation of the heme-porphyrin ring. If the π -complex attaches to the alkene at the internal carbon, the terminal carbon of the double bond can irreversibly N-alkylate the pyrrole nitrogen of the porphyrin ring.²⁹ The heme adduct formation is observed mostly with monosubstituted, unconjugated alkenes (i.e., 17 α -ethylenic steroids and 4-ene metabolite of valproic acid) (Fig. 3.9).

In addition to the formation of epoxides, heme adducts, and hydroxylated products, carbonyl products are also created. These latter products result from the migration of atoms to adjacent carbons (i.e., 1,2-group migration). For example, during the P450-catalyzed oxidation of trichloroethylene, a 1,2-shift of chloride occurs to yield chloral (Fig. 3.9).

Like the alkenes, alkynes (acetylenes) are readily oxidized, but usually faster.²⁹ Depending on which of the two alkyne carbons are attacked, different products are obtained. If attachment of P450 occurs on the terminal alkyne carbon, a R2 group migrates, forming a ketene intermediate that readily hydrolyzes with water to form an acid or that alkylates nucleophilic protein side chains (i.e., lysinyl or cysteinyl) to form a protein adduct (Fig. 3.10). The effect of attaching the ferryl oxygen at the internal alkenyl carbon is N-alkylation of a pyrrole nitrogen in the porphyrin ring by the terminal acetylene carbon, with the formation of a keto heme adduct (Fig. 3.11). This mechanism has been proposed for the irreversible inactivation of CYP3A4 with 17 α -alkenyl steroids (i.e., 17 α -ethinyl estradiol).

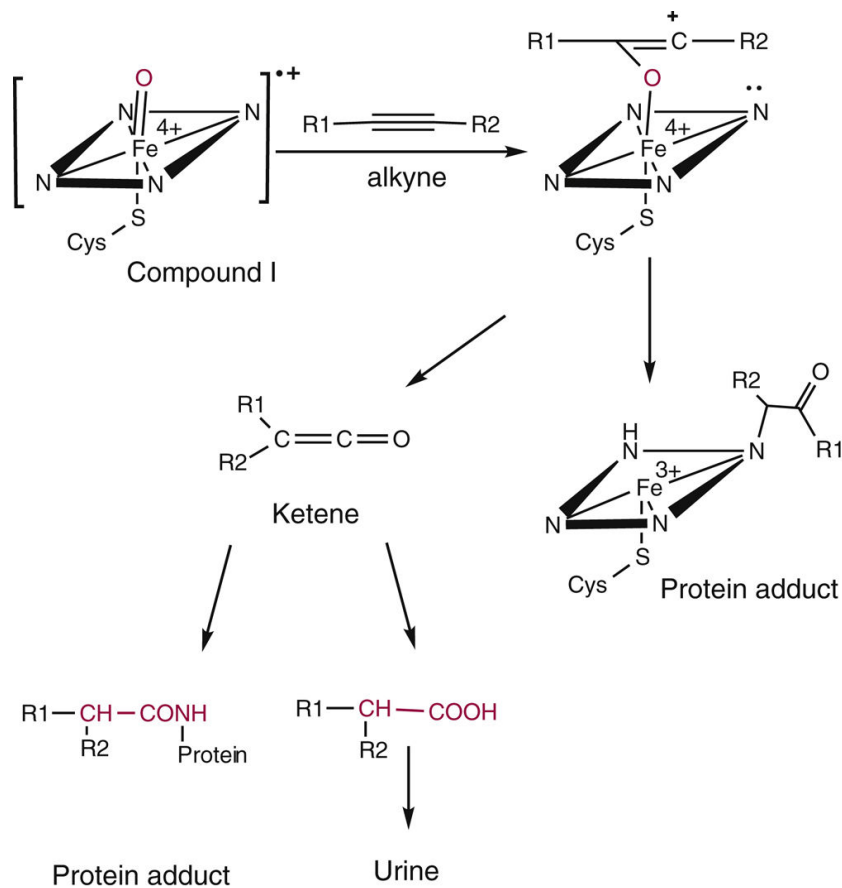


FIGURE 3.10 Alkyne oxidation catalyzed by CYP450.

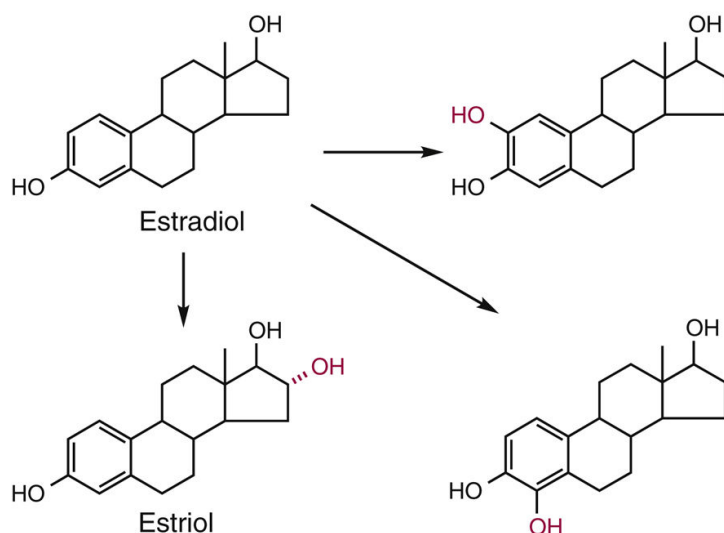
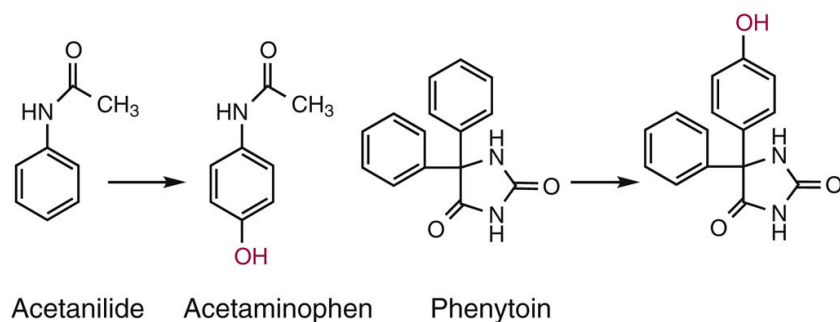


FIGURE 3.11 Examples of oxidative metabolism of aryl compounds catalyzed by CYP450.

Aromatic Hydroxylation

In the case of aromatic oxidations (Fig. 3.8),^{6,25,26} following the initial formation of an arene CYP450 π -complex, one-electron transfer yields either a π -complex or a radical σ -complex. The radical σ -complex can collapse to the arene epoxide (Fig. 3.8, step d) or the π -complex can proceed to a σ -complex followed by a NIH shift (1,2-group migration) to a phenolic product (Fig. 3.8, step e). Arene oxides are highly unstable entities and rearrange (NIH shift) nonenzymatically to phenols or hydrolyze enzymatically with epoxide hydrolase to 1,2-dihydrodiols (*trans* configuration) (Fig. 3.8, step f), which subsequently are dehydrogenated to 1,2-diphenols (catechols). The oxidation of aromatic compounds can be highly specific to individual CYP450 isoforms, suggesting that substrate binding and orientation at the active site can dominate the mechanism of oxidative catalysis.

The metabolic oxidation of aromatic carbon atoms by P450 depends on both the isoform catalyzing the oxidation and the oxidation potential of the aromatic compound. The products are typically phenolic and the position of hydroxylation can be activated/deactivated by the type of substituents on the ring, according to the theories of aromatic electrophilic substitution (Fig. 3.8). For example, electron-donating substituents enhance *o*- and *p*-hydroxylation, whereas electron-withdrawing substituents reduce or prevent *o*- or *p*-hydroxylation. Moreover, steric factors must also be considered, since oxidation occurs mostly at the least hindered position. For monosubstituted benzene compounds, *p*-hydroxylation frequently predominates, with some ortho product also formed (Fig. 3.11). When more than one phenyl ring is present, only one ring is typically hydroxylated (e.g., phenytoin).

Traditionally, hydroxylation of aromatic compounds by P450 has been thought to be mediated by an arene oxide (epoxide) intermediate, followed by the NIH shift as discussed previously^{6,26} (Fig. 3.8). The formation of phenols and the isolation of urinary dihydrodiols, catechols, and Phase 2 glutathione conjugates (mercapturic acid derivatives), implicate arene oxides as intermediates in the metabolism of both benzene and substituted benzenes. Arene oxides are also prone to conjugate with glutathione to form premercapturic acids (see section Glutathione Conjugation and Mercapturic Acid Synthesis).

The CYP1A and CYP3A subfamilies are important contributors to 2- and 4-hydroxylation of estradiol, and CYP3A4 is an important contributor to 2-hydroxylation of the synthetic estrogens (e.g., 17 α -ethinyl estradiol). The principal metabolite (as much as 50%) for estradiol is 2-hydroxyestradiol, with 4-hydroxy and 16 α -hydroxyestradiol as minor metabolites (Fig. 3.11). The 2-hydroxy metabolite of both estradiol and ethinyl estradiol have limited or no estrogenic activity, whereas the C4 and C16 α -hydroxy metabolites have a potency similar to that of estradiol. In humans, 16 α -hydroxyestradiol (estriol) is the primary estrogen metabolite both in pregnancy and in breast cancer. The metabolites 16 α -hydroxyestrone and 4-hydroxyestrone can be carcinogenic in specific cells since they are capable of damaging cellular proteins and DNA after activation to quinone intermediates.

Xenobiotic-metabolizing enzymes not only detoxify xenobiotics but also cause the formation of active intermediates (bioactivation), which in certain circumstances can elicit a multitude of toxicities, including mutagenesis, carcinogenesis, and hepatic necrosis.^{29,30} In addition to glutathione, some nucleophiles such as other sulfhydryl compounds (most effective), alcohols, and phosphates can react with arene oxides. Many of these nucleophiles are found in proteins and nucleic acids. The covalent binding of these bioactive epoxides to intracellular macromolecules provides a molecular basis of these toxic effects (see the discussion of toxicity due to oxidative metabolism).

N-Dealkylation, Oxidative Deamination, and N-Oxidation

The accepted mechanism of oxidative N-, O-, and S-dealkylations involves two competing mechanisms: single electron transfer (SET) or hydrogen atom abstraction (HAT) (Fig. 3.12).^{6,25,26} Heteroatom-containing substrates most commonly undergo hydroxylation adjacent (α) to the heteroatom, as compared to other positions. Reactions of this type include N-, O-, and S-dealkylations as well as dehydrohalogenations and oxidative deamination reactions. The SET pathway involves abstraction of an electron from the heteroatom to produce a radical cation, while the HAT pathway involves hydrogen abstraction. Both pathways are followed by the loss of the α -proton from the more labile α carbon to generate a carbon radical which can recombine with the ferric hydroxyl radical intermediate (oxygen rebound) to generate an unstable geminal hydroxy heteroatom-substituted intermediate (e.g., carbinolamine, halohydrin, hemiacetal, hemiketal, or hemithioketal) that breaks down, releasing the dealkylated heteroatom and forming a carbonyl compound. The resultant carbon radical is stabilized by the heteroatom. However, if the recombination reaction with the ferric hydroxyl radical intermediate (oxygen rebound) is faster than the deprotonation of the α -proton from the adjacent α -carbon, the oxidation will simply result in oxidation of the heteroatom, as in the formation of N-oxides or oxidation of sulfides to sulfoxides and/or sulfones. Therefore, whether N-dealkylation metabolism occurs by HAT or SET depends on the substrate.

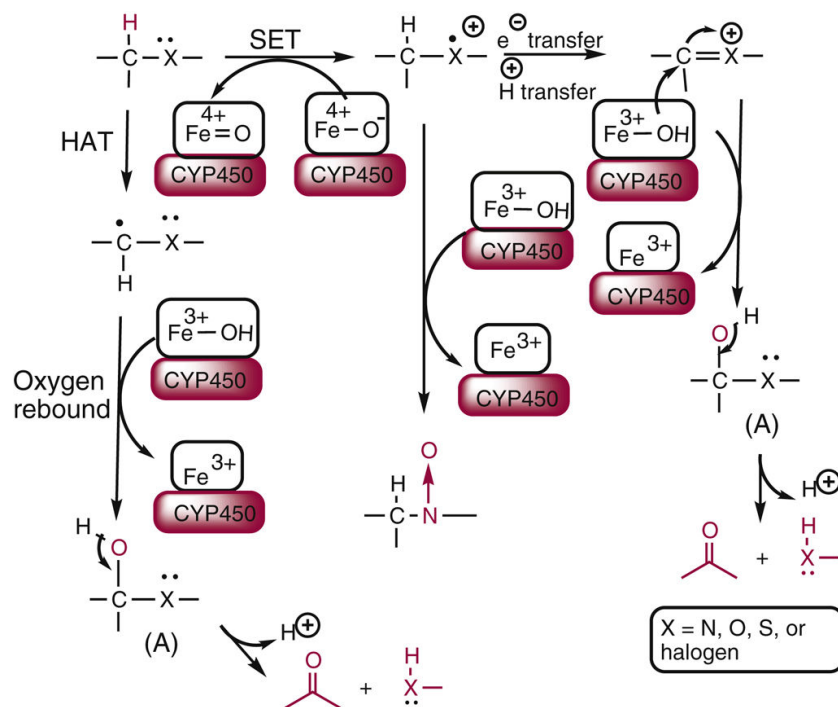


FIGURE 3.12 Proposed mechanism for heteroatom-compound oxidation, dealkylation, and dehalogenation.

Xenobiotics containing heteroatoms (N and S) can also be metabolized by heteroatom oxidation to their corresponding heteroatom oxides (tertiary amines to N-oxides, sulfides to sulfoxides and/or sulfones). Heteroatom oxidation can also be attributed to a microsomal flavin-containing monooxygenase (FMO). As is the case with heteroatom α -hydroxylation, one-electron oxidation of the heteroatom occurs as the first step to form the heteroatom ferric-hydroxide radical intermediate. This reaction is favored by the absence of α -hydrogens and by the stability of the heteroatom radical-cation, which cannot lose a proton, and collapses to generate the heteroatom oxide.

N-Dealkylation.

Dealkylation of secondary and tertiary amines to yield primary and secondary amines, respectively, is one of the most important and frequently encountered reactions in drug metabolism.^{25,26} The proposed mechanism of oxidative N-dealkylation involves α -hydrogen abstraction or an electron abstraction from the nitrogen by the ferryl oxenoid and has been previously discussed (Fig. 3.12).

Typical N-substituents removed by oxidative dealkylation are methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, allyl, and benzyl. Dealkylation occurs most readily with a smaller alkyl group. Substituents that are more resistant to dealkylation include *t*-butyl (no α -hydrogen) and cyclopropylmethyl. In general, tertiary amines are dealkylated to secondary amines faster than secondary amines are dealkylated to primary amines. This rate difference has been correlated with lipid solubility. Appreciable amounts of secondary and primary amines accumulate as metabolites which are more polar than the parent amine, thus slowing the rate at which the amines diffuse across membranes and reducing their accessibility to receptors. Frequently, these amine metabolites contribute to the pharmacological activity of the parent substance (e.g., imipramine) (Fig. 3.13), or they produce unwanted side effects, such as hypertension that results from the N-dealkylation of N-isopropylmethoxamine to methoxamine. The design of an analogous drug without these unwanted metabolites can be achieved by proper choice of replacement substituents, such as substitution of the N-isopropyl group in N-isopropylmethoxamine with a tert-butyl (N-tert-butylmethoxamine or butoxamine).

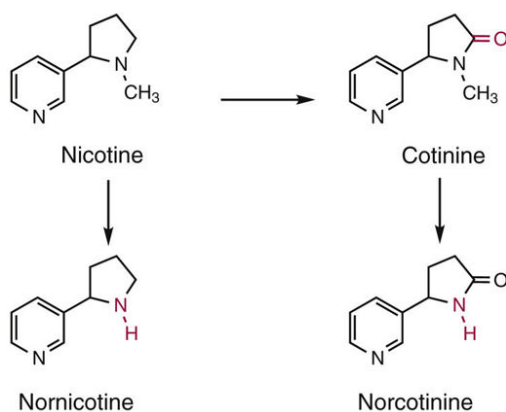
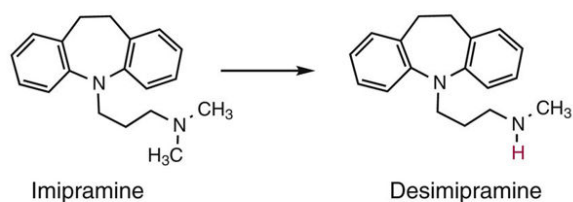
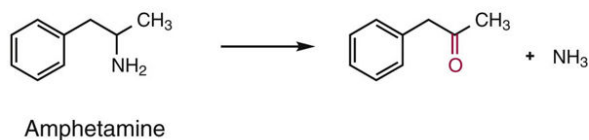
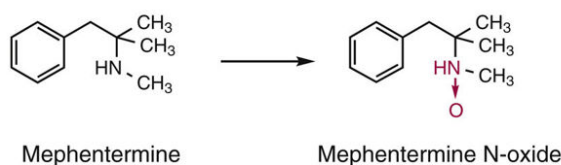
N-dealkylation:**N-deamination:****N-oxidation:**

FIGURE 3.13 Examples of N-dealkylation, oxidative deamination, and N-oxidation reactions catalyzed by CYP450.

N-dealkylation of substituted amides and aromatic amines occurs in a similar manner to aliphatic amines. N-substituted nonaromatic nitrogen heterocycles undergo oxidation on the α -carbon to a lactam (e.g., nicotine to cotinine) as well as N-dealkylation (e.g., nicotine to nornicotine, cotinine, and norcotinine) (Fig. 3.13).

Oxidative Deamination

The mechanism of oxidative deamination follows a pathway similar to that of N-dealkylation. Initially, oxidation to the imminium ion occurs, followed by decomposition to the carbonyl metabolite and ammonia. Oxidative deamination can occur with α -substituted amines, exemplified by amphetamine (Fig. 3.13). Disubstitution of the α -carbon inhibits deamination (e.g., phentermine). Some secondary and tertiary amines, as well as amines substituted with bulky groups, can undergo deamination directly without N-dealkylation (e.g., fenfluramine). Apparently, this behavior is associated with increased lipid solubility.

N-Oxidation.

In general, N-oxygenation of amines forms stable N-oxides with tertiary amines and amides and hydroxylamines with primary and secondary amines. This reaction predominates when no α -protons are available (e.g., mephentermine and arylamines) (Fig. 3.13). Tertiary amines having one or more hydrogens on the adjacent carbon dealkylate via the N-oxide. Rearrangement of the N-oxide forms a carbinolamine, which subsequently collapses to produce the secondary amine. Amine metabolites can be N-conjugated, increasing their rate of excretion.

O- and S-Dealkylation.

Oxidative O-dealkylation of ethers is a common metabolic reaction with a mechanism analogous to N-dealkylation; oxidation of the α -carbon and subsequent decomposition of the unstable hemiacetal to an alcohol (or phenol) and a carbonyl product. S-dealkylation of thioethers to hemithioacetals is a minor pathway in comparison to the major direct CYP450 oxidation of sulfur to sulfoxides and/or sulfones.

The majority of ether groups in drug molecules are aromatic ethers (e.g., codeine, prazosin, and verapamil). For example, codeine is O-demethylated to morphine (Fig. 3.14). The rate of O-dealkylation is a function of chain length (i.e., increasing chain length or branching reduces the rate of dealkylation). Steric factors and aromatic ring substituents influence the rate of dealkylation but are complicated by electronic effects. Some drug molecules contain more than one ether group, in which case, only one ether is dealkylated. The methylenedioxy group undergoes variable rates of dealkylation to the 1,2-diphenolic metabolite. Metabolism of such a group is also being capable of forming a stable complex with (and inhibiting) CYP450.

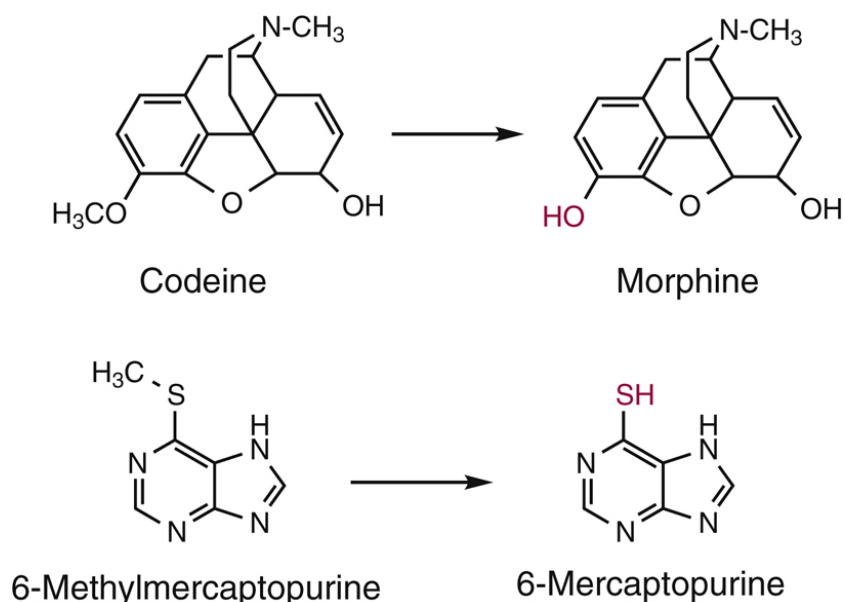


FIGURE 3.14 Examples of O- and S-dealkylations catalyzed by CYP450.

By analogy to O- and N-dealkylations, esters and amides can also be oxidized by CYP450. In these reactions, the carbon next to the ether oxygen of an ester or the nitrogen of an amide (termed the α -carbon) is attacked by the enzyme. This reaction is minor due to the significantly more common hydrolysis of esters and amides.

Aliphatic and aromatic methyl thioethers undergo S-dealkylation to thiols and carbonyl compounds. For example, 6-methylthiopurine is demethylated to give the active anticancer drug 6-mercaptopurine (Fig. 3.14). Other thioethers are oxidized to sulfoxides or sulfones (see N- and S-oxidations).

Oxidative Dehalogenation

Many halogenated hydrocarbons, such as insecticides, pesticides, general anesthetics, plasticizers, flame retardants, and commercial solvents, undergo a variety of different dehalogenation biotransformations.^{31,32} Because of our potential exposure to these halogenated compounds as drugs and environmental pollutants in air, soil, water, or food, it is important to understand the interactions between metabolism and toxicity.

When the carbon bearing the halogen also contains a hydrogen, carbon-centered hydroxylation (C-OH) occurs. The hydroxylated products are unstable and react with loss of the halogen to give an aldehyde or a ketone. Halogenated hydrocarbons differ in their chemical reactivity because of the electron-withdrawing properties of the halogens on adjacent carbon atoms, resulting in the α -carbon developing electrophilic character that promotes carbon-centered oxidation (C-OH). The halogen atoms also have the ability to stabilize α -carbon cations, free radicals, carbanions, and carbenes. If other halogens are also present in the molecule, then acyl halides result (Fig. 3.12). Acyl halides can react with macromolecules (protein) and cause hepatotoxicity or immunogenicity. These acyl halides have been implicated in the toxicity of chloroform and halothane. Some halogenated hydrocarbons form glutathione or mercapturic acid conjugates, whereas others undergo oxidative dehydrohalogenation (elimination of hydrogen halide) and reductive dehalogenation catalyzed by CYP2E1. In many cases, reactive intermediates, including radicals, anions, and cations, are produced that can react with a variety of molecules in tissues, resulting in hepatotoxicity or immunogenicity.

Oxidative dehydrohalogenation is a common metabolic pathway of many halogenated hydrocarbons. P450-catalyzed oxidative dehydrohalogenation generates the transient *gem*-halohydrin (analogous to alkane hydroxylation) that can eliminate the hydrohalic acid to form carbonyl derivatives (aldehydes, ketones, acyl halides, and carbonyl halides) (Fig. 3.15). This reaction requires the presence of at least one halogen and one α -hydrogen. *gem*-Trihalogenated hydrocarbons are more readily oxidized than are the *gem*-dihalogenated and monohalogenated compounds. The acyl and carbonyl halides formed are reactive metabolites that can react either with water to form carboxylic acids or nonenzymatically with tissue molecules (with a potential for eliciting increased toxicity).

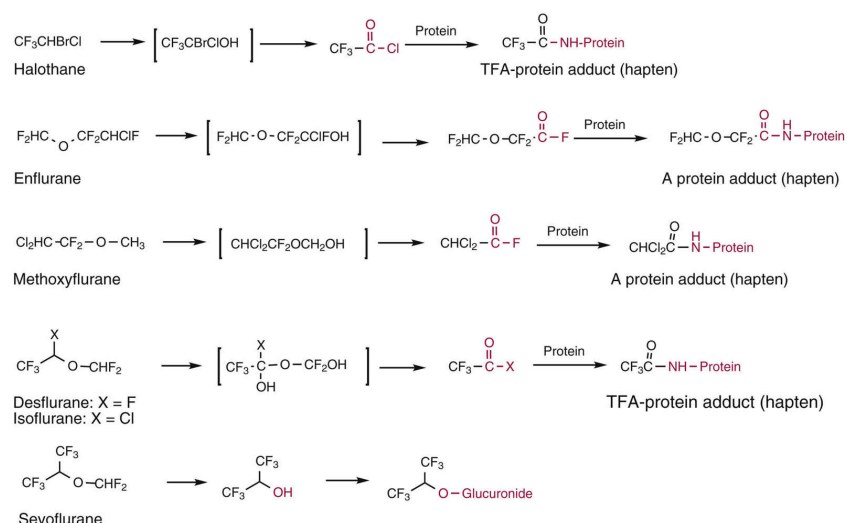


FIGURE 3.15 CYP2E1-catalyzed metabolism of fluorinated volatile anesthetics to antigenic proteins.

Oxidative dehydrohalogenation leading to significant hepatotoxicity and nephrotoxicity is seen with the fluorinated inhalation anesthetics halothane, enflurane, isoflurane, desflurane, and methoxyflurane, but not sevoflurane^{31,32} (Fig. 3.15). The fluranes are metabolized by CYP2E1 to reactive intermediates that produce trifluoroacetylated immunogenic protein adducts. The severity of hepatotoxicity is associated

with the degree of hepatic metabolism. The hepatotoxicity of halothane and the other fluranes is related to their metabolism to either an acid chloride (or fluoride) or a trifluoroacetate intermediate.^{31,32} The hydroxylated intermediate decomposes spontaneously to reactive intermediates, an acid chloride (or fluoride) or trifluoroacetate, that can either react with water to form halide anions and a fluorinated carboxylic acid or bind covalently to tissue proteins to produce an acylated protein. The acylated protein becomes a “hapten,” stimulating an immune response and a hypersensitivity reaction.

Halothane has received the most attention because of its ability to cause “halothane-associated” hepatitis. This immunologic reaction occurs after repeated exposure in surgical patients to trifluoroacetyl protein. After subsequent exposure to a fluorinated anesthetic, the antigenic trifluoroacetyl protein stimulates an immunogenic response, producing halothane-like hepatitis. For this reason, halothane is no longer used in general anesthesia in humans.

Because of the common metabolic pathway involving CYP2E1 for enflurane, isoflurane, desflurane, and methoxyflurane, halothane-exposed patients who have halothane hepatitis can show cross-sensitization to one of the other fluranes, triggering an idiosyncratic hepatotoxicity. The formation of antigenic protein is related to the amount of CYP2E1-catalyzed metabolism of each agent: halothane (20%-40%) > enflurane (2%-8%) > isoflurane (0.2%-1.0%) > desflurane (<0.1%).^{31,32} Enough fluoride ion is generated from oxidative dehalogenation during flurane anesthesia to produce subclinical nephrotoxicity. For patients with preexisting liver dysfunction, isoflurane, desflurane, or sevoflurane may be better choices of general anesthetic.

Unlike halothane and the other halogenated anesthetics, sevoflurane is not significantly metabolized to hepatotoxic trifluoroacetylated proteins but, rather, is metabolized by O-dealkylation to hexafluoroisopropanol and readily eliminated as the glucuronide. Approximately 90% of administered sevoflurane is eliminated intact in the patient’s expired air. The glucuronide is mostly excreted in urine within 12 hours after anesthesia, whereas trifluoroacetic acid is detectable in urine for up to 12 days after anesthesia.

In today’s environment, most humans have been exposed to many CYP2E1-inducing agents (including recreational, industrial, and agricultural chemicals and alcohol). This exposure has an unknown effect on hepatic toxicity from administered volatile anesthetics. Enhanced activity of CYP2E1 has been observed in obesity, isoniazid therapy, ketogenic diets, and alcoholism.

Azo and Nitro Reduction

In addition to the oxidative systems, liver microsomes also contain enzyme systems that catalyze the reduction of azo and nitro compounds to primary amines. A number of azo compounds, such as prontosil and sulfasalazine (Fig. 3.16) are converted to aromatic primary amines by azoreductase, an NADPH-dependent enzyme system in the liver microsomes. Evidence exists for the participation of CYP450 in some reductions. Nitro compounds (e.g., chloramphenicol and nitrobenzene) are reduced to aromatic primary amines by a nitroreductase, presumably through nitrosamine and hydroxylamine intermediates. These reductases are not solely responsible of reduction of azo and nitro compounds; reduction by the bacterial flora in the anaerobic environment of the intestine can also occur.

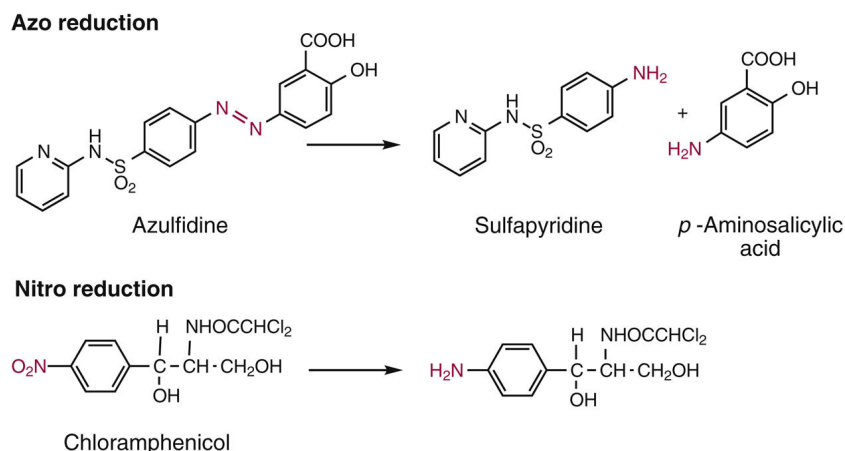


FIGURE 3.16 CYP450-catalyzed reduction of azo and nitro compounds.

In summary, all known oxidative reactions catalyzed by CYP450 monooxygenases can be described in the context of a mechanistic scheme which involves the ability of a high-valent ferric iron oxenoid species to bring about the stepwise one-electron oxidation through the abstraction of hydrogen atoms, abstraction of electrons from heteroatoms, or addition to π -bonds. A series of radical recombination reactions completes the oxidation process.

Induction and Inhibition of Cytochrome P450 Isoforms

Induction

Many of the enzymes involved in drug metabolism can be upregulated by exposure to drugs and environmental chemicals or by other coingested/inhaled compounds. This leads to increased rates of drug metabolism, thereby altering pharmacological and toxicological effects.³³⁻³⁵ Prolonged administration of some drugs or xenobiotics can enhance their own metabolism, along with a wide variety of other compounds. This phenomenon is known as enzyme induction, a dose-dependent phenomenon.

Drugs and xenobiotics exert this effect by inducing transcription of CYP450 mRNA and synthesis of xenobiotic-metabolizing enzymes in the smooth ER of the liver and other extrahepatic tissues.³³⁻³⁵

Enzyme induction is an adaptive response associated with increases in liver weight, induction of gene expression, and morphological changes in hepatocytes. Induction is the process whereby the rate of enzyme synthesis is increased relative to the rate of enzyme synthesis in the uninduced organism. In many older studies of mammalian systems, the term “induction” was inferred from an increase in enzyme activity, but the amount of enzyme protein had not been determined.

Enzyme induction is important to interpret the results of chronic toxicities, mutagenicities, or carcinogenesis and to explain certain unexpected drug-drug interactions in patients. Enzyme inducers trigger pathways involving the constitutive androstane receptor (CAR), the peroxisome proliferator-activated receptor (PPAR), the aryl hydrocarbon receptor (AhR), and the pregnane-X-receptor (PXR).³⁵ CAR is a member of the nuclear receptor superfamily and, along with PXR, upregulates the expression of P450 proteins responsible for the metabolism and excretion of these xenobiotics, and thus it is a key regulator of xenobiotic CYP450 metabolism.

Many drugs and xenobiotics induce/stimulate the upregulation of CYP450 isoforms, as shown in [Table 3.13](#). These inducers have nothing in common so far as their pharmacological activities or chemical structures are concerned, but they are all metabolized by one or more of the CYP450 isoforms. Most are lipid soluble at physiological pH. Polycyclic aromatic hydrocarbons in cigarette smoke, xanthines and

flavones in foods, halogenated hydrocarbons in insecticides, polychlorinated biphenyls, and food additives are but a few of the environmental chemicals that alter the activities of CYP450 enzymes.³⁴

Table 3.13
Drugs That Induce the Expression of P450 Isoforms^a

<i>Amprenavir</i> smoke Clotrimazole Ethanol <i>Efavirenz</i> Erythromycin Glucocorticoids (Dexamethasone, prednisone) Griseofulvin Lansoprazole Mephenytoin	3A4, 2C9, 2C19, 2B6, 1A2, 3A4, 2C8, 2C9, 2D6, 2B6, 1A1/2, 1A1/2, 1A1/2, 3A4, 2D6, 2E13, 4A3, 4A4, 2A6, 2C19, 3A4, 1A1/2, 3A4, 2B6	4-Methylpyrazole Modafinil N-aromatic hydrocarbons Rifampin

^aDrugs in bold italic have been reported to cause drug-drug interaction.

Enzyme induction, which can last as long as 1-3 weeks to allow for drug blood levels to decrease, can alter the pharmacokinetics and pharmacodynamics of a drug, with clinical implications for the therapeutic actions of the drug as well as increased potential for drug-drug interactions. Because of induction, a drug can be metabolized more rapidly to metabolites that are more potent, more toxic, or less active than the parent drug. Induction can also enhance the activation of procarcinogens or promutagens.

Not all inducing agents enhance their own metabolism; for example, phenytoin induces CYP3A4 but is hydroxylated by CYP2C9. Some of the more common enzyme inducers of CYP450 subfamilies, which can also be substrates for the same P450 isoforms, include phenobarbital (CYP2B6, CYP2C, and CYP3A4), rifampicin (CYP3A4), and cigarette smoke (CYP1A1/2) (Table 3.13). The broad range of drugs metabolized by these CYP450 subfamilies (Table 3.12) that are also affected by these enzyme inducers raise the issue of clinically significant drug-drug interactions and their clinical implications. Examples of a clinically significant CYP450 drug-drug interaction and an herbal drug-drug interaction are rifampin and oral contraceptives, and St. John's wort and oral contraceptives, respectively. Both rifampin and St. John's wort induce the expression of CYP3A4. This reduces the serum levels of the oral contraceptive due to increased oxidative metabolism of the oral contraceptives by CYP3A4 to less active metabolites, thereby increasing the risk for pregnancy. Drugs poorly metabolized by CYP450 enzymes are less affected by enzyme induction.

Inducers of CYP450 isoforms also stimulate oxidative metabolism and the synthesis of endogenous substances: for example, the hydroxylation of androgens, estrogens, progestational steroids (synthetic oral contraceptives), and glucocorticoids, which decreases their biological activity. These enzyme inducers might also be implicated in deficiencies associated with these steroids. For example, the induction of C2 hydroxylation of estradiol and synthetic estrogens by phenobarbital, dexamethasone, or cigarette smoking in women results in the increased formation of the principal and less active metabolite of these estrogenic substances, reducing their effectiveness. Thus, cigarette smoking in premenopausal women could result in an estrogen deficiency, increasing the risks of osteoporosis and early menopause. Postmenopausal women who smoke and take estrogen replacement therapy risk decreasing the effectiveness of the estrogen.

In addition to enhancing the metabolism of other drugs, many compounds, when chronically administered, stimulate their own metabolism, thereby decreasing their therapeutic activity and producing a state of apparent tolerance. This self-induction can explain some of the changes in drug toxicity observed during prolonged treatments. The duration of the sedative action of phenobarbital, for

example, becomes shorter with repeated doses, and this can be explained, in part, on the basis of increased metabolism via enzyme induction.

The time course of induction varies with different inducing agents and with different P450 isoforms. CYP1A induction always involves the AhR. Increased transcription of CYP450 mRNA has been detected as early as 1 hour after administration of phenobarbital, with maximum induction occurring after 48-72 hours. After administration of PAH such as 3-methylcholanthrene and benzo[a]pyrene, maximum induction of the CYP1A subfamily is reached within 24 hours. Less potent inducers of hepatic drug metabolism can take as long as 6-10 days to produce maximum induction.³³

Exposure to a variety of different xenobiotics can increase the hepatic content of specific isoforms of CYP450.³³⁻³⁵ Therefore, the process of enzyme induction involves the adaptive increase in the content of specific enzymes in response to the enzyme-inducing agent. Other inducible Phase 2 metabolizing enzymes include uridine diphosphate (UDP)-glucuronosyl transferase and glutathione transferase.

Specific Inducers

Phenobarbital and Rifampin.

Phenobarbital and rifampin are enzyme inducers which have been most extensively studied. These drugs alter the pharmacokinetics and pharmacodynamics of many concurrently administered drugs listed in [Tables 3.7](#) (CYP2C) and [3.10](#) (CYP3A4), which raises the issue of clinically significant drug-drug interactions.

Cigarette Smoke

Cigarette smoke has been shown to increase the hydrocarbon-inducible isoforms CYP1A1 and CYP1A2 in the lungs, liver, small intestine, and placentas. A stimulation of the CYP1A1/2 metabolism of several drugs, and a decrease in their pharmacological action, is the end result. Cigarette smoking has been reported to lower blood levels of drugs metabolized by CYP1A1/2, including theophylline, imipramine, estradiol, pentazocine, and propoxyphene. Smoking also decreases urinary excretion of nicotine and the drowsiness caused by chlorpromazine, diazepam, and chlordiazepoxide.

Alcohol

Alcoholics show an increase in CYP2E1 enzyme activity, leading to more rapid clearance of drugs and xenobiotics that are substrates for this isoform. As discussed previously, hepatic CYP2E1 oxidizes ethanol, and chronic ethanol intake increases the activity of CYP2E1 through enzyme induction.¹⁹ The changes in drug metabolism in alcoholics can also be attributed to other factors, such as malnutrition, coadministered drugs, and the trace chemicals that are sources of flavors and odors of particular alcoholic beverages. Heavy drinkers metabolize phenobarbital, tolbutamide, and phenytoin more rapidly than nonalcoholics, which can be clinically important when adjusting drug therapies for alcoholics.

Inhibition

Another method to alter in vivo effects of xenobiotics metabolized by P450s is the use of inhibitors ([Table 3.11](#)). The CYP450 inhibitors can be divided into three categories according to their mechanisms of action: reversible inhibition, metabolite intermediate complexation of P450, or mechanism-based inactivation of CYP450.^{35,36} The polysubstrate nature of CYP450 is responsible for the large number of documented interactions associated with the inhibition of drug oxidation and drug biotransformation.

Reversible Inhibition

Reversible inhibition of CYP450 is the result of reversible interactions at the heme-iron active center of P450, the lipophilic sites on the apoprotein, or both. The interaction occurs before the oxidation steps of the catalytic cycle and the effects dissipate quickly when the inhibitor is discontinued. The most effective reversible inhibitors are those that interact strongly with both the apoprotein and the heme iron. It is widely accepted that inhibition has an important impact on the oxidative metabolism and

pharmacokinetics of drugs which have a metabolism that cosegregates with that of an inhibitor (Tables 3.3 and 3.7-3.10).^{35,36} Some drugs that interact reversibly with CYP450 are shown in Table 3.11. The imidazole-based azole antifungals are potent inhibitors of CYP3A4 and of the CYP450-mediated biosynthesis of endogenous steroid hormones. The azole antifungals noted in this Table exert their fungistatic effects through inhibition of fungal P450, inhibiting the oxidative biosynthesis of lanosterol to ergosterol, thereby affecting the integrity and permeability of the fungal membranes.

CYP450 Complexation Inhibition

Some alkylamine drugs have the ability to undergo P450-mediated oxidation to nitrosoalkane metabolites, which have high affinities for the P450 heme iron active center and form stable complexes with the reduced (ferrous) heme-P450 intermediates of the CYP2B, CYP2C, and CYP3A subfamilies. This process is termed “metabolite intermediate complexation.”^{6,8} Thus the CYP450 isoform is unavailable for further oxidation, and synthesis of the new enzyme is required to restore CYP450 activity. The process relies on at least one iteration of the CYP450 catalytic cycle to generate the required heme intermediate.

The macrolide antibiotics troleandomycin, erythromycin, and clarithromycin, as well as their analogues, are selective inhibitors of CYP3A4 and are capable of inducing expression of hepatic and extrahepatic CYP3A4 mRNA and of inducing their own biotransformation into nitrosoalkane metabolites. The clinical significance of this inhibition with CYP3A4 is the long-lived impairment of metabolism of a large number of coadministered substrates of this isoform, and the potential for drug-drug interactions and time-dependent nonlinearities in their pharmacokinetics upon long-term administration (Tables 3.11 and 3.12). For macrolides to be so metabolized, they must possess an unhindered dimethylamino sugar and the compound itself must be lipophilic. A second example is methylenedioxyphenyl compounds (i.e., the antidepressant paroxetine, the insecticide synergist piperonyl butoxide, and the flavoring agent isosafrole), which generate metabolite intermediates which form stable complexes with both the ferric and ferrous state of P450.

Mechanism-Based Inhibition

Certain drugs that are not inhibitors of CYP450 contain functional groups that, when oxidized by P450, generate metabolites that irreversibly bind to the enzyme.^{34,37} This process is termed “mechanism-based inhibition” (“suicide inhibition”) and requires at least one catalytic CYP450 cycle either during or subsequent to the oxygen-transfer step, when the drug is activated to the inhibitory species. Alkenes and alkynes were the first functionalities found to inactivate P450 by generation of a radical intermediate that alkylates the heme structure (see section Alkene and Alkyne Hydroxylation). Iron is lost from the heme and abnormal N-alkylated porphyrins are produced. Drugs that are mechanism-based inhibitors of CYP450 include (1) the 17 α -acetylenic estrogen 17 α -ethinyl estradiol,³⁷ the 17 α -acetylenic progestin norethindrone (norethisterone), and their radical intermediates that N-alkylate the heme of CYP3A4; (2) cyclophosphamide and its generated acrolein and phosphoramidate mustard that alkylates CYP2B6 apoprotein; (3) spironolactone and its 7-thio metabolite that alkylates heme; (4) 8-methoxypsoralen (a furocoumarin) and its epoxide metabolite that alkylates the CYP450 apoprotein of CYP2A6; (5) nicotine (CYP1A6 and 1A13 isoforms); (6) isoniazide that alkylates CYP1A2, 2A6, 2C19, and 3A4 isoforms; (7) 21-halosteroids; (8) halocarbons; and (9) secobarbital. The selectivity of CYP450 isoform destruction by several of these inhibitors indicates involvement of specific isoforms in the bioactivation of such drugs.^{38,39}

Oxidations Catalyzed by Flavin Monooxygenase

Flavin-containing monooxygenase (FMO) oxidizes drugs, xenobiotics, and environmental chemicals containing a soft-nucleophile, usually nitrogen or sulfur. A soft-nucleophile is a group that is less electronegative, is more polarizable, and has a weak tendency to donate or share their electrons. FMO is

a monooxygenase but, unlike P450, utilizes the two electron reducing equivalents of NADPH (as hydride anion) in one step to reduce one atom of molecular oxygen to water, while the other oxygen atom is used to oxidize the substrate. Because oxygen activation occurs in two steps before substrate addition, any compound binding to 4 α -hydroperoxyflavin, the enzyme-bound monooxygenating FMO intermediate, is a potential substrate. The products formed from FMO-catalyzed oxidation are consistent with a two-electron oxidation of the heteroatom. Unlike P450, FMO does not catalyze epoxidation reactions or hydroxylation reactions at unactivated carbon atoms of xenobiotics. FMO and P450 also exhibit similar tissue and cellular location, molecular weight, and substrate specificity and exist as multiple enzymes.

Humans express five different flavin monooxygenases (FMO1, FMO2, FMO3, FMO4, and FMO5) in a tissue-specific manner, with different substrate specificities. Unlike P450, the human FMO functional gene family consists of five families each expressing a single protein. Three of the five expressed human FMO genes, *FMO1*, *FMO2*, and *FMO3*, exhibit genetic polymorphisms. FMO3 is the prominent form in adult human liver that is likely to be associated with the bulk of FMO-mediated metabolism and contributes to the disease known as trimethylaminuria. FMO2 is largely found in the lung. Because of their low expression, little is known about the substrate specificity for FMO3 and FMO4. Thus, these enzymes do not contribute significantly (<10%) to human drug metabolism.

The catalytic cycle of FMO and P450 is very different. For example, FMO does not require a reductase to transfer electrons from NADPH. Another distinction is the lack of induction of FMOs by xenobiotics. In general, P450 is the major contributor to oxidative xenobiotic metabolism. However, FMO activity may be of significance in a number of reactions involving N or S (see below) and should not be overlooked. FMO and P450 have overlapping substrate specificities but often yield different metabolites with potentially diverse toxicological/pharmacological consequences.

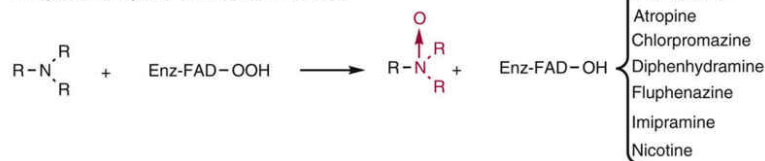
The physiological function(s) of FMO are poorly understood.⁴⁰ Unlike P450 which exhibits interindividual variation in expression to both genetic and environmental factors, FMO is not regulated by environmental factors and interindividual variability in FMO enzyme expression would be predominately genetic in origin.

N- and S-Oxidations

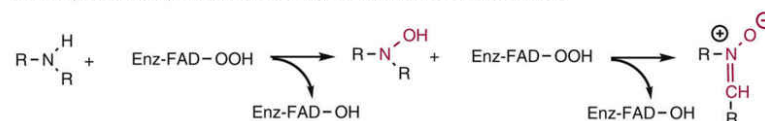
FMO constitutes an alternative biotransformation pathway for converting N- and S-containing lipophilic drugs and xenobiotics to more polar metabolites that are more efficiently excreted in the urine. Typically, FMO catalyzes oxygenation of the N- and S-heteroatoms (soft nucleophiles) (Fig. 3.17), excluding heteroatom dealkylation reactions. FMO is not normally inducible by phenobarbital, nor is it affected by P450 inhibitors. With few exceptions, however, xenobiotic substrates of FMO are also substrates of the P450 isoforms, producing similar oxidation products. Which monooxygenase is responsible for the oxidation can be readily determined, because FMO is thermally labile in the absence of NADPH, whereas P450 is stable.

Nitrogen compounds:

t-Acyclic and cyclic amines to N-oxides

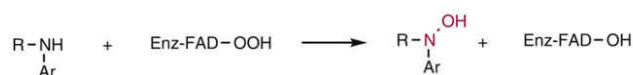


sec-Acyclic and cyclic amines to hydroxylamines and nitrones

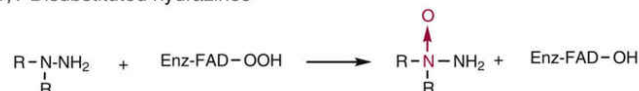


Drugs: desmethylinipramine; desmethyiltrifluoperazine

N-Alkyl and N,N-dialkylaryl amines to hydroxylamines

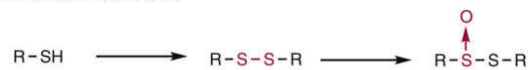


1,1-Disubstituted hydrazines



Sulfur compounds:

Thiols and disulfides



Thioethers/sulfides

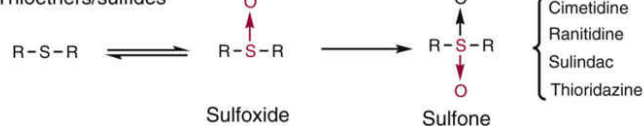


FIGURE 3.17 Examples of flavin monooxygenase (FMO) oxidations.

Of the many nitrogen functional groups in xenobiotics, only secondary and tertiary acyclic, cyclic and arylamines, as well as hydroxylamines and hydrazines, are oxidized by FMO and excreted in urine (Fig. 3.17). The tertiary amines form stable amine oxides (N-oxides) which are highly polar, hydrophilic, and less basic compounds, and which can be reduced back to the parent tertiary amine in the GIT. On the other hand, secondary amines are sequentially oxidized to hydroxylamines, nitrones, and a complex mixture of products. Secondary N-alkylaryl amines can be N-oxygenated to reactive N-hydroxylated metabolites, which are responsible for the toxic, mutagenic, and carcinogenic activity of these compounds. For example, the chemically unstable hydroxylamine intermediates of aromatic amines degrade into bladder carcinogens (see discussion of this type of toxic mechanism in section Glucuronic Acid Conjugation), and the hydroxamic acid intermediates of N-arylacetamides are bioactivated into liver carcinogens. Hepatic FMO, however, will not catalyze the oxidation of primary alkyl- or arylamines, except for the carcinogenic N-hydroxylated derivatives of 2-aminofluorene, 2-aminoanthracene, and other amino PAHs.

S-oxidation occurs almost exclusively by FMO (Fig. 3.17). Sulfides are oxidized to sulfoxides and sulfones; thiols to disulfides and thiocarbamates; mercaptopyrimidines and mercaptoimidazoles (i.e., the antithyroid drug methimazole) via sulfenates (RSOH) to sulfinates (R-SO-OH), all of which are eliminated in urine. FMO S-oxygenates a number of sulfur-containing substrates. The hepatotoxicity of thioureas (e.g., the antimycobacterial drug ethionamide) is the result of metabolic bioactivation through FMO3-dependent S-oxidation to produce toxic sulfinic acid metabolites. Metabolic bioactivation of ethionamide by bacterial FMOs may be its antimycobacterial mechanism of action. Primary aromatic amines and amides, aromatic heterocyclic amines and imines, and the aliphatic primary amine phentermine are N-oxidized by P450 to hydroxylamines.

Catalytic Cycle for Flavin Monooxygenase

The major steps in the catalytic cycle for FMO are shown in Figure 3.18.⁴⁰⁻⁴² Like most of the other monooxygenases, FMO requires NADPH and oxygen as cofactors to catalyze the oxidation of the xenobiotic substrate. Unlike P450, however, the xenobiotic being oxidized does not need to be bound to the 4 α -hydroperoxyflavin intermediate (FAD-OOH) for oxygen activation to occur. FMO is present within the cell in its enzyme-bound activated hydroperoxide (Enz-FAD-OOH) state, ready to oxidize any suitable lipophilic substrate that binds to it. The FMO uses a nonradical, nucleophilic displacement type of mechanism binding dioxygen with a reduced flavin. The reactive oxygen intermediate is a reactive derivative of hydrogen peroxide, flavin-4 α -[SEPP]hydroperoxide (Fig. 3.18, insert), which is reactive enough to successfully attack a lone electron pair on a heteroatom, such as nitrogen or sulfur, but not reactive enough to abstract a hydrogen atom from a typical C-H bond. Studies suggest the xenobiotic substrate interacts with the 4 α -hydroperoxyflavin form of FMO and is oxidized by oxygen transfer from Enz-FAD-OOH to form the oxidized product. Steps 2-5 in Figure 3.18 simply regenerate the oxygenating agent Enz-FAD-OOH from Enz-FAD-OH, NADPH, oxygen, and a proton. Any compound readily crossing cell membranes and penetrating to the FMO-bound hydroperoxyflavin intermediate is a potential substrate, thus explaining the broad substrate specificity exhibited by FMO. The fact that the xenobiotic substrate is not required for activation of the FMO-hydroperoxyflavin state distinguishes FMO from P450 monooxygenases, in which substrate binding initiates the P450 catalytic cycle and activation of oxygen to the ferryl oxenoid intermediate. It is not unusual for FMO oxidation products to undergo reduction to the parent xenobiotic, which can enter into repeated redox reactions (termed "metabolic cycling").

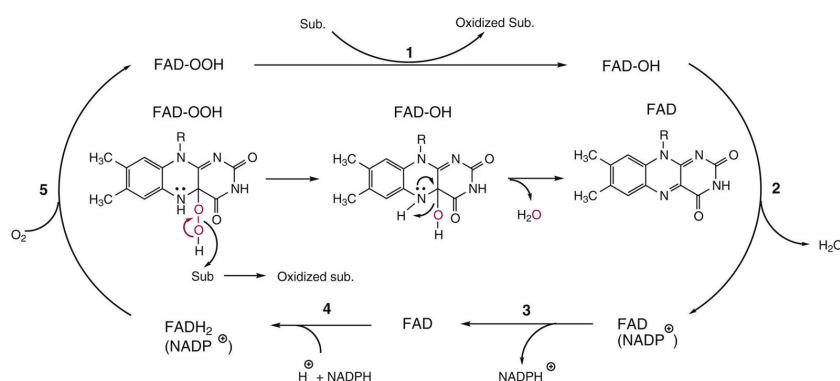


FIGURE 3.18 Flavin monooxygenase (FMO) catalytic cycle. Oxygenated substrate is formed by nucleophilic attack of a substrate (Sub.) by the terminal oxygen of the enzyme-bound hydroperoxyflavin (FAD-OOH), followed by heterolytic cleavage of the peroxide (1). The release of H₂O (2) or of NADP[•] (3) is rate-limiting for reactions catalyzed by liver FMO. Reduction of flavin by NADPH (4) and addition of oxygen (5) complete the cycle by regeneration of the oxygenated FAD-OOH.

Results of substrate specificity studies suggest that the number of ionic groups on endogenous substrate is an important factor in enabling FMO to distinguish between xenobiotic and endogenous substrates, thus preventing the indiscriminate oxidation of physiologically important amine and sulfur compounds.⁴³ Without exception, FMO readily catalyzes the oxidation of uncharged amines or sulfur compounds (in equilibrium with its respective monocation or monoanion; for sulfur compounds, the charge is on sulfur atom). The FMO will not catalyze the oxidation of dianions (e.g., thiamine pyrophosphate), dications (e.g., polyamines), dipolar ions (e.g., amino acids and peptides), or other polyionic compounds with one or more anionic groups (e.g., COO⁻) distal to the heteroatom (e.g., coenzyme A).

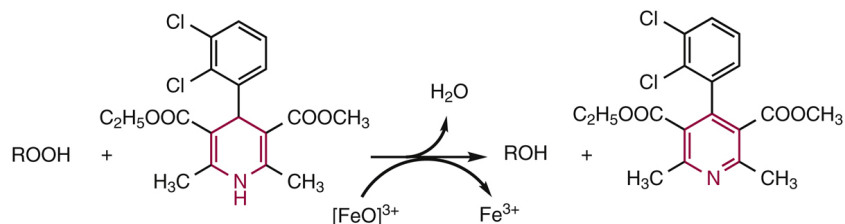
Unlike the P450 system, only three isoforms of hepatic FMO have been characterized in the adult human liver (FMO1, FMO2, FMO3).^{42,43} FMO1 is the major form in fetal liver. The availability of different forms of FMO can be of clinical importance in the pharmacological and toxicological properties of FMO-dependent drug oxidations.

The substrate specificity for FMO3 is distinct from that of FMO1. FMO3 N-oxygenates primary, secondary, and tertiary amines, whereas FMO1 is only efficient at N-oxygenating tertiary amines. FMO3 is sensitive to steric features of the substrate, and aliphatic amines with linkages of at least five carbons between the nitrogen atom and a large aromatic group (e.g., phenothiazines) are N-oxygenated significantly more efficiently than substrates with a two or three carbon spacer. Amines with smaller aromatic substituents (e.g., phenethylamines) are often efficiently N-oxygenated by FMO3. Interindividual variation in FMO3-dependent metabolism of drugs, xenobiotics, and environmental chemicals is therefore more likely because of genetic differences, rather than environmental effects.

Certain mutations of the FMO3 gene have been associated with deficient N-oxygenation of trimethylamine, which results in an inherited genetic condition called trimethylaminuria. Allelic variations of FMO3 might cause abnormal metabolism of drugs, which has clinical implications for human drug metabolism. For example, (S)-Nicotine N1'-oxide formation is a highly stereoselective product of FMO3 for adult humans who smoke cigarettes. Thus, FMO3 genetic polymorphism and poor metabolism phenotype in certain human populations could contribute to adverse drug reactions or exaggerated clinical response to certain medications. FMO3 may be another example of an environmental gene that participates in a protective mechanism to help shield humans from potentially toxic exposure to chemicals.

Peroxidases and Other Monooxygenases

Peroxidases are hemoproteins that are closely related enzymes to P450 monooxygenase.^{44,45} The differences between peroxidase and P450 include the replacement of the cysteine axial ligand of P450 with a histidine residue and the inclusion of polar amino acids within proximity of the heme-active site. These polar amino acid differences in the peroxidase heme-active site allow the peroxidase to rapidly catalyze the reduction of hydroperoxides to alcohol (water in the case of hydrogen peroxide) and the simultaneous reoxidation of peroxidase. The normal course of peroxide (ROOH)-catalyzed oxidation involves the formation of the ferryl-oxo-intermediate, analogous to the ferryl oxenoid complex in P450. Thus, the catalytic cycle of peroxidases is simpler than that of P450. Unlike P450 which is capable of abstracting a hydrogen atom from almost any type of substrate, peroxidase-catalyzed oxidation of drugs and xenobiotics is limited to electron-rich substrates that are easily oxidized (e.g., heteroatom oxygenation, aromatization [oxidation] of 1,4-dihydropyridines [calcium channel blockers] and arylamines).



Cyclooxygenase (COX, also known as prostaglandin synthase or prostaglandin endoperoxide synthetase) is a widely-distributed heme-peroxidase enzyme responsible for the formation of important biological mediators, called prostanoids, from arachidonic acid. These essential prostanoids include prostaglandins, prostacyclin, and thromboxane. COX catalyzes the heme-peroxidation of arachidonic acid by means of hydrogen atom abstraction by a tyrosine radical generated by a ferryl-oxo-heme-^[L-SEP] intermediate to hydroperoxy endoperoxide prostaglandin G₂ (PGG₂), which is then reduced to prostaglandin H₂, regenerating the COX. Two oxygen molecules then react with the arachidonic acid radical to produce PGG₂. During the reduction of prostaglandin G₂ (PGG₂) to prostaglandin H₂, drug substrates can be oxidized. Three COX isoenzymes are known: COX-1, COX-2, and COX-3. Inhibitors of COX are the nonsteroidal anti-inflammatory drugs (NSAIDs).

Myeloperoxidase (MPO) is a heme-peroxidase enzyme most abundantly present in neutrophil granulocytes (a subtype of white blood cells). MPO differs from P450 and other peroxidases in that the substrates are hydrogen peroxide and chloride anion. The chloride anion is oxidized to hypochlorous acid (HOCl) in the granulocyte, which is cytotoxic and very effective in killing bacteria, viruses, and other pathogens: the natural function of neutrophils. HOCl is also used to disinfect drinking water. HOCl can oxidize drugs to reactive intermediates (e.g., nitrenium ions) which can be responsible for causing agranulocytosis. Examples of HOCl-generated reactive intermediates associated with agranulocytosis are the atypical antipsychotic clozapine, the antimalarial amodiaquine, and the phosphodiesterase cardiotonic vesnarinone.

Other peroxidases include horseradish peroxidase (found in plants), lactoperoxidase found in breast milk, and thyroid peroxidase found in the thyroid gland to produce thyroid hormones from iodide.

Other monooxygenases catalyzing oxidation reactions similar to P450 include dopamine β-monooxygenase, a mammalian copper-containing enzyme catalyzing carbon hydroxylation, epoxidation, S-oxygenation, and N-dealkylation reactions, and nonheme iron-containing enzymes from bacteria and plants.

Nonmicrosomal Oxidations

In addition to the microsomal monooxygenases, other oxidases and dehydrogenases that catalyze oxidation reactions are present in the mitochondrial and soluble fractions of tissue homogenates.

Oxidation of Alcohols

Alcohol dehydrogenases (ADH) are a family of NAD-specific dehydrogenase enzymes that occur in many organisms and are responsible for catalyzing the oxidation of primary and secondary alcohols to aldehydes and ketones, respectively, with concurrent reduction of NAD⁺ to NADH. ADHs can also catalyze the reverse reaction, oxidizing NADH to NAD⁺. In humans and many other animals, genetic evidence suggests that early on in evolution, ADHs were effective for metabolizing endogenous and exogenous formaldehyde and metabolizing naturally occurring toxic alcohols contained in foods or produced by bacteria in the digestive tract.

In humans, ADH exists as a dimer with a mass of 80 kDa and is encoded by at least seven different genes. There are five classes (1-5) of alcohol dehydrogenases, but the hepatic form that is primarily present in humans is class 1. Class 1 consists of A, B, and C subunits that are encoded by the genes

ADH1A, *ADH1B*, and *ADH1C*. The human genes that encode class 2, 3, 4, and 5 ADHs are *ADH4*, *ADH5*, *ADH7*, and *ADH6*, respectively. The large number of ADH classes gives the body the capability to metabolize/detoxify a variety of primary and secondary alcohols.

Human ADH is present at high levels in the liver and the lining of the stomach and exhibits a broad specificity for alcohols. Most primary alcohols are readily oxidized to their corresponding aldehydes. Some secondary alcohols are oxidized to ketones, whereas other secondary and tertiary alcohols are excreted either unchanged or as their glucuronide conjugate metabolite. Some secondary alcohols show also mixed activity due to steric factors and a lack of substrate affinity for the enzyme. The mechanism of dehydrogenation involves abstraction and transfer of a hydride anion from the alcohol to NAD⁺, reducing NAD⁺ to NADH, with the subsequent formation of an aldehyde. ADH contains a two Zn⁺² ions in each subunit (Fig. 3.19). One of those Zn ions is located at the catalytic site and binds the hydroxyl group of the alcohol in place for dehydrogenation to occur. Alcohol dehydrogenase is located in the soluble fraction of tissue homogenates.

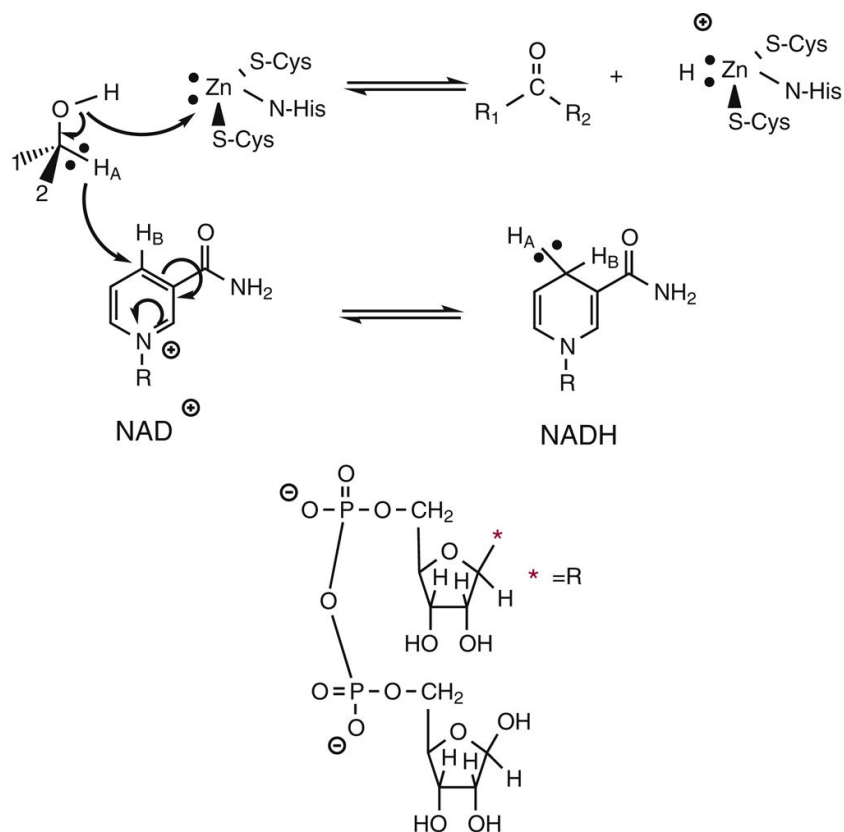


FIGURE 3.19 Alcohol dehydrogenase oxidation mechanism.

Oxidation by ADH is the principal pathway for ethanol metabolism, but the microsomal isoform CYP2E1 has also a significant role in ethanol metabolism and tolerance. Two-thirds of ingested ethanol is oxidized by ADH and the remainder by CYP2E1 (Fig. 3.20). During intoxication, however, ethanol induces the expression of CYP2E1. The induction of CYP2E1 contributes to the activation of other drugs and xenobiotics, increasing the vulnerability of heavy drinkers to anesthetic drugs, over-the-counter analgesics, prescription drugs, and chemical carcinogens. In turn, the excessive amounts of acetaldehyde generated cause hepatotoxicity, lipid peroxidation of membranes, formation of protein adducts, and other hepatocellular changes.

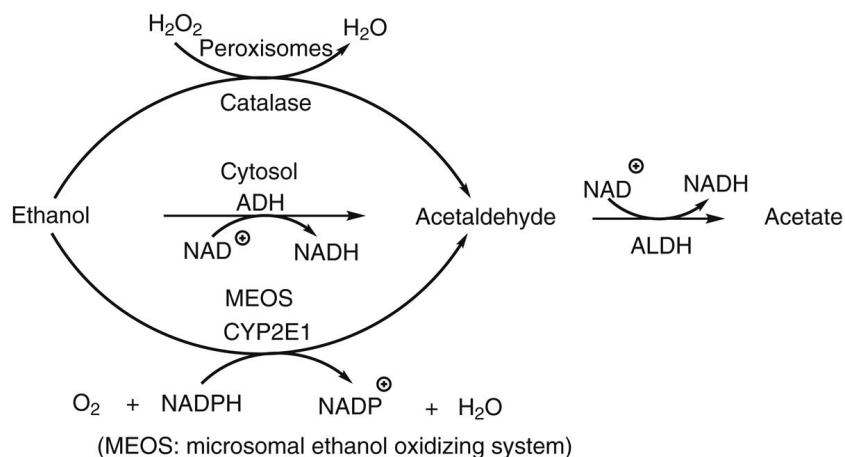


FIGURE 3.20 Ethanol metabolism in humans.

The toxicity of methanol and ethylene glycol in humans has long been recognized, but frequent reports of such toxicity are not surprising given the number of consumer products containing these alcohols (e.g., automotive antifreeze). Methanol (wood alcohol or methyl alcohol) is commonly used as a solvent in organic synthetic procedures and is available to consumers in a variety of products, ranging from solid fuels (Sterno), paint removers, motor fuels, antifreeze to alcoholic beverages (an adulterant in wines or an unintentional ingredient). Oral methanol toxicity in humans is characterized by its rapid absorption from the gut, followed by a latent period of many hours before metabolic acidosis (lowered blood pH and bicarbonate levels) and ocular toxicity are evident. The metabolic acidosis and blindness result from the excessive accumulation of formic acid and the inability of the hepatic tetrahydrofolate pathway to oxidize formate to carbon dioxide. The rate of elimination of methanol from the blood is relatively slow compared to the rapid rate for ethanol, accounting for its long latency period. Its half-life ranges from 2 to 3 hours at low blood concentration to 27 hours at high blood concentration. Research supports the singular role of liver ADH in the metabolism of methanol to formaldehyde, although it is oxidized slowly by ADH (approximately one-sixth the rate of ethanol).

The demonstration that methanol is a substrate for ADH provides a rational basis for the use of ethanol in the treatment of methanol toxicity. Ethanol depresses the rate of methanol oxidation by acting as a competitive substrate for ADH, reducing the formation of formaldehyde. On the other hand, formaldehyde is not customarily detected in the blood because of its rapid metabolism by ADH to formate. Although human exposure to methanol vapor is less prevalent, methanol is rapidly absorbed through the skin or by inhalation, and depending on the severity of exposure, this can result in methanol poisoning.

Ethylene glycol is oxidized to hydroxyacetaldehyde and glyoxal and, subsequently, to oxalate by ADH. When eliminated into the urine, oxalate forms calcium oxalate crystals that can block the renal tubules. 4-Methylpyrazole (Fomepizole) is an alcohol dehydrogenase inhibitor that is used as an antidote for the treatment of methanol or ethylene glycol poisoning. 1,4-Butanediol is a solvent that has become known to the public as a date-rape drug and drug-of-abuse due to its metabolism to γ -hydroxybutyrate. The oxidized metabolite binds to the γ -hydroxybutyrate receptor, which in turn produces CNS sedation with amnesia.

Alcohol dehydrogenase functions also as a reductase when it catalyzes the reduction of an aldehyde or ketone to an alcohol. In addition, other NADP- or NAD-dependent dehydrogenases in the cytosol are capable of reducing a variety of ketones. Ketones are stable to further oxidation and, consequently, yield reduction products as major metabolites. Examples of reduction include the sedative-hypnotic chloral hydrate to trichloroethanol, the opioid antagonist naltrexone to 6 β -hydroxynaltrexol, the opioid

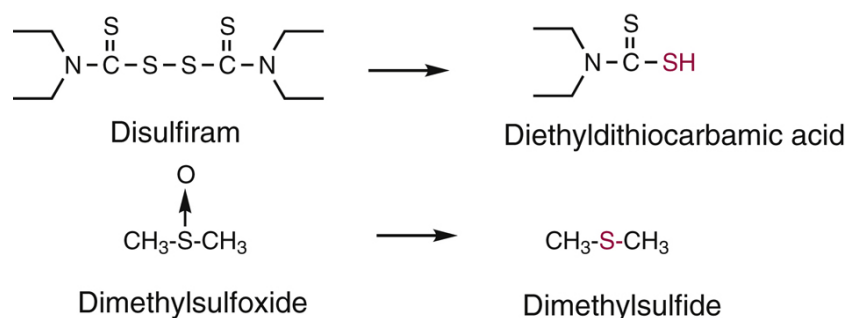
analgesic methadone to α -methadol, the antipsychotic haloperidol to hydroxyhaloperidol, and the antiemetic dolasetron to dihydrodolasetron. These alcohol metabolites are all pharmacologically active.

Aldehyde Dehydrogenase

Aldehyde dehydrogenases (ALHD) (not to be confused with aldehyde oxidase) are a family of polymorphic NAD⁺-specific enzymes that catalyze the NAD⁺-dependent oxidation (dehydrogenation) of aldehydes to carboxylic acids, which are subsequently metabolized by the body's muscle and heart. There are three mammalian classes of these enzymes: class 1 (low K_m , cytosolic ALDH1), class 2 (low K_m , mitochondrial ALDH2), and class 3 (high K_m , such as those expressed in tumors, stomach, and cornea). In all the three classes, constitutive (an enzyme whose activity is constant and active) and inducible forms exist. ALDH1 and ALDH2 are the most important enzymes for aldehyde oxidation [SEP] and both are tetrameric enzymes composed of 54 kDa subunits. These enzymes are found in many tissues of the body but are at the highest concentration in the liver.

ALDH1, along with ADH, is responsible for the metabolism of retinol to retinoic acid. ALDH2 catalyzes the bioactivation of nitroglycerin (glyceryl trinitrate) in blood vessels, resulting in vasodilation by nitric oxide (NO). ALDH2 has a major role in the hepatic detoxication of acetaldehyde and other aldehydes produced from oxidation of ethanol and other primary alcohols, and the oxidation of endogenous aldehydes, such as those produced by the oxidation of biogenic amines (norepinephrine, serotonin, dopamine). These aldehydes can be toxic and health problems arise when the aldehyde cannot be cleared. The accumulation of unmetabolized acetaldehyde in the blood has been shown to be a carcinogen in lab animals.

For example, when high levels of acetaldehyde occur in the blood, symptoms of facial flushing, light-headedness, palpitations, nausea, and general "hangover" symptoms occur. These symptoms are indicative of a disease known as "Asian flush" or "Oriental flushing syndrome," which is due to a mutant form of ALDH2 termed *ALDH2*2*. In the protein expressed by this mutant, a lysine residue replaces a glutamate in the active [SEP] site, yielding an enzyme with about 8% of the activity of the wild-type (normal) *ALDH2* allele. The mutant allele shows a higher k_m for NAD⁺ than the wild-type allele. This mutation is common in Japan, where 41% of a nonalcoholic control group were *ALDH2* (wild-type) deficient, and where only 2%-5% of an alcoholic group were ALDH2 deficient. In Taiwan, the numbers are similar, with 30% of the nonalcoholic control group showing the wild-type deficiency and 6% of alcoholics displaying the deficiency. The ALDH2 wild-type deficiency is manifested by slow acetaldehyde removal and low alcohol tolerance, which may lead to a lower frequency of alcoholism. These acetaldehyde toxicity symptoms are similar to those observed in people who drink ethanol while being treated with the drug disulfiram (Antabuse), which is why it is used to treat alcoholism in patients seeking recovery. These patients show higher blood levels of acetaldehyde and become violently ill upon consumption of even small amounts of alcohol. Several other drugs (e.g., metronidazole) cause a similar reaction known as "disulfiram-like reaction."



Molybdenum Hydroxylases

Molybdenum hydroxylases are non-P450 enzymes capable of catalyzing the oxidation of drugs. The molybdenum hydroxylases, which include aldehyde oxidase, xanthine oxidase, and xanthine dehydrogenase, are more commonly found in the cytosol of mammalian liver and carry out the oxidation and detoxification of structurally different azaheterocycles⁴⁶⁻⁴⁸ (Fig. 3.21). The efficient oxidation of endogenous purine nucleosides suggests that their metabolism and detoxification might be an important physiological role of the molybdenum hydroxylases. Among the azaheterocycles metabolized are derivatives of pyridine, quinoline, pyrimidine, purine, quinazoline, and pteridines. These hydroxylases as a rule oxidize the α -carbon to the nitrogen of the azaheterocycle to oxo metabolites (also known as lactams).

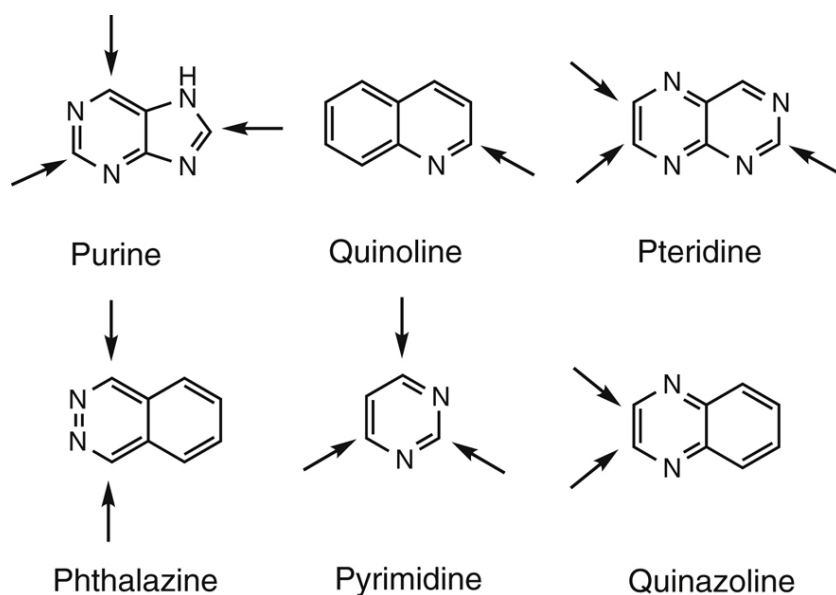


FIGURE 3.21 Heterocycle substrates for aldehyde oxidase.

The molybdenum hydroxylases are cytosolic with a common electron-transfer system in each enzyme subunit: a molybdopterin (a pyranopteridine ligand) cofactor that binds the single molybdenum (Mo) ion, two iron-sulfur centers, and an FAD molecule. The Mo is an essential component of the enzyme and, along with FAD, is required for enzyme catalysis. The enzyme is biologically inactive until it becomes complexed by the pterin to form the tetracyclic pteridine complex termed “MoCo.” The pterin moiety positions the catalytic Mo correctly within the active site of the enzyme to participate in electron transfer to and from the Mo atom. The molybdenum hydroxylases catalyze their reactions differently than P450 and other hydroxylase enzymes, requiring water rather than molecular oxygen as the source of the oxygen atom incorporated into the metabolite, and with the concomitant reduction of molecular oxygen to superoxide.⁴⁷ The active sites possess a catalytically labile $\text{Mo}^{\text{V}}\text{-OH}$ group that is transferred to the substrate during the hydroxylation reaction.

Aldehyde Oxidase

Aldehyde oxidase (AO) is an enzyme located in the cytosol of cells and is similar to the xanthine oxidase (XO) family of molybdoenzymes that requires a molybdenum cofactor (MoCo) for the catalytic activity. The physiological role of AO remains unclear, but in humans, it plays an important role in the clearance of drug compounds containing aldehydes and N-containing heterocyclic aromatic compounds. It generates carboxylic acids from aldehydes in the presence of oxygen. Although aldehydes are not often present in drug compounds, they can be a product of biotransformation reactions by P450s and MAO,

which can be subsequently oxidized to a carboxylic acid by AO. In addition to metabolizing some aldehydes, aldehyde oxidase also oxidizes a variety of azaheterocycles but excludes thia- or oxaheterocycles. Of the various purine nucleosides metabolized by aldehyde oxidase, the 2-hydroxy- and 2-amino derivatives are more efficiently metabolized. The typical order of N9 substituent preference of the acyclic nucleosides is as follows: 9-[(hydroxy-alkyloxy)methyl]-purines > 2'-arabinofuranosyl > H. The kinetic rate constants for purine analogues reveal that the pyrimidine portion of the purine ring system is more important for substrate affinity than the imidazole portion. Aldehyde oxidase is inhibited by potassium cyanide and menadione (synthetic vitamin K).

Aldehyde oxidase metabolizes an assortment of azaheterocycles, including the short-acting sedative-hypnotic drug zaleplon (a pyrazolo[1,5 α] pyrimidine derivative) to its 5-oxo metabolite; the anticancer drug thioguanine to 8-oxothioguanine; the α_2 -adrenergic agonist brimonidine (a pyrimidine derivative) to its 2-oxo-, 3-oxo-, and 2,3-dioxo- metabolites; quinine and quinidine to their 2-quinolone metabolites; the pro-antiviral drug famciclovir (a purine derivative) to its active 6-oxo metabolite (penciclovir); O6-benzylguanine to its 8-oxo metabolite (also formed primarily from CYP3A4); and the antiseizure drug zonisamide (a 1,2-benzisoxazole derivative), primarily by reductive cleavage of the 1,2-benzisoxazole ring to 2-sulfamoylacetylphenol. Although the azaheterocycles thiazole and oxazole are not metabolized by aldehyde oxidase, their carbocyclic analogues, benzothiazole, benzoxazole, and 1,2-benzisoxazole, are. On the other hand, the heterocycles, benzothiophene and benzofuran, which do not contain a nitrogen atom, are not metabolized by, nor do they inhibit, aldehyde oxidase. Both aldehyde oxidase and xanthine oxidase contribute to the first-pass hepatic metabolism of orally administered methotrexate (a 2,4-diaminopteridine) to its 7-hydroxymethotrexate metabolite.

Potent inhibitors of aldehyde oxidase include the selective estrogen receptor modulator raloxifene, tamoxifen, estradiol, and ethinyl estradiol. Other classes of drugs that demonstrate inhibition of aldehyde oxidase included phenothiazines, tricyclic antidepressants, tricyclic atypical antipsychotic agents, dihydropyridine calcium channel blockers, loratadine, ondansetron, propafenone, domperidone, quinacrine, verapamil, and salmeterol. Thus, the possibility of clinical drug interactions mediated by inhibition of this aldehyde oxidase could be observed between these drugs, and drugs for which aldehyde oxidase is the primary route of metabolism.

Xanthine Oxidase and Xanthine Dehydrogenase

The properties of AO and XO are closely related. Both enzymes are present in the cytosol. The primary difference between the two enzymes is that XO can exist in two interconvertible forms, xanthine oxidase (XO) and xanthine dehydrogenase, while AO exists only in the oxidase form. AO utilizes only molecular oxygen as an electron acceptor in contrast to XO, which can transfer electrons to both oxygen and NAD⁺. The key physiological function of XO has been recognized as the oxidative metabolism of purines into the terminal metabolite uric acid (Fig. 3.22).

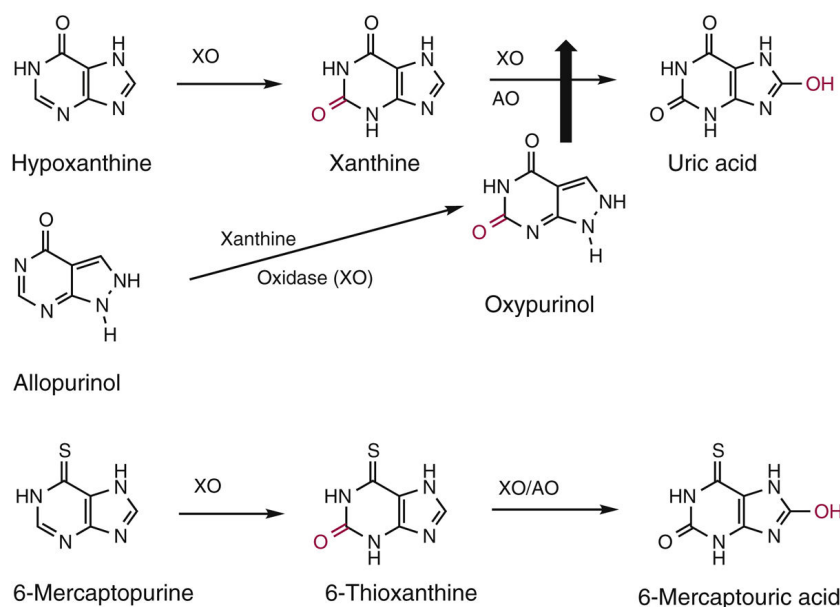


FIGURE 3.22 Xanthine oxidase reactions.

XO and xanthine dehydrogenase represent different forms of the same gene product. Xanthine dehydrogenase can be interconverted to XO by reversible sulfhydryl oxidation. These two enzyme forms and their reactions often are referred to as xanthine oxidoreductase. XO is the rate-limiting enzyme in human purine catabolism of hypoxanthine to uric acid and can also generate reactive oxygen species. XO is a cytosolic 370 kDa molybdoflavoenzyme that requires a molybdopterin cofactor, two iron-sulfur centers, and FAD.

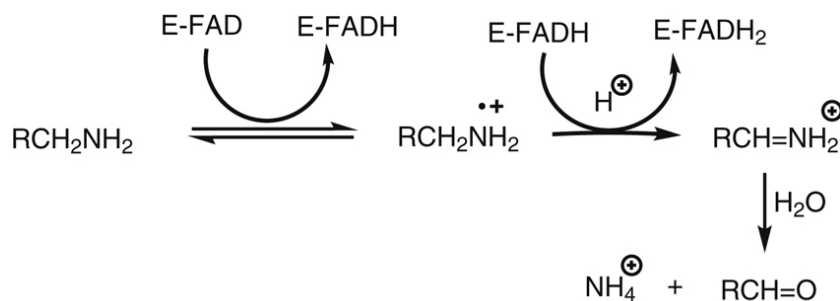
Both XO and xanthine dehydrogenase have important roles in the metabolism of purine anticancer drugs to their active and inactive metabolites. Although XO is strongly inhibited by the antigout drug allopurinol, AO also oxidizes allopurinol to oxypurinol. Only xanthine dehydrogenase requires NAD^+ as an electron acceptor for the oxidation of azaheterocycles. 6-Mercaptopurine is metabolized by XO to 6-mercaptopuric acid. In humans, XO is normally found in the liver and not free in the blood. Xanthinuria is a rare genetic disorder where the lack of XO leads to high concentration of xanthine in blood and can cause health problems such as renal failure. There is no specific treatment for this disorder except to avoid foods high in purine and to maintain a high fluid intake.

Oxidative Deamination of Amines

Monoamine oxidase (MAO) catalyzes the oxidative deamination of amines to aldehydes in the presence of oxygen. The aldehyde products can be metabolized further to the corresponding acid or alcohol by aldehyde oxidase or dehydrogenase.

Monoamine Oxidase

MAO is a mitochondrial membrane flavin-containing enzyme that catalyzes the oxidative deamination of monoamines, where oxygen is used to remove an amine group from a monoamine substrate resulting in the formation of the corresponding aldehyde and ammonia⁴⁸ (Fig. 3.23).



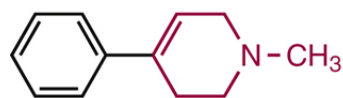
Regeneration of E-FAD:



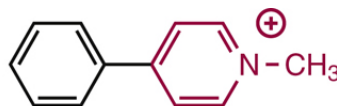
FIGURE 3.23 MAO oxidation of monoamines.

Substrates for this enzyme include primary amines and secondary and tertiary amines in which the amine substrates are methyl groups. The amine must be attached to an unsubstituted methylene group. Compounds having a single substitution at the α -carbon atom are poor substrates for MAO (e.g., aniline, amphetamine, and ephedrine) but can be oxidized by the microsomal P450 enzymes. For secondary and tertiary amines, alkyl groups larger than a methyl and branched alkyl groups (i.e., isopropyl, *t*-butyl, or β -phenylisopropyl) inhibit MAO oxidation, but such substrates can function as reversible inhibitors of this enzyme. Nonselective irreversible inhibitors of MAO include hydrazides (phenelzine) and tranylcypromine and the MAO-B selective inhibitors pargyline and selegiline. Monoamine oxidase is important in regulating the metabolic degradation of catecholamines and serotonin in neural tissues. Hepatic MAO has a crucial defensive role in inactivating circulating monoamines, or those originating in the GIT and absorbed into the systemic circulation (e.g., tyramine). There are two types of MAO: MAO-A and MAO-B. They show dissimilar substrate preferences and different sensitivities to inhibitors. MAO-A, found mainly in peripheral adrenergic nerve terminals but also in the liver, GIT, and placenta, shows substrate preference for 5-hydroxytryptamine, norepinephrine, and epinephrine. MAO-B is found principally in platelets and shows selectivity for nonphenolic, lipophilic β -phenethylamines. Common substrates to both MAO-A and MAO-B are dopamine, tyramine, and other monophenolic phenylethylamines.

A contaminant in the synthesis of reversed esters of meperidine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), was discovered to be a highly selective neurotoxin for dopaminergic cells, producing parkinsonism.⁴⁹ The neurotoxicity of MPTP is associated with cellular destruction in the substantia nigra along with severe reductions in the concentration of dopamine, norepinephrine, and serotonin. The remarkable neurotoxic action for MPTP involves a sequence of events beginning with the metabolic activation of MPTP to the toxic metabolite MPP⁺ (1-methyl-4-phenylpyridinium ion) by MAO-B, specific uptake and accumulation of MPP⁺ in the nigrostriatal dopaminergic neurons, and ending with the inhibition of oxidative phosphorylation. This inhibition results in mitochondrial injury depriving the sensitive nigrostriatal cells of oxidative phosphorylation with their eventual cell death (neurotoxic actions of MPP⁺). The MAO-B inhibitors (e.g., deprenyl) blocked this biotransformation.



MPTP



MPP⁺

Because of the vital role that MAOs have in the inactivation of neurotransmitters, MAO dysfunction (too much or too little MAO activity) is thought to be responsible for a number of neurological disorders. For example, unusually high or low levels of MAOs in the body have been associated with depression, schizophrenia, substance abuse, attention deficit disorder, and migraines. Monoamine oxidase inhibitors (MAOIs) are one of the major classes of drugs prescribed for the treatment of depression, although they are last-line treatment due to risk of the drug's interaction with dietary constituents (e.g., tyramine) or other drugs. Excessive levels of catecholamines (epinephrine, norepinephrine, and dopamine) may lead to a hypertensive crisis, and excessive levels of serotonin may lead to serotonin syndrome, both of which are potentially fatal.

Miscellaneous Reductions

Various studies on the biotransformation of xenobiotic ketones have established that ketone reduction is an important metabolic pathway in mammalian tissue.⁵⁰ Because carbonyl compounds are lipophilic and can be retained in tissues, their reduction to the hydrophilic alcohols and subsequent conjugation are critical to their elimination. Although ketone reductases (carbonyl reductases) can be closely related to the alcohol dehydrogenases, they have distinctly different properties and use NADPH as the cofactor. Carbonyl reductase participates in arachidonic acid metabolism. The metabolism of xenobiotic ketones to free alcohols or conjugated alcohols has been demonstrated for aromatic, aliphatic, alicyclic, and unsaturated ketones (e.g., naltrexone, naloxone, hydromorphone, and daunorubicin). The carbonyl reductases are distinguished by the stereospecificity of their alcohol metabolites.

Hydrolysis

In general, esters and amides are hydrolyzed by enzymes in the blood, liver microsomes, intestine, kidneys, and other tissues. Esters and certain amides are rapidly hydrolyzed by a group of enzymes termed "carboxylesterases." The more lipophilic the amide, the more favorable it is as a substrate for this enzyme. In most cases, the hydrolysis of an ester or amide bond in a toxic substance results in bioinactivation to hydrophilic metabolites that are readily excreted. Some of these metabolites can yield conjugated metabolites (i.e., glucuronides). Amides are very common in food as proteins and peptides we eat. Not surprisingly, there are a large number of proteolytic enzymes in the GIT called amino endopeptidases and amino exopeptidases that hydrolyze ingested proteins into amino acids.

Carboxylesterases include cholinesterase (pseudocholinesterase), arylcarboxylesterases, liver microsomal carboxylesterases, and other unclassified liver carboxylesterases. Cholinesterase hydrolyzes choline-like esters (e.g., acetylcholine, succinylcholine) and procaine as well as acetylsalicylic acid. Genetic variant forms of cholinesterase have been identified in human serum (e.g., succinylcholine toxicity results when administered as a ganglionic blocker for muscle relaxation to patients with the variant form of cholinesterase). Meperidine is hydrolyzed only by liver microsomal carboxylesterases (Fig. 3.24). Diphenoxylate is hydrolyzed to its active metabolite diphenoxylate acid within 1 hour (Fig. 3.24). Presumably, the lack of a central pharmacological action of diphenoxylate is attributed to the formation of a zwitterionic diphenoxylate acid, which is readily eliminated in the urine.

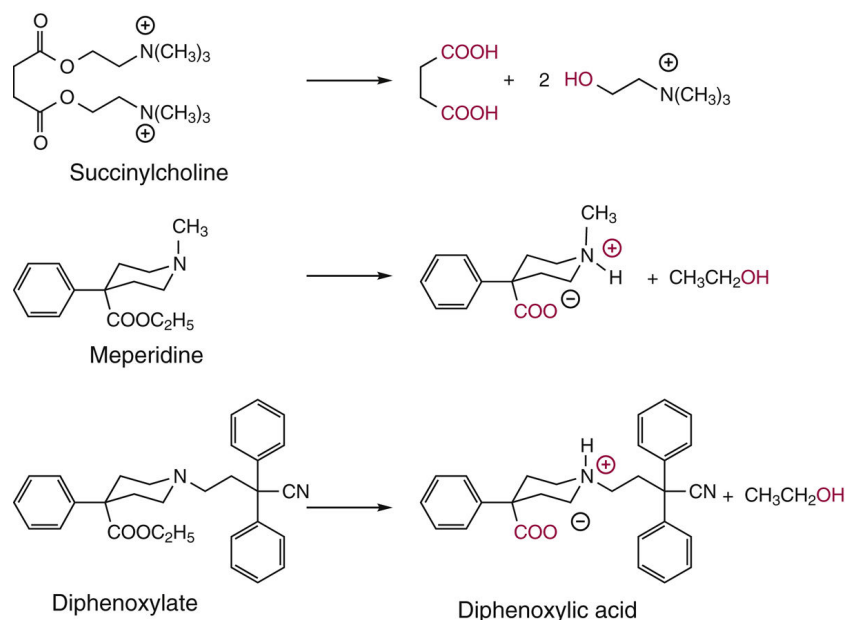


FIGURE 3.24 Examples of hydrolysis reactions.

Sterically hindered esters are hydrolyzed more slowly and can appear unchanged in the urine. For example, approximately 50% of a dose of atropine appears unchanged in the urine of humans, and the remainder consists of unhydrolyzed biotransformed products.

As a rule, amides are more stable to hydrolysis than are esters and it is not surprising to find amides of drugs excreted largely unchanged. This fact has been exploited in developing the antiarrhythmic drug procainamide. Procaine is not useful as an antiarrhythmic because of its rapid esterase-catalyzed hydrolysis, but 60% of a dose of procainamide was recovered unchanged from the urine of humans, with the remainder being mostly N-acetylprocainamide. On the other hand, the deacylated metabolite of indomethacin (a tertiary amide) is one of the major metabolites detected in human urine. Amide hydrolysis of phthalylsulfathiazole and succinylsulfathiazole by bacterial enzymes in the colon releases the antibacterial agent sulfathiazole.

Summary

In summary, Phase 1 metabolic transformations introduce new and polar functional groups into the molecule, which can produce one or more of the following changes:

1. Decreased pharmacological activity (deactivation)
2. Increased pharmacological activity (activation)
3. Increased toxicity (carcinogenesis, mutagenesis, cytotoxicity)
4. Altered pharmacological activity

Drugs exhibiting increased activity or activity different from the parent drug usually undergo further metabolism and conjugation resulting in deactivation and excretion of the inactive conjugates.

Drug Conjugation Pathways (Phase 2)

Conjugation reactions (Fig. 3.25) represent probably the most important xenobiotic biotransformation. Xenobiotics are as a rule lipophilic, well absorbed into tissues from the blood, but excreted slowly in the urine.⁵ Only after conjugation (Phase 2) reactions have added an ionic hydrophilic moiety, such as glucuronic acid, sulfate ester, or glycine to the xenobiotic, is water solubility significantly increased and lipid solubility decreased enough to make urinary elimination possible. The major proportion of the administered drug dose is excreted as conjugates into the urine and bile. Conjugation reactions can be preceded by Phase 1 reactions. For xenobiotics with a functional group available for Phase 2 metabolism, conjugation can be its fate.

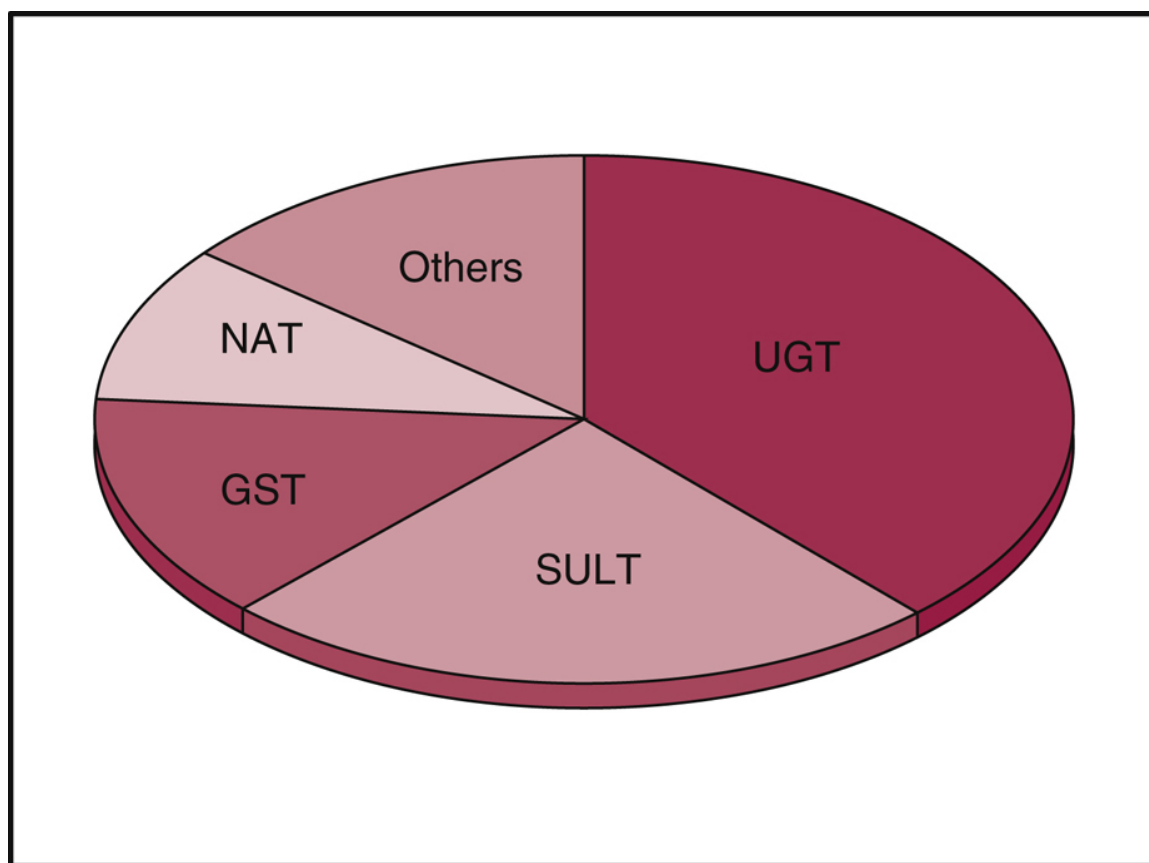


FIGURE 3.25 Phase 2 pathways contributing to drug metabolism (new).

Common Features of Phase 2 Pathways

Phase 2 conjugation commonly creates anionic metabolites (e.g., glucuronides, sulfate esters, glycine conjugates, glutathione conjugates) that are then actively excreted into the bile via biliary transporters, or into the renal proximal tubules by renal transporters.^{5,7,51} Conjugated metabolites often have higher elimination clearance than the parent drug, due in part to active excretion/transport into urine and/or bile, and are expected to have a half-life shorter than that of the parent drug. Cofactor depletion can occur at high drug doses. Biliary excretion of glucuronide and sulfate ester conjugates with higher molecular weight (e.g., >325) is increased.

The major conjugation reactions (glucuronidation and sulfonation) were traditionally thought to terminate pharmacological activity by transforming the parent drug or Phase 1 metabolites into readily excreted ionic polar products. Moreover, these terminal metabolites were assumed to have no significant pharmacological activity due to poor cellular diffusion and poor affinity for the active drug's receptor. This long-established view has changed with the discoveries that morphine 6-glucuronide has more analgesic activity than morphine in humans, and that minoxidil sulfate is the active metabolite for the antihypertensive minoxidil. For most xenobiotics, however, conjugation is an inactivating and detoxification mechanism. However, some compounds could form reactive intermediates that have been implicated in carcinogenesis, allergic reactions, and tissue damage.

Sequential conjugation of the same drug substance produces multiple conjugated products, for example, *p*-aminosalicylic acid in [Figure 3.26](#). Thus, the drug or xenobiotic can be a substrate for more than one metabolizing enzyme. Different conjugation pathways could compete for the same functional group with the outcome being an array of metabolites excreted in the urine or feces. Factors determining the outcome of this interplay include availability of cofactors, enzyme kinetics ($V_{\max}$), affinity of the drug/xenobiotic (K_m) for the metabolizing enzyme, and type of tissues (e.g., hepatic, kidney, lung). When a cofactor is low or depleted, the competing conjugation reactions can take control. The reactivity of the

functional group is determined also by all subsequent conjugation reactions. For example, major competing conjugation reactions are sulfonation, ether glucuronidation, and methylation for the phenolic hydroxyl groups; acetylation, sulfonation, and glucuronidation for amine groups; and amino acid conjugation, CoA conjugation, and ester glucuronidation for carboxyl groups.

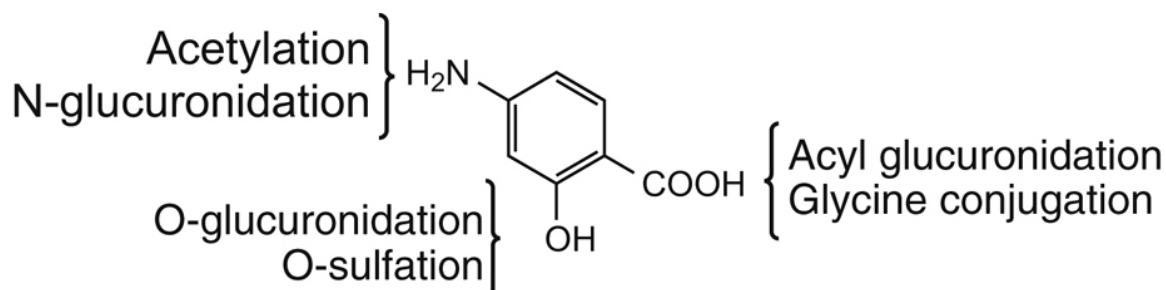


FIGURE 3.26 Sequential conjugation pathways for *p*-aminosalicylic acid.

Conjugation enzymes can show stereospecificity toward enantiomers when a racemic drug is administered. The metabolite pattern of a drug when administered orally can be different when the same drug is administered intravenously because of the avoidance of presystemic intestinal conjugation via the latter route.

Glucuronic Acid Conjugation

Glucuronide formation is the most common route for Phase 2 metabolism to water-soluble metabolites, accounting for approximately 35% of drug conjugation and the major share of the conjugated metabolites found in the urine and bile.⁵¹ The significance of glucuronidation lies in the available supply of glucuronic acid (oxidized glucose) in the liver and in the many functional groups capable of forming glucuronide conjugates (e.g., phenols, alcohols, carboxylic acids, and amines).

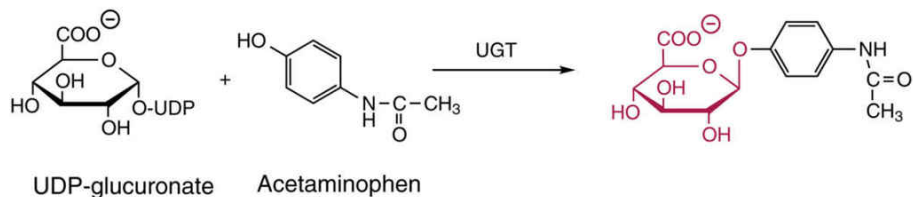
Mechanism of Glucuronide Conjugation

Glucuronide conjugation involves the direct condensation of the xenobiotic (or its Phase 1 metabolite) with the activated form of glucuronic acid, UDP-glucuronic acid (UDPGA). The overall scheme of the glucuronide conjugation reaction is shown in [Figure 3.27](#). The S_N2 reaction

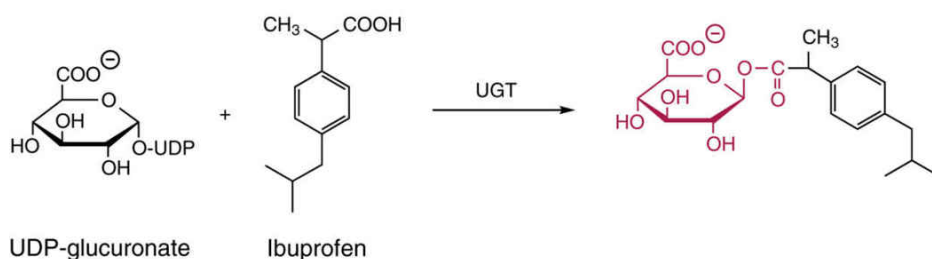
between UDPGA requires a nucleophile or “handle” on the drug or xenobiotic acceptor compound and is catalyzed by a family of UDP-glucuronosyltransferases (UGT), a multigene family of isozymes located along the ER of the liver, epithelial cells of the intestine, and other extrahepatic tissues.⁵ Its unique location in the ER, along with the P450 isoforms, has important physiologic effects in the conjugation of reactive metabolites generated by the P450 isoforms and in controlling the levels of reactive metabolites present in these tissues. Transporters carry the UDPGA and xenobiotics from the cytosol into the ER lumen and then transport the glucuronide metabolite from the ER lumen into the cytosol. The presence of the active site for UGT toward the ER lumen catalyzes the reaction between the substrate and UDPGA.⁵¹



O-glucuronidation:



Acylglucuronidation:



N-glucuronidation:

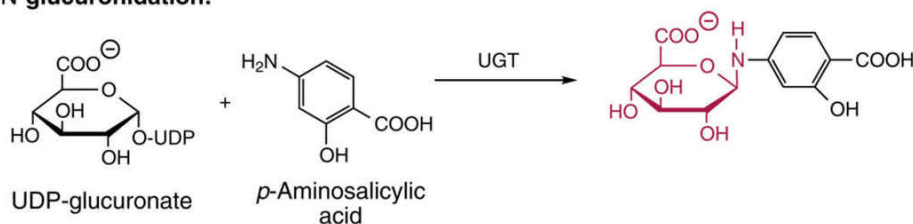


FIGURE 3.27 Glucuronidation pathways catalyzed by UDP-glucuronosyltransferases (UGTs).

Glucuronidation is a low-affinity (a higher concentration of the xenobiotic is required for full enzyme activity) and high-capacity (high rate of xenobiotic turnover) Phase 2 reaction. The resultant glucuronide has the β -configuration about carbon 1 of glucuronic acid. With the attachment of the hydrophilic carbohydrate moiety containing an easily ionizable carboxyl group ($\text{pK}_a = 3\text{--}4$), a lipid-soluble substance is converted into a conjugate that is reabsorbed poorly by the renal system.

Endogenous substances that readily conjugate with glucuronic acid include catecholamines, steroids, bilirubin, and thyroxine. Not all glucuronides are excreted by the kidneys. Some are excreted into the

intestinal tract with bile (enterohepatic cycling), where β -glucuronidase in the intestinal flora hydrolyzes the C¹-O-glucuronide to the aglycone (xenobiotic or their metabolites) for reabsorption into the portal circulation (see section Enterohepatic Cycling of Drugs). A summary of drug functional groups commonly glucuronidated is provided in [Figure 3.28](#).

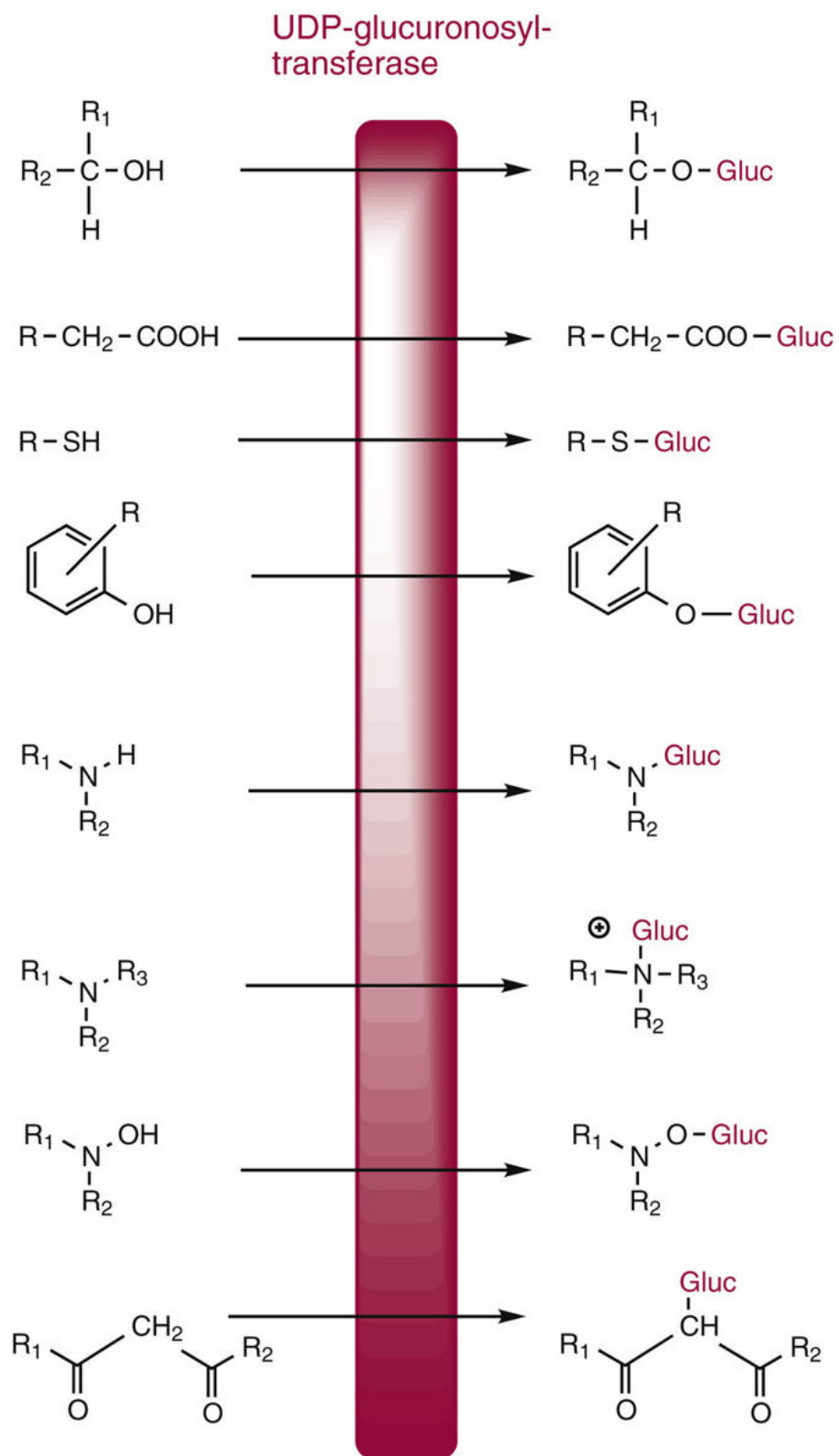


FIGURE 3.28 Phase 2 glucuronidation pathways contributing to drug metabolism (new).

UGT Families

UGTs have been classified into families according to similarities in amino acid sequences, analogous to the P450 family.⁵¹ The human UGT family is divided into two subfamilies, UGT1 (UGT1A1, 1A2, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9, and 1A10) and UGT2 (UGT2B4, 2B7, 2B10, 2B11, 2B15, 2B17, and 2B28).^{5,7,51} Considerable overlap in substrate specificities exists between the two families. The UGT1A1 isoform is primarily responsible for the glucuronidation of bilirubin, estradiol, and other estrogenic steroids; UGT1A3 and UGT1A4 catalyze the glucuronidation of drugs with tertiary amines to form quaternary glucuronides and hydroxylated xenobiotics; UGT1A6 exhibits limited substrate specificity for planar phenolic substances; UGT1A9 has a wide range of substrate specificity and can glucuronidate nonplanar phenols, plant substances (e.g., anthraquinones and flavones), steroids, other phenolic drugs, and C-glucuronidate phenylbutazone; and UGT1A10 glucuronidates mycophenolic acid, an inhibitor of inosine monophosphate dehydrogenase. Human family 2 isoform UGT2B4 is homologous to UGT2B7 and catalyzes the glucuronidation of the 6 α -hydroxyl group of bile acids; UGT2B7 glucuronidates the largest number of substrates including the 3- and 6-glucuronidation of morphine and 6-glucuronidation of codeine; UGT2B11 glucuronidates a wide range of planar phenols, bulky alcohols, and polyhydroxylated estradiol metabolites; and UGT2B15 catalyzes the glucuronidation of the 17 α -hydroxyl group of dihydrotestosterone and other steroidal compounds. UGT1A isoforms are inducible with 3-methylcholanthrene and cigarette smoking, and the UGT2B family is inducible by barbiturates. Approximately 40% of the glucuronides are produced by UGT2B7, 20% by UGT1A4, and 15% by UGT1A1.

UGT Distribution

The human liver is the most important tissue for all routes of Phase 1 and Phase 2 metabolism. The UGTs expressed in the intestine include UGT1A1 (bilirubin-glucuronidating isoform), UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, and UGT1A3. Substrate specificities of intestinal UGT isoforms are comparable to those in the liver. The UGT isoforms in the

intestine can glucuronidate orally administered drugs, such as morphine, acetaminophen, α - and β -adrenergic agonists, and other phenolic phenethanolamines, as well as other dietary xenobiotics, reducing their oral bioavailability (first-pass metabolism). Although UGT isoforms are found in the kidney, brain, nasal epithelia, and lung, they are not uniformly distributed, with UGT1A6 being the isoform that is ubiquitous in extrahepatic tissue.

O-, N-, and S-Glucuronides

The xenobiotics that contain an electron-rich nucleophilic (heteroatom) O, N, or S or some extremely electrophilic carbons form glucuronides. In humans, almost 40%-70% of clinically used drugs are subjected to O-glucuronidation, with alcohols and phenols producing ether O-glucuronides. O-phenolic glucuronides predominate over alcohol hydroxy O-glucuronidation because of the greater nucleophilicity of phenols (pK_a 7-10), efficiencies, and turnover rates. Aromatic and some aliphatic carboxylic acids form ester (acyl) glucuronides. Aromatic amines form N-glucuronides and sulfhydryl compounds form S-glucuronides, both of which are more labile to acid as compared with the O-glucuronides (Fig. 3.27). Some tertiary amines (e.g., tripeleminamine) have been reported to form quaternary ammonium N-glucuronides. Substances containing a 1,3-dicarbonyl structure (e.g., phenylbutazone) can undergo formation of C-glucuronides by direct conjugation without previous metabolism. The acidity of the methylene carbon of the 1,3-dicarbonyl group determines the degree of C-glucuronide formation.

Acyl Glucuronides

Drug-acyl glucuronides are reactive conjugates of carboxylic acids at physiologic pH.⁵² The acyl group of the C1-acyl glucuronide can migrate via transesterification from the original C1 position of the glucuronic acid to the C2, C3, or C4 positions (Fig. 3.27). The resulting positional isomers are not hydrolyzed by β -glucuronidase, giving the appearance of a new unknown conjugate. However, under physiologic or weakly alkaline conditions, the C1-acyl glucuronide can hydrolyze in the urine to produce the parent substance (aglycone) or undergo acyl migration to an acceptor

macromolecule, forming an immunoreactive hapten. The pH-catalyzed migration of the acyl group from the drug C1-O-acyl glucuronide to a protein or other cellular constituent occurs with the formation of a covalent bond to the protein. The acylated protein becomes a “hapten” and could stimulate an immune response against the drug, resulting in the expression of a hypersensitivity reaction or other forms of immunotoxicity. A high incidence of idiosyncratic/immunotoxic reactions has been reported for several NSAIDs; for example, benoxaprofen, zomepirac, indoprofen, alclofenac, ticrynafen, and ibufenac, all of which have been removed from the market. All of these NSAIDs are metabolized by humans to acyl glucuronides. Similar reactions have been reported for other NSAIDs, including tolmetin, sulindac, ibuprofen, ketoprofen, and acetylsalicylic acid. The frequency of the immunotoxic response can be related to the stability of the acyl glucuronide, the chemical rate kinetics for the migration of the acyl group, and the concentration and stability/half-life of the antigenic protein.

When the acyl glucuronide is the primary metabolite in patients with decreased renal function (i.e., elderly individuals) or when probenecid is coadministered, renal cycling of the unconjugated (aglycone) parent drug or metabolite is likely to occur, resulting in the plasma accumulation of the aglycone. The reduced elimination of the acyl glucuronide increases its hydrolysis to either the aglycone or the migration of the C1-O-acyl group to an acceptor macromolecule, as previously described.

Bioactivation and Toxic Glucuronides

As a rule, glucuronides are biologically and chemically less reactive than their parent molecules and are readily eliminated without interaction with intracellular substances. However, some glucuronide conjugates are more pharmacologically active than the parent drug.⁵³ Morphine, for example, forms the 3-O- and 6-O-glucuronides in the intestine and in the liver. The 3-O-glucuronide is the primary glucuronide metabolite of morphine with a blood concentration 20-fold that of morphine. Pharmacologically, it is an opioid antagonist. On the other hand, 6-O-glucuronide is a more potent μ -receptor agonist than morphine and, whether administered orally or parentally, is 650-fold more analgesic than morphine in humans. Thus, the

analgesic effects of morphine are the result of a complex interaction of the drug and its two glucuronide metabolites with the opioid receptor. The 6-O-glucuronide is transported into the brain via an anion-transport system. Glucuronidation is also capable of causing hepatotoxicity and carcinogenesis by facilitating the formation of reactive electrophilic (electron-deficient) intermediates and their transport into target tissues.⁵⁴ As previously described, the major metabolic biotransformations for carboxylic acids include conjugation with glucuronic acid, which are bioactivated via UGT-catalyzed conjugation with glucuronic acid to acyl glucuronides. The reactive acyl glucuronides are electrophilic and, therefore, can contribute to the acylation of target proteins (see section Reactive Metabolites Resulting from Bioactivation), resulting in idiosyncratic reactions.

The induction of bladder carcinogenesis by aromatic amines can result from the O-glucuronidation of the N-hydroxylarylamine⁵⁴ (Fig. 3.29). These O-glucuronides become concentrated in the urine, where they are either hydrolyzed by the acid pH of the urine to produce N-hydroxylarylaminines or eliminate water from the N-hydroxylarylamine under these acidic conditions to form an electrophilic arylnitrenium species (see Fig. 3.29, metabolite 4). This reactive species binds covalently with endogenous cellular constituents (e.g., nucleic acids and proteins), initiating carcinogenesis.

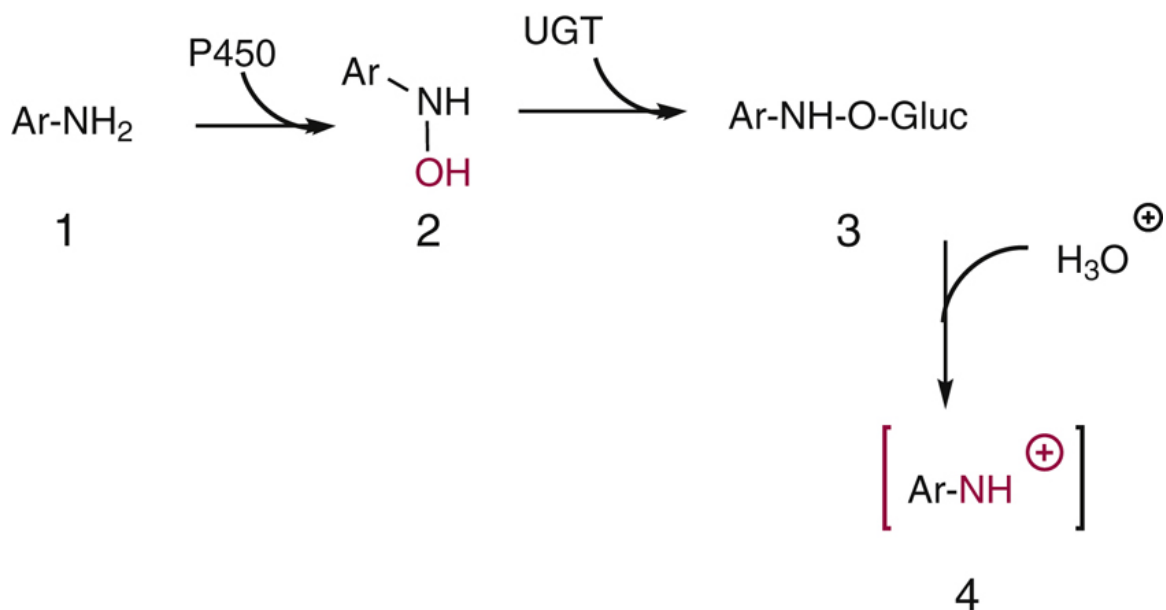


FIGURE 3.29 Bioactivation of arylamines.

Sulfonation and glucuronidation occur side by side, often competing for the same substrate (most commonly phenols, i.e., acetaminophen). The balance between sulfonation and glucuronidation is influenced by factors such as animal species, dose, availability of cofactors, and inhibition and induction of the respective transferases.

Sulfonation (Sulfoconjugation)

Sulfonation (sulfoconjugation) is an important conjugation reaction in the biotransformation of steroid hormones, catecholamine neurotransmitters, thyroxine, bile acids, phenolic drugs, and other xenobiotics.^{7,55,56} The major physiologic consequence of sulfonation of a drug or xenobiotic is its increased aqueous solubility and excretion. Because the pK_a of the sulfate group is approximately 1, sulfate esters are completely ionized in physiologic solution and possess a smaller volume of distribution than unconjugated steroids and drugs. Thus a lipid-soluble substance is converted into a conjugate that is reabsorbed poorly by the renal system. For some drugs, sulfonation can result in their bioactivation to reactive electrophiles (molecules that accept an electron pair to make a covalent bond) or therapeutically active conjugates (e.g., minoxidil sulfate). Cytosolic sulfotransferases are, in general, associated with the sulfonation

of phenolic steroids, neurotransmitters, and xenobiotics. Membrane-bound sulfotransferases are localized in the Golgi apparatus of most cells and are responsible for the sulfonation of glycosaminoglycans, glycoproteins, and the tyrosinyl group of peptides and protein but are usually not associated with xenobiotic metabolism.

Mechanism of Sulfonation

Xenobiotics are sulfonated by the transfer of a sulfonic group ($-\text{SO}_3^-$) from 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to the acceptor molecule, a cytosolic reaction catalyzed by a family of multigene sulfotransferases (Fig. 3.30); PAPS is formed enzymatically from adenosine triphosphate (ATP) and inorganic sulfate. Sulfonation is a reaction principally of phenols and to a lesser extent, of alcohols to form highly ionic and hydrophilic sulfate esters ($\text{R-O-SO}_2\text{H}$). The availability of PAPS and its precursor inorganic sulfate determines the sulfonation reaction rate.

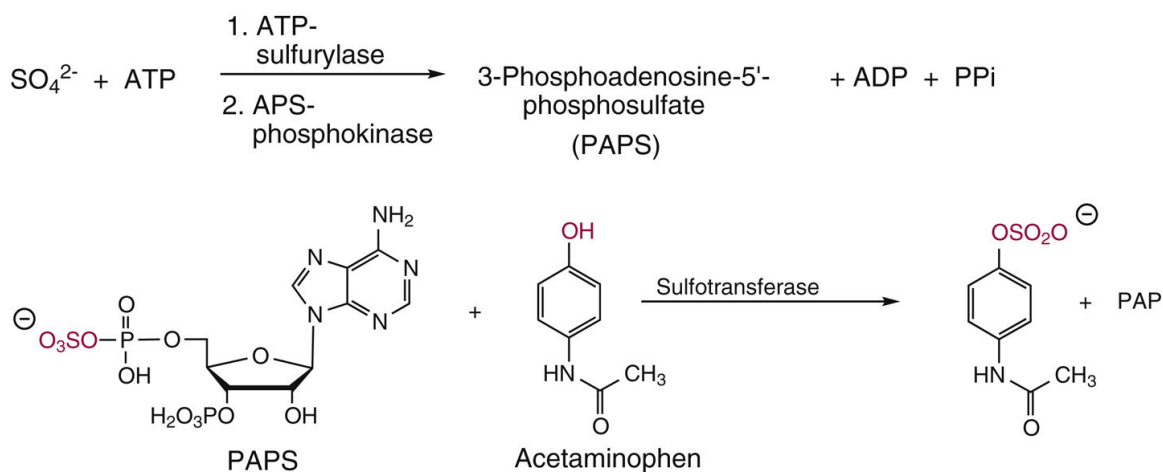


FIGURE 3.30 Sulfonation pathways.

The total pool of sulfate is frequently limited and can be readily exhausted due to the large number of substrates. Sulfonation is a high-affinity and low-capacity Phase 2 reaction which works in conjunction with glucuronidation on overlapping substrates. Thus, sulfonation predominates at low substrate concentrations and glucuronidation at high substrate concentrations.⁵⁵ When sulfonation becomes saturated from increasing doses of a drug, sulfonation becomes a less predominant

pathway and the rate of glucuronidation increases until it reaches saturation. At that point, either the drug is excreted unchanged or other metabolism pathways become activated. For example, at high doses of acetaminophen, glucuronidation predominates over sulfonation, which prevails at low doses. When PAPS, inorganic sulfate, or the sulfur amino acids are low or depleted, or when a substrate for sulfonation is given in high doses, competing reactions with glucuronidation can take control. Additionally, O-methylation is a competing reaction for a catechol.

Sulfotransferase Family

In humans, sulfotransferases are divided into two families, SULT1 and SULT2.⁵⁵ SULT1A1, SULT1A2, and SULT1A3 catalyze the sulfonation of many phenolic drugs, catecholamine, hormones, aromatic amines, and other xenobiotics. SULT1A3 displays stereoselectivity in the sulfonation of chiral phenolic phenethanolamines. This isoform can be responsible, in part, for the enantiomer-specific metabolism observed for the β -adrenergic agonists. For example, the (+)-enantiomers of terbutaline and isoproterenol and the (–)-enantiomer of albuterol are selectively sulfonated.

SULT1A1/2 (formerly known as phenol sulfotransferase thermally stable) preferentially sulfonates small planar phenols in the micromolar concentration range, estradiol and synthetic estrogens, phytoestrogens, acetaminophen, N-hydroxyaromatic and heterocyclic amines. The crystal structure for SULT1A1 has provided insights into this enzyme's substrate specificity and catalytic function, including its role in the sulfonation of endogenous substrates such as estrogens. SULT1A3 (formerly known as phenol sulfotransferase thermally labile) selectively sulfonates the catecholamines dopamine, norepinephrine, and epinephrine, as well as the N-oxide of minoxidil, thyroid hormones, but not estrogenic steroids and other hydroxy steroids. SULT1B1 catalyzes the sulfonation of the thyroid hormones, while SULT1C1 is involved with the bioactivation of procarcinogens via sulfonation. SULT1E1 (formerly known as estrogen sulfotransferase) preferentially sulfonates estradiol in the nanomolar range. SULT2A1 (formerly known as dehydroepiandrosterone [DHEA] sulfotransferase) conjugates DHEA, estradiol (micromolar range), the synthetic estrogens, and other estrogen metabolites. Finally, SULT2B1

(formerly known as hydroxysteroid sulfotransferase) sulfonates DHEA and pregnenolone. A summary of drug functional groups commonly sulfonated is provided in [Figure 3.31](#).

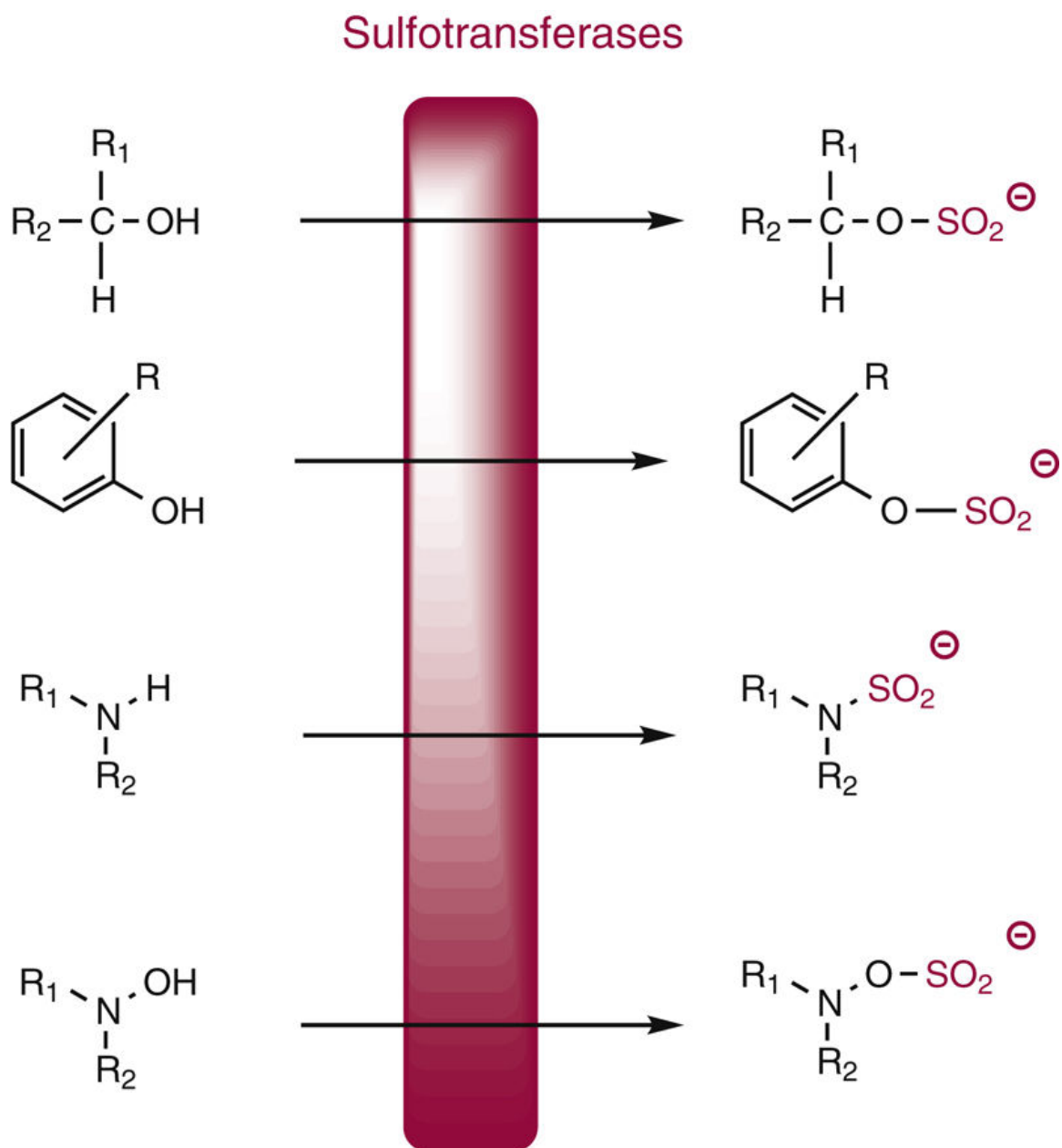


FIGURE 3.31 Phase 2 sulfonation pathways contributing to drug metabolism (new).

Sulfonation is an important reaction in the transport and metabolism of steroids. Sulfonation decreases the biological activity of the steroid because the steroid sulfate esters are not capable of binding to their

receptors. It provides for the transport of an inactive form of the steroid to its target tissue, where the active steroid is regenerated by sulfatases at the target tissue.

Sulfotransferase Distribution

The SULT1A families are abundantly expressed in the liver, small intestine, brain, kidneys, and platelets.⁵⁵ For example, phenol is sulfonated by a sulfotransferase in the liver, kidneys, and intestines, whereas steroids are sulfonated only in the liver. The broad diversity of compounds sulfonated in human tissues results, in part, from the multi-isoforms of the cytosolic sulfotransferases and their overlapping substrate specificities. Sulfate esters are almost totally ionized and, therefore, are excreted mostly in the urine, via transporters but biliary elimination is common for steroids. When biliary sulfate esters are hydrolyzed in the intestine by sulfatases, the parent drug (or xenobiotic) or its metabolites can be reabsorbed into the portal circulation to be resulfonated for eventual elimination in the urine as a sulfate ester (enterohepatic cycling). The rate of sulfonation appears to be age dependent, decreasing with age.

An important site of sulfonation, especially after oral administration, is the intestine. The result is a presystemic first-pass effect, decreasing drug bioavailability of several drugs for which the primary route of conjugation is sulfonation. Drugs such as isoproterenol, albuterol, steroid hormones, α -methyldopa, acetaminophen, and fenoldopam are sulfonated in the gut. Competition for intestinal sulfonation between coadministered substrates can influence their bioavailability with either an enhancement of or a decrease in therapeutic effects. An example would be the coadministration of a 500-mg dose of acetaminophen and 0.03-mg dose of the oral contraceptive ethinyl estradiol. This interaction could result in increased toxicity of ethinyl estradiol because of less sulfonation in the gut.

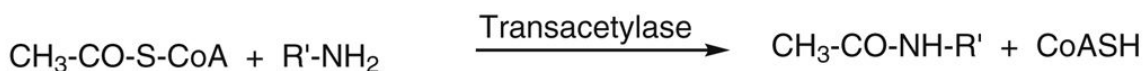
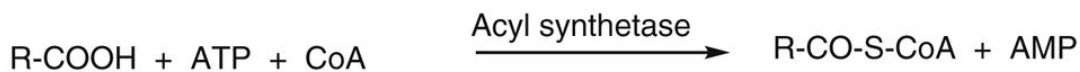
Bioactivation and Toxicity

Sulfonation is a common final step in the biotransformation of xenobiotics and is traditionally associated with inactivation, although sulfate esters have been reported to be pharmacologically active (e.g., minoxidil sulfate, dehydroepiandrosterone sulfate, and morphine 6-sulfate). However, the sulfate ester group is electron-withdrawing and may be cleaved off in

some molecules, leading to an electrophilic cation forming reactive intermediates implicated in carcinogenesis and tissue damage.⁵⁶ Sulfotransferase-mediated toxicity has been demonstrated for numerous benzylic alcohols derived from polycyclic aromatic hydrocarbons and various aromatic hydroxylamines. For example, 1'-hydroxysafrole (an allylic/benzylic alcohol) is bioactivated to reactive metabolites by sulfotransferases. SULT1A1 can also sulfonate procarcinogens such as hydroxymethyl polycyclic aromatic hydrocarbons, producing reactive intermediates capable of forming DNA adducts, potentially resulting in mutagenesis. Sulfonation of an alcohol generates a good leaving group and can be an activation process for alcohols to produce a reactive electrophilic species.⁵⁵ However, like the N-glucuronides, N-sulfates are capable of promoting cytotoxicity by facilitating the formation of reactive electrophilic intermediates. Sulfonation of N-oxygenated aromatic amines is an activation process for some arylamines which can eliminate sulfate to produce an electrophilic species capable of reacting with proteins or DNA (e.g., 2-acetylaminofluorene). N-sulfonation of arylamines to arylsulfamic acids (R-NHSO₃H) is a minor pathway.

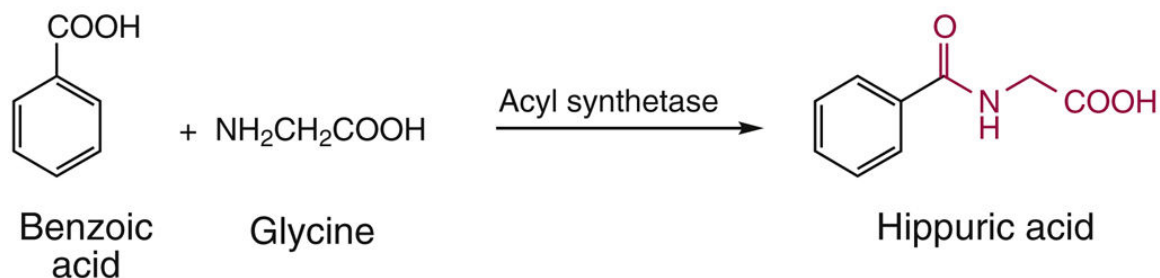
Conjugation with Amino Acids

Conjugation with amino acids is an important metabolic route in the metabolism of drug or xenobiotic carboxylic acids before elimination involving CoA thioesters.^{5,7} Glycine, the most common amino acid, forms water-soluble ionic conjugates with aromatic, arylaliphatic, and heterocyclic carboxylic acids. These glycine conjugates are usually less toxic than their precursor acids and are readily excreted into the urine and bile. These reactions involve the formation of an amide or peptide bond between the xenobiotic carboxylic acid and the amino group of glycine. The xenobiotic is first activated to its CoA thioester before reacting with the amino group (Fig. 3.32). The formation of the xenobiotic acyl CoA thioester is of critical importance in intermediary metabolism of lipids, as well as intermediate- and long-chain fatty acids.



Examples:

Glycine conjugation:



Acetylation:

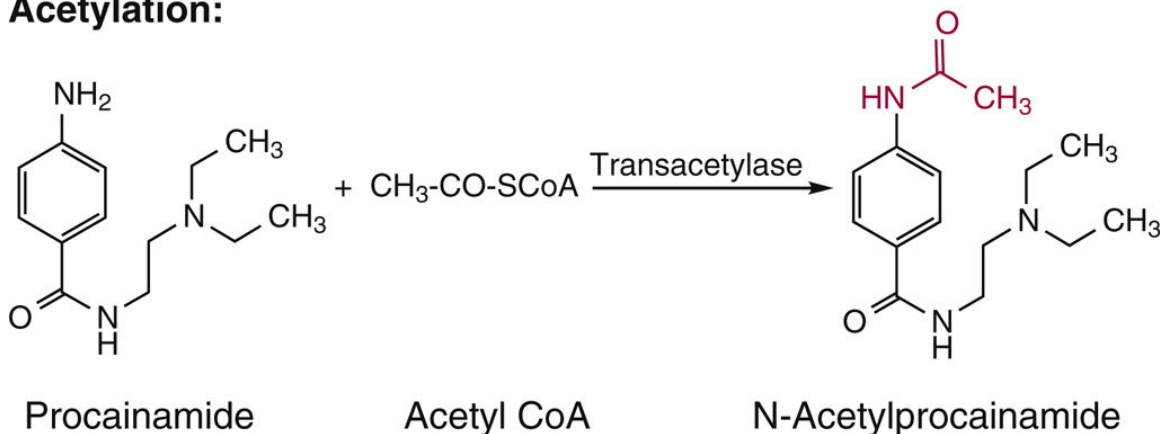


FIGURE 3.32 Amino acid conjugation pathway of carboxylic acids with glycine and acetylation pathway catalyzed by acetyltransferases.

The major metabolic biotransformations for xenobiotic carboxylic acids include conjugation with either glucuronic acid or glycine. Carboxylic acids can be bioactivated via two distinct pathways: UGT-catalyzed conjugation with glucuronic acid to acyl glucuronides, or acyl CoA synthetase-catalyzed formation of acyl CoA thioesters.^{52,57} The reactive acyl glucuronides and CoA thioester intermediates of carboxylic acids are electrophilic and, therefore, can contribute to the acylation of target

proteins (see section Reactive Metabolites Resulting from Bioactivation). Acyl CoA thioester serves as an obligatory intermediate in the formation of glycine conjugates and carnitine esters, which are involved in mitochondrial acyl transfer or elimination of acyl groups into urine. Therefore, their appearance in metabolism studies and urine is of significance because they serve as biomarkers for the formation acyl CoA thioesters, which can provide the link between protein-reactive acyl CoA thioesters and the rare and unpredictable idiosyncratic drug reactions in humans. Unlike CYP and UGT, which are localized in the SER, the enzymes of amino acid conjugation reside in mitochondria of the liver and kidney.

Glycine conjugation has generally been assumed to be a detoxification mechanism, increasing the water solubility of organic acids in order to facilitate their urinary excretion as compared to glucuronidation. However, glycine conjugation does not significantly increase the water solubility of aromatic acids. The role of glycine conjugation is to dispose of the end products of benzoic acid metabolism into hippuric acid products.⁵⁷ The metabolic fate of these carboxylic acids depends on the size and type of substituents adjacent to the carboxyl group. Most unbranched aliphatic acids are completely oxidized by β -oxidation to acetic acids and do not typically form conjugates. Branched aliphatic and arylaliphatic acids are resistant to β -oxidation and form glycine or acyl glucuronide conjugates: substitution of the α -carbon favors glucuronidation over glycine conjugation. Benzoic and heterocyclic aromatic acids are mostly conjugated with glycine. Glycine conjugation is preferred for xenobiotic carboxylic acids at low doses, and glucuronidation is preferred at high doses with broad substrate selectivity. In humans, glutamine can also form a conjugate with phenylacetic acids and related arylacetic acids. Bile acids form conjugates with glycine and taurine by the action of enzymes in the microsomal fraction rather than in the mitochondria.

In contrast to the enhanced reactivity and toxicity of the various glucuronides, sulfate ester, acetyl and glutathione conjugates, amino acid conjugates have not proven to be toxic. However, organic acids can be toxic if they are not a substrate for glycine conjugation with CoASH.⁵⁷

Carboxylic acids have been associated with adverse reactions linked to the metabolic activation of the carboxylic acid moiety of the compounds. It has been proposed that amino acid conjugation is a detoxication pathway for reactive acyl CoA thioesters.

Several carboxylic acid-containing drugs (e.g., zomiperac and benzoxaprofen) have been implicated in rare but serious adverse reactions. These carboxylic acids were withdrawn in the late 1980s from the market as a result of unpredictable idiosyncratic reactions that could have been caused by carboxylic acid-protein adducts formed by reaction of their reactive acyl glucuronide or acyl CoA thioesters with endogenous proteins.

Acetylation

Acetylation is principally a reaction of amino groups involving the transfer of acetyl CoA to primary aliphatic and aromatic amines, amino acids, hydrazines, or sulfonamide groups. The liver is the primary site of acetylation, although extrahepatic sites have been identified. Antimicrobial sulfonamides (e.g., sulfisoxazole), being difunctional, can form either N1 or N4 acetyl derivatives. Secondary amines are not acetylated. Acetylation can produce conjugates that retain the pharmacological activity of the parent drug (e.g., N-acetylprocainamide) ([Fig. 3.32](#)).

Acetylation, a nonmicrosomal form of metabolism, also exhibits polymorphisms and was first demonstrated in the acetylation of isoniazid. Several forms of N-acetyltransferase (NAT1 and NAT2) occur in humans. NAT1 (arylamine N-acetyltransferase) is widely distributed and catalyzes the N-acetylation of acidic arylamines, such as *p*-aminobenzoic acid and *p*-aminosalicylic acid; therefore its expression levels in the body have toxicological importance with regard to drug toxicity and cancer risk (e.g., bladder cancer and colorectal cancer).

NAT2 is found predominately in the liver. Clinically used drugs undergoing NAT2-catalyzed N-acetylation include isoniazid, procainamide, hydralazine, phenelzine, dapsone, caffeine, and the carcinogenic secondary N-alkylarylamines (2-aminofluorene, benzidine, and 4-aminobiphenyl). Substituents ortho to the amino group sterically block acetylation. Polymorphisms in the NAT2 gene in human populations can be segregated into fast, intermediate, and slow acetylator phenotypes. Polymorphisms in NAT2 are also associated with higher incidences of cancer and drug toxicity. Intestinal N-acetyltransferase appears not to be polymorphic (i.e., 5-aminosalicylic acid). The proportion of the fast acetylation phenotype is approximately 30%-45% in Caucasians, 89%-90% in the Oriental population, and 100% in Canadian Eskimos. Drug-induced systemic lupus erythematosus from chronic procainamide therapy is more likely to appear with slow acetylators.

Acetylation polymorphism has been associated with differences in human drug toxicity between slow and fast acetylator phenotypes. Slow

acetylators are more prone to drug-induced toxicities and accumulate higher blood concentrations of the unacetylated drug than do fast acetylators. Examples of toxicities that can result include hydralazine- and procainamide-induced lupus erythematosus, isoniazid-induced peripheral nerve damage, and sulfasalazine-induced hematologic disorders. Fast acetylators eliminate the drug more rapidly by conversion to its relatively nontoxic N-acetyl metabolite. However, for some drug substances, fast acetylators can pose a greater risk of liver toxicity than slow acetylators because fast acetylators produce toxic metabolites more rapidly. The possibility arises that genetic differences in acetylating capacity can confer differences in susceptibility to chemical carcinogenicity from arylamines. The tumorigenic activity of arylamines (1 in Fig. 3.33) can be the result of a complex series of sequential metabolic reactions commencing with N-acetylation (2 in Fig. 3.33), subsequent oxidation to arylhydroxamic acids (3 in Fig. 3.33), and metabolic transformation to acetoxarylamine by N,O-acetyltransferase (4 in Fig. 3.33).⁵⁴ The acetoxarylamine can eliminate the acetox group to form the reactive arylnitrenium ion (5 in Fig. 3.33), which is capable of covalently binding to nucleic acids and proteins, thus increasing the risk for development of bladder and liver tumors.⁵⁴ The rapid acetylator phenotype is expected to form the acetoxarylamine metabolite at a greater rate than the slow acetylator and thereby, to present a greater risk for development of tumors compared with the slow acetylator.

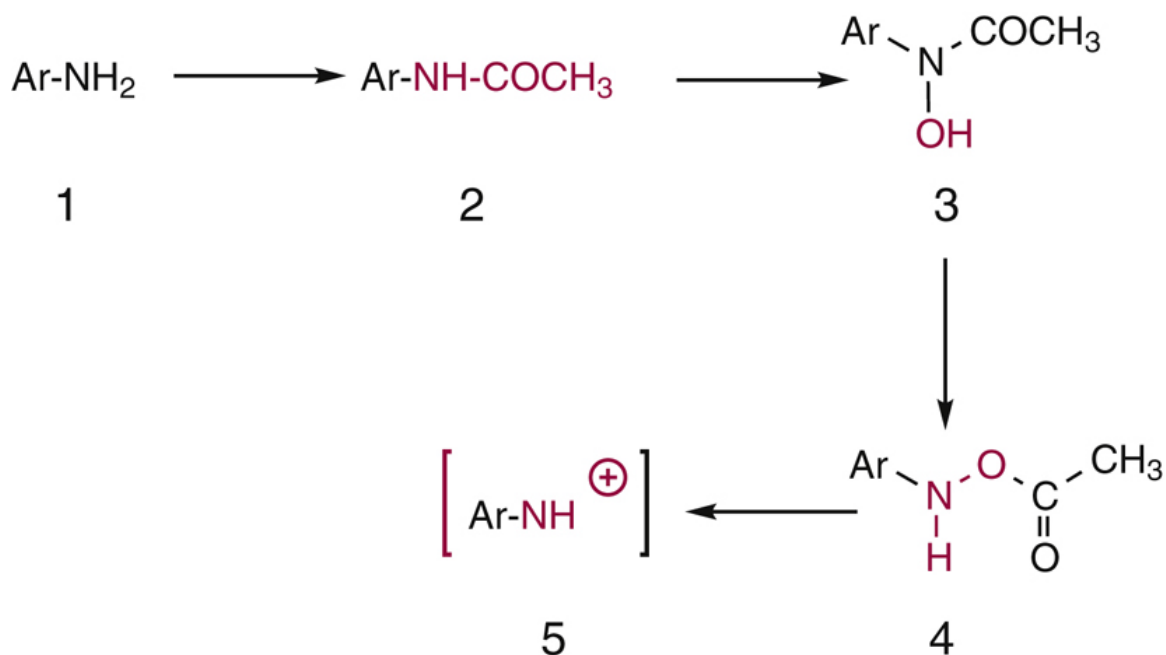


FIGURE 3.33 Bioactivation of acetylated arylamines.

Glutathione Conjugation and Mercapturic Acid Synthesis

Mercapturic acids are S-derivatives of N-acetylcysteine synthesized from glutathione (GSH).^{5,58,59} The mercapturic acid pathway appears to have evolved as a protective (scavenger) mechanism against xenobiotic-induced hepatotoxicity or carcinogenicity, serving to detoxify a large number of noxious substances that we inhale or ingest or that are produced daily in the human body. Most xenobiotics that are metabolized to mercapturic acids first undergo conjugation with GSH catalyzed by the enzyme glutathione S-transferase (GST), a multigene isoenzyme family that is abundant in the soluble supernatant liver fractions. In humans, the GST family consists of cytosolic dimeric isoenzymes of 45-55 kDa size that have been assigned to at least seven classes: alpha (five members), kappa (one member), mu (six members), omega (two members), pi (one member), theta (two members), zeta (one member), and microsomal (three members). The principal drug substrates for the mu family are the nitrosourea and mustard-type anticancer drugs. The theta isoform metabolizes small organic molecules, such as solvents, halocarbons, and electrophilic compounds (e.g., α,β -unsaturated carbonyl compounds). The

GSH conjugation reaction to mercapturic acid metabolites is depicted in Figure 3.34.

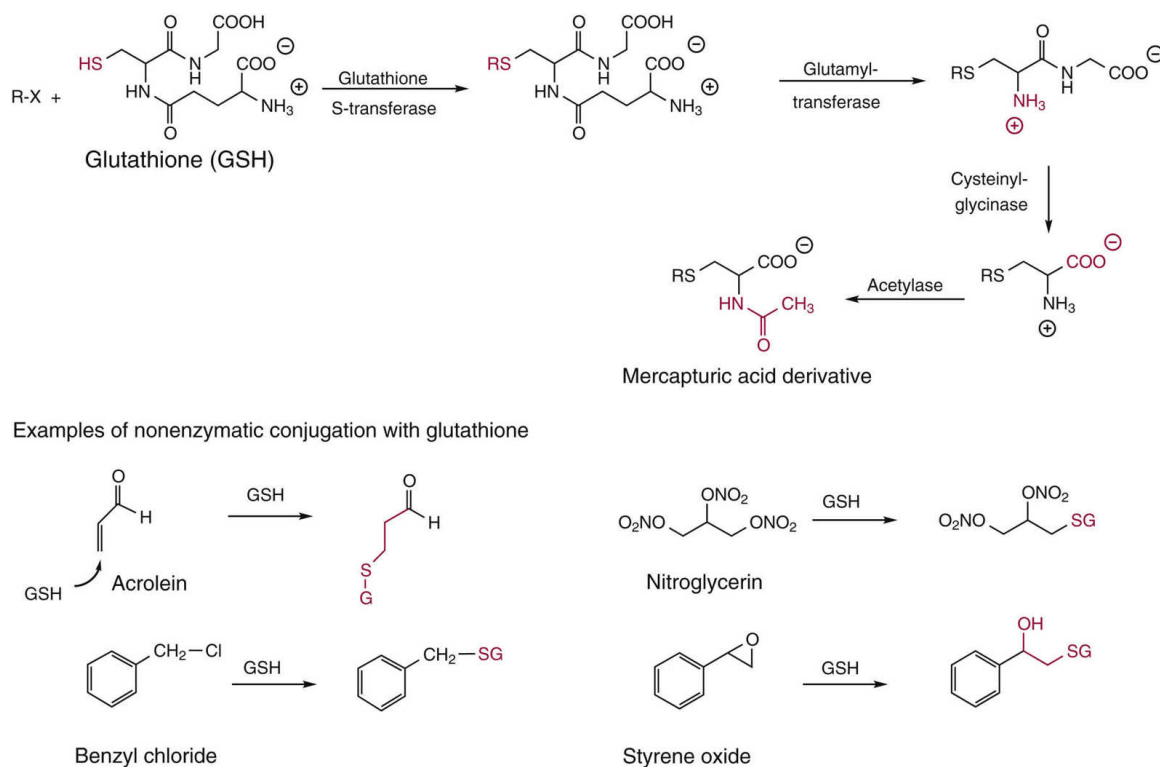


FIGURE 3.34 Glutathione and mercapturic acid conjugation pathways.

GST increases the ionization of the thiol group of GSH, increasing its nucleophilicity toward electrophiles (a group or ion that accepts an electron pair to make a covalent bond such as a carbocation or acyl ion) and thereby increasing the rate of conjugation with these potentially harmful electrophiles. In this way, GSH conjugation protects other vital nucleophilic centers in the cell, such as nucleic acids and proteins, from these electrophiles. Glutathione is also capable of reacting nonenzymatically with nucleophilic sites on neighboring macromolecules (Fig. 3.34) (see also section Metabolic Activation). Once conjugated with GSH, the electrophiles are excreted in the bile and urine.

A range of functional groups yields thioether conjugates of GSH, as well as products other than thioethers (Fig. 3.34). The nucleophilic attack by GSH occurs on electrophilic carbons with leaving groups (e.g., halogen [alkyl, alkenyl, aryl, or aralkyl halides], sulfonates [alkylmethanesulfonates] and

nitro [alkyl nitrates] groups), ring opening of small ring ethers (epoxides and β -lactones, e.g., β -propiolactone), and the Michael-type addition to the activated β -carbon of an α,β -unsaturated carbonyl compound (e.g., acrolein) (see section Reactive Metabolites Resulting from Bioactivation). The lack of substrate specificity gives argument to the fact that glutathione transferase has undergone adaptive changes to accommodate the variety of xenobiotics to which it is exposed. The conjugation of an electrophilic compound with GSH is usually a reaction of detoxication, but in some cases carcinogens have been activated through conjugation with GSH.^{58,59} The enzymatic conjugation of GSH with epoxides provides a mechanism for protecting the liver from injury caused by certain bioactivated intermediates (see section Reactive Metabolites Resulting from Bioactivation). Not all epoxides are substrates for this enzyme, but the more chemically reactive epoxides appear to be better substrates. Important among the epoxides that are substrates for this enzyme are those produced from halobenzenes and polycyclic aromatic hydrocarbons through the action of a P450 monooxygenase. Epoxide formation represents bioactivation because the epoxides are reactive and potentially toxic, whereas their GSH conjugates are inactive. Conjugation of GSH with the epoxides of aryl hydrocarbons eventually results in the formation of hydroxymercapturic acids (premercapturic acids) which undergo acid-catalyzed dehydration to the mercapturic acids. The halobenzenes are typically conjugated in the *p*-position.

Monohalogenated, *gem*-dihalogenated, and vicinal dihalogenated alkanes undergo glutathione transferase-catalyzed conjugation reactions to produce S-substituted glutathione derivatives that are metabolically transformed into the more stable and less toxic mercapturic acids. This common route of metabolism occurs through nucleophilic displacement of a halide ion by the thiolate anion of glutathione. The mutagenicity of the 1,2-dihaloethanes (e.g., the pesticide and fumigant ethylene dibromide) has been attributed to GSH displacing bromide with the formation of the S-(2-haloethyl) glutathione, which subsequently rearranges to a reactive episulfonium ion electrophile that, in turn, alkylates DNA. Many of the

halogenated hydrocarbons exhibiting nephrotoxicity undergo the formation of similar S-substituted cysteine derivatives.

A correlation exists between the hepatotoxicity of acetaminophen and levels of GSH in the liver. The probable mechanism of toxicity that has emerged from animal studies is that acetaminophen is oxidized by CYP1A2 and CYP2E1 to the N-acetyl-p-benzoquinonimine intermediate, which conjugates with and depletes hepatic GSH levels (Fig. 3.35). This action allows the benzoquinonimine to bind covalently to tissue macromolecules. The mercapturic acid derivative of acetaminophen represents approximately 2% of the administered dose of acetaminophen. Thus, the possibility exists that those toxic metabolites that are mostly detoxified by conjugating with GSH exhibit their hepatotoxicity (or, perhaps, carcinogenicity) because the liver has been depleted of GSH and is incapable of inactivating them. Pretreatment of animals with phenobarbital often hastens the depletion of GSH by increasing the formation of epoxides or other reactive intermediates.

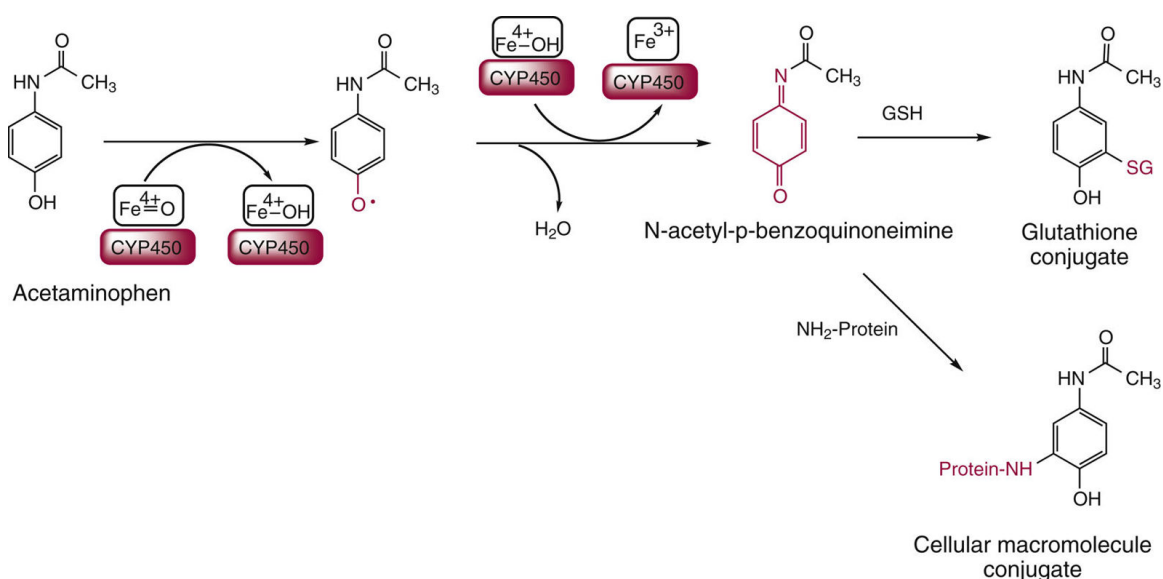


FIGURE 3.35 Proposed mechanism for the CYP450-catalyzed oxidation of acetaminophen to its N-acetyl-p-benzoquinoneimine intermediate, which can further react with either glutathione (GSH) or cellular macromolecules (NH₂-protein).

Methylation

Methylation is a common biochemical reaction but appears to be of greater significance in the metabolism of endogenous compounds than for drugs and other xenobiotics. Methylation differs from other conjugation processes in that the O-methyl metabolites formed can, in some cases, have as great or greater pharmacological activity and lipophilicity than the parent molecule (e.g., the conversion of norepinephrine to epinephrine). Methionine is involved in the methylation of endogenous and exogenous substrates, because it transfers its methyl group via the activated intermediate S-adenosylmethionine to the substrate under the influence of methyltransferases (Fig. 3.36). Methylation results principally in the formation of O-methylated, N-methylated, and S-methylated products.

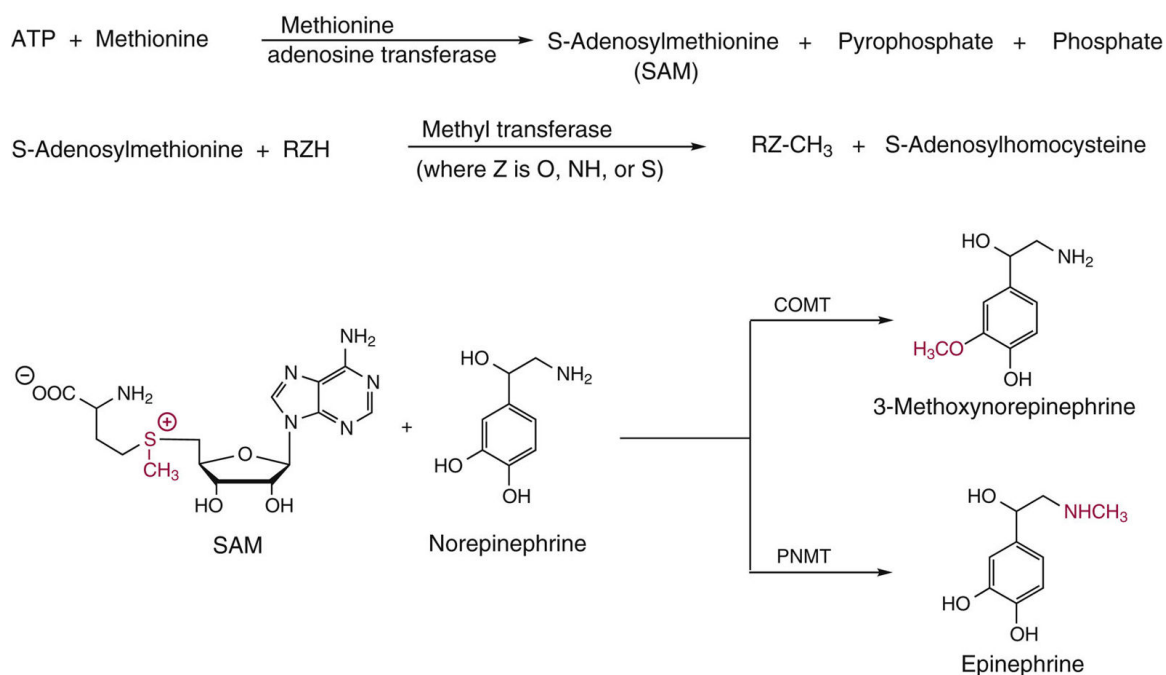


FIGURE 3.36 Methylation pathways.

O-Methylation

O-methylation is catalyzed by the magnesium-dependent enzyme catechol-O-methyltransferase (COMT), which transfers a methyl group primarily to the *m*- or 3-hydroxy group of the catechol moiety (3,4-dihydroxyphenyl moiety) of norepinephrine, epinephrine, or dopamine, resulting in inactivation. Less frequently, the *p*- or 4-hydroxy group of

these catecholamines is methylated (regioselectivity), along with their deaminated metabolites. COMT does not methylate monophenols or other dihydroxy non-catechol phenols. The *m:p* product ratio depends greatly on the type of substituent attached to the catechol ring. Substrates specific for COMT include the aforementioned catecholamines norepinephrine, epinephrine, and dopamine; the catechol amino acids L-DOPA and α -methyl-L-DOPA; and 2- and 4-hydroxyestradiol (catechol-like) metabolites of estradiol. It is found in liver, kidneys, nervous tissue, and other tissues. Hydroxyindole-O-methyltransferase, which O-methylates N-acetylserotonin, serotonin, and other hydroxyindoles, is found in the pineal gland and is involved in the formation of melatonin, a hormone associated with the dark-light diurnal cycle in humans. This enzyme differs from COMT in that it does not methylate catecholamines and has no requirement for magnesium ion.

N-Methylation

N-Methylation is among several conjugation pathways for metabolizing amines. Specific N-methyltransferases catalyze the transfer of active methyl groups from S-adenosylmethionine to the acceptor substance. Phenylethanolamine-N-methyltransferase methylates a number of endogenous and exogenous phenylethanolamines (e.g., normetanephrine, norepinephrine, and norephedrine) but does not methylate phenylethylamines. Histamine-N-methyltransferase methylates specifically histamine, producing the inactive metabolite N¹-methylhistamine. Amine-N-methyltransferase will N-methylate a variety of primary and secondary amines from a number of sources, including endogenous biogenic amines (serotonin, tryptamine, tyramine, and dopamine) and drugs (desmethylinipramine, amphetamine, and normorphine). Amine-N-methyltransferases seem to have a role in recycling N-demethylated drugs.

S-Methylation

As a rule, thiols are toxic, and the role of thiol S-methyltransferases is a nonoxidative detoxification pathway of these compounds. S-methylation

of sulfhydryl compounds also involves a microsomal enzyme requiring S-adenosylmethionine. Although a wide range of exogenous sulfhydryl compounds are S-methylated by this microsomal enzyme, none of the endogenous sulfhydryl compounds (e.g., cysteine and GSH) can function as substrates. S-methylation represents a detoxication step for thiols. Dialkyldithiocarbamates (e.g., disulfiram) and the antithyroid drugs (e.g., 6-propyl-2-thiouracil), mercaptans, and hydrogen sulfide (from thioglycosides as natural constituents of foods, mineral sulfides in water, fermented beverages, and bacterial digestion) are S-methylated. Other drugs undergoing S-methylation include captopril, thiopurine, azaprime, penicillamine, and 6-mercaptopurine.

Thiopurine methyltransferase or thiopurine S-methyl-transferase (TPMT) catalyzes the S-methylation of thiopurine drugs, such as azathioprine, 6-mercaptopurine, and 6-thioguanine. Interindividual variations in sensitivity and toxicity to thiopurine are correlated with TPMT genetic polymorphisms. Defects in the TPMT gene result in decreased methylation and decreased inactivation of the thiopurine drugs, leading to enhanced bone marrow toxicity. The clinical result may be myelosuppression, anemia, bleeding tendency, leukopenia, and infection. Approximately, 5% of all thiopurine therapies will fail due to toxicity related to TPMT polymorphism.

Elimination Pathways

Most xenobiotics are lipid-soluble and are altered chemically by the metabolizing enzymes, usually into less toxic and more water-soluble substances, before being excreted into the urine or, in some cases, bile. The formation of conjugates with sulfonate, amino acids, and glucuronic acid is particularly effective in increasing the hydrophilicity of drug molecules. The principal route of excretion of drugs and their metabolites is via the urine. If drugs and other compounds foreign to the body are not metabolized in this manner, substances with a high lipid-water partition coefficient could be reabsorbed readily from the urine through the renal tubular membranes and into the plasma. Therefore, such substances would continue to be recirculated and their pharmacological or toxic effects would be prolonged. Very polar or highly ionized drug molecules often are excreted in the urine unchanged. Urine is not the only route for excreting drugs and their metabolites from the body. Other routes include bile, saliva, lungs, sweat, and milk. The bile and biliary efflux transporters have been recognized as major routes of excretion for many endogenous and exogenous compounds.

Enterohepatic Cycling of Drugs

The liver is the principal organ for the metabolism and eventual elimination of xenobiotics from the human body in either the urine or the bile. When eliminated in the bile, steroid hormones, bile acids, drugs, and their respective conjugated metabolites are available for reabsorption from the duodenal-intestinal tract into the portal circulation, undergoing the process of enterohepatic cycling (EHC) (Fig. 3.37).⁶⁰ Nearly all drugs are excreted in the bile, but only a few are concentrated in the bile. For example, the bile salts are efficiently concentrated in the bile and reabsorbed from the GIT, such that the entire body pool of bile acids/salts are recycled multiple times per day. Therefore, EHC is responsible for the conservation of bile acids, steroid hormones, thyroid hormones, and other endogenous substances. In humans, compounds excreted into the bile typically have a molecular weight greater than 500 Da. In humans,

metabolites with a molecular weight between 300 and 500 Da are excreted in both urine and bile. Compounds excreted into bile are, as a rule, hydrophilic substances that can be either charged (anionic) or uncharged (e.g., cardiac glycosides and steroid hormones). Biotransformation of these compounds by means of Phase 1 and Phase 2 reactions would produce a conjugated metabolite, which is anionic and hydrophilic with a molecular weight greater than that of the parent compound. The conjugated metabolites are most often glucuronides, because glucuronidation adds 176 Da to the molecular weight of the parent compound. Unchanged drug in the bile is either excreted with the feces, metabolized by the bacterial flora in the intestinal tract, or reabsorbed into the portal circulation via EHC.

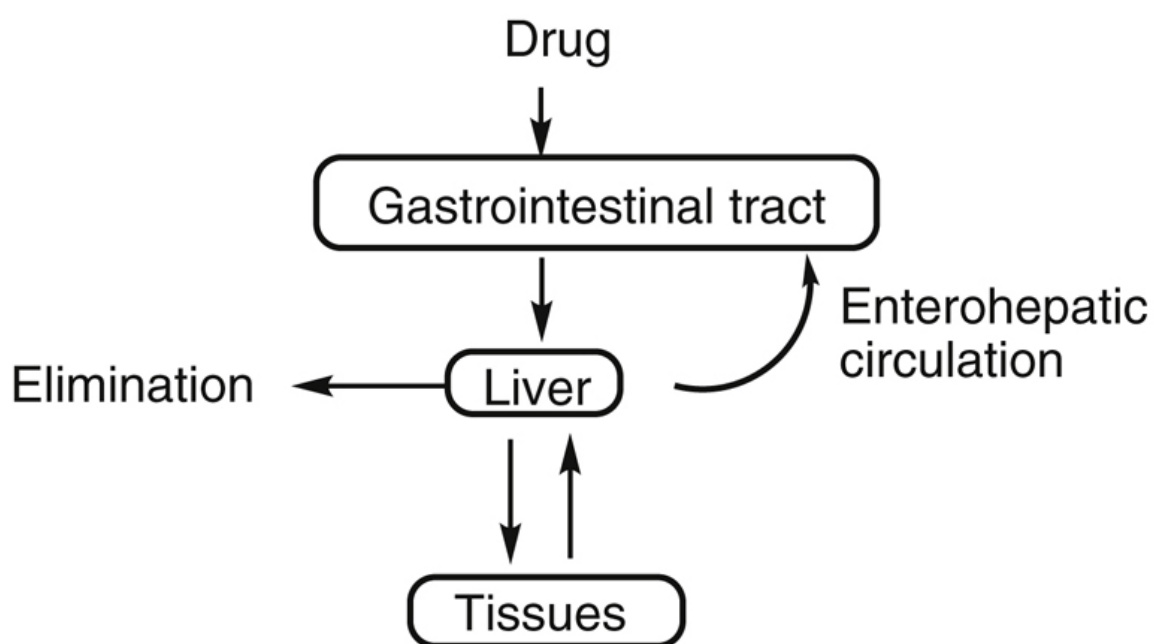


FIGURE 3.37 Enterohepatic cycling.

The bacterial intestinal flora is directly involved in EHC and the recycling of drugs through the portal circulation.⁶¹ A conjugated drug and metabolites excreted via the bile can be hydrolyzed by enzymes of the bacterial flora, releasing the parent drug or its Phase 1 metabolite for reabsorption into the portal circulation. Among the numerous compounds metabolized in the enterohepatic circulation are the estrogenic and

progestational steroids, digitoxin, indomethacin, diazepam, pentaerythritol tetranitrate, mercurials, arsenicals, and morphine. The oral ingestion of xenobiotics inhibiting the gut flora (i.e., nonabsorbable antibiotics) can affect the pharmacokinetics of drugs.

The impact of EHC on the pharmacokinetics and pharmacodynamics of a drug depends on the importance of biliary excretion relative to renal clearance and on the efficiency of gastrointestinal absorption. The EHC becomes dominant when biliary excretion is the major clearance mechanism for the drug. Because the majority of the bile is stored in the gallbladder and released on the ingestion of food, intermittent spikes in the plasma drug concentration is observed following reentry of the drug from the bile via EHC. From a pharmacodynamic point of view, the net effect of EHC is to increase the duration of a drug in the body and to prolong its pharmacological action.

Drug Metabolism and Age

Metabolism in the Elderly

The widespread use of medications in the elderly increases the potential for an increased incidence of drug-related interactions, which can be related to changes in drug metabolism and clearance from the body (Table 3.14).^{62,63} The interpretation of the age-related alteration in drug response must consider the contributions of absorption, distribution, metabolism, and excretion. Drug therapy in the elderly is expected to become one of the more significant problems for clinical medicine. It has been well documented that the metabolism of many drugs and their elimination is impaired in the elderly.

TABLE 3.14
Effect of Age on the Clearance of Some Drugs

No Change	Decrease
Acetaminophen ^a	Alprazolam
Aspirin	Amitriptyline
Diclofenac	Chlordiazepoxide
Diphenhydramine	Chlormethiazole
Ethanol	Diazepam
Flunitrazepam	Labetalol
Midazolam	Lidocaine
Nitrazepam	Lorazepam
Oxazepam	Morphine
Phenytoin ^a	Meperidine
Prazosin	Nifedipine and other dihydropyridines
Propylthiouracil	Norepinephrine
Temazepam	Nortriptyline
Thiopental ^a	Phenytoin
Tolbutamide ^a	Piroxicam
Warfarin	Propranolol
	Quinidine

No Change	Decrease
	Quinine
	Theophylline
	Verapamil

^aDrugs for which clearance is disputable but can be increased

The decline in drug metabolism due to advancing age is associated with physiological changes that have pharmacokinetic implications affecting the steady-state plasma concentrations and renal clearance for the parent drug and its metabolites. Those changes relevant to the bioavailability of drugs in the elderly are decreases in hepatic blood flow, glomerular filtration rate, hepatic P450 activity, plasma protein binding, and body mass. Because the rate of a drug's elimination from the blood through hepatic metabolism is determined by hepatic blood flow, protein binding, and intrinsic clearance, a reduction in hepatic blood flow can lead to an increase in drug bioavailability and decreased clearance, with the symptoms of drug overdose and toxicity as the outcome (e.g., warfarin in patients with congestive heart failure^{63,64}). Those orally administered drugs exhibiting a reduction in first-pass metabolism in the elderly include antihypertensives, diuretics, and antidepressants.

Age-related changes in drug metabolism are a complicated interplay between the age-related physiological changes, genetics, environmental influences (diet and nutritional status, smoking, and enzyme induction), concomitant diseases states, and drug intake. In most studies, the elderly appear to be just as responsive to drug-metabolizing enzyme activity (Phase 1 and Phase 2) as young individuals. Given the number of factors that determine the rate of drug metabolism, it is not surprising that the effects of aging on drug elimination by metabolism have yielded variable results, even for the same drug. The fact that drug elimination can be altered in old age suggests that doses of metabolized drugs should be initially reduced in older patients and then adjusted according to the clinical

response. A decrease in hepatic drug metabolism coupled with age-related alterations in clearance, volume of distribution, and receptor sensitivity can lead to prolonged plasma half-life and increased drug toxicity.

Those P450 substrates reported to cause drug-drug interactions are shown in [Tables 3.3-3.10](#) in bold italics.

Drug Metabolism in the Fetus and During Pregnancy

Over two-thirds of women receive prescription drugs while pregnant, with treatment and dosing strategies based on data from healthy male volunteers and nonpregnant women, and with little adjustment for the complex physiology of pregnancy and its unique disease states. Pregnancy influences hepatic metabolism of drugs in a CYP-dependent manner. Hormone levels in the plasma which rise during pregnancy are capable of regulating expression of hepatic drug metabolism enzymes. These hormones include estrogens, progesterone, cortisol, and prolactin. Studies are needed to examine the role of pregnancy hormones in altered drug metabolism during pregnancy.

The activities of CYP3A4, CYP2A6, CYP2D6, and CYP2C9 are all increased during pregnancy. Changes in CYP3A4 activity lead to increased metabolism of drugs ([Table 3.10](#)). By contrast, CYP1A2 and CYP2C19 appear to undergo a gradual decrease in activity with advancing gestation,^{63,64} though with uncertain effects on drug therapy. The activity of Phase 2 enzymes, including uridine 5'-diphosphate glucuronosyltransferases (UGTs), is also altered during pregnancy, with a 200% increase in UGT1A4 activity during the first and second trimesters and a 300% increase during the third trimester.^{63,64} This change leads to lower concentrations of UGT1A4 substrates such as lamotrigine, leading directly to poorer seizure

control with advancing gestation in the absence of appropriate dose titration.

Knowledge of drug transporter function during pregnancy is necessary for a complete understanding of drug absorption, distribution, elimination, and effect.⁶³ In addition, fetal development is dependent upon the transport of nutrients by the placenta toward the fetal side, and that of products of fetal metabolism transported away from the fetus for elimination by the mother.⁶⁵ The placenta produces and secretes hormones that affect the maternal physiology and endocrine state. Compounds transported between the mother and the fetus are carried by the maternal circulation within the uterine vasculature.

The ability of the human fetus and placenta to metabolize xenobiotics is well established. Drug-metabolizing enzymes expressed in the placenta include CYP1A1, 2E1, 3A4/5, 3A7, 4B1, epoxide hydrolase, alcohol dehydrogenase, glutathione transferase, UGTs, sulfotransferases, and N-acetyltransferase. The majority of the drugs used in pregnancy readily cross the placenta, thus exposing the fetus to a large number of xenobiotic agents. The human fetus is at special risk from these substances due to the presence of the P450 system which is capable of metabolizing xenobiotics during the first part of gestation. Placentas of tobacco smokers have shown a significant increase in the rate of placental CYP1A activity. Concern for this CYP1A activity is increasing because this enzyme system is known to catalyze the formation of reactive metabolites capable of covalently binding to macromolecules, producing permanent effects (e.g., teratogenicity, hepatotoxicity, or carcinogenicity) in the fetus and newborn. A more disturbing fact is that the conjugation enzymes (i.e., glucuronosyltransferases, glutathione transferase, and sulfotransferase), which are important for the formation of Phase 2 conjugates of these reactive metabolites, are found in low to

negligible levels in the fetus and newborn, increasing the exposure of these infants to these potentially toxic metabolites.

The placenta is not a barrier protecting the fetus from xenobiotics; almost every drug present in the maternal circulation will cross the placenta and reach the fetus. For some drugs, however, the placental efflux transport protein P-gp functions as a maternofetal barrier, pumping drugs and Pgp substrates out of the fetal circulation back into the maternal circulation⁶⁵ and protecting the fetus from exposure to potentially harmful teratogenic xenobiotics/drugs and endogenous substances that have been absorbed through the placenta. The P-gp inhibitors should be carefully evaluated for their potential to increase fetal susceptibility to drug/chemical-induced teratogenesis. On the other hand, selective inhibition of P-gp could be used clinically to improve pharmacotherapy of the unborn child. Because metabolites are usually more water soluble than the parent substance, drug metabolites formed in the fetus can be trapped and accumulate on the fetal side of the placenta. Such accumulation can result in drug-induced toxicities or developmental defects. The activity of CYP3A isoenzymes in the human fetal liver is similar to that seen in adult liver microsomes. The fetal activity for CYP3A7 is unusual, as most other fetal isoenzymes of P450 exhibit activity 5%-40% of the adult isoenzymes. Fetal and neonatal drug-metabolizing enzyme activities can differ from those in the adult.

Most xenobiotics cross the placental barrier by simple diffusion. Protein binding, degree of ionization, lipid solubility, and molecular weight can affect placental transport. In fact, small, lipid-soluble, ionized, and poorly protein-bound molecules cross the placenta easily. For other substrates, the placenta facilitates maternal to fetal transport through the polarized expression of various transporters.⁶⁵ Transporters enable transport of specific endogenous substrates (such as cytokines, nucleoside analogues, and steroid hormones);

however, exogenous compounds with similar structures may also interact with them.

From the day of birth, the neonate is exposed to drugs and other foreign compounds persisting from pregnancy, as well as those transferred via breast milk. Fortunately, many of the drug-metabolizing enzymes operative in the neonate developed during the fetal period. The routine use of therapeutic agents during labor and delivery, as well as during pregnancy, is widespread, and consideration must be given to the fact that potentially harmful metabolites can be generated by the fetus and newborn. Consequently, the use of drugs capable of forming reactive metabolic intermediates should be avoided during pregnancy, delivery, and the neonatal period. The activity of Phase 1 and Phase 2 drug-metabolizing enzymes is high at birth but decreases to normal levels with increasing age. Evidence suggests increased activity of drug-metabolizing enzymes in liver microsomes of neonates resulting from treatment of the mother during the pregnancy with enzyme inducers (e.g., phenobarbital).

Genetic Polymorphism

The reality of drug therapy is that many drugs do not work in all patients.⁶⁶ By current estimates, the percentage of patients who will react favorably to a specific drug ranges from 20% to 80%. Drugs have been developed and dosage regimens prescribed under the old paradigm that “one dose fits all,” which largely ignores the fact that humans are genetically different, resulting in interindividual differences in drug metabolism and disposition.⁶⁶ It is widely accepted that genetic factors have an important impact on the oxidative metabolism and pharmacokinetics of drugs. Genotype-phenotype correlation studies (pharmacogenetics) have shown that inherited mutations in P450 genes (alleles) result in distinct phenotypic subgroups. For example, mutations in the *CYP2D6* gene result in poor (PM), intermediate (IM), extensive (EM), and ultrarapid (UM) metabolizers of *CYP2D6* substrates⁶⁷ (Table 3.8). Each of these phenotypic subgroups experiences different responses to drugs extensively metabolized by the *CYP2D6* pathway, ranging from severe toxicity to complete lack of efficacy. Genetic studies confirm that “one dose does not fit all,” leaving the question of why we would continue to develop and prescribe drugs under the old paradigm. The regulatory agencies have recognized that identifying genetic polymorphisms might allow the safe dosing, marketing, and approval of drugs that would otherwise not be approved and advised pharmaceutical companies to incorporate the knowledge of genetic polymorphisms into drug development. Importantly, pharmacogenomic testing (the study of heritable traits affecting patient response to drug treatment) can significantly increase the likelihood of developing drug regimens that benefit most patients without severe adverse events. Pharmacists play a vital role in the

development of patient-specific dosing regimens based on metabolizing phenotype.

Polymorphisms are expressed for a number of metabolizing enzymes, but the polymorphic P450 isoforms that are most important for drug metabolism include CYP2A6, CYP2A13, CYP2B6, CYP2C9, CYP2C19, and CYP2D6.⁶⁸⁻⁷¹ [1]
[SEP] These isoforms have been studied extensively and, despite their low abundance in the liver, they have been found to catalyze the metabolism of many drugs. Most gene variants are expressed into inactive, truncated proteins or they fail to express any protein. There is no clear information about CYP1A1 and CYP3A4/5 polymorphism. These polymorphic isoforms give rise to phenotypic subgroups in the population differing in their ability to perform clinically significant biotransformation reactions with obvious clinical ramifications.⁶⁶ Metabolic polymorphism can have several consequences; for example, when enzymes that metabolize drugs used either therapeutically or socially are deficient, adverse or toxic drug reactions can occur in these individuals.

The discovery of genetic polymorphism resulted from the observation of increased frequency of adverse effects or no drug effects after normal doses of drugs to some patients (e.g., hyper-CNS response from the administration of the antihistamine doxylamine or no analgesic response with codeine). Polymorphism is a difference in DNA sequence found at 1% or greater in a population and expressed as an amino acid substitution in the protein sequence of an enzyme resulting in changes in its rate of activity ($V_{\max}$) or affinity (K_m). Thus, mutant DNA sequences can lead to interindividual differences in drug metabolism. Furthermore, the polymorphisms do not occur with equivalent frequency in all racial or ethnic groups. Because of these differences, it is important to be aware of a person's race and ethnicity when giving drugs that are metabolized differently by different populations, although practitioners must guard against

stereotyping.⁶⁶ Because no other way may exist to adequately clear these drugs from the body, PMs can be at greater risk for adverse drug reactions or toxic overdoses. The signs and symptoms of these overdoses are primarily extensions of the drug's common adverse effects or pharmacological effects (Table 3.15).⁶⁶ The level of adverse reactions or overdosage depends very much on the overall contribution of the mutant isoform to the drug's metabolism. Perhaps the most interesting explanation for the various mutant isoforms is that they evolved as protective mechanisms against alkaloids and other common substances in the food chain for the different ethnicities. Although much effort has gone into finding polymorphisms of *CYP3A4* and *CYP1A2* genes, none has yet to be discovered.

TABLE 3.15
Impact of Human P450 Polymorphisms on Drug Treatment in Poor Metabolizers

Polymorphic Enzyme	Decreased Clearance	Adverse Effects (Overdosage)	Reduced Activation of Coadministered Prodrug
CYP2C9	S-warfarin	Bleeding	Losartan
	Phenytoin	Ataxia	
	Losartan		
	Tolbutamide	Hypoglycemia	
	NSAIDs	GI bleeding	
CYP2C19	Omeprazole		Proguanil
	Diazepam	Sedation	
CYP2D6	Tricyclic antidepressants	Cardiotoxicity	Tramadol
	SSRIs	Serotonin syndrome	Codeine
	Anti-arrhythmic drugs	Arrhythmias	Ethylmorphine
	Perhexiline	Neuropathy	
	Haloperidol	Parkinsonism	
	Perphenazine		
	Zuclopenthixol		
	S-Mianserin		

Polymorphic Enzyme	Decreased Clearance	Adverse Effects (Overdosage)	Reduced Activation of Coadministered Prodrug
	Tolterodine		
CYP2A6	Nicotine		

Occasionally, one derives benefit from an unusual P450 phenotype. For example, cure rates for peptic ulcer treated with omeprazole are substantially greater in individuals with defective CYP2C19 due to the sustained high plasma levels achieved.

CYP2A6

CYP2A6 is of particular importance because it activates a number of procarcinogens to carcinogens and is the major isoform metabolizing the anticoagulant warfarin and nicotine.⁶⁹ Approximately 15% of Asians express the *CYP2A6*4del* allele and 2% of Caucasians express the other *CYP2A6*2* allele. Both these alleles express either no enzyme or a nonfunctional enzyme, and individuals carrying these alleles are referred to as having the PM phenotype. A benefit from being a PM of CYP2A6 substrates might be the protection against some carcinogens, including cigarette smoke due to the high plasma levels of nicotine achieved with fewer cigarettes.

CYP2B6

CYP2B6 is of special interest due to its wide interindividual variability in expression and activity.⁷⁰ Such a large variability for an enzyme that is not highly expressed is probably because of genetic polymorphisms and exposure to drugs that are inducers or inhibitors of CYP2B6. Variant alleles resulting from splicing defects or gene deletions have been identified in PM individuals with altered metabolic activity or impaired enzyme function. At least 28 allelic variants and some subvariants of *CYP2B6* have been shown to have an effect on drug clearance and drug response. For example, HIV-infected African-American individuals with a defective variant

of *CYP2B6* are PMs of efavirenz and have plasma levels approximately 3-fold higher than individuals with the normal *CYP2B6* gene. A different variant of *CYP2B6* is associated with approximately 2-fold greater plasma levels of nevirapine in HIV-infected patients. A benefit from being a PM of *CYP2B6* antiviral drugs might be enhanced HIV protection because of the higher plasma levels of the antiviral drugs achieved with lower dosages. Smokers with another variant of *CYP2B6* may be more vulnerable to abstinence symptoms and relapse following treatment with bupropion (a *CYP2B6* substrate) as a smoking cessation agent.

CYP2C9 and CYP2C19

CYP2C9 and *CYP2C19* metabolize approximately 15% of clinically used drugs, such as phenytoin, *S*-warfarin, tolbutamide, losartan, and many NSAIDs.⁷¹ Although *CYP2C19* metabolizes fewer drugs than *CYP2D6*, the drugs that *CYP2C19* does metabolize are clinically important (Table 3.7). The deficit of *CYP2C19* found in the PM phenotype is only seen in 8%-13% of Caucasians, 20%-30% of the Asian population (11%-23% of Japanese and 5%-37% of Chinese), up to 20% of the black African-American population, 14%-15% of Saudi Arabians and Ethiopians, and up to 70% of Pacific Islanders.⁷¹ The more common mutant allele in these individuals is *CYP2C19**2, which expresses an inactive enzyme. The large interindividual variability observed in the therapeutic response to the antiseizure drug mephenytoin is attributed to *CYP2C19* polymorphism, which catalyzes the *p*-hydroxylation of the *S*-stereoisomer.⁷¹ The *R*-enantiomer is *N*-demethylated by *CYP2C8* with no difference in its metabolism between PMs and EMs.

CYP2C9 is highly polymorphic with at least 33 variants identified. *CYP2C9**2 is frequent among Caucasians with ~1% of the population being homozygous carriers and 22% heterozygous. The

corresponding homozygous/heterozygous figures for the *CYP2C9**3 allele are 0.4% and 15%, respectively. Clinical studies have addressed the importance of the *CYP2C9**2 and *3 alleles as a determining factor for drug clearance and drug response. P450 2C9 polymorphisms are relevant for the efficacy and adverse effects of numerous NSAIDs, sulfonylurea antidiabetic drugs, and, most critically, oral anticoagulants belonging to the class of vitamin K epoxide reductase inhibitors (e.g., warfarin).⁷¹ A deficiency of this isoform is seen in 8%-13% of Caucasians, 2%-3% of African-Americans, and 1% of Asians. Individuals with the PM phenotype who possess this deficient isoform variant are ineffective in clearing S-warfarin (so much so that they can be fully anticoagulated on just 0.5 mg of warfarin per day) and in the clearance of phenytoin, which has a potentially very toxic narrow therapeutic range. On the other hand, the prodrug losartan will be poorly activated and ineffective.

CYP2D6

CYP2D6 is of specific importance because it metabolizes a wide range of commonly prescribed drugs, including antidepressants, antipsychotics, β -adrenergic blockers, and antiarrhythmics ([Table 3.8](#)).^{67,68} CYP2D6 deficiency is a clinically important genetic variation of drug metabolism characterized by three phenotypes: UM, EM, and PM. About 10% of Caucasians have gene variants that result in a functionally deficient CYP2D6 enzyme, and these PMs often respond inadequately to drugs, such as codeine, that are activated by CYP2D6. The PM phenotype is inherited as an autosomal recessive trait, with 5 of 30 of the known *CYP2D6* gene mutations leading to either zero expression or the expression of a nonfunctional enzyme.⁶⁸ Approximately 12%-20% of Caucasians express the *CYP2D6**4 allele and 5% express the other *CYP2D6* alleles. Up to 34% of African-Americans express the *CYP2D6**17 allele and 5% express the other

CYP2D6 alleles. Up to 50% of Chinese express the *CYP2D6**10 allele and 5% express the other *CYP2D6* alleles (these individuals are all referred to as PM).^{67,68} Conversely, the 20%-30% of Saudi Arabians and Ethiopians who express the *CYP2D6**2XN allele are known as UMs of *CYP2D6* substrates, because they express excess enzyme because of having multicopies of the gene.⁷² Inasmuch as *CYP2D6* is not inducible, many individuals of Ethiopian and Saudi Arabian descent have developed a genetically different strategy to cope with the (presumed) high load of alkaloids and other substances in their diet, thus the high expression of *CYP2D6* using multiple copies of the gene.

Those individuals who are deficient in *CYP2D6* will be predisposed to adverse effects or drug toxicity from antidepressants or neuroleptics caused by inadequate metabolism, leading to long half-lives, but the metabolism of *CYP2D6*-dependent prodrugs in these patients will be ineffective due to lack of activation (e.g., codeine, which must be metabolized by O-demethylation to morphine). In contrast, individuals with the UM phenotype will require a dose of drugs inactivated by *CYP2D6* that is much higher than normal to attain therapeutic drug plasma concentrations (e.g., one patient required a daily dose of approximately 300 mg of nortriptyline to achieve therapeutic plasma levels; the usual dose is 25 mg) or a lower dose for prodrugs that require metabolic activation. Individuals with the PM phenotype are also characterized by loss of *CYP2D6* stereoselectivity in hydroxylation reactions. It can be anticipated that large differences in steady-state concentration for *CYP2D6* substrates will occur between individuals with the different phenotypes when they receive the same dose. Depending on the drug and reaction type, a 10- to 30-fold difference in blood concentrations can be observed in the PM-phenotype polymorphism.⁶⁸

Other Polymorphic Metabolizing Enzymes

CYP2E1 polymorphism is expressed more frequently in individuals of Chinese ancestry than in Caucasians. Those with the CYP2E1 PM phenotype exhibit tolerance to alcohol and less toxicity from halohydrocarbon solvents.

Polymorphism has been associated with serum cholinesterases, alcohol dehydrogenases, aldehyde dehydrogenases, epoxide hydrolase and xanthine oxidase. Approximately 50% of the Oriental population lack aldehyde dehydrogenase, resulting in high levels of acetaldehyde following ethanol ingestion and causing nausea and flushing. People with genetic variants of cholinesterase respond abnormally to succinylcholine, procaine, and other related choline esters. The clinical consequence of reduced enzymatic activity of cholinesterase is that succinylcholine and procaine are not hydrolyzed in the blood, resulting in prolongation of their respective pharmacological activities (e.g., skeletal muscle paralysis in the case of succinylcholine).

Increasing knowledge of genetic polymorphism has contributed a great deal to our understanding about interindividual variation in the metabolism of drugs, including how to change dose regimens accordingly to minimize drug toxicity and improve therapeutic efficacy.

Presystemic First-Pass Metabolism

Although hepatic metabolism continues to be the most important route for xenobiotics, the ability of the liver and intestine to metabolize substances to either pharmacologically inactive or bioactive metabolites before reaching the systemic circulation is called presystemic first-pass metabolism. This metabolic process results in low systemic availability for orally susceptible drugs. Sulfonation and glucuronidation are major pathways of presystemic intestinal first-pass metabolism in humans. For example, oral acetaminophen, phenylephrine, terbutaline, albuterol, fenoterol, or isoproterenol exhibits approximately 5% bioavailability because of sulfonation and glucuronidation.

The discovery that CYP3A4 is found in the mucosal enterocytes of the intestinal villi signifies its role as a key determinant in the oral bioavailability of its numerous drug substrates ([Table 3.10](#)). Drugs known to be substrates for CYP3A often have a low and/or variable oral bioavailability that can be explained by presystemic first-pass metabolism by the small intestine P450 isoforms.⁶⁸ The concentration of functional intestinal CYP3A is influenced by genetic disposition, induction, and inhibition, which to a great extent determines drug blood levels and therapeutic response. Xenobiotics when ingested orally can modify the activity of intestinal CYP3A enzymes by induction and inhibition. By modulation of the isoform pattern in the intestine, a xenobiotic could alter its own metabolism and that of others in a time- and dose-dependent manner.

The concentration of CYP3A in the intestine is comparable to that of the liver. The oral administration of dexamethasone induces the formation of CYP3A and erythromycin inhibits it. The glucocorticoid inducibility of CYP3A4 can also be a factor in differences of metabolism between males and females. Studies have suggested that

intestinal CYP3A4 C2 hydroxylation of estradiol contributed to the oxidative metabolism of endogenous estrogens circulating with the enterohepatic recycling pool.⁷² Norethisterone has a low oral bioavailability of 42% due to oxidative CYP3A first-pass metabolism, but levonorgestrel is completely available in women, having no conjugated metabolites.

Several clinically relevant drug-drug interactions between orally coadministered drugs and CYP3A4 can be explained by a modification of drug metabolism at the P450 level. If a drug has high presystemic elimination (low bioavailability) and is metabolized primarily by CYP3A4, then coadministration with a CYP3A4 inhibitor can be expected to alter the drug's pharmacokinetics by reducing its metabolism, thus increasing its plasma concentration. Drugs and some foods (e.g., grapefruit juice) that are known inhibitors, inducers, or substrates for intestinal CYP3A4 can potentially interact with the metabolism of a coadministered drug, affecting its area under the curve and rate of clearance (Tables 3.10-3.12). Inducers can reduce absorption and oral bioavailability, whereas these same factors are increased by inhibitors. For example, erythromycin can enhance the oral absorption of another drug by inhibiting its metabolism in the small intestine by CYP3A4. Because they are competitive substrates for CYP3A4, prednisone, prednisolone, and methylprednisolone (but not dexamethasone) are competitive inhibitors of synthetic glucocorticoid metabolism. This is because a major metabolic pathway for synthetic glucocorticoids involves CYP3A4. In addition to coadministered drugs, metabolic interactions with exogenous CYP3A4 substrates secreted in the bile are possible. The poor oral bioavailability for cyclosporine is attributed to a combination of intestinal metabolism by CYP3A4 and efflux by P-gp.⁷³

Because the intestinal mucosa is enriched with glucuronosyltransferases, sulfotransferases, and glutathione

transferases, presystemic first-pass metabolism for orally administered drugs susceptible to these conjugation reactions results in their low oral bioavailability. Presystemic metabolism often exceeds liver metabolism for some drugs. For example, more than 80% of intravenously administered albuterol is excreted unchanged in urine, with the balance presenting as glucuronide conjugates, whereas when albuterol is administered orally, less than 5% is systemically absorbed because of intestinal sulfonation and glucuronidation. Presystemic metabolism is a major pathway in humans for most β -adrenergic agonists, such as glucuronides or sulfate esters of terbutaline, fenoterol, albuterol and isoproterenol, morphine (3-O-glucuronide), acetaminophen (O-sulfate and O-glucuronide), and estradiol (3-O-sulfate). The bioavailability of orally administered estradiol or ethinyl estradiol in females is approximately 50% because of conjugation. Mestranol (3-methoxyethinyl estradiol) has greater oral bioavailability because, as a nonphenol, it is not significantly conjugated. Levodopa has a low oral bioavailability because of its metabolism by intestinal l-aromatic amino acid decarboxylase. Drugs subject to first-pass metabolism are included in [Table 3.16](#).

TABLE 3.16
Examples of Drugs Exhibiting Presystemic Metabolism

Acetaminophen	Isoproterenol	Oxprenolol
Albuterol	Lidocaine	Pentazocine
Alprenolol	Meperidine	Propoxyphene
Aspirin	Methyltestosterone	Propranolol
Cyclosporin	Metoprolol	Salicylamide
Desmethylinipramine	Dihydropyridines (nifedipine)	Terbutaline
Fluorouracil	Nortriptyline	Verapamil
Hydrocortisone	Organic nitrates	
Imipramine		

The extensive presystemic first-pass sulfonation of phenolic drugs, for example, can lead to increased bioavailability of other drugs by competing for the available sulfate pool, resulting in the possibility of drug toxicity. Concurrent oral administration of acetaminophen with phenolic drugs could result in an increase in drug blood levels. Ascorbic acid, which is sulfonated, also increases the bioavailability of concurrently administered phenolic drugs. Sulfonation and glucuronidation occur side by side, often competing for the same substrate, and the balance between sulfonation and glucuronidation is influenced by several factors, such as species, doses, availability of cosubstrates, inhibition, and induction of the respective transferases.

Extrahepatic Metabolism

Because the liver is the primary tissue for xenobiotic metabolism, it is not surprising that our understanding of mammalian P450 monooxygenase is based chiefly on hepatic studies. Although the tissue content of P450s is highest in the liver, P450 enzymes are found in the lung, nasal epithelium, intestinal tract, kidney, adrenal tissues, and brain. It is possible that the expression of the polymorphic genes and induction of the isoforms in the extrahepatic tissues can affect the activity of the P450 isoforms in the metabolism of endogenous steroids, drugs, and other xenobiotics. Therefore, characterization of P450, UGT, SULT, and other polymorphic drug-metabolizing enzymes in extrahepatic tissues is important to our overall understanding about the biological importance of these isoform families to improved drug therapy, design of new drugs and dosages forms, toxicity, and carcinogenicity.

The mucosal surfaces of the GIT, the nasal passages, and the lungs are major portals of entry for xenobiotics into the body and, as such, are continuously exposed to a variety of orally ingested or inhaled airborne xenobiotics, including drugs, plant toxins, environmental pollutants, and other chemical substances. As a consequence of this exposure, these tissues represent a major target for tumorigenesis and other chemically induced toxicities. Many of these toxins and chemical carcinogens are relatively inert substances that must be bioactivated to exert cytotoxicity and tumorigenicity. The epithelial cells of these tissues are capable of metabolizing a wide variety of exogenous and endogenous substances, and these cells provide the principal and initial source of biotransformation for these xenobiotics during the absorptive phase. The consequences of such presystemic biotransformation is either (1) a decrease in the amount of xenobiotics available for systemic absorption by facilitating the

elimination of polar metabolites or (2) toxification by activation to carcinogens, which can be one determinant of tissue susceptibility for the development of intestinal cancer. The risk of colon cancer can depend on dietary constituents that contain either procarcinogens or compounds modulating the response to carcinogens.

Intestinal Metabolism

As noted earlier, many of the clinically relevant aspects of P450 can, in fact, occur at the level of the intestinal mucosa and can account for differences among patients in dosing requirements. The intestinal mucosa is enriched especially with the CYP3A4 isoform, glucuronosyltransferases, sulfotransferases, and GSTs, making it particularly important for orally administered drugs susceptible to oxidation and/or Phase 2 glucuronidation, sulfonation, or glutathione conjugation pathways. The highest concentrations of P450s occur in the duodenum, with gradual tapering into the ileum. In the human intestine, CYP2E, CYP3A, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 have been identified. Therefore, intestinal P450 isoforms provide potential presystemic first-pass metabolism of ingested xenobiotics affecting their oral bioavailability (e.g., hydroxylation of naloxone) or bioactivation of carcinogens or mutagens. It is not surprising that dietary factors can affect the intestinal P450 isoforms. For example, a 2-day dietary exposure to cooked Brussels sprouts significantly decreased the 2 α -hydroxylation of testosterone, yet induced CYP1A2 activity for PAH metabolism. An 8-oz. glass of grapefruit juice inhibited the sulfoxidation metabolism of omeprazole (CYP3A4) but not its hydroxylation (CYP2C19), thus increasing its systemic blood concentrations. These types of interactions between a drug and a dietary inhibitor could result in a clinically significant drug interaction.

Intestinal UGT isoforms can glucuronidate orally administered drugs, such as morphine, acetaminophen, α - and β -adrenergic agonists, and other phenolic phenethanolamines and dietary xenobiotics, resulting in a reduction of their oral bioavailability (increasing first-pass metabolism), thus altering their pharmacokinetics and pharmacodynamics. The UGTs expressed in the intestine include UGT1A1 (bilirubin-glucuronidating isoform), UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, and UGT1A3. Substrate specificities of intestinal UGT isoforms are comparable to those in the liver. Glucuronidase hydrolysis of biliary glucuronide conjugates in the intestine can contribute to EHC of the parent drug. Likewise, the sulfotransferases in the small intestine can metabolize orally administered drugs and xenobiotics for which the primary route of conjugation is sulfonation (e.g., isoproterenol, albuterol, steroid hormones, α -methyldopa, acetaminophen, and fenoldopam), decreasing their oral bioavailability and, thus, altering their pharmacokinetics and pharmacodynamics. Competition for intestinal sulfonation between coadministered substrates can influence their bioavailability with either an enhancement or a decrease of therapeutic effects. Sulfatase hydrolysis of biliary sulfate esters in the intestine can contribute to EHC of the parent drug.

Plants contain a variety of protoxins, promutagens, and procarcinogens, and the occurrence of intestinal P450 enzymes and bacterial enzymes in the microflora allows the metabolic conversion of relatively stable environmental pollutants and food-derived xenobiotics into mutagens and carcinogens. For example, cruciferous vegetables (Brussels sprouts, cabbage, broccoli, cauliflower, and spinach) are all rich in indole compounds (e.g., indole 3-carbinol) which, with regular and chronic ingestion, are capable of inducing some intestinal P450s (CYP1A subfamily) and inhibiting others (CYP3A subfamily). It is likely that these vegetables would also alter the metabolism of food-derived mutagens and carcinogens. For

example, heterocyclic amines produced during the charbroiling of meat are P450 N-hydroxylated and become carcinogenic in a manner similar to arylamines.

Intestinal Microflora

When drugs are orally ingested, or there is considerable biliary excretion of a drug or its metabolites into the GIT, such as with a parentally administered drug (EHC or recirculation), the intestinal bacterial microflora can have a significant metabolic role. The microflora has an important role in the enterohepatic recirculation of xenobiotics via their conjugated metabolites (e.g., digoxin, the oral contraceptives norethisterone and ethinyl estradiol and chloramphenicol) and endogenous substances (steroid hormones, bile acids, folic acid, and cholesterol), which reenter the gut via the bile. Compounds eliminated in the bile are conjugated with glucuronic acid, glycine, sulfate, and glutathione and, once secreted into the small intestine, the bacterial β -glucuronidase, sulfatase, nitroreductases, and various glycosidases catalyze the hydrolysis of the conjugates. The activity of orally administered conjugated estrogens (e.g., Premarin) involves the hydrolysis of the sulfate esters by sulfatases, releasing estrogens to be reabsorbed from the intestine into the portal circulation. The clinical use of oral antibiotics (e.g., erythromycin, penicillin, clindamycin, and aminoglycosides) has a profound effect on the gut microflora and the enzymes responsible for the hydrolysis of drug conjugates undergoing EHC. Bacterial reduction includes nitro reduction of nitroimidazole, azo reduction of azides (sulfasalazine to 5-aminosalicylic acid and sulfapyridine), and reduction of the sulfoxide to its sulfide. The sulfoxide of sulindac is reduced by both gut microflora and hepatic P450s. Other ways in which bacterial flora can affect metabolism include the following: (1) production of toxic metabolites, (2) formation of carcinogens from inactive precursors, (3) detoxication,

(4) exhibition of species differences in drug metabolism, (5) exhibition of individual differences in drug metabolism, (6) production of pharmacologically active metabolites from inactive precursors, and (7) production of metabolites not formed by animal tissues. In contrast to the predominantly hepatic oxidative and conjugative metabolism of the liver, gut microflora is largely degradative, hydrolytic, and reductive, with a potential for both metabolic activation and detoxication of xenobiotics.

Lung Metabolism

Some of the hepatic xenobiotic biotransformation pathways are also operative in the lung. Because of the differences in organ size, the total content of the pulmonary xenobiotic-metabolizing enzyme systems is usually lower than in the liver, creating the impression of a minor role for the lung in xenobiotic elimination. CYP2E1 is expressed in the lung to the greatest extent. The expression of other P450s, FMO, epoxide hydrolase, and the Phase 2 conjugation pathways are comparable to those in the liver. Thus, the lungs can have a significant role in the metabolic elimination or activation of low-molecular-weight inhaled xenobiotics.

When drugs are injected intravenously, intramuscularly, or subcutaneously, or after skin absorption, the drug initially enters the pulmonary circulation, after which the lung becomes the organ of first-pass metabolism ^[1]for the drug. The blood levels and therapeutic response of the drug are influenced by genetic disposition, induction, and inhibition of the pulmonary metabolizing enzymes. By modulation of the P450 isoform pattern in the lung, a xenobiotic could alter its own metabolism and that of others in a time- and dose-dependent manner. Because of its position in the circulation, the lung provides a second-pass metabolism for xenobiotics and their metabolites exiting from the liver, but it is also

susceptible to the cytotoxicity or carcinogenicity of hepatic activated metabolites. Antihistamines, β -blockers, opioids, and tricyclic antidepressants are among the basic amines known to accumulate in the lungs because of their binding to surfactant phospholipids in lung tissue. The significance of this relationship to potential pneumotoxicity remains to be seen.

Nasal Metabolism

The nasal olfactory mucosa is recognized as a first line of defense for the lung against airborne environmental xenobiotics because the mucosa is constantly exposed to the airborne external environment.⁷⁴ Drug metabolism in the olfactory nasal epithelium represents a major metabolic pathway for protecting the CNS and brain against the entry of drugs, inhaled environmental pollutants, or other volatile chemicals. In some instances, these enzymes can biotransform a given drug or environmental or airborne xenobiotic into more reactive and potentially toxic metabolites, increasing the risk of carcinogenesis in the nasopharynx and lung (e.g., procarcinogens and nitrosamines in cigarette smoke). P450 activity is high in olfactory tissue and olfactory-specific isoforms have been identified, which include CYP1A2, CYP2A6, CYP2A13, CYP2E1, CYP3A4, and the olfactory-specific CYP2G1 isoform, as well as several isoforms of the flavin-dependent monooxygenases. Phase 2 enzymes found in the olfactory mucosa include UGTs, glutathione-S-transferases, carboxylesterases, aldehyde oxidases, and epoxide hydrolases. The most striking feature of the nasal epithelium is that the P450 catalytic activity per gram of tissue is higher than in any other extrahepatic tissue or in the liver. Nasal decongestants, essences, anesthetics, alcohols, nicotine, and cocaine have been shown to be metabolized in vitro by P450 enzymes from the nasal epithelium. Because the P450s in the nasal mucosa are active, first-

pass metabolism should be considered when delivering susceptible drugs intranasally.

The nasal mucosa is also exposed to a wide range of odorants. Odorants, which are mostly small lipophilic molecules, enter the mucosa and reach the odorant receptors on sensory neurons by a transient process requiring signal termination, which could be provided by biotransformation of the odorant in the epithelial supporting cells. Thus metabolism of odorants could be involved in both the termination and initiation of olfactory stimuli.

Brain Metabolism

The metabolism of endogenous neurochemicals and neurotransmitters by brain CYPs may help explain variations in mood, aggression, personality disorders, and other psychiatric conditions.⁷⁵⁻⁷⁷ The role of brain CYP metabolism in neurotoxicity may also contribute to our understanding of the underlying mechanisms of diseases such as Parkinson's disease. Knowledge of CYP isoforms and transporter function in the blood-brain barrier (BBB) may increase our ability to deliver active forms of drugs specifically to the brain.

The isoforms of P450s and their regulation in the brain are of interest in defining the possible involvement of P450s in CNS toxicity and carcinogenicity. The expression level of P450 in the brain is approximately 0.5%-2% of that in liver, too low to significantly influence the overall pharmacokinetics of drugs and hormones in the periphery.^{75,77} Brain CYPs can impact acute and chronic drug response and susceptibility to damage by neurotoxins and are associated with altered personality, behavior, and risk of neurological disease. The functional role for brain CYP enzyme activity can impact CNS drug activation, protection against neurotoxicity, regulate neurotransmitter metabolism, chronic (e.g.,

nicotine dependence) response to drugs, and are associated with altered personality and behavior, and risk of neurological disease. Expression on a g/tissue basis can be as high as levels in hepatocytes. However, while hepatic CYPs are expressed primarily in the endoplasmic reticulum, brain expression is found in the mitochondrial and plasma membrane fraction and other cell membrane compartments. CYP1A1, CYP2B6, CYP2D6, CYP2E1, and CYP3A, are predominantly found in neurons and glial cells. CYP3A4 and CYP2C19 are expressed in human prefrontal cortex, hippocampus, and amygdala, areas that may mediate the behavioral effects of testosterone. Human CYP1B1 is found at the blood-brain interface, where it may act in conjunction with transporters, such as ATP-binding cassette transporters, to regulate passage of xenobiotics in and/or out of the brain. The interaction of a variety of transporters, in addition to local CYP-mediated metabolism, may play an important role in regulating the levels of centrally acting drugs in the brain and, thereby, their therapeutic effects.

The expression of brain CYPs levels vary among different brain regions. For example, in the human brain, CYP2B6 protein expression varies significantly among brain regions with a 2.5-fold range.⁷⁵ Brain CYP2D6 can metabolize endogenous neurochemicals, such as tyramine to dopamine and 5-methoxytryptamine to serotonin, while the metabolism of the endogenous cannabinoid anandamide, the neurosteroid progesterone, and testosterone (to 16 α -hydroxytestosterone) is catalyzed by CYP2B6.⁷⁵ Thus these enzymes may play a functional role in the brain. CYP2C19 can metabolize the sex hormones testosterone, progesterone, and estradiol that are known to affect brain function and personality traits, such as aggression and anxiety. CYP2E1 can metabolize the fatty acid neural signaling molecule arachidonic acid, which is abundant in the brain and is required for neurologic health, and CYP19 aromatase can metabolize testosterone to estradiol, both of

which can influence personality traits, including aggression, impulsivity, and anxiety. The differences in expression of specific brain CYPs can cause shifts in the neurochemical homeostasis in the brain.

Individuals with a CYP2D6 PM genotype are at greater risk for parkinsonism, and this risk is even greater with exposure to neurotoxic pesticides.⁷⁵ CYP2D6 metabolizes and inactivates a number of compounds that can cause parkinsonian symptoms, including MPTP, a Parkinson-causing compound, and its neurotoxic metabolite MPP+. CYP2D6 is expressed in human brain regions affected by Parkinson's disease, such as the substantia nigra. The impaired ability of CYP2D6 PMs to inactivate these neurotoxic metabolites may contribute to their increased risk for Parkinson's disease. In contrast to CYP2D6 PMs, smokers are at lower risk for Parkinson's disease since nicotine is neuroprotective. In humans, CYP2D6 expression levels are higher in the brains of smokers than in those of nonsmokers, while induction of brain CYP2D6 by nicotine or smoking may reduce an individual's relative risk for the disease. These data support a contributing role for lower brain CYP2D6 in the increased risk for Parkinson's disease, likely through modulation of local neurotoxin metabolism.⁷⁵

Nicotine is the main component of cigarette smoke that causes tobacco dependence, and genetic variation in brain CYP2B6, CYP2D6, and CYP2E1 can affect smoking behaviors.^{75,76} CYP2B6 slow metabolizers (IMs) progress to tobacco dependence more quickly and have more difficulty quitting than normal (EM) metabolizers. Brain CYP2B6 metabolizes nicotine and other endogenous substrates, such as serotonin and neurosteroids. Nicotine and smoking induces the brain expression of CYP2B6, CYP2D6, and CYP2E1, all of which affect smoking behaviors. Smokers have higher levels of brain CYP2D6, but unchanged levels of hepatic CYP2D6. Individuals exposed to nicotine through

smoking or through nicotine replacement therapy are likely to have increased CYP2D6-mediated brain metabolism of centrally acting drugs, neurotoxins, and endogenous neurochemicals, increasing risk of drug interactions and neurotoxicity. Brain levels of CYP2D6 enzymes and activity may be contributing factors to the clinical observations of personality differences with chronic nicotine administration between CYP2D6 EM and PM phenotypes. Preliminary data suggest that reducing brain CYP2B and CYP2D6 activity increases the rewarding properties of nicotine by altering brain levels of nicotine and its metabolites.

Metabolism in Other Tissues

P450s in the kidney and adrenal tissues include isoforms primarily involved in the hydroxylation of steroids, arachidonic acid, and 25-hydroxycholecalciferol.

Stereochemical Aspects of Drug Metabolism

In addition to the physicochemical factors that affect xenobiotic metabolism, stereochemical factors have an important role in the biotransformation of drugs. This involvement is not unexpected, because the xenobiotic-metabolizing enzymes are also the same enzymes that metabolize certain endogenous substrates which, for the most part, are chiral molecules. Most of these CYP isoforms show stereoselectivity but not stereospecificity; in other words, one stereoisomer enters into biotransformation pathways preferentially but not exclusively. Metabolic stereochemical reactions can be categorized as follows: substrate stereoselectivity, in which two enantiomers of a chiral substrate are metabolized by CYP isoforms at different rates; product stereoselectivity, in which a new chiral center is created in a symmetric molecule and one enantiomer is metabolized preferentially; and substrate-product stereoselectivity, in which a new chiral center of a chiral molecule is metabolized preferentially to one of two possible diastereomers.

An example of substrate stereoselectivity is the preferred decarboxylation of (*S*)- α -methyldopa to (*S*)- α -methyldopamine, with almost no reaction for (*R*)- α -methyldopa. Another example is the (*S*)-ketamine enantiomer, which is preferentially N-demethylated by CYP2B6 to (*S*)-norketamine, whereas the (*R*) enantiomer is N-demethylated by CYP3A4. The reduction of ketones to stereoisomeric alcohols and the hydroxylation of enantiotropic protons or phenyl rings by P450 are examples of product stereoselectivity. For example, phenytoin undergoes aromatic *p*-hydroxylation of only one of its two phenyl rings to create a chiral center at C5 of the hydantoin ring; methadone is reduced preferentially to its α -diastereometric alcohol and naltrexone is reduced to its 6- β -alcohol. Two examples of substrate-product

stereoselectivity are the reduction of the enantiomers of warfarin, and the β -hydroxylation of (*S*)- α -methyldopamine to (1*R*,2*S*)- α -methylnorepinephrine ((*R*)- α -methyldopamine is hydroxylated only to a negligible extent). In vivo studies of this type often can be confused by the further biotransformation of one stereoisomer, giving the false impression that only one stereoisomer was formed preferentially. Moreover, some compounds show stereoselective absorption, distribution, and excretion, which proves the importance of performing in vitro studies.

Although studies regarding the stereoselective biotransformation of drug molecules are not yet extensive, those that have been done indicate that stereochemical factors have an important role in drug metabolism. In some cases, stereoselective biotransformation could account for the differences in pharmacological activity and duration of action between enantiomers.

Metabolic Bioactivation: Role in Hepatotoxicity, Idiosyncratic Reactions, and Chemical Carcinogenesis

Drug-Induced Hepatotoxicity

Drug-induced hepatotoxicity is the leading cause of hepatic injury, accounting for approximately half of all cases of acute liver failure in the U.S. Recent studies have shown that drug-induced hepatotoxicity represents a larger percentage of adverse drug reactions than reported previously, and that the incidence and severity of drug-induced liver injury is underestimated among the general population.

Acetaminophen overdose is the leading cause for calls to poison control centers (>100,000 calls/year) and accounts for more than 56,000 emergency room visits, 2600 hospitalizations, and an estimated 458 deaths from acute liver failure each year. Among the listed drugs in Table 3.17, acetaminophen is the most frequently implicated hepatotoxic agent and can cause extensive hepatic necrosis with as little as 10-12 g (30-40 tablets). Chronic alcohol intake enhances acetaminophen hepatotoxicity more than five times as compared to acute alcohol intake, yet acetaminophen is heavily marketed for its safety as compared to nonsteroidal analgesics (NSAIDs). U.S. drug manufacturers continue to market and promote extra-strength acetaminophen products (500-750 mg/tablet) and a variety of Extra-strength acetaminophen-drug combination products. Self-poisoning with acetaminophen (paracetamol) is also a common cause of hepatotoxicity in the Western [SEP] world. To reduce the number of acetaminophen poisonings in the UK, over-the-counter (nonprescription) sales of acetaminophen are limited to 16 tablets per packet.

TABLE 3.17

Some Drugs Causing Hepatic Injury^a

Acarbose	<i>Felbamate</i>	Methotrexate	Ritonavir
Acetaminophen	Fenofibrate	Methsuximide	Rosiglitazone
Allopurinol	<i>Fluconazole</i>	Methyldopa	Rosuvastatin

Amiodarone	<i>Flutamide</i>	Nabumetone	<i>Saquinavir</i>
Amprenavir	Fluvastatin	Naproxen	Simvastatin
Anagrelide	Gemfibrozil	Nefazodone (2005)	Sulindac
<i>Atomoxetine</i>	Gemtuzumab	<i>Nevirapine</i>	<i>Tacrine</i>
Atorvastatin	Griseofulvin	Niacin (SR)	Tamoxifen
Azathioprine	<i>Halothane</i>	Nitrofurantoin	Tasosartan (1998)
Bicalutamide	Imatinib	Olanzapine	<i>Terbinafine</i>
Bosentan	Indinavir	Oxaprozin	Testosterone
Bromfenac (1998)	<i>Infliximab</i>	Pemoline (2005)	Thioguanine
Carbamazepine	Isoflurane	Pentamidine	Tizanidine
Celecoxib	Isotretinoin	Pioglitazone	<i>Tolcapone</i>
Dapsone	Itraconazole	Piroxicam	Troglitazone (2000)
<i>Diclofenac</i>	<i>Ketoconazole</i>	Pravastatin	<i>Trovafloxacin</i>
Disulfiram	<i>Ketorolac</i>	<i>Pyrazinamide</i>	<i>Valproic acid</i>
<i>Duloxetine</i>	Lamivudine	Ribavirin	Voriconazole
Efavirenz	<i>Leflunomide</i>	Rifabutin	Ximelagatran (2004)
Ethotoin	Lovastatin	<i>Rifampin</i>	<i>Zileuton</i>
Ethosuximide	Meloxicam	Riluzole	<i>Zafirlukast</i>

^aDrugs in **bold** have exhibited severe drug-induced hepatotoxicity and were withdrawn either voluntarily or by a regulatory agency (year given). Drugs in *italics* have exhibited moderate-severe drug-induced hepatotoxicity requiring a black box warning restricting their use. The other drugs have exhibited mild to moderate drug-induced hepatotoxicity that can need frequent liver transaminase testing for those at risk (see [Table 3.18](#)).

Drug-induced hepatotoxicity is also the most frequent reason that new therapeutic agents are not approved by the FDA (e.g., ximelagatran in 2004) and the most common adverse drug reaction leading to withdrawal of a drug from the market ([Table 3.17](#)). Hepatotoxicity almost always involves metabolism with Phase 1 P450 enzymes rather than Phase 2 enzymes. More than 600 drugs, chemicals, and herbal remedies can cause hepatotoxicity, of which more than 30 drugs have either been withdrawn from the U.S. market because of hepatotoxicity or have carried a black box warning for hepatotoxicity since 1990. [Table 3.17](#) includes some of the more common drugs that have exhibited drug-induced hepatotoxicity ranging from severe, requiring the drug's regulatory withdrawal from the market (bold in [Table 3.17](#)); moderate to severe, requiring black box warning restrictions (*italics* in [Table 3.17](#)); or mild to moderate, requiring frequent liver function monitoring.

However, Watkins⁷⁸ reported that one-third of 106 patients taking a maximum daily acetaminophen dose of 4 g for 8 days, either alone or in

combination with hydrocodone, exhibited a 3-fold increase in liver enzymes associated with acetaminophen-induced liver injury. This 3-fold increase in transaminase levels is a signal for potential liver safety concerns in those individuals who are at risk of acetaminophen-induced liver toxicity. Drugs reported to cause hepatocellular necrosis include acetaminophen, methyldopa, valproic acid, trazodone, nefazodone, venlafaxine, and lovastatin. Drug-induced liver damage occurs after a prolonged period of drug administration.

The most commonly used indicators of hepatotoxicity (i.e., liver injury) are increased levels of the liver transaminases, aspartate aminotransferase (AST), and alanine aminotransferase (ALT).⁷⁹ Drug-induced hepatotoxicity can develop rapidly, often before abnormal laboratory tests are noticed, which are characterized by rapid elevations in ALT and AST of 8-500 times the upper normal limit, with variable elevations in bilirubin. Drugs causing acute liver injury (hepatocellular necrosis) exhibit elevations in hepatic transaminases ranging from 50 to 100 times higher than the normal level. On the other hand, the elevations of ALT and AST in alcoholic liver disease are two to three times higher than normal. Some hepatotoxins, however, do not elevate transaminases, whereas nonhepatic toxins can elevate ALT.

Most drug-induced hepatotoxicity is of an idiosyncratic nature, occurring in a small percentage of patients (1 in 5000) who ingest the drug.⁸⁰⁻⁸² These reactions tend to be of two distinct types: (1) hypersensitivity reactions that are immune mediated, occurring within the first 4-6 weeks and are associated with fever, rash, eosinophilia, and a hepatitis-like picture (e.g., phenytoin, sulindac, and allopurinol); and (2) metabolic idiosyncratic reactions that tend to occur at almost any time during the first year of treatment (e.g., troglitazone). The incidence of overt idiosyncratic liver diseases varies with the drug, ranging from approximately 1 in 1000 with phenytoin, to 1 in 10,000 or more with sulindac and troglitazone and 1 in 100,000 with diclofenac. To detect a single case of drug-induced hepatotoxicity with 95% confidence requires the number of patients studied to be 3-fold the incidence of the reaction (i.e., for one adverse drug reaction in 10,000 patients, at least 30,000 patients need to be evaluated).

Thus, many drugs are approved before liver toxicity is observed. It is the responsibility of postmarketing surveillance and monitoring of liver transaminases to identify potential cases of liver-adverse drug reactions.

Risk factors (Table 3.18) for drug-induced liver injury, such as age, gender, genetic predisposition, multiple drugs, or dietary supplements and degree of alcohol consumption, appear to increase the susceptibility to drug-induced hepatotoxicity.^{80,81} However, the drugs in Table 3.17 should be used with caution in these high-risk patients, because such patients can have altered metabolism of these drugs and, therefore, can be at increased risk for liver injury. The coadministration of drugs in Table 3.17 with enzyme inducers, such as phenobarbital, phenytoin, ethanol, and/or cigarette smoke, can induce hepatic enzymes, resulting in the enhancement of hepatotoxicity.

TABLE 3.18
Risk Factors for Drug-Induced Liver Injury

Race	Some drugs exhibit different toxicities based on race because of individual P450 polymorphism. For example, blacks and Hispanics can be more susceptible to isoniazid (INH) toxicity.
Age	Elderly persons are at increased risk of hepatic injury due to decreased clearance, drug-drug interactions, reduced hepatic blood flow, variation in drug binding, and lower hepatic volume. In addition, poor diet, infections, and multiple hospitalizations are important reasons for drug-induced hepatotoxicity. Hepatic drug reactions are rare in children (e.g., acetaminophen, halogenated general anesthetics).
Gender	Although the reasons are unknown, hepatic drug reactions are more common in females. Females are more susceptible to hepatotoxicity from acetaminophen, halothane, nitrofurantoin, diclofenac, and sulindac.
Alcohol	Alcoholics are susceptible to drug toxicity because alcohol induces liver injury and cirrhotic changes that alter drug metabolism. Alcohol causes depletion of glutathione (hepatoprotective) stores, making the person more susceptible to toxicity by drugs (e.g., acetaminophen, statins).
Liver disease	Patients with chronic liver disease are not uniformly at increased risk of hepatic injury. Although the total P450 level is reduced, some patients can be affected more than others. The modification of doses in persons with liver disease should be based on knowledge of the specific P450 isoform involved in the metabolism. Patients with HIV infection who are coinfectd with hepatitis B or C virus are at increased risk for hepatotoxic effects. Similarly, patients with cirrhosis are at increased risk to hepatotoxic drugs (e.g., methotrexate, methyl dopa, valproic acid).

Genetic factors	Genetic (polymorphic) differences in the formation of P450 isoforms (2C family and 2D6) can result in abnormal reactions to drugs, including idiosyncratic reactions
Other comorbidities	Patients with AIDS, renal disease, diabetes mellitus, persons who are malnourished, and persons who are fasting can be susceptible to drug reactions because of low glutathione stores.
Pharmacokinetics	Long-acting drugs can cause more injury than shorter-acting drugs, as well as sustained-release drug product formulation.
Drug adulterants	Contaminants are often found in noncertified herbal supplements (e.g., hepatitis C).

Most hepatic adverse effects associated with drugs occur in adults rather than children. Drug-induced liver injury occurs at a higher rate in patients older than 50 years and drug-associated jaundice occurs also more frequently in the geriatric population.^{80,81} This age-risk can be the result of increased frequency of drug exposure, multidrug therapy, and age-related changes in drug metabolism.

For reasons that are unclear, drug-induced liver injury affects females more than males. Females accounted for approximately 79% of all reactions to acetaminophen and 73% of all idiosyncratic drug-induced reactions. Females exhibit increased risk of hepatic injury from drugs such as atorvastatin, nitrofurantoin, methyldopa, and diclofenac.⁸¹

Genetic factors because of enzyme polymorphism in affected individuals can decrease the ability to metabolize or eliminate drugs, thus increasing their duration of action and drug exposure and/or decreasing the ability to modulate the immune response to drugs or metabolites. Chronic ingestion of alcohol can also predispose many patients to increased hepatotoxicity from drugs by lowering the store of glutathione (a detoxifying mechanism), which prevents trapping of the toxic metabolites as mercapturate conjugates that are excreted in the urine.

Drug-induced hepatotoxicity can be categorized as intrinsic (predictable) or idiosyncratic (unpredictable) reactions.⁸² Most drugs involved in hepatotoxicity belong to the idiosyncratic group. Intrinsic hepatotoxins produce liver injury in a dose-related manner when a toxic amount of drug is ingested without bioactivation, such as the toxins found in the *Amanita* mushroom. Idiosyncratic hepatotoxicity is the result of the toxic effects of a drug's metabolites.

The common trigger for both mild and severe forms of hepatotoxicity is bioactivation of relatively inert functional groups to reactive electrophilic intermediates, which is considered to be an obligatory event in the etiology of many drug-induced idiosyncratic hepatotoxicity.^{83,84} Research now shows that reactive metabolites are formed from drugs known to cause idiosyncratic hepatotoxicity, but how these toxic species initiate and propagate tissue damage remains poorly understood. However, the relationship between bioactivation and the occurrence of hepatic injury is not simple. For example, many drugs at therapeutic doses undergo bioactivation in the liver but are not hepatotoxic. The tight coupling of bioactivation with bioinactivation pathways can be one reason for the lack of hepatotoxicity with these drugs. Examples of bioinactivation (detoxification) pathways include glutathione conjugation of quinones by glutathione S-transferases (GSTs) and hydration of arene oxides to dihydrodiols by epoxide hydrolases. When reactive metabolites are poor substrates for such detoxifying enzymes, they can escape bioinactivation and, thereby, damage proteins and nucleic acids, prompting hepatotoxicity.

Most drugs, however, are not directly chemically reactive but, through the normal process of drug metabolism, can form electrophilic, chemically reactive metabolites. Formation of chemically reactive metabolites is mainly catalyzed by P450 enzymes (Phase 1), but products of Phase 2 metabolism (e.g., acylglucuronides, acyl CoA thioesters, or N-sulfates) can also lead to toxicity. However, if Phase 1 drug bioactivation is closely coupled with Phase 2 bioinactivation (e.g., glutathione conjugation to mercapturates), then the net chemical process is one of detoxification if the final product is rapidly cleared.

Toxicity can accrue when accumulation of a chemically reactive metabolite, if not detoxified, leads to covalent modification of biological macromolecules. The identity of the target macromolecule and the functional consequence of its modification will dictate the resulting toxicological response. P450 enzymes are present in many organs, mainly the liver but also the kidney and lung, and thus can bioactivate chemicals to cause organ-specific toxicity. Evidence for the formation of reactive

metabolites was found for 5 of the 6 drugs that have been withdrawn from the market since 1995 and for 8 of the 15 drugs that have black box warnings (Table 3.17). Evidence for reactive metabolite formation has been found for acetaminophen, bromfenac, diclofenac, clozapine and troglitazone. Acetaminophen is the most studied hepatotoxin.

The current hypotheses of how reactive metabolites lead to liver injury suggest that hepatic (target) proteins can be modified by reactive metabolites. Much more clinically important is the identification of the target proteins modified by these toxic metabolites and how these reactions alter the function of those proteins. Additionally, it is important to note that the toxicity of reactive metabolites can also be mediated by noncovalent binding mechanisms, which can have profound effects on normal liver physiology. Such information should dramatically improve our understanding of drug-induced hepatotoxic reactions. Moreover, covalent binding per se does not necessarily lead to drug hepatotoxicity. The regioisomer of acetaminophen, 3-hydroxyacetanilide, becomes covalently bound to hepatic proteins in rodents without inducing hepatotoxicity.⁸⁵

It therefore is necessary to identify targets for these reactive metabolites (i.e., covalently modified macromolecules) that are critical to the toxicological process. Unfortunately, no simple rules predict the target macromolecules for a particular chemically reactive metabolite or the biological consequences of a particular modification. Furthermore, noncovalent interactions also have a role, because covalent binding of hepatotoxins is not indiscriminate with respect to proteins. Even within a single protein, there can be selective modification of an amino acid side chain found repeatedly in the primary structure. Thus, the microenvironment (e.g., pK_a and hydrophobicity) of the amino acid in the tertiary structure appears to be the crucial determinant of selective binding and, therefore, the impact of covalent binding on protein function. In turn, the extent of binding and the biochemical role of the protein will determine the toxicological insult of drug bioactivation. The resulting pathological consequences will be a balance between the rates of protein damage and the rates of protein replacement and cellular repair.

Drug-Induced Idiosyncratic Reactions

Idiosyncratic drug reactions (IDR; type B adverse drug reactions) occur in from 1 in 1000 to 1 in 50,000 patients, are not predictable from the known pharmacology or toxicology of the drug, are not produced experimentally in vitro and in vivo, and are dose independent. The occurrence of IDRs during late clinical trials or after a drug has been released can lead to severe restriction of its use and even its withdrawal. IDRs do not as a rule result from the drug itself because most people can tolerate the drug, but rather from a unique set of patient characteristics, including gender, age, genetic predisposition, and a lack of drug-metabolizing enzymes that can increase the risk of these adverse drug reactions. Most IDRs are caused by hypersensitivity reactions and can result in hepatocellular injury. The hepatic injury occurs within 1 week to 12 months after initiation of drug therapy and is often accompanied by systemic characteristics of allergic drug reactions, such as rash and fever. Signs of hepatic injury reappear with subsequent administration of the same drug with only one or two doses. Hypersensitivity reactions can be severe and associated with fatal reactions as a multiorgan clinical syndrome characterized by the following: (1) fever; (2) rash; (3) gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea, or abdominal pain); (4) generalized malaise, fatigue, or achiness; and (5) respiratory symptoms (e.g., dyspnea, cough, or pharyngitis). Examples of drugs causing IDRs through a hypersensitivity mechanism include penicillin, methyldopa, chlorpromazine, erythromycin, azathioprine toxicity in thiopurine methyltransferase-deficient individuals, sulfonamide and acetaminophen hepatotoxicity in alcoholics and in UGT1A6-deficient cats, ivermectin neurotoxicity in Collie dogs deficient in P-gp, perhexilene hepatotoxicity in CYP2D6-deficient individuals, phenytoin toxicity in CYP2C9-deficient individuals, and valproic acid hepatotoxicity.

The clinical features of some cases of drug-induced idiosyncratic hepatotoxicity strongly suggest an involvement of the immune system.^{83,84} These clinical characteristics include the following: (1) concurrence of rash, fever, and eosinophilia; (2) delay of the initial reaction (1-8 weeks) or requirement of repeated exposure to the culprit drug; (3) rapid recurrence

of toxicity on reexposure to the drug; and (4) presence of antibodies specific for native or drug-modified hepatic proteins. Our current understanding of drug-induced adaptive immune responses is largely based on the hapten hypothesis.

Idiosyncratic drug reactions that are connected with hepatotoxicity involve the formation of reactive metabolites.^{83,84} Such reactions are not predictable, but current bioanalytical technology have enabled the in vivo identification of the formation of reactive metabolites, as evidenced by the detection of biomarkers (i.e., mercapturate or cysteine adducts) in urine, drug-specific antibodies, or antibodies to P450 isoforms.⁸⁴ As a result, some drugs known to cause hepatic injury continue to be used because the drug's benefit outweighs its risk and no alternative efficacious drug exists. Additionally, NSAIDs, including cyclooxygenase-2 inhibitors (e.g., celecoxib), commonly are associated with idiosyncratic liver injury. Most of the idiosyncratic toxins listed in Table 3.19 that have been studied to date produce reactive metabolites.

TABLE 3.19
Some Examples of Idiosyncratic Toxins

Abacavir	Hypersensitivity
Acetaminophen	Hepatotoxicity
Amiodarone	Hepatotoxicity
Aromatic anticonvulsants	Hypersensitivity
Cefaclor	Hepatotoxicity
Clozapine	Agranulocytosis
Diclofenac	Hepatotoxicity
Felbamate	Aplastic anemia
Fibrates	Hepatotoxicity
Halothane	Hepatotoxicity
Indomethacin	Hepatotoxicity
Isoniazid	Hepatotoxicity
Levamisole	Hepatotoxicity
Nefazodone	Hepatotoxicity
Nevirapine	agranulocytosis
Oral contraceptives	Hepatotoxicity
Paroxetine	Hepatotoxicity
Penicillinamine ^a	Hypersensitivity
Phenytoin	Hepatotoxicity

Statins	Hepatotoxicity
Sulfonamides	Stevens-Johnson syndrome
Tamoxifen	Hepatotoxicity
Tacrine	Hepatotoxicity
Tienilic acid	Hypersensitivity
Ticlopidine	Agranulocytosis
Troglitazone	Hepatotoxicity
Valproic acid	Hepatotoxicity
Vesnarinone	Agranulocytosis

^aDoes not produce reactive metabolites.

Current hypotheses regarding IDRs suggest that metabolic activation of a drug to a reactive metabolite is a necessary step in the generation of an idiosyncratic reaction.⁸³ Evidence for this hypothesis comes from drugs that are associated with hepatotoxicity (Table 3.19) and the detection of drug-metabolite specific antibodies in affected patients.

For the other drugs that have been associated with idiosyncratic hepatotoxicity but that do not have black box warnings, either evidence for hepatotoxicity was not available or suitable studies had not been carried out. High doses increase the risk for an IDR (e.g., clozapine at 300 mg/d vs. olanzapine 20 mg/d).

The hapten hypothesis proposes that the reactive metabolites of hepatotoxic drugs act as haptens and bind covalently to endogenous proteins to form immunogenic drug-protein adducts triggering either antibody or cytotoxic T-cell responses.⁸⁴ The hapten hypothesis is supported by the detection of antibodies that recognize drug-modified hepatic proteins in the sera of drug-induced liver injury patients. For example, antibodies that recognize trifluoroacetate-altered hepatic proteins have been detected in the sera of patients with halothane-induced hepatitis. Such drug-specific antibodies that recognize native liver proteins have also been found in patients with liver injury caused by other drugs, such as diclofenac. Most drugs are small molecules and are unlikely to form haptens. Electrophilic acylators can react with the lysine ω -amino residues of the target protein or guanosine residues of DNA. Halothane is the most studied molecule for supporting this hypothesis regarding IDRs.⁸⁴ Therefore, it is not surprising that irreversible chemical modification of a protein, which has a profound effect on function, is a

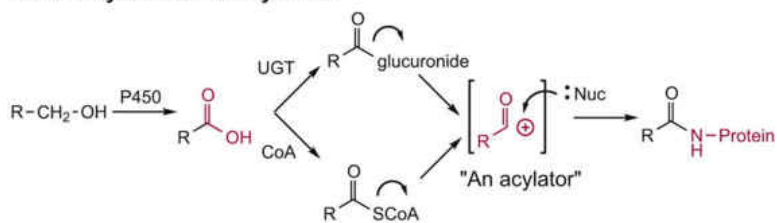
mechanism of idiosyncratic hepatotoxicity. However, it is important to note that a number of drugs (e.g., penicillins, aspirin, and omeprazole) rely on covalent binding to proteins for their efficacy; thus, prevention of their covalent binding through chemical modification of the compound can also, inadvertently, lead to loss of efficacy.

Reactive Metabolites Resulting From Bioactivation

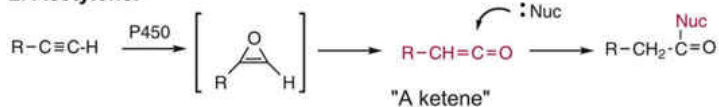
Electrophiles

The concept that small organic molecules can undergo bioactivation to electrophiles (an electron-deficient group or ion that accepts an electron pair to make a covalent bond, such as carbocations and acyl ions) and free radicals, and can elicit toxicity by chemical modification of cellular macromolecules, has its basis in chemical carcinogenicity and the pioneering work of the Millers⁸⁶ and Gillette et al.⁸⁷ Electrophiles are reactive because they possess electron-deficient centers (polarization-activated double bonds or positive-charge acylators) (Fig. 3.38) and can form covalent bonds with electron-rich biological nucleophiles such as the thiol groups in either glutathione or cysteine residues within proteins. A number of different types of reactive metabolites exist; however, they can be broadly classified as either electrophiles (Fig. 3.38) or free radicals (Fig. 3.39).⁸⁸ These reactive metabolites are short-lived, with half-lives of usually less than 1 minute, and they are not normally detectable in plasma or urine except as Phase 2 conjugates or other biomarkers.

1. Carboxylic acids as acylators:



2. Acetylene:



Example: Ethinylestradiol

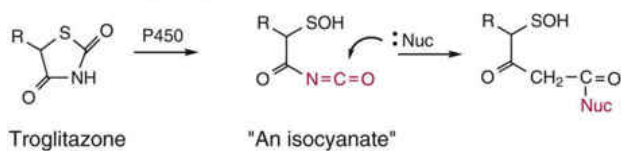
3. Halogenated hydrocarbon:



Haloethane

Trifluoroacetyl halide
(An acylator)

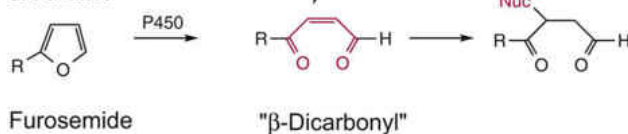
4. Thiazolidinedione:



Troglitazone

"An isocyanate"

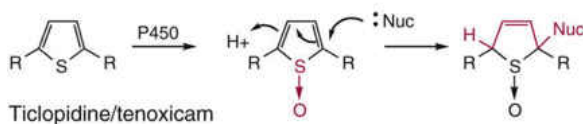
5. Furans:



Furosemide

" β -Dicarbonyl"

6. Thiophene:



Ticlopidine/tenoxicam

7. Aromatics and olefins:

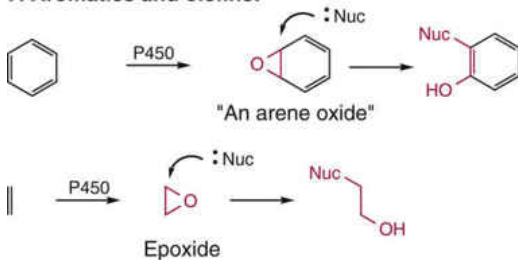
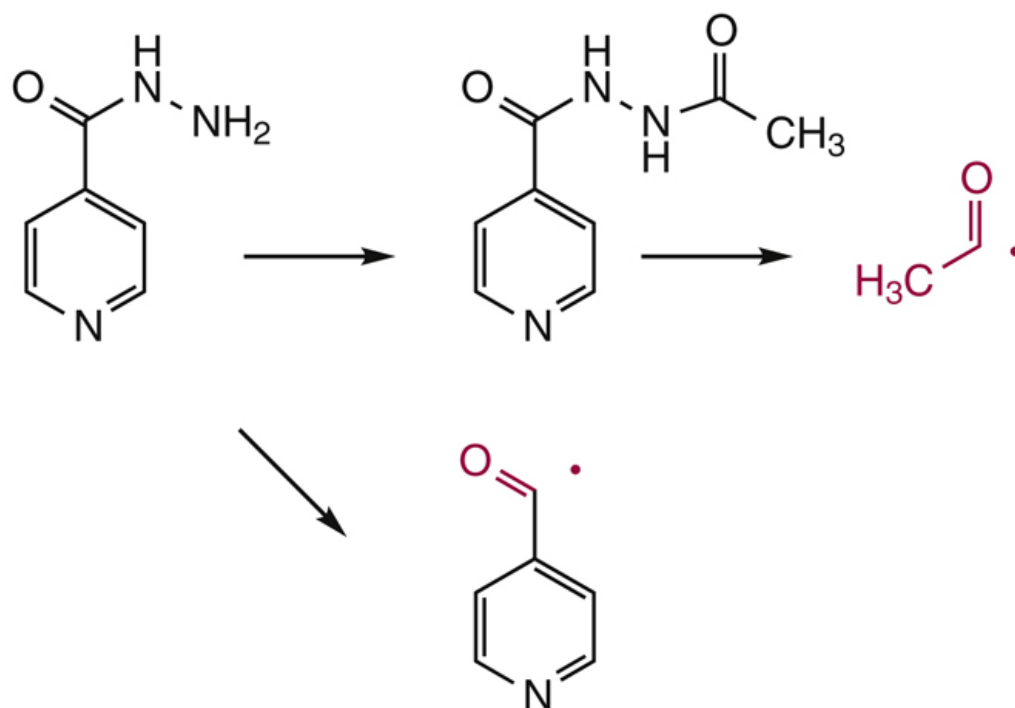


FIGURE 3.38 Some examples of electrophilic intermediates resulting from bioactivation. *NUC*, nucleophiles.

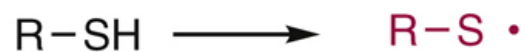
1. Isoniazid



2. Hydrazines



3. Sulfhydryl

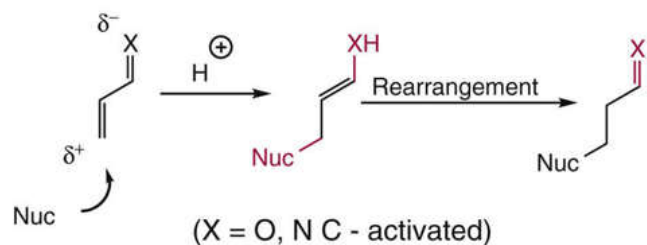


Captopril

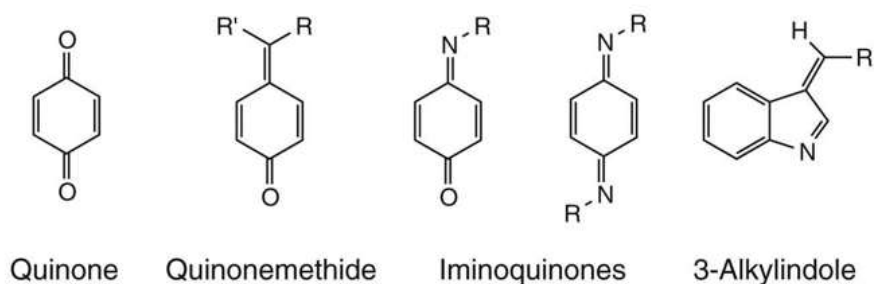
FIGURE 3.39 Drug bioactivation to free radicals.

Activated double bonds are electrophilic intermediates. Examples of activated double bond electrophiles include α,β -unsaturated carbonyl compounds, quinones, quinoneimines, quinonemethides, and diiminoquinones as shown in Figure 3.40. These electrophilic intermediates are highly polarized and can react with nucleophiles in a 1,4-Michael-type addition at the more electrophilic β -carbon of the activated double bond intermediate to generate the addition product (Fig. 3.40A). Specific examples of activated double bond electrophiles that have been proposed for the anticancer drug leflunomide, acetaminophen, the antiandrogen flutamide, the anticonvulsant felbamate, and the cytotoxic cyclophosphamide are shown in Figure 3.40C. The bioinactivation pathways for these electrophilic intermediate can involve direct addition, with or without GSH or epoxide transferases, depending upon the degree of polarization and reactivity of the electrophilic intermediate.⁸⁹

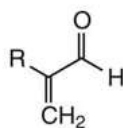
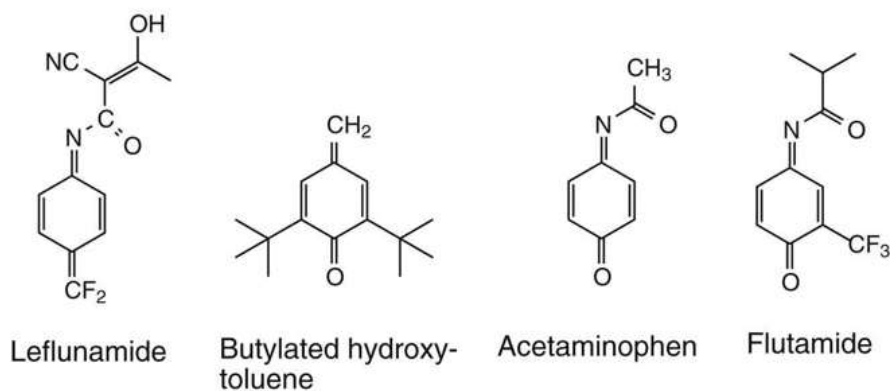
A. 1,4-Addition of a nucleophile in a Michael-type reaction:



B. Examples of activated double bonds:



C. Reactive intermediates from drugs that form activated double bonds:



R = phenyl - felbamate

R = H - acrolein from cyclophosphamide

FIGURE 3.40 Examples of activated double bonds to electrophilic reactive intermediates.

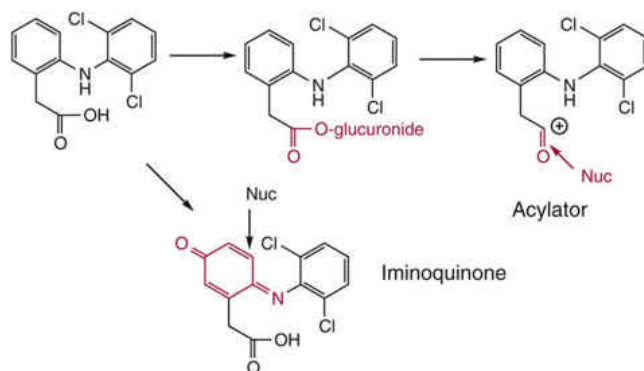
Other commonly found electrophilic intermediate for drug molecules in [Figure 3.38](#) include ketenes from the bioactivation of acetylenic groups (e.g., ethinyl estradiol) (2 in [Fig. 3.38](#)); isocyanates from thiazolidinediones (e.g., the “glitazones”) (4 in [Fig. 3.38](#))⁸⁸; acyloxonium ions from halogenated hydrocarbons (e.g., haloethane) (3 in [Fig. 3.38](#))⁸⁸ and carboxylic acids; β -dicarbonyl from furans (e.g., furosemide) (5 in [Fig. 3.38](#))⁸⁸; activated thiophene-S-oxide from thiophenes, such as ticlopidine and tenoxicam which cause an IDR, agranulocytosis (6 in [Fig. 3.38](#))⁸⁸; and epoxides and arene oxides from olefins and aromatic compounds (7 in [Fig. 3.38](#)).⁸⁸

Drugs possessing structural features prone to metabolic epoxidations are abundant. Therefore the incidence of epoxide metabolites in mediating adverse biologic effects has aroused concern about clinically used drugs known to be metabolized to epoxides. Metabolically produced epoxides have been reported for allobarbitol, secobarbitol, protriptyline, carbamazepine, and cyproheptadine and are implicated with 8-methoxypsoralen and other furanocoumarins (6,7-dihydroxybergamottin in grapefruit juice), phenytoin, phenoximide, phenobarbitol, mephobarbitol, lorazepam, and imipramine.^{89,90} The alarming biologic effects of some epoxides, however, do not imply that all epoxides have similar effects. Epoxides vary greatly in molecular geometry, stability, electrophilic reactivity, and relative activity as substrates for epoxide-transforming enzymes (e.g., epoxide hydrolase, glutathione S-transferase, and others).

Some carboxylic acid-containing drugs have been implicated in rare IDRs, which was the basis for the market withdrawal of the NSAIDs zomiperac and benzoxaprofen.^{57,91} These drugs (e.g., NSAIDs, fibrates, “statins,” and valproic acid) can be bioactivated to acyl glucuronides or acyl CoA thioesters⁵⁷ (1 and 5 in [Fig. 3.41](#)). These products are electrophilic acylators that can acylate target proteins if they escape inactivation by S-glutathione-thioester formation.⁹² A crucial factor is the concentration of acyl glucuronides in hepatocytes due to their transport by conjugate export pumps, where acylglucuronides can selectively acylate hepatic membrane proteins. Acyl CoA esters can be either rapidly hydrolyzed or further metabolized in hepatocytes. Evidence is accumulating that acyl

glucuronides can alter cellular function by haptening of peptides, target protein acylation or glycation, or direct stimulation of neutrophils and macrophages. The role of acyl CoA reactive metabolites is less clear. It should be noted that some noncarboxylic acid drugs can be biotransformed by oxidative metabolism in the liver to the respective carboxylic acids.⁹³

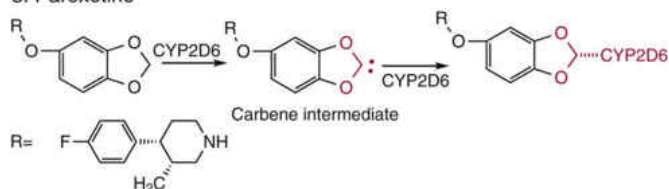
1. Diclofenac



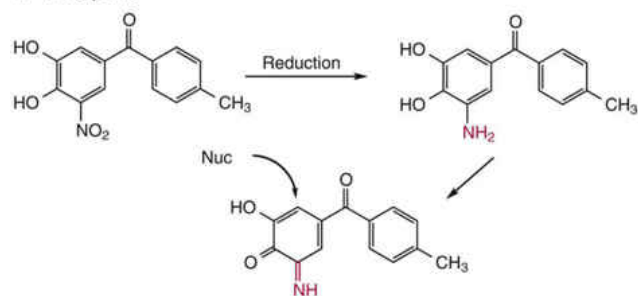
2. Carbamazepine



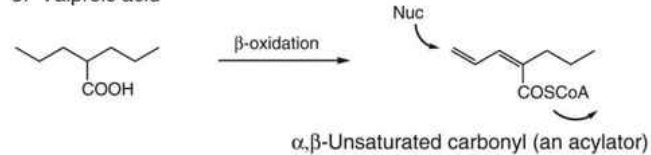
3. Paroxetine



4. Tolcapone



5. Valproic acid



6. Clozapine

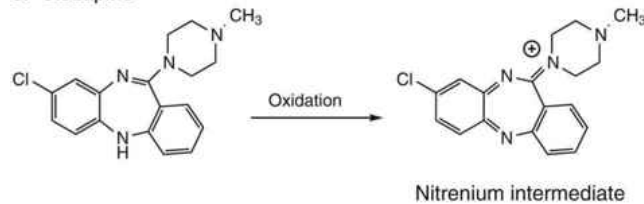


FIGURE 3.41 Examples of drug bioactivation to hepatotoxic intermediates.

Free Radicals

P450 activates molecular dioxygen to generate reactive oxygen species (ROS), such as singlet oxygen ($^1\text{O}_2$) or superoxide. Reactive metabolites that possess an odd unpaired electron are free radicals that can react with molecular oxygen (ground state triplet) to generate intracellular oxidative stress damage.⁹⁴ Free radicals abstract a hydrogen atom from other molecules rather than becoming covalently bound. Free radical reactions can be self-propagating by abstracting a hydrogen atom from the double bond of a lipid that initiates a chain reaction leading to lipid peroxidation, oxidative stress, or other types of modification of biological molecules.

Some examples of free radicals generated by the bioactivation of drug molecules are shown in Figure 3.39. Isoniazid is acetylated to its major metabolite acetylisoniazid, which is hydrolyzed to acetylhydrazine and isonicotinic acid (1 in Fig. 3.39). Acetylhydrazine is further metabolized by the CYP2E1 to an N-hydroxy intermediate that hydrates into an acetyl radical, which can then initiate the process that leads to hepatic necrosis. Other carbon-centered radicals are formed from hydrazines such as the antihypertensive hydralazine and thio-radicals from the ACE inhibitor captopril (3 in Fig. 3.39).

Bioinactivation Mechanisms

Several enzyme systems exist as cellular defense (detoxification) pathways against the chemically reactive metabolites generated by P450 metabolism. These include GST, epoxide hydrolase, and quinone reductase, as well as catalase, glutathione peroxidase, and superoxide dismutase, which detoxify the peroxide and superoxide by-products of metabolism. The efficiency of the bioinactivation process is dependent on the inherent chemical reactivity of the electrophilic intermediate, its affinity and selectivity of the reactive metabolite for the bioinactivation enzymes, the tissue expression of these enzymes, and the rapid upregulation of these enzymes, and cofactors mediated by the cellular sensors of chemical stress. The reactive metabolites that can evade these defense systems can damage target proteins and nucleic acids by either oxidation or covalent modification.

The most abundant agents of cellular defense are thiols. Glutathione will only react noncatalytically with electrophiles, such as activated double bonds (Fig. 3.40). Glutathione conjugation to mercapturates is one of the most important defenses against hepatocellular injury.^{58,59} Glutathione protects cellular enzymes and membranes from toxic metabolites, and its inadequate stores can compromise efficient detoxification of the reactive metabolites. The subsequent inability to detoxify the reactive metabolites can result in hepatocellular injury. The rate-limiting factor for glutathione synthesis is the intracellular concentration of cysteine. N-acetylcysteine is often used as an alternative to glutathione to trap the iminoquinone intermediate in the treatment of acute acetaminophen toxicity and in preventing nephrotoxicity from ifosfamide-generated chloroacetaldehyde. Glutathione has a protective role in the hepatic tissue injury produced by acetaminophen, but not by furosemide.

The relationship between bioactivation, bioinactivation, and DNA adduct formation has been well established for a number of hepatocarcinogens.⁹⁴ Glutathione conjugation of carcinogens becomes more efficient when catalyzed by GSTs, an important example being the detoxification of the hepatocarcinogen aflatoxin. Aflatoxin, a hepatocarcinogen and a hepatotoxin found in mold growing on peanuts, is converted into aflatoxin B1 epoxide in rodents, which is more readily detoxified by GST enzymes than by epoxide hydrolase. The balance between these transferase reactions explains the greater DNA damage in humans compared with rodents, because human forms of GST are less able to catalyze the conjugation of aflatoxin epoxide compared with the rodent forms. Transgenic knockout mice have been used to establish the role of bioactivation by P450 and bioinactivation by GSTs for a number of carcinogenic polyaromatic hydrocarbons.

Substances that detoxify free radicals include the antioxidants vitamin C, vitamin E, and carotene, which scavenge free radicals, including reactive metabolites and reactive oxygen species generated as a consequence of chemical stress.

Specific Examples

Some examples of bioactivation to hepatotoxic or IDR electrophilic intermediates are shown in Figure 3.41. Bioactivation can occur by both oxidation and conjugation reactions, such as those with diclofenac, which undergoes the formation of an acyl glucuronide to produce iminoquinones via formation of a phenol intermediary (1 in Fig. 3.41).⁹² The anticonvulsant carbamazepine is 2-hydroxylated and the elimination of the amide group yields the reactive quinoneimine intermediate (2 in Fig. 3.41). The antidepressant paroxetine and other xenobiotics with the common methylenedioxyphenyl nucleus undergo methylene oxidation to a *p*-quinoid intermediate (3 in Fig. 3.41). The nitro group of the COMT inhibitor tolcapone, used in the treatment of parkinsonism, is first reduced to an amine, then oxidized to an *o*-quinoneimine (4 in Fig. 3.41). The mitochondrial/hepatotoxicity of the anticonvulsant valproic acid results from the formation of an activated α,β -unsaturated CoA thioester via mitochondrial β -oxidation, most commonly associated with the oxidation of fatty acids (5 in Fig. 3.41). The agranulocytosis resulting from the ingestion of the antipsychotic clozapine is bioinitiated by oxidation by hypochlorous acid in neutrophils to a nitrenium intermediate (6 in Fig. 3.41).⁹³

The effect of structure modification for troglitazone that reduced its hepatotoxicity is shown in Figure 3.42. The *p*-dihydroxy groups of the chroman ring nucleus (outlined in red in Fig. 3.42) of troglitazone is bioactivated to an activated double bond (*p*-quinone) was replaced with a pyridine ring that is not bioactivated, although the thiazolidone ring can be bioactivated to an isocyanate (Fig. 3.42).⁹¹

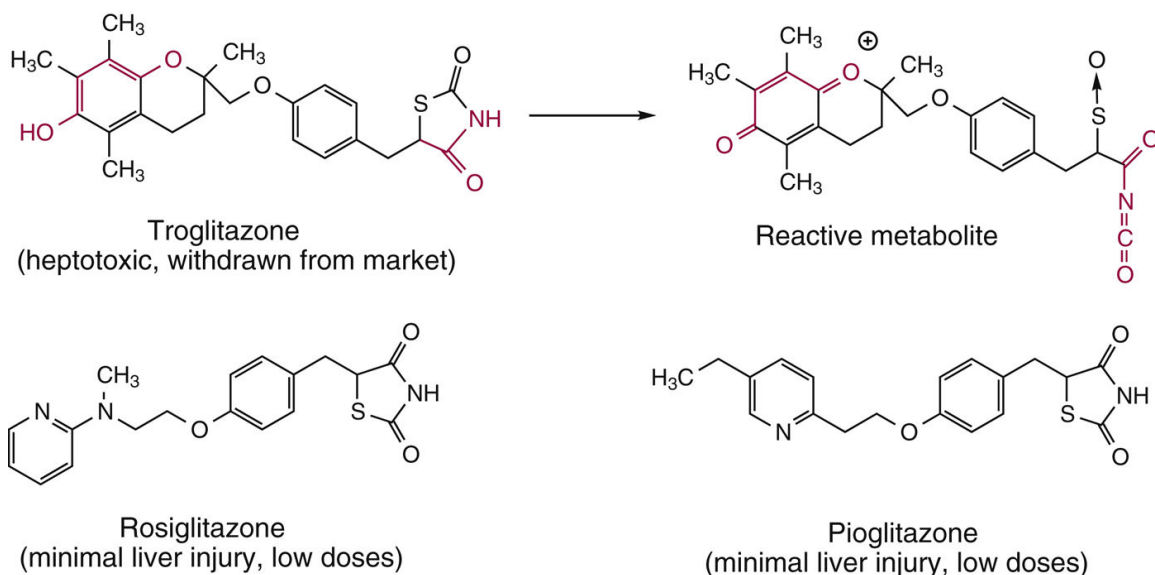


FIGURE 3.42 The effect of structure modification on drug-induced hepatotoxicity of troglitazone.

The oxidation of acetaminophen to the chemically reactive N-acetyl-p-benzoquinoneimine (Fig. 3.36) is catalyzed by the isoforms CYP1A2 and CYP2E1. The reactive quinoneimine can react covalently either with glutathione to form an inactive product or with cellular macromolecules, initiating the processes leading to hepatic necrosis (Fig. 3.36).⁹³ The usual route for acetaminophen metabolism is glucuronidation. If insufficient UDP-glucuronic acid is present, then bioactivation will dominate.

Furosemide, a frequently used diuretic drug, is reportedly a human hepatocarcinogen. The hepatic toxicity apparently results from metabolic activation of the furan ring to a β -dicarbonyl intermediate (5 in Fig. 3.38).⁹³ Ticlopidine and tenoxicam, reported to cause agranulocytosis, do so via metabolic activation of the thiophene ring to an S-oxide (6 in Fig. 3.38).⁹² The agranulocytosis resulting from the ingestion of clozapine is via its bioactivation to a nitrenium ion intermediate (6 in Fig. 3.41).^{93,94}

Drug-Induced Chemical Carcinogenesis

The mechanism whereby xenobiotics are transformed into chemical carcinogens is usually accepted as bioactivation to reactive metabolites, which are responsible for initiating carcinogenicity.^{86,87} Many carcinogens elicit their cytotoxicity through a covalent linkage to DNA. This process

can lead to mutations and, potentially, to cancer. Most chemical carcinogens of concern are chemically inert but require activation by the xenobiotic-metabolizing enzymes before they can undergo reaction with DNA or proteins (cytotoxicity). There are many ways to bioactivate procarcinogens, promutagens, plant toxins, drugs, and other xenobiotics (Fig. 3.43).

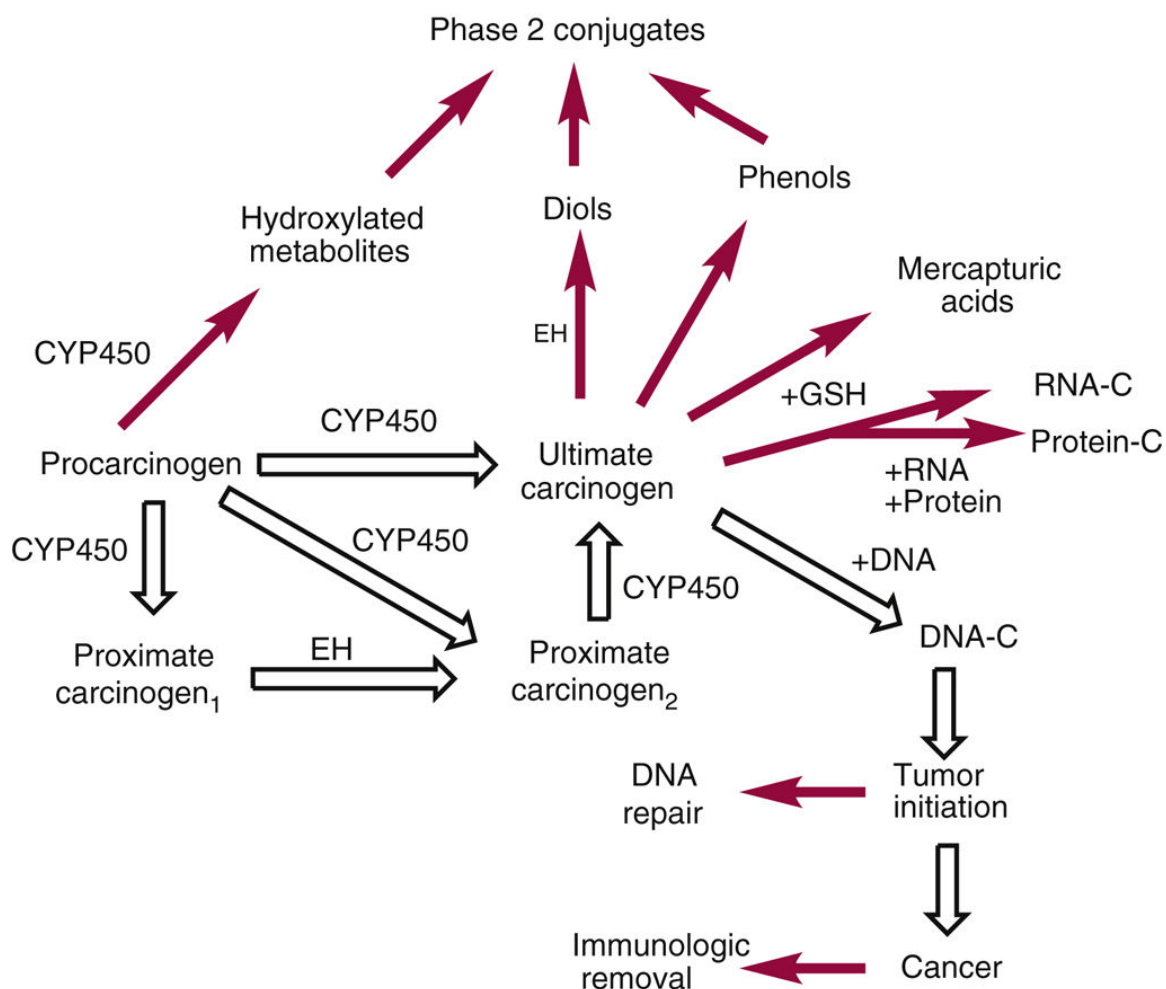


FIGURE 3.43 The bioactivation of procarcinogens and a proposed mechanism of chemical carcinogenesis.

Oxidative bioactivation reactions are by far the most studied and common. Conjugation reactions (Phase 2), however, are also capable of activating these xenobiotics to produce electrophiles, in which the conjugating derivative acts as a leaving group. These reactive metabolites are mostly

electrophiles, such as epoxides, quinones, or free radicals formed by the P450 enzymes or FMO. The reactive metabolites tend to be oxygenated in sterically hindered positions, making them poor substrates for subsequent bioinactivation enzymes, such as epoxide hydrolase and GST. Therefore, their principal fate is formation of covalent linkage to intracellular macromolecules, including enzyme proteins and DNA.^{91,92} Experimental studies indicate that the CYP1A subfamily can oxygenate aromatic hydrocarbons (e.g., PAHs) in sterically hindered positions to arene oxides. Activation by N-hydroxylation of polycyclic aromatic amines (e.g., aryl N-acetamides) appears to depend on either FMO or P450 isoforms. The formation of chemically reactive metabolites is important, because they frequently cause a number of different toxicities, including tumorigenesis, mutagenesis, tissue necrosis, and hypersensitivity reactions.

Our understanding of these reactions was advanced by the studies of the Millers⁸⁶ and Gillette et al.⁸⁷ They proposed that the proportion of the dose that binds covalently to critical macromolecules could depend on the quantity of the reactive metabolite is formed.

A scheme illustrating the complexities of drug-induced chemical carcinogenesis is shown in [Figure 3.43](#). Reactions that proceed via the open arrows eventually lead to neoplasia. Some carcinogens can form the “ultimate carcinogen” directly through P450 isoform bioactivation; others, like the PAHs (e.g., benzo[a]pyrene), appear to involve a multistep reaction sequence forming an epoxide, reduction to a diol by epoxide hydrolase, and perhaps the formation of a second epoxide group on another part of the molecule. Other procarcinogens form the N-hydroxy intermediate that requires transferase-catalyzed conjugation (e.g., O-glucuronide and O-sulfate) to form the “ultimate carcinogen.” The quantity of “ultimate carcinogen” formed should relate directly to the proportion of the dose that binds or alkylates DNA.

The solid-arrow reaction sequences in [Figure 3.43](#) are intended to show detoxification mechanisms, which involve several steps. First, the original chemical can form less active products (phenols, diols, mercapturic acids, and other conjugates). Second, the “ultimate carcinogen” can rearrange to be prevented from its reaction with DNA (or whatever the critical

macromolecule is). Third, the covalently bound DNA can be repaired. Fourth, immunologic removal of the tumor cells can occur. Several mechanisms within this scheme could regulate the quantity of covalently bound carcinogen: (1) The activity of the rate-limiting enzyme, such as epoxide hydrolase, P450 isoform, or one of the transferases, could be involved; (2) the availability of cofactors, such as glutathione, UDP-glucuronic acid, or PAPS, can be rate-limiting; (3) relative P450 activities for detoxification and activation must be considered; (4) availability of alternate reaction sites for the ultimate carcinogen (e.g., RNA and protein can be involved); (5) and possible specific transport mechanisms that deliver either the procarcinogen or its ultimate carcinogen to selected molecular or subcellular sites.

It is well established that numerous organic compounds that are essentially nontoxic as long as their structure is preserved can be converted into cytotoxic, teratogenic, mutagenic, or carcinogenic compounds by normal biotransformation pathways in both animals and humans. The reactive electrophilic intermediate involves reaction with cellular DNA constituents forming either detoxified products or covalent bonding with essential macromolecules, initiating processes that eventually lead to the toxic effect. A better understanding of the mechanisms underlying these reactions can lead to more rational approaches to the development of nontoxic therapeutic drugs. Special attention to risk factors is required for drugs that will be used for long periods in the same patient.

Drug-Drug Interactions

Drug-drug interactions represent a common clinical problem, which has been compounded by the introduction of many new drugs and the expanded use of herbal medicines. Such interactions do significant harm, prompting anywhere from 1.9 to 5 million patient visits to hospital emergency departments per year according to data from the Medication Safety Program at the Centers for Disease Control and Prevention. Approximately 50% of these serious drug-drug interactions involved P450 inhibition. The problem is likely to grow in the future as the population ages and more people take multiple medications.

Drug-drug interactions occur when the efficacy or toxicity of a medication is changed by coadministration of another substance, drug, food (i.e., grapefruit), or herbal product.^{95,96} Pharmacokinetic interactions often occur because of a change in drug metabolism. For example, CYP3A4 oxidizes more than 30% of the clinically used drugs with a broad spectrum of structural features, and its location in the small intestine and liver permits an effect on both presystemic and systemic drug disposition. Some drug-drug interactions with CYP3A4 substrates/inhibitors can also involve inhibition of P-gp. Other clinically important sequelae due to drug-drug interactions resulting from coadministration of CYP3A4 substrates or inhibitors include rhabdomyolysis with the coadministration of some 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors ("statins"), symptomatic hypotension with some dihydropyridine calcium antagonists, excessive sedation from benzodiazepine or nonbenzodiazepine hypnotics, ataxia with carbamazepine, and ergotism with ergot alkaloids.

The clinical importance of any drug-drug interaction depends on factors that are drug-, patient-, and administration-related. Drugs with low oral bioavailability or high first-pass metabolism are particularly susceptible to drug-drug interactions when inhibitors that alter absorption, distribution, and elimination are coadministered. In general, a doubling or more of the plasma drug concentration has the potential for enhanced adverse or beneficial drug response. Less pronounced pharmacokinetic drug interactions can still be clinically important for drugs with a steep concentration-response relationship or narrow therapeutic index. In most

cases, the extent of drug interaction varies markedly among individuals; this is likely to be dependent on interindividual differences in P450 content (polymorphism), preexisting medical conditions, and possibly age. Drug-drug interactions can occur under single-dose conditions or only at steady state. The pharmacodynamic consequences may or may not closely follow pharmacokinetic changes. Drug-drug interactions can be most apparent when patients are stabilized on the affected drug and the P450 substrates or inhibitors are then added to the regimen. One reason for the increased incidence of drug-drug interactions is the practice of simultaneously prescribing several potent drugs, as well as concurrently ingesting nonprescription products and herbal products that compete for, inhibit, or induce metabolizing enzymes.

Although drug-drug interactions constitute only a small proportion of adverse drug reactions, they have become an important issue in health care. Many of the drug-drug interactions can be explained by alterations in the metabolic enzymes in the liver and other extrahepatic tissues, and many of the major pharmacokinetic interactions between drugs are caused by hepatic P450 isoenzymes being affected by coadministration of other drugs. Some drugs act as potent enzyme inducers, whereas others are inhibitors. Drug-drug interactions involving enzyme inhibition, however, are much more common. Understanding these mechanisms of enzyme inhibition or induction is extremely important to appropriately administer multidrug therapies. Individuals at greatest risk for drug-drug interactions and adverse events need to be identified and treated based upon their specific pharmacogenomic-related needs.

P450s have a dominant role in the metabolism and elimination of drugs from the body, and their substrates are shown in [Tables 3.3 and 3.7-3.10](#). Drugs in ***bold italics*** have been associated with clinically relevant drug-drug interactions. Inhibitors of P450 are shown in [Table 3.11](#). Pharmacokinetic interactions can arise when the biotransformation and elimination of a drug are impaired by coadministered drugs. Thus, drugs can compete for biotransformation by a common P450. Adverse drug reactions, including toxicity, can occur if elimination is dependent on a P450 that exhibits defective gene variants. Thus, the genetic makeup of the individual (see

section Genetic Polymorphism) has a major influence on the duration of drug action, as well as on drug efficacy and safety.

P450 pharmacogenetics affects the tendency for certain drug-drug interactions to occur. Thus, the future safe use of drug combinations in patients can require genotyping and phenotyping of individuals before the commencement of therapy. Identification of subjects who metabolize drugs in a different fashion from the general population should minimize the impact of pharmacogenetic variation on drug pharmacokinetics.

Metabolism-Based Enzyme Inhibition

Many drug-drug interactions are the result of inhibition or induction of P450 enzymes. Metabolism-based enzyme inhibition involves mostly reversible competition between two drugs for the enzyme-active site. Metabolic drug-drug interactions occur when drug A (or its metabolite) alters the pharmacokinetics of a coadministered drug B by inhibiting, activating, or inducing the activity of the enzymes that metabolize drug B. Inhibitory drug-drug interactions could result in serious adverse effects, including fatalities in patients receiving multiple medications. This process is competitive and begins with the first dose of the inhibitor, with the extent of inhibition correlating with their relative affinities for the enzymes and the metabolic half-lives of the drugs involved. On the other hand, mechanism-based (irreversible) inhibition results from a metabolite that binds irreversibly to the enzyme through a covalent bond, rendering the enzyme inactive.³⁶

Enzyme-specific P450 inhibitors, including metabolism- and mechanism-based inhibitors, or drugs that are metabolized by specific P450 isoforms, are by and large excluded from further consideration for new drug development. Not only is CYP3A4 the most abundant isoform in human liver, it also metabolizes more than 30% of the drugs in clinical use, which renders CYP3A4 highly susceptible to both metabolism- (reversible) and mechanism-based inhibition. The CYP3A subfamily is involved in many clinically significant drug-drug interactions and therefore metabolism-based inhibition of CYP3A can cause clinically significant drug-drug interactions, such as those involving nonsedating antihistamines and the GI motility stimulant cisapride that can result in cardiac dysrhythmias. An excellent example of a metabolism-based inhibition drug-drug interaction that resulted in a life-

threatening ventricular arrhythmia associated with QT prolongation (torsades de pointes) occurred when CYP3A4 substrates or inhibitors were coadministered with terfenadine, astemizole, cisapride, or pimozide. This potentially lethal drug interaction led to the withdrawal of terfenadine and cisapride from clinical use and to the approved marketing of fexofenadine, the active metabolite of terfenadine, which does not have this interaction.

Inhibitors of CYP3A4 can increase the risk of toxicity from many drugs, including carbamazepine, cyclosporine, ergot alkaloids, lovastatin, protease inhibitors, rifabutin, simvastatin, tacrolimus, and vinca alkaloids. Furthermore, inhibitors of CYP1A2 can increase the risk of toxicity from clozapine or theophylline. Inhibitors of CYP2C9 can increase the risk of toxicity from phenytoin, tolbutamide, and oral anticoagulants (e.g., warfarin). Inhibitors of CYP2D6 can increase risk of toxicity of many antidepressants, opioid analgesics, and psychotherapeutic agents. Examples of enzyme inducers include barbiturates, carbamazepine, glutethimide, griseofulvin, phenytoin, primidone, rifabutin, and rifampin. Some drugs, such as ritonavir, can act as either an enzyme inhibitor or an enzyme inducer, depending on the situation. Drugs metabolized by CYP3A4 or CYP2C9 are particularly susceptible to enzyme induction.

Mechanism-Based Enzyme Inhibition

Mechanism-based inhibition differs from metabolism-based (reversible) inhibition in that the inhibitors require enzymatic activation by the target enzyme prior to exerting their inhibitory effect.³⁶ This initial activation step leads to the formation of an active inhibitor, often referred to as the metabolite-intermediate complex (MIC). The MIC can then exert its inhibitory effect by either forming a direct covalent link with the enzyme or forming a noncovalent tight binding complex. Mechanism-based inhibition is characterized by NADPH-, time-, and concentration-dependent enzyme inactivation, occurring when some drugs are converted by P450s to reactive metabolites.³⁶ Mechanism-based inactivation of CYP3A4 by drugs can be the result of chemical modification of the heme, the apoprotein, or both, because of covalent binding of the modified heme to the protein. The clinical pharmacokinetic effect of a mechanism-based CYP3A4 inhibitor is a function of its enzyme kinetics (i.e., K_m and V_{max}) and the rate of synthesis of new or

replacement enzyme. Predicting drug-drug interactions involving CYP3A4 inactivation is possible when pharmacokinetic principles are followed. Such prediction can become difficult, however, because the clinical outcomes of CYP3A4 inactivation depend on many factors associated with the enzyme, the drugs, and the patients.

Some of the clinically important drugs that have been identified to be mechanism-based CYP3A4 inhibitors include antibacterials (e.g., clarithromycin, erythromycin, isoniazid), anticancer drugs (e.g., irinotecan, tamoxifen, raloxifene), antidepressants (e.g., fluoxetine and paroxetine), anti-HIV agents (e.g., ritonavir and delavirdine), antihypertensives (e.g., dihydralazine and verapamil), steroids and their receptor modulators (e.g., ethinyl estradiol, gestodene, and raloxifene), dihydrotestosterone reductase inhibitors (e.g., finasteride), and some herbal constituents (e.g., bergamottin and glabridin). Drugs inactivating CYP3A4 often possess several common moieties such as a tertiary amine, furan ring, or an acetylene group. The chemical properties of a drug critical to CYP3A4 inactivation include formation of reactive metabolites by P450 isoenzymes, P450 inducers, P-gp substrates, or inhibitors and the occurrence of clinically significant pharmacokinetic interactions with coadministered drugs.

Compared to the more common metabolism-based (reversible) inhibition, mechanism-based inhibitors of CYP3A4 more frequently cause pharmacokinetic-pharmacodynamic drug-drug interactions and are often the cause for unfavorable drug-drug interactions because the inactivated CYP3A4 must be replaced by newly synthesized CYP3A4 protein.^{95,96} The resultant drug interactions may lead to adverse drug effects, including some fatal events. For example, raloxifene, a drug approved for the treatment of osteoporosis and chemoprevention of breast cancer in postmenopausal women at high risk for invasive breast cancer, has demonstrated the ability to act as a mechanism-based inhibitor of CYP3A4 by forming adducts with the apoprotein. It has been established that 3'-hydroxyraloxifene is produced exclusively via CYP3A4-mediated oxygenation to produce a reactive diquinone methide, excluding the alternative arene oxide pathway.

However, predicting drug-drug interactions involving CYP3A4 inactivation is difficult, since the clinical outcomes depend on a number of factors that are associated with drugs and patients. The apparent pharmacokinetic effect of a

mechanism-based inhibitor of CYP3A4 would be a function of its enzyme kinetics and the synthesis rate of new or replacement enzyme. Most CYP3A4 inhibitors are also P-gp substrates/inhibitors, confounding the in vitro to in vivo extrapolation. The clinical significance of CYP3A inhibition for drug safety and efficacy warrants an in-depth understanding of the mechanisms for each inhibitor. Furthermore, such inactivation may be exploited for therapeutic gain in certain circumstances. Clinicians should have good knowledge about these CYP3A4 inhibitors and avoid their combination.

Although CYP2D6 constitutes a relatively minor fraction of the total hepatic P450 content (Fig. 3.2), the contribution of this isoform is significant because of its role in the metabolism and clearance of many therapeutic agents that target the cardiovascular and CNS (Fig. 3.3) (Table 3.8). In addition, clinically significant polymorphisms in the CYP2D6 gene have been identified in a variety of populations with altered metabolic activity as PMs or individuals with impaired enzyme function resulting from splicing defects or gene deletions. On the other hand, EMs (individuals with normal enzyme function) are heterozygous or homozygous for the wild-type allele. In vivo clearance of CYP2D6 substrates in PMs is usually much lower than in EMs, leading to higher plasma concentrations and the potential for clinical toxicities with therapeutic doses. For example, paroxetine is a selective serotonin reuptake inhibitor that is both a substrate for and an inhibitor of CYP2D6. Paroxetine is metabolized by CYP2D6 via the formation of a carbene intermediate of the methylenedioxy group yielding an irreversible complex with CYP2D6 (Fig. 3.41).⁹⁷ Also, an in vitro study with 3,4-methylenedioxy-methamphetamine (MDMA, ecstasy) suggested that a typical recreational MDMA dose could inactivate most hepatic CYP2D6 within an hour, and the return to a basal level of CYP2D6 could take at least 10 days, impacting its pharmacokinetics.

Because of the pivotal role of P450 isoenzymes in drug metabolism, significant inactivation of these isoforms, and particularly the major human hepatic and intestinal CYP3A4 and hepatic CYP2D6, could result in drug-drug interactions and adverse drug reactions. Compared with the reversible inhibition of CYP3A4 and CYP2D6, mechanism-based inhibitors of these isoenzymes more frequently cause pharmacokinetic/pharmacodynamic drug-drug interactions, as the inactivated isoenzyme has to be replaced by newly

synthesized P450 protein. Pharmacokinetic interactions often occur because of a change in drug metabolism. For example, the macrolide antibiotics increase the plasma concentrations of therapeutic agents that are substrates of CYP3A4 (Table 3.10). Diltiazem has been shown to inhibit the metabolism of a variety of coadministered drugs including carbamazepine, quinidine, midazolam, and lovastatin (Table 3.10). Inhibition of CYP3A by ritonavir explains, in part, the remarkable elevation of blood concentrations and area under the plasma concentration-time curve (AUC) of other concomitantly administered drugs that are extensively metabolized by CYP3A4 and have significant first-pass metabolism. These drugs include rifabutin (400%), clarithromycin (77%), ketoconazole, saquinavir (5000%), amprenavir (210%), nelfinavir (152%), lopinavir (7700%), and indinavir (380%).⁹⁶ Furthermore, such inactivation can be exploited for therapeutic gain in certain circumstances, e.g., extended duration of action of the protease inhibitors ritonavir/indinavir used in the treatment of AIDS.

Beneficial Drug-Drug Interactions

By understanding the unique functions and characteristics of these isoenzymes, health care practitioners can better anticipate and manage drug-drug interactions. They can also predict or explain an individual's response to a particular therapeutic regimen. A beneficial drug interaction, for example, is the coadministration of a CYP3A4 inhibitor with cyclosporine. By inhibiting CYP3A4, the plasma concentrations of cyclosporine (a CYP3A4 substrate) are increased which allows a reduction of the cyclosporine dosage thereby improving clinical efficacy and reducing its cost. Similarly, certain HIV protease inhibitors, such as saquinavir, have a low oral bioavailability due to intestinal CYP3A4 metabolism. The oral bioavailability of saquinavir can be profoundly increased by the addition of a low dose of the mechanism-based CYP3A4 inhibitor, ritonavir. This concept of altering drug pharmacokinetics by adding a low, subtherapeutic dose of a mechanism-based CYP3A4 inhibitor (ritonavir) to increase the oral bioavailability of another protease inhibitor, lopinavir (CYP3A4 substrate), led to the marketing of Kaletra, a new drug combination of lopinavir and ritonavir. Another beneficial mechanism-based inhibition is of the P2Y₁₂ receptor (a protein found on the surface of blood platelet cells and an important

regulator in blood clotting) by the antiaggregating platelet prodrugs, clopidogrel and prasugrel. Clopidogrel is bioactivated to its reactive metabolite by CYP2C19, CYP1A2, and CYP3A4, whereas prasugrel is bioactivated by CYP3A4 and CYP2B6. The bioactivated metabolites can then react with the P2Y₁₂ receptor protein.

Grapefruit Juice-Drug Interactions

Historical Significance of Grapefruit Juice

The discovery that grapefruit juice can markedly increase the oral bioavailability of CYP3A4 drugs was based on an unexpected observation from an interaction study between the dihydropyridine calcium channel antagonist felodipine and ethanol in which grapefruit juice was used to mask the taste of the ethanol. Subsequent investigations confirmed that grapefruit juice significantly increased the oral bioavailability of felodipine by reducing presystemic felodipine metabolism through selective inhibition of CYP3A4 expression in the intestinal wall.⁹⁸

Grapefruit juice is a beverage often consumed at breakfast for its health benefits and to mask the taste of drugs or foods. Unlike other citrus fruit juices, however, grapefruit juice can significantly increase the oral bioavailability of drugs that are metabolized primarily by intestinal CYP3A4, causing an elevation in their serum concentrations (Table 3.20). Those drugs with high oral bioavailabilities (>60%), however, are all likely safe to be taken with grapefruit juice because their high oral bioavailability leaves little room for elevation by grapefruit juice. The importance of the interaction appears to be influenced by individual patient susceptibility, type and amount of grapefruit juice, and administration-related factors.

TABLE 3.20

Some CYP3A4 Substrates and Interactions With Grapefruit Juice

Drug	Interaction ^a	Drug	Interaction ^a	Drug	Interaction ^a
Calcium channel blockers		HIV protease inhibitors		CNS drugs	
Amlodipine	Y	Indinavir	N?	Buspirone	Y
Felodipine	Y	Nelfinavir	N?	Carbamazepine	Y
Nifedipine	Y	Ritonavir	N?	Diazepam	Y
Nimodipine	Y	Saquinavir	Y	Midazolam	Y
Nisoldipine	Y	Macrolides		Triazolam	Y

Drug	Interaction ^a	Drug	Interaction ^a	Drug	Interaction ^a
Nitrendipine	Y	Clarithromycin	N	Immunosuppressants	
Pranidipine	Y	HMG-CoA reductase inhibitors		Cyclosporine	Y
Antiarrhythmics		Atorvastatin	Y	Tacrolimus	Y?
Diltiazem	N	Fluvastatin	N?	Other	
Verapamil	N	Lovastatin	Y	Methadone	Y
Quinidine	N	Pravastatin	N?	Sildenafil	Y
Antihistamines		Simvastatin	Y		
Ebastine	Y?				
Loratadine	Y?				

^a Y (yes) and N (no) indicate published evidence of the presence or absence of an interaction with grapefruit juice. Y? and N? indicate expected findings based on available data. Those drugs with Y or Y? should not be consumed with grapefruit juice in an unsupervised manner.

Grapefruit juice can alter oral drug pharmacokinetics by different mechanisms. Irreversible inactivation of intestinal CYP3A4, which can persist up to 24 hours, is produced by grapefruit juice given as a single, normal, 200- to 300-mL drink or by whole fresh fruit segments ([Table 3.20](#)). As a result, presystemic metabolism is reduced and oral drug bioavailability increased. Enhanced oral drug bioavailability can occur up to 24 hours after juice consumption, so taking CYP3A4 vulnerable drugs with water or other liquids within 24 hours of consuming grapefruit juice can still result in an interaction. Inhibition of P-gp is a possible mechanism by which grapefruit juice, and perhaps grapefruit oil, increases oral drug bioavailability by reducing intestinal and/or hepatic efflux transport. Inhibition of organic anion-transporting polypeptides by grapefruit juice and apple juice has been observed; intestinal uptake transport appeared to be decreased as oral drug bioavailability was reduced.

Numerous medications used in the prevention or treatment of coronary artery disease and its complications have been observed or predicted to interact with grapefruit juice. Such drug-drug interactions can increase the risk of rhabdomyolysis when dyslipidemia is treated with the HMG-CoA reductase inhibitors (“statins”). Such drug-drug interactions could also cause excessive vasodilatation when hypertension is managed with the dihydropyridines felodipine, nifedipine, nisoldipine, or nitrendipine. An alternative agent could be amlodipine. The therapeutic effect of the angiotensin II type I receptor antagonist losartan can be reduced

by grapefruit juice. Grapefruit juice interacting with the antidiabetic agent repaglinide can cause hypoglycemia, and interaction with the appetite suppressant sibutramine can cause elevated blood pressure and heart rate. In angina pectoris, administration of grapefruit juice could result in atrioventricular conduction disorders with verapamil or attenuated antiplatelet activity with clopidogrel. Grapefruit juice can enhance the drug toxicity for antiarrhythmic agents, such as amiodarone, quinidine, disopyramide, or propafenone and for the congestive heart failure drug carvedilol. Some drugs used for the treatment of peripheral or central vascular disease also have the potential to interact with grapefruit juice. Interaction with sildenafil, tadalafil, or vardenafil for erectile dysfunction can cause serious systemic vasodilatation, especially when combined with a nitrate. In stroke, interaction with nimodipine can cause systemic hypotension.

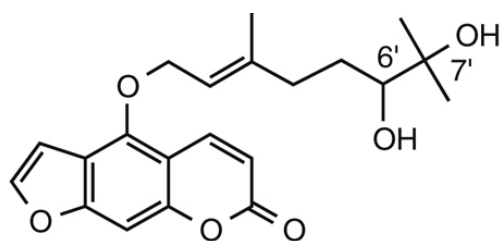
If a drug has low inherent oral bioavailability from presystemic metabolism by CYP3A4 or efflux transport by P-gp and the potential to produce serious overdose toxicity, avoidance of grapefruit juice entirely during pharmacotherapy appears mandatory. Although altered drug response is variable among individuals, the outcome is difficult to predict and avoiding the combination will guarantee that toxicity is prevented.

The mechanism by which grapefruit juice produces its effect is through inhibition of the enzymatic activity and a decrease in the intestinal expression of CYP3A4. The P-gp efflux pump also transports many CYP3A4 substrates; thus, the presence of inhibitors of P-gp in grapefruit juice (e.g., 6',7'-dihydroxybergamottin and other furanocoumarins) could be a related factor for drug-grapefruit juice interactions.⁹⁹ Numerous studies have shown that grapefruit juice consumed in normal (single glass) quantities acts on intestinal CYP3A4, not at the hepatic level.

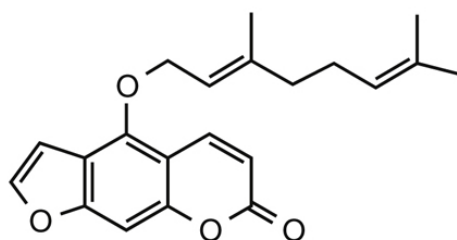
Does the quantity of juice matter? The majority of the presystemic CYP3A4 inhibition is obtained following ingestion of one glass of grapefruit juice; however, 24 hours after ingestion of a glass of grapefruit juice, 30% of its effect is still present.⁹⁹ The reduction in intestinal CYP3A4 concentration is rapid: a 47% decrease occurred in a healthy volunteer within 4 hours after consuming grapefruit juice. Daily ingestion of grapefruit juice results in a loss of CYP3A4 from the small intestinal epithelium. Consumption of very large

quantities of grapefruit juice (six to eight glasses/day) can lead to inhibition of hepatic CYP3A4.

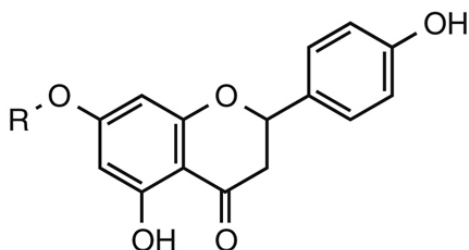
The active constituents found in grapefruit juice responsible for its effects on CYP3A4 include flavonoids (e.g., naringenin and naringin) and furanocoumarins (e.g., bergamottin and 6',7'-dihydroxybergamottin).⁹⁰



6',7'-Dihydroxybergamottin



Bergamottin



Naringin R: rhamnose-glucoside-
Naringenin R: H-

The majority of studies to date have used either freshly squeezed grapefruit juice, reconstituted frozen juice, commercial grapefruit juice, grapefruit segments, or grapefruit extract; all are capable of causing drug-drug interactions with CYP3A4 substrates (blended grapefruit juices have not yet been investigated). The active constituents in grapefruit juice are present not just in the juice but also in the pulp, peel, and core of the fruit and are responsible for its flavor. Bergamottin and 6',7'-dihydroxybergamottin are potent mechanism-based inhibitors of CYP3A4 and naringenin isomers are competitive inhibitors of CYP3A4. Higher concentrations of 6',7'-dihydroxybergamottin are present in grapefruit segments. Thus, any therapeutic concern for a drug interaction with grapefruit juice should now be extended to include whole fruit and other products derived from the grapefruit peel. The difference in the in vitro CYP3A4 inhibition between grapefruit juice and orange juice is that orange juice contained no measurable amounts of 6',7'-dihydroxybergamottin. The nutraceutical grapefruit oil

contains minor amounts of bergamottin, therefore grapefruit oil has the potential to cause drug interactions with CYP3A4 substrates.

If a patient has been taking medication with grapefruit juice for some time without ill effects, is it safe to continue to do so? Much of this unpredictability results from the inconsistency of the juice concentrations and the sporadic manner in which grapefruit juice is consumed, suggesting that this approach cannot be entirely safe. Given the unpredictability of the effect of grapefruit juice on the oral bioavailability of the drugs in [Table 3.20](#), patients should be advised to avoid this combination, thus preventing the onset of potential adverse effects. Each patient's situation should be considered, and advice should be based on consumption history and the specific medications involved. The benefits of increased and controlled drug bioavailability by grapefruit juice can, in the future, be achieved through either standardizing the constituents or coadministration of the isolated active ingredients. This would then lead to a safe, effective, and cost-saving means to enhance the absorption of many therapeutic agents.

P-Glycoprotein-Drug Interactions

P-gp-mediated transport has an important role in pharmacokinetic-mediated drug-drug interactions. The effect of P-gp inhibition is to increase the oral bioavailability of CYP3A4 substrates so that the later actions of CYP3A4 inhibition will be increased. One of the best examples is the interaction between digoxin and quinidine. Quinidine blocks P-gp in the intestinal mucosa and in the proximal renal tubule; thus, digoxin elimination into the intestine and urine is inhibited, increasing the plasma digoxin concentration to toxic levels. Another example is loperamide, which is an opioid antidiarrheal normally kept out of the brain by the P-gp pump. Inhibition of P-gp allows accumulation of loperamide in the brain, leading to respiratory depression as a result of central μ opioid receptor agonism.

The components of grapefruit juice reportedly inhibit P-gp, and this can be one of the mechanisms for the increase in bioavailability of drugs that are substrates for P-gp.

Drug-Dietary Supplement Interactions

The increasing use of dietary supplements presents a special challenge in health care; thus, there is an increasing need to predict and avoid these potential adverse drug-dietary supplement interactions.⁹⁹ The present interest and widespread use of herbal remedies has created the possibility of interaction between them and over-the-counter or prescription drugs if they are used simultaneously. As herbal medicines become more popular, herbal hepatotoxicity is being increasingly recognized. Females appear to be predisposed to hepatotoxicity, and coadministered agents that induce P450 enzymes (e.g., St. John's wort) can also increase individual susceptibility to some dietary supplements. Nearly one in

five adults taking prescription medicines is also taking at least one dietary supplement. The mechanisms for drug-dietary supplement interactions are similar to those for drug-drug interactions affecting the pharmacokinetics of the respective drug. Little is known regarding the pharmacokinetic properties of many of the substances in dietary supplements. Therefore, the potential for drug-dietary supplement interactions has greatly increased.

A commonly reported drug-dietary supplement interaction is between St. John's wort and HIV protease inhibitors, leading to drug resistance and treatment failure. ^{[1][SEP]}St. John's wort is a popular dietary supplement often used for depression. Of the two substances found in St. John's wort, hypericin and hyperforin, hyperforin appears to be the main constituent, with in vitro selective serotonin reuptake inhibitor (SSRI) activity. Hyperforin appears also to be the more potent inducer of CYP3A enzymes based on in vitro and in vivo studies.

Other Dietary Supplements Exhibiting Drug-Induced Hepatotoxicity

DHEA and androstenedione are dietary testosterone precursors that have been associated with hepatic toxicity and should not be taken by those with hepatic disease. Co-ingestion with other potentially hepatotoxic products or enzyme inducers that might increase the risk of liver damage should also be avoided.

Black cohosh, commonly used by women for menopausal symptoms, including hot flashes and sleep disorders, forms quinone metabolites in vitro, but no mercapturate conjugates were detected in urine samples from women who consumed multiple oral doses of up to 256 mg of a standardized black cohosh extract. At moderate doses of black cohosh, the risk of liver injury is minimal.

Silybum marianum (milk thistle) is used in the treatment of chronic or acute liver disease, as well as in protecting the liver against toxicity. *Silybum* is cited as one of the oldest known herbal medicines. The active constituents of milk thistle are flavonolignans, which are known collectively as silymarin.

Miscellaneous Drug-Drug Interactions

The ability of drugs and other foreign substances to induce metabolism of other drugs has already been discussed. Phenobarbital, for example, stimulates metabolism of a variety of drugs (e.g., phenytoin and coumarin anticoagulants). Stimulation of bishydroxycoumarin metabolism can create a problem in patients undergoing anticoagulant therapy. If phenobarbital administration is stopped, the rate of metabolism of the anticoagulant decreases, resulting in greater plasma concentrations of bishydroxycoumarin and enhanced anticoagulant activity, increasing the possibility of hemorrhage. Serious side effects have resulted from this type of interaction. These observations indicate that combined therapy of a potent drug (e.g., bishydroxycoumarin) and an inducer of drug metabolism (e.g., phenobarbital) can create a hazardous situation if the enzyme inducer is withdrawn and therapy with the potent drug is continued without an appropriate decrease in dose.

Serious reactions have been reported in patients treated with an MAO inhibitor such as tranylcypromine because they are, as a rule, sensitive to a subsequent dose of a sympathomimetic amine (e.g., amphetamine) or a tricyclic antidepressant (e.g., amitriptyline), which is metabolized by MAO.

Allopurinol, a xanthine oxidase inhibitor used for the treatment of gout, inhibits metabolism of 6-mercaptopurine and other drugs metabolized by this enzyme. A serious drug interaction results from the concurrent use of allopurinol for gout and 6-mercaptopurine to

block the immune response from a tissue transplant or as antimetabolite in neoplastic diseases.

Gender Differences in Drug Metabolism

Although numerous gender differences have been described in humans, so far most clinical research has been carried out with the questionable view that the male can fulfill the function of the true representative of the human species. However, in spite of the increasing evidence pointing out physiological and pathological differences between the sexes, beyond those related to reproduction, women differ from men in gene expression and regulation, in the susceptibility to and risk for many medical conditions, and in the response to numerous drugs.^{100,101} Gender difference in drug response may explain, at least in part, the interindividual variations occurring in therapeutic response and toxicity, especially considering that female sex has been shown to be a risk factor for the development of adverse drug reactions (ADRs). Nonetheless, current studies indicate that gender differences in pharmacological response are more widespread than originally believed and involve pharmacodynamics and pharmacokinetics, with pharmacokinetic being the most investigated.

The role of pharmacokinetics versus pharmacodynamics is not yet completely appreciated, and only a few contributions have evaluated the impact of genetics, hormonal variations, and their relative interactions.¹⁰⁰ The role of gender as a contributor to variability in xenobiotic metabolism and ADRs, which are more common in women than in men, is not clear, but increasing numbers of reports show differences in metabolism between men and women, raising the intriguing possibility that endogenous sex hormones, hydrocortisone, or their synthetic equivalents can influence the activity of inducible CYP3A. N-demethylation of erythromycin was significantly higher in females than males, which was persistent

throughout adulthood. In contrast, males exhibited unchanged N-demethylation values.

Gender-dependent differences of metabolic rates have been detected for some drugs. Side-chain oxidation of propranolol was 50% faster in males than in females, but no differences between genders were noted in aromatic ring hydroxylation. N-demethylation of meperidine was depressed during pregnancy and for women taking oral contraceptives. CYP1A2 shows a higher activity in men, and therefore, clearance of antidepressant, antipsychotic drugs, and theophylline is faster in men than in women. CYP1A2 activity is decreased during pregnancy. Other examples of drugs cleared by CYP3A4 more rapidly in men than in women include chlordiazepoxide and lidocaine. Diazepam, prednisolone, caffeine, and acetaminophen are metabolized slightly faster by women than by men. No gender differences have been observed in the clearance of phenytoin, nitrazepam, and trazodone, which interestingly are not substrates for the CYP3A subfamily. CYP3A4 activity is higher in women than in men. Drugs such as cyclosporine, erythromycin, nimodipine, and cortisol are substrates of CYP3A4, showing faster clearance among women. Although CYP3A4 is also responsible for about 60% of CYP-mediated metabolism of zolpidem, its overall clearance is actually slower in women than in men, requiring a FDA-recommended 50% dose reduction. Gender differences in the rate of glucuronidation have been noted. Men show a faster clearance of drugs that are primarily eliminated by glucuronidation. Thus, oxazepam, metabolized mainly by UGT2B15, has a longer half-life in women than in men. Gender differences are also found in other pharmacokinetic parameters such as drug absorption, drug distribution, and excretion.

Historically, women were less enrolled in clinical trials because both the pharmacokinetics and pharmacodynamics of a drug can be influenced by menstrual cycle phases, hormonal fluctuations, use of

oral contraceptives, and hormonal therapy. There was also concern about life events such as pregnancy and lactation, including the potential negative impact of investigational drugs on the fetus or nursing neonate.¹⁰⁰ The number of trials enrolling women has increased after an FDA request to include a fair representation of both genders as participants, but overall, women are still underrepresented. Women are generally treated with doses that essentially reflect the results obtained by trials carried out mainly in men.

Despite the differences in drug pharmacokinetics between men and women, sex-specific recommendations in dosage do not exist for most drugs.¹⁰¹ Pharmacists need to recognize the underrepresentation of women in clinical trials and take the responsibility to inform consumers and emphasize to clinicians that women can differ significantly from men with respect to metabolism, absorption, distribution, and excretion of drugs. Pharmacists also need to be aware that pregnancy, oral contraceptive use, and hormonal replacement therapy can significantly change drug metabolism and drug clearance. If a woman consistently experiences more adverse drug events or less therapeutic effect from a particular drug, it may be necessary to discuss with her physician the possibility of changing the dosing regimen or switching to a different medication. Even in postmenopausal women, CYP3A function can be altered and influenced by the lack of estrogen or the presence of androgens.

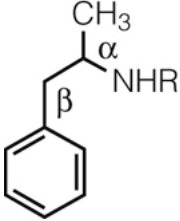
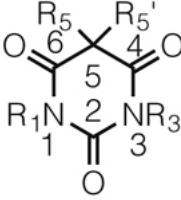
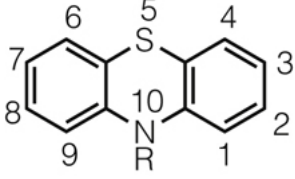
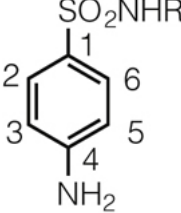
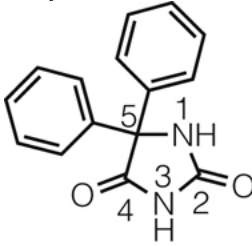
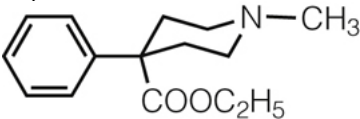
Compared to men, there are more women in the population, more women with chronic diseases, and more women visiting physicians. It is, therefore, obvious that there needs to be more interest in knowing how women react to drugs. Ultimately, a better understanding of sex-related influences on drug responses will help to improve drug safety and efficacy and will also permit to

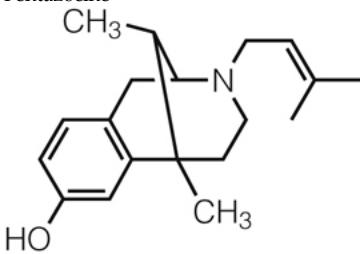
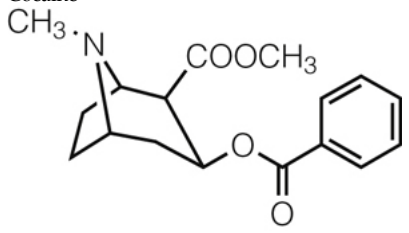
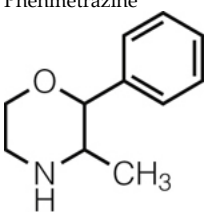
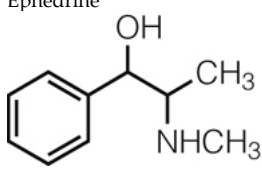
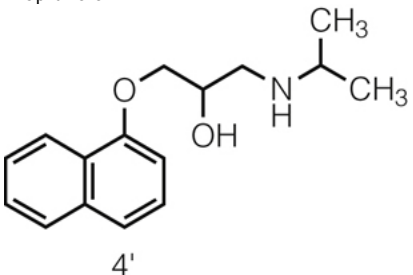
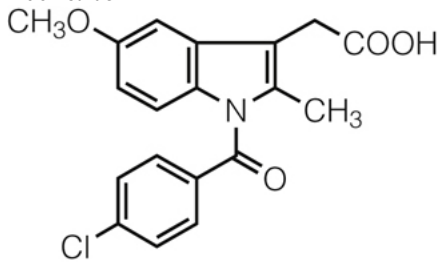
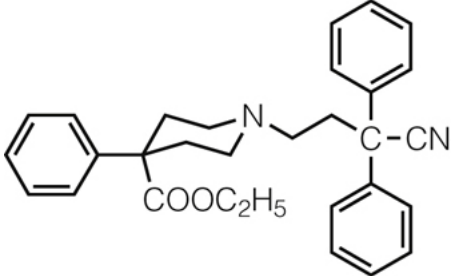
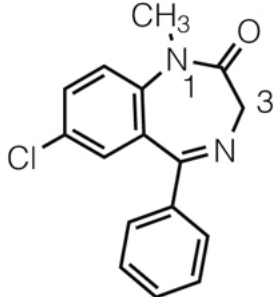
practitioners to “tailor” pharmacological treatments both in men and women.

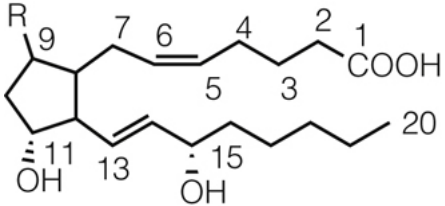
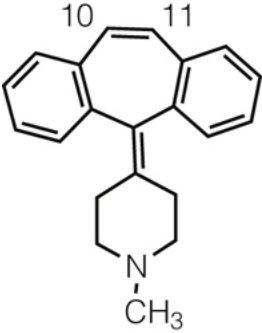
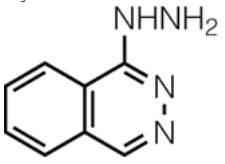
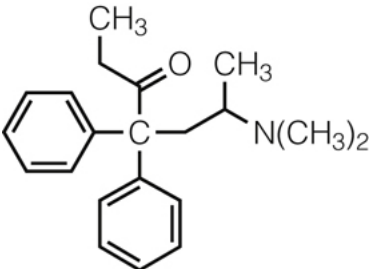
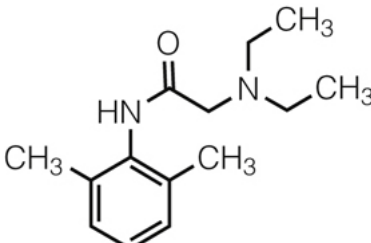
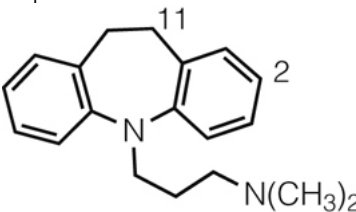
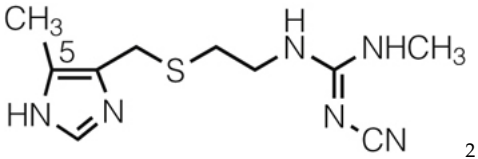
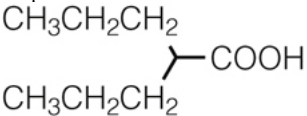
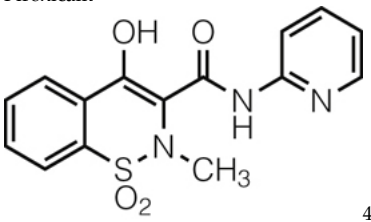
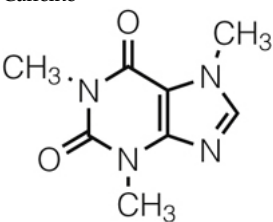
Major Pathways of Metabolism

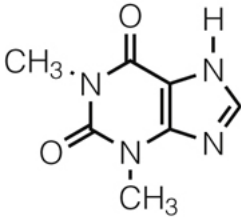
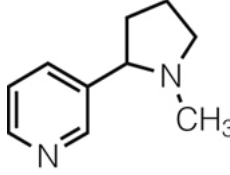
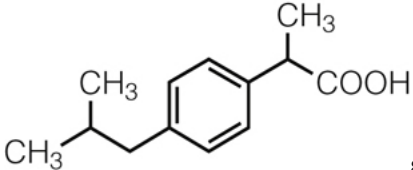
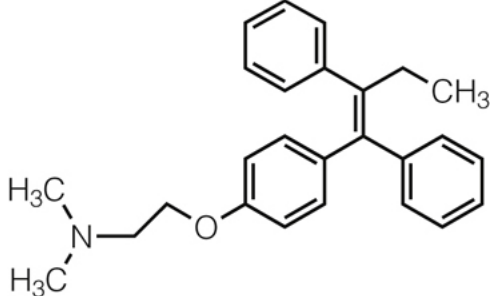
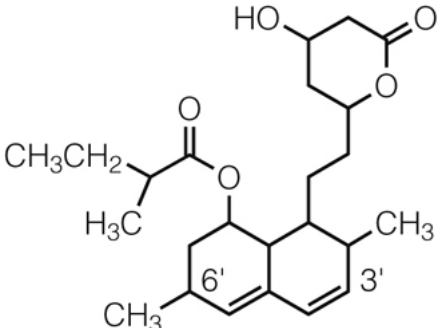
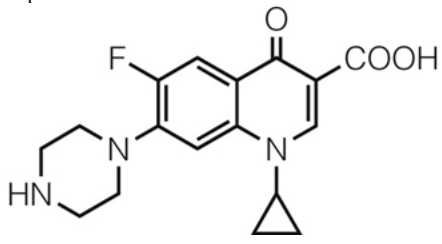
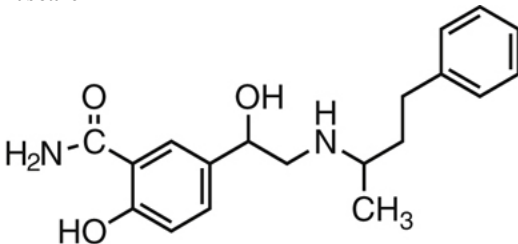
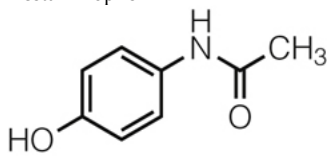
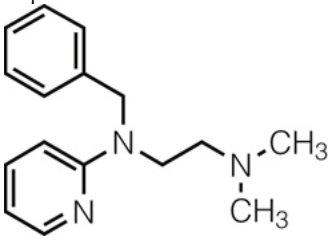
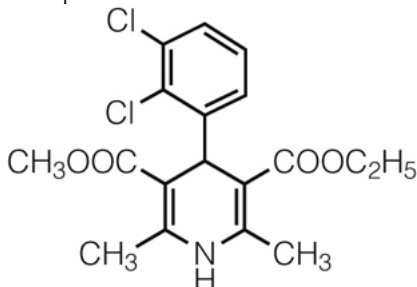
Table 3.21 contains an extensive list of commonly used drugs and the P450 isoforms that catalyze their metabolism. In addition, Phase 1 and Phase 2 metabolic pathways for some common drugs are listed in Table 3.21.

TABLE 3.21
Metabolic Pathways of Common Drugs

Drug	Pathway	Drug
Amphetamines 	Deamination (followed by oxidation and reduction of the ketone formed) N-oxidation N-dealkylation Hydroxylation of the aromatic ring Hydroxylation of the β-carbon atom Conjugation with glucuronic acid of the acid and alcohol products from the ketone formed by deamination	Barbiturates 
Phenothiazines 	N-dealkylation in the N10 side chain N-oxidation in the N10 side chain Oxidation of the heterocyclic S atom to sulfoxide or sulfone Hydroxylation of one or both aromatic rings Conjugation of phenolic metabolites with glucuronic acid or sulfate Scission of the N10 side chain	Sulfonamides 
Phenytoin 	Hydroxylation of one aromatic ring Conjugation of phenolic products with glucuronic acid or sulfate Hydrolytic scission of the hydantoin ring at the bond between carbons 3 and 4 to give 5,5- diphenylhydantoic acid	Meperidine 

Drug	Pathway	Drug
Pentazocine 	Hydroxylation of terminal methyl groups of the alkenyl side chain to give <i>cis</i> and <i>trans</i> (major) alcohols Oxidation of hydroxymethyl product of the alkenyl side chain to carboxylic acids Reduction of alkenyl side chain and oxidation of terminal methyl group	Cocaine 
Phenmetrazine 	Oxidation to lactam Aromatic hydroxylation N-oxidation Conjugation of phenolic products	Ephedrine 
Propranolol 	Aromatic hydroxylation at C4' N-dealkylation Oxidative deamination Oxidation of deaminated product to naphthoxylactic acid Conjugation with glucuronic acid O-dealkylation	Indomethacin 
Diphenoxylate 	Hydrolysis of ester to acid Hydroxylation of one aromatic ring attached to the N-alkyl side chain	Diazepam 

Drug	Pathway	Drug
Prostaglandins 	Reduction of double bonds at carbons 5 and 6 and 13 and 14 Oxidation of 15-hydroxyl to ketone β -Oxidation of carbons 3, 5 and 7 ω -Oxidation of carbon 20 to acid	Cyproheptadine 
Hydralazine 	N-acetylation with cyclization to a methyl-S-triazolophthalazine N-formylation with cyclization to an S-triazolophthalazine Aromatic hydroxylation of benzene ring Oxidative loss of hydrazinyl group to 1-hydroxy Hydroxylation of methyl of methyl-S-triazolophthalazine Conjugation with glucuronic acid	Methadone 
Lidocaine 	N-dealkylation Oxidative cyclization to a 4-imidazolidone N-oxidation of amide N Aromatic hydroxylation ortho to methyl Hydrolysis of amide	Imipramine 
Cimetidine 	S-oxidation Hydroxylation of 5-methyl	Valproic acid 
Piroxicam 	Pyridine 3'-hydroxylation Hydrolysis of amide Decarboxylation	Caffeine 

Drug Theophylline 	Pathway N3-demethylation N1-demethylation C8 oxidation to uric acids Imidazole ring opened 1-Me xanthine to 1-Me uric acid – xanthine oxidase	Drug Nicotine 
Ibuprofen 	CoA thioester and epimerization of R- to S+ enantiomer Methyl hydroxylation to CH₂OH CH₂OH to COOH Acylglucuronide	Tamoxifen 
Lovastatin 	6'-Hydroxylation 3'-Side chain hydroxylation 3'-Hydroxylation β-oxidation of lactone O-glucuronides	Ciprofloxacin 
Labetalol 	O-sulfate (major) O-glucuronide	Acetaminophen 
Tripelennamine 	p-Hydroxylation Benzylic C-hydroxylation N-depyridinylation N-debenzylation	Felodipine 

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Acetylation

Acetylation is principally a reaction of amino groups involving the transfer of acetyl CoA to primary aliphatic and aromatic amines, amino acids, hydrazines, or sulfonamide groups. The liver is the primary site of acetylation, although extrahepatic sites have been identified. Antimicrobial sulfonamides (e.g., sulfisoxazole), being difunctional, can form either N1 or N4 acetyl derivatives. Secondary amines are not acetylated. Acetylation can produce conjugates that retain the pharmacological activity of the parent drug (e.g., N-acetylprocainamide) ([Fig. 3.32](#)).

Acetylation, a nonmicrosomal form of metabolism, also exhibits polymorphisms and was first demonstrated in the acetylation of isoniazid. Several forms of N-acetyltransferase (NAT1 and NAT2) occur in humans. NAT1 (arylamine N-acetyltransferase) is widely distributed and catalyzes the N-acetylation of acidic arylamines, such as *p*-aminobenzoic acid and *p*-aminosalicylic acid; therefore its expression levels in the body have toxicological importance with regard to drug toxicity and cancer risk (e.g., bladder cancer and colorectal cancer).

NAT2 is found predominately in the liver. Clinically used drugs undergoing NAT2-catalyzed N-acetylation include isoniazid, procainamide, hydralazine, phenelzine, dapsone, caffeine, and the carcinogenic secondary N-alkylarylamines (2-aminofluorene, benzidine, and 4-aminobiphenyl). Substituents ortho to the amino group sterically block acetylation. Polymorphisms in the NAT2 gene in human populations can be segregated into fast, intermediate, and slow acetylator phenotypes. Polymorphisms in NAT2 are also associated with higher incidences of cancer and drug toxicity. Intestinal N-acetyltransferase appears not to be polymorphic (i.e., 5-aminosalicylic acid). The proportion of the fast acetylation phenotype is approximately 30%-45% in Caucasians, 89%-90% in the Oriental population, and 100% in Canadian Eskimos. Drug-induced systemic lupus erythematosus from chronic procainamide therapy is more likely to appear with slow acetylators.

Acetylation polymorphism has been associated with differences in human drug toxicity between slow and fast acetylator phenotypes. Slow

acetylators are more prone to drug-induced toxicities and accumulate higher blood concentrations of the unacetylated drug than do fast acetylators. Examples of toxicities that can result include hydralazine- and procainamide-induced lupus erythematosus, isoniazid-induced peripheral nerve damage, and sulfasalazine-induced hematologic disorders. Fast acetylators eliminate the drug more rapidly by conversion to its relatively nontoxic N-acetyl metabolite. However, for some drug substances, fast acetylators can pose a greater risk of liver toxicity than slow acetylators because fast acetylators produce toxic metabolites more rapidly. The possibility arises that genetic differences in acetylating capacity can confer differences in susceptibility to chemical carcinogenicity from arylamines. The tumorigenic activity of arylamines (1 in Fig. 3.33) can be the result of a complex series of sequential metabolic reactions commencing with N-acetylation (2 in Fig. 3.33), subsequent oxidation to arylhydroxamic acids (3 in Fig. 3.33), and metabolic transformation to acetoxarylamine by N,O-acyltransferase (4 in Fig. 3.33).⁵⁴ The acetoxarylamine can eliminate the acetox group to form the reactive arylnitrenium ion (5 in Fig. 3.33), which is capable of covalently binding to nucleic acids and proteins, thus increasing the risk for development of bladder and liver tumors.⁵⁴ The rapid acetylator phenotype is expected to form the acetoxarylamine metabolite at a greater rate than the slow acetylator and thereby, to present a greater risk for development of tumors compared with the slow acetylator.

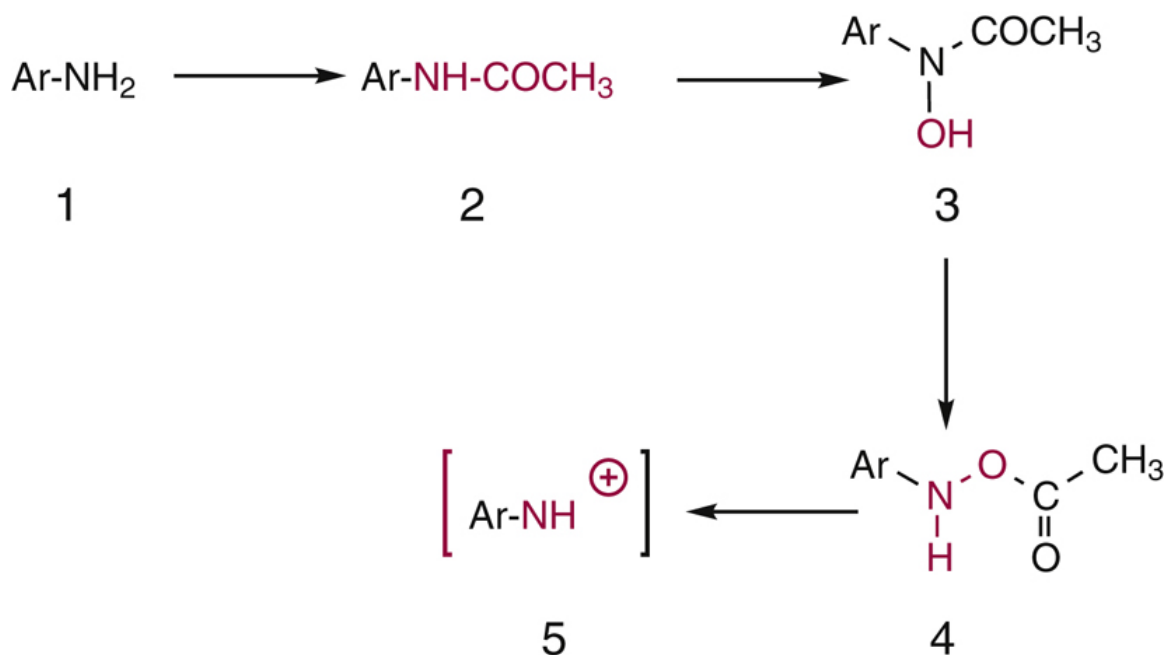


FIGURE 3.33 Bioactivation of acetylated arylamines.

Glutathione Conjugation and Mercapturic Acid Synthesis

Mercapturic acids are S-derivatives of N-acetylcysteine synthesized from glutathione (GSH).^{5,58,59} The mercapturic acid pathway appears to have evolved as a protective (scavenger) mechanism against xenobiotic-induced hepatotoxicity or carcinogenicity, serving to detoxify a large number of noxious substances that we inhale or ingest or that are produced daily in the human body. Most xenobiotics that are metabolized to mercapturic acids first undergo conjugation with GSH catalyzed by the enzyme glutathione S-transferase (GST), a multigene isoenzyme family that is abundant in the soluble supernatant liver fractions. In humans, the GST family consists of cytosolic dimeric isoenzymes of 45-55 kDa size that have been assigned to at least seven classes: alpha (five members), kappa (one member), mu (six members), omega (two members), pi (one member), theta (two members), zeta (one member), and microsomal (three members). The principal drug substrates for the mu family are the nitrosourea and mustard-type anticancer drugs. The theta isoform metabolizes small organic molecules, such as solvents, halocarbons, and electrophilic compounds (e.g., α,β -unsaturated carbonyl compounds). The

GSH conjugation reaction to mercapturic acid metabolites is depicted in Figure 3.34.

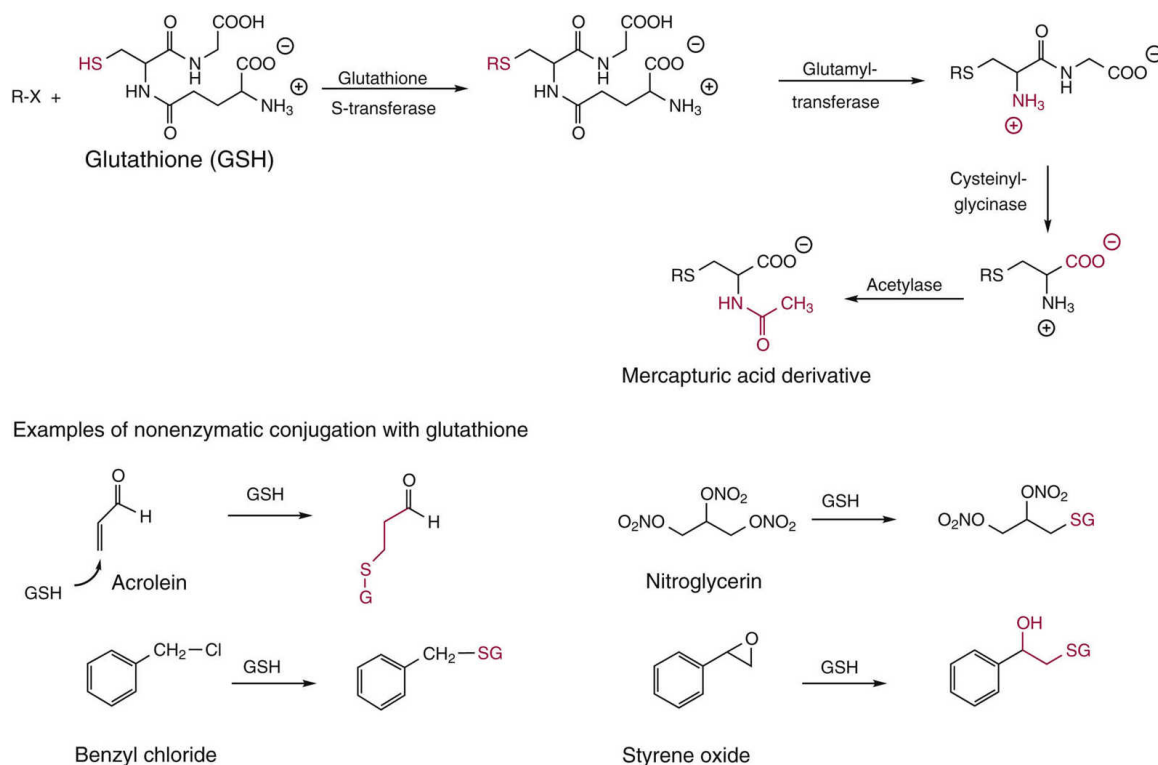


FIGURE 3.34 Glutathione and mercapturic acid conjugation pathways.

GST increases the ionization of the thiol group of GSH, increasing its nucleophilicity toward electrophiles (a group or ion that accepts an electron pair to make a covalent bond such as a carbocation or acyl ion) and thereby increasing the rate of conjugation with these potentially harmful electrophiles. In this way, GSH conjugation protects other vital nucleophilic centers in the cell, such as nucleic acids and proteins, from these electrophiles. Glutathione is also capable of reacting nonenzymatically with nucleophilic sites on neighboring macromolecules (Fig. 3.34) (see also section Metabolic Activation). Once conjugated with GSH, the electrophiles are excreted in the bile and urine.

A range of functional groups yields thioether conjugates of GSH, as well as products other than thioethers (Fig. 3.34). The nucleophilic attack by GSH occurs on electrophilic carbons with leaving groups (e.g., halogen [alkyl, alkenyl, aryl, or aralkyl halides], sulfonates [alkylmethanesulfonates] and

nitro [alkyl nitrates] groups), ring opening of small ring ethers (epoxides and β -lactones, e.g., β -propiolactone), and the Michael-type addition to the activated β -carbon of an α,β -unsaturated carbonyl compound (e.g., acrolein) (see section Reactive Metabolites Resulting from Bioactivation). The lack of substrate specificity gives argument to the fact that glutathione transferase has undergone adaptive changes to accommodate the variety of xenobiotics to which it is exposed. The conjugation of an electrophilic compound with GSH is usually a reaction of detoxication, but in some cases carcinogens have been activated through conjugation with GSH.^{58,59} The enzymatic conjugation of GSH with epoxides provides a mechanism for protecting the liver from injury caused by certain bioactivated intermediates (see section Reactive Metabolites Resulting from Bioactivation). Not all epoxides are substrates for this enzyme, but the more chemically reactive epoxides appear to be better substrates. Important among the epoxides that are substrates for this enzyme are those produced from halobenzenes and polycyclic aromatic hydrocarbons through the action of a P450 monooxygenase. Epoxide formation represents bioactivation because the epoxides are reactive and potentially toxic, whereas their GSH conjugates are inactive. Conjugation of GSH with the epoxides of aryl hydrocarbons eventually results in the formation of hydroxymercapturic acids (premercapturic acids) which undergo acid-catalyzed dehydration to the mercapturic acids. The halobenzenes are typically conjugated in the *p*-position.

Monohalogenated, *gem*-dihalogenated, and vicinal dihalogenated alkanes undergo glutathione transferase-catalyzed conjugation reactions to produce S-substituted glutathione derivatives that are metabolically transformed into the more stable and less toxic mercapturic acids. This common route of metabolism occurs through nucleophilic displacement of a halide ion by the thiolate anion of glutathione. The mutagenicity of the 1,2-dihaloethanes (e.g., the pesticide and fumigant ethylene dibromide) has been attributed to GSH displacing bromide with the formation of the S-(2-haloethyl) glutathione, which subsequently rearranges to a reactive episulfonium ion electrophile that, in turn, alkylates DNA. Many of the

halogenated hydrocarbons exhibiting nephrotoxicity undergo the formation of similar S-substituted cysteine derivatives.

A correlation exists between the hepatotoxicity of acetaminophen and levels of GSH in the liver. The probable mechanism of toxicity that has emerged from animal studies is that acetaminophen is oxidized by CYP1A2 and CYP2E1 to the N-acetyl-p-benzoquinonimine intermediate, which conjugates with and depletes hepatic GSH levels (Fig. 3.35). This action allows the benzoquinonimine to bind covalently to tissue macromolecules. The mercapturic acid derivative of acetaminophen represents approximately 2% of the administered dose of acetaminophen. Thus, the possibility exists that those toxic metabolites that are mostly detoxified by conjugating with GSH exhibit their hepatotoxicity (or, perhaps, carcinogenicity) because the liver has been depleted of GSH and is incapable of inactivating them. Pretreatment of animals with phenobarbital often hastens the depletion of GSH by increasing the formation of epoxides or other reactive intermediates.

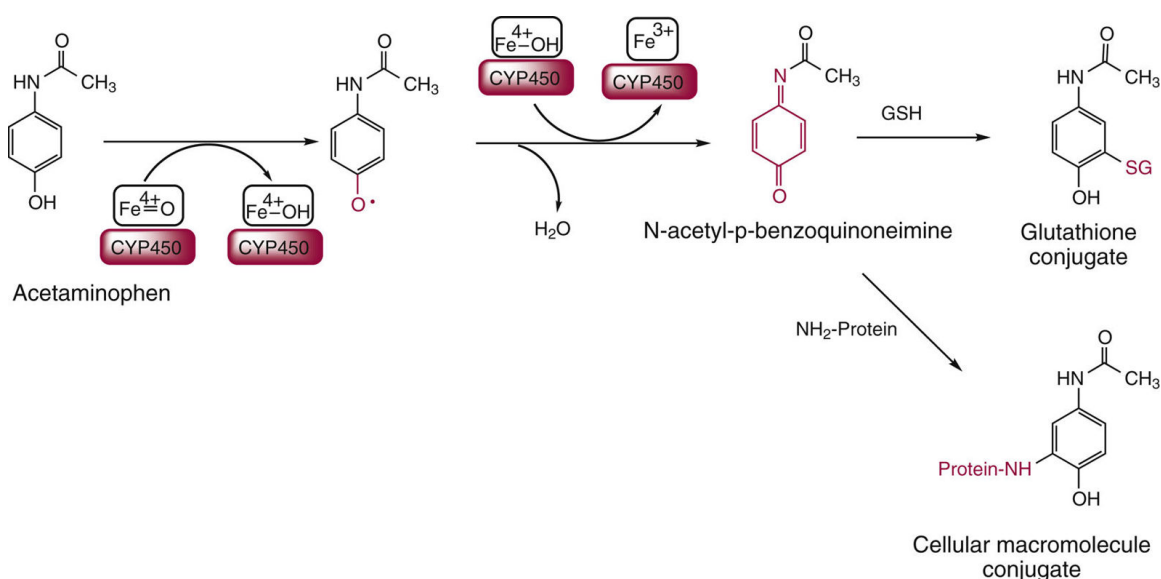


FIGURE 3.35 Proposed mechanism for the CYP450-catalyzed oxidation of acetaminophen to its N-acetyl-p-benzoquinoneimine intermediate, which can further react with either glutathione (GSH) or cellular macromolecules (NH₂-protein).

Methylation

Methylation is a common biochemical reaction but appears to be of greater significance in the metabolism of endogenous compounds than for drugs and other xenobiotics. Methylation differs from other conjugation processes in that the O-methyl metabolites formed can, in some cases, have as great or greater pharmacological activity and lipophilicity than the parent molecule (e.g., the conversion of norepinephrine to epinephrine). Methionine is involved in the methylation of endogenous and exogenous substrates, because it transfers its methyl group via the activated intermediate S-adenosylmethionine to the substrate under the influence of methyltransferases (Fig. 3.36). Methylation results principally in the formation of O-methylated, N-methylated, and S-methylated products.

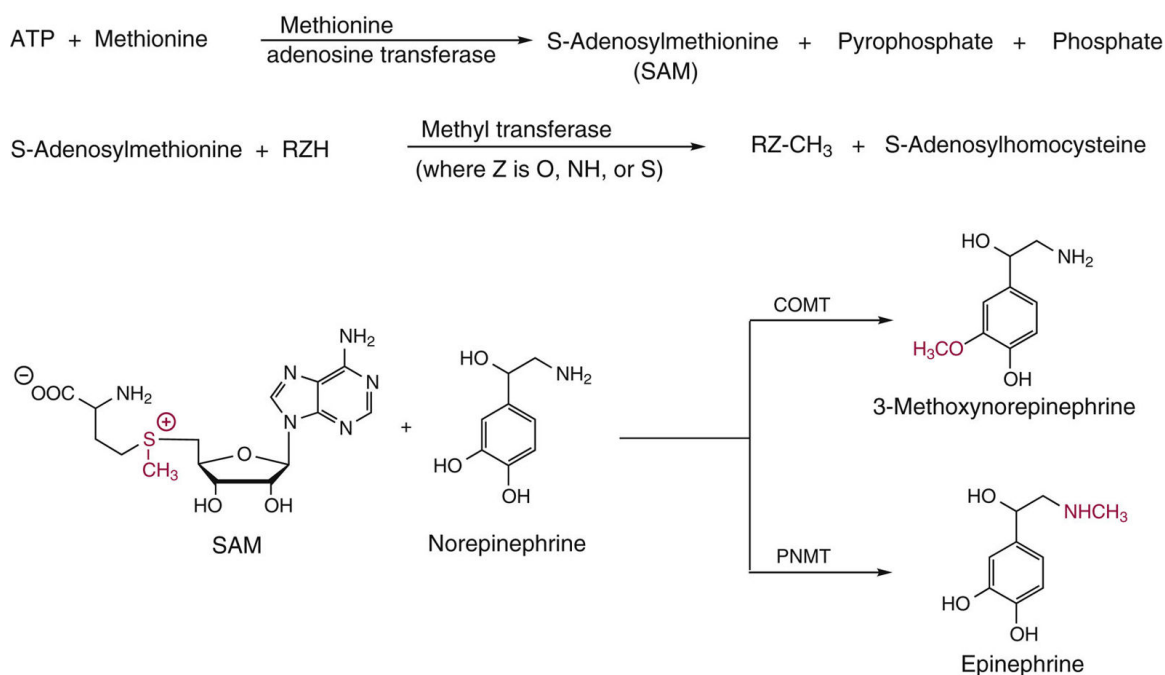


FIGURE 3.36 Methylation pathways.

O-Methylation

O-methylation is catalyzed by the magnesium-dependent enzyme catechol-O-methyltransferase (COMT), which transfers a methyl group primarily to the *m*- or 3-hydroxy group of the catechol moiety (3,4-dihydroxyphenyl moiety) of norepinephrine, epinephrine, or dopamine, resulting in inactivation. Less frequently, the *p*- or 4-hydroxy group of

these catecholamines is methylated (regioselectivity), along with their deaminated metabolites. COMT does not methylate monophenols or other dihydroxy non-catechol phenols. The *m:p* product ratio depends greatly on the type of substituent attached to the catechol ring. Substrates specific for COMT include the aforementioned catecholamines norepinephrine, epinephrine, and dopamine; the catechol amino acids L-DOPA and α -methyl-L-DOPA; and 2- and 4-hydroxyestradiol (catechol-like) metabolites of estradiol. It is found in liver, kidneys, nervous tissue, and other tissues. Hydroxyindole-O-methyltransferase, which O-methylates N-acetylserotonin, serotonin, and other hydroxyindoles, is found in the pineal gland and is involved in the formation of melatonin, a hormone associated with the dark-light diurnal cycle in humans. This enzyme differs from COMT in that it does not methylate catecholamines and has no requirement for magnesium ion.

N-Methylation

N-Methylation is among several conjugation pathways for metabolizing amines. Specific N-methyltransferases catalyze the transfer of active methyl groups from S-adenosylmethionine to the acceptor substance. Phenylethanolamine-N-methyltransferase methylates a number of endogenous and exogenous phenylethanolamines (e.g., normetanephrine, norepinephrine, and norephedrine) but does not methylate phenylethylamines. Histamine-N-methyltransferase methylates specifically histamine, producing the inactive metabolite N¹-methylhistamine. Amine-N-methyltransferase will N-methylate a variety of primary and secondary amines from a number of sources, including endogenous biogenic amines (serotonin, tryptamine, tyramine, and dopamine) and drugs (desmethylinipramine, amphetamine, and normorphine). Amine-N-methyltransferases seem to have a role in recycling N-demethylated drugs.

S-Methylation

As a rule, thiols are toxic, and the role of thiol S-methyltransferases is a nonoxidative detoxification pathway of these compounds. S-methylation

of sulfhydryl compounds also involves a microsomal enzyme requiring S-adenosylmethionine. Although a wide range of exogenous sulfhydryl compounds are S-methylated by this microsomal enzyme, none of the endogenous sulfhydryl compounds (e.g., cysteine and GSH) can function as substrates. S-methylation represents a detoxication step for thiols. Dialkyldithiocarbamates (e.g., disulfiram) and the antithyroid drugs (e.g., 6-propyl-2-thiouracil), mercaptans, and hydrogen sulfide (from thioglycosides as natural constituents of foods, mineral sulfides in water, fermented beverages, and bacterial digestion) are S-methylated. Other drugs undergoing S-methylation include captopril, thiopurine, azaprine, penicillamine, and 6-mercaptopurine.

Thiopurine methyltransferase or thiopurine S-methyl-transferase (TPMT) catalyzes the S-methylation of thiopurine drugs, such as azathioprine, 6-mercaptopurine, and 6-thioguanine. Interindividual variations in sensitivity and toxicity to thiopurine are correlated with TPMT genetic polymorphisms. Defects in the TPMT gene result in decreased methylation and decreased inactivation of the thiopurine drugs, leading to enhanced bone marrow toxicity. The clinical result may be myelosuppression, anemia, bleeding tendency, leukopenia, and infection. Approximately, 5% of all thiopurine therapies will fail due to toxicity related to TPMT polymorphism.

Elimination Pathways

Most xenobiotics are lipid-soluble and are altered chemically by the metabolizing enzymes, usually into less toxic and more water-soluble substances, before being excreted into the urine or, in some cases, bile. The formation of conjugates with sulfonate, amino acids, and glucuronic acid is particularly effective in increasing the hydrophilicity of drug molecules. The principal route of excretion of drugs and their metabolites is via the urine. If drugs and other compounds foreign to the body are not metabolized in this manner, substances with a high lipid-water partition coefficient could be reabsorbed readily from the urine through the renal tubular membranes and into the plasma. Therefore, such substances would continue to be recirculated and their pharmacological or toxic effects would be prolonged. Very polar or highly ionized drug molecules often are excreted in the urine unchanged. Urine is not the only route for excreting drugs and their metabolites from the body. Other routes include bile, saliva, lungs, sweat, and milk. The bile and biliary efflux transporters have been recognized as major routes of excretion for many endogenous and exogenous compounds.

Enterohepatic Cycling of Drugs

The liver is the principal organ for the metabolism and eventual elimination of xenobiotics from the human body in either the urine or the bile. When eliminated in the bile, steroid hormones, bile acids, drugs, and their respective conjugated metabolites are available for reabsorption from the duodenal-intestinal tract into the portal circulation, undergoing the process of enterohepatic cycling (EHC) (Fig. 3.37).⁶⁰ Nearly all drugs are excreted in the bile, but only a few are concentrated in the bile. For example, the bile salts are efficiently concentrated in the bile and reabsorbed from the GIT, such that the entire body pool of bile acids/salts are recycled multiple times per day. Therefore, EHC is responsible for the conservation of bile acids, steroid hormones, thyroid hormones, and other endogenous substances. In humans, compounds excreted into the bile typically have a molecular weight greater than 500 Da. In humans,

metabolites with a molecular weight between 300 and 500 Da are excreted in both urine and bile. Compounds excreted into bile are, as a rule, hydrophilic substances that can be either charged (anionic) or uncharged (e.g., cardiac glycosides and steroid hormones). Biotransformation of these compounds by means of Phase 1 and Phase 2 reactions would produce a conjugated metabolite, which is anionic and hydrophilic with a molecular weight greater than that of the parent compound. The conjugated metabolites are most often glucuronides, because glucuronidation adds 176 Da to the molecular weight of the parent compound. Unchanged drug in the bile is either excreted with the feces, metabolized by the bacterial flora in the intestinal tract, or reabsorbed into the portal circulation via EHC.

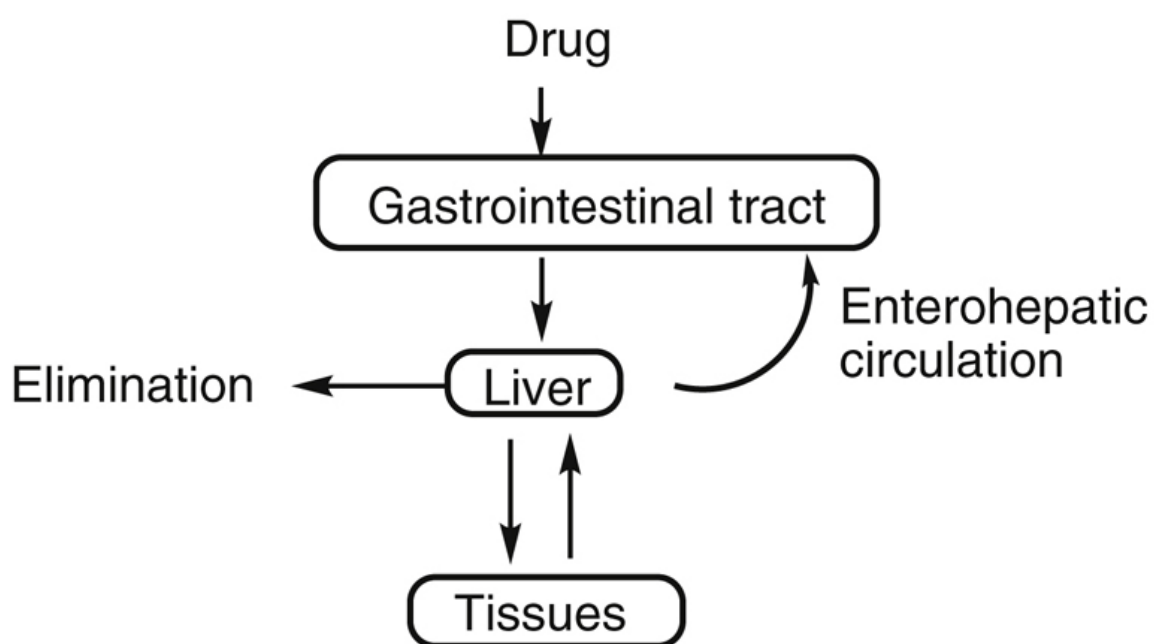


FIGURE 3.37 Enterohepatic cycling.

The bacterial intestinal flora is directly involved in EHC and the recycling of drugs through the portal circulation.⁶¹ A conjugated drug and metabolites excreted via the bile can be hydrolyzed by enzymes of the bacterial flora, releasing the parent drug or its Phase 1 metabolite for reabsorption into the portal circulation. Among the numerous compounds metabolized in the enterohepatic circulation are the estrogenic and

progestational steroids, digitoxin, indomethacin, diazepam, pentaerythritol tetranitrate, mercurials, arsenicals, and morphine. The oral ingestion of xenobiotics inhibiting the gut flora (i.e., nonabsorbable antibiotics) can affect the pharmacokinetics of drugs.

The impact of EHC on the pharmacokinetics and pharmacodynamics of a drug depends on the importance of biliary excretion relative to renal clearance and on the efficiency of gastrointestinal absorption. The EHC becomes dominant when biliary excretion is the major clearance mechanism for the drug. Because the majority of the bile is stored in the gallbladder and released on the ingestion of food, intermittent spikes in the plasma drug concentration is observed following reentry of the drug from the bile via EHC. From a pharmacodynamic point of view, the net effect of EHC is to increase the duration of a drug in the body and to prolong its pharmacological action.

Drug Metabolism and Age

Metabolism in the Elderly

The widespread use of medications in the elderly increases the potential for an increased incidence of drug-related interactions, which can be related to changes in drug metabolism and clearance from the body (Table 3.14).^{62,63} The interpretation of the age-related alteration in drug response must consider the contributions of absorption, distribution, metabolism, and excretion. Drug therapy in the elderly is expected to become one of the more significant problems for clinical medicine. It has been well documented that the metabolism of many drugs and their elimination is impaired in the elderly.

TABLE 3.14
Effect of Age on the Clearance of Some Drugs

No Change	Decrease
Acetaminophen ^a	Alprazolam
Aspirin	Amitriptyline
Diclofenac	Chlordiazepoxide
Diphenhydramine	Chlormethiazole
Ethanol	Diazepam
Flunitrazepam	Labetalol
Midazolam	Lidocaine
Nitrazepam	Lorazepam
Oxazepam	Morphine
Phenytoin ^a	Meperidine
Prazosin	Nifedipine and other dihydropyridines
Propylthiouracil	Norepinephrine
Temazepam	Nortriptyline
Thiopental ^a	Phenytoin
Tolbutamide ^a	Piroxicam
Warfarin	Propranolol
	Quinidine

No Change	Decrease
	Quinine
	Theophylline
	Verapamil

^aDrugs for which clearance is disputable but can be increased

The decline in drug metabolism due to advancing age is associated with physiological changes that have pharmacokinetic implications affecting the steady-state plasma concentrations and renal clearance for the parent drug and its metabolites. Those changes relevant to the bioavailability of drugs in the elderly are decreases in hepatic blood flow, glomerular filtration rate, hepatic P450 activity, plasma protein binding, and body mass. Because the rate of a drug's elimination from the blood through hepatic metabolism is determined by hepatic blood flow, protein binding, and intrinsic clearance, a reduction in hepatic blood flow can lead to an increase in drug bioavailability and decreased clearance, with the symptoms of drug overdose and toxicity as the outcome (e.g., warfarin in patients with congestive heart failure^{63,64}). Those orally administered drugs exhibiting a reduction in first-pass metabolism in the elderly include antihypertensives, diuretics, and antidepressants.

Age-related changes in drug metabolism are a complicated interplay between the age-related physiological changes, genetics, environmental influences (diet and nutritional status, smoking, and enzyme induction), concomitant diseases states, and drug intake. In most studies, the elderly appear to be just as responsive to drug-metabolizing enzyme activity (Phase 1 and Phase 2) as young individuals. Given the number of factors that determine the rate of drug metabolism, it is not surprising that the effects of aging on drug elimination by metabolism have yielded variable results, even for the same drug. The fact that drug elimination can be altered in old age suggests that doses of metabolized drugs should be initially reduced in older patients and then adjusted according to the clinical

response. A decrease in hepatic drug metabolism coupled with age-related alterations in clearance, volume of distribution, and receptor sensitivity can lead to prolonged plasma half-life and increased drug toxicity.

Those P450 substrates reported to cause drug-drug interactions are shown in [Tables 3.3-3.10](#) in bold italics.

Drug Metabolism in the Fetus and During Pregnancy

Over two-thirds of women receive prescription drugs while pregnant, with treatment and dosing strategies based on data from healthy male volunteers and nonpregnant women, and with little adjustment for the complex physiology of pregnancy and its unique disease states. Pregnancy influences hepatic metabolism of drugs in a CYP-dependent manner. Hormone levels in the plasma which rise during pregnancy are capable of regulating expression of hepatic drug metabolism enzymes. These hormones include estrogens, progesterone, cortisol, and prolactin. Studies are needed to examine the role of pregnancy hormones in altered drug metabolism during pregnancy.

The activities of CYP3A4, CYP2A6, CYP2D6, and CYP2C9 are all increased during pregnancy. Changes in CYP3A4 activity lead to increased metabolism of drugs ([Table 3.10](#)). By contrast, CYP1A2 and CYP2C19 appear to undergo a gradual decrease in activity with advancing gestation,^{63,64} though with uncertain effects on drug therapy. The activity of Phase 2 enzymes, including uridine 5'-diphosphate glucuronosyltransferases (UGTs), is also altered during pregnancy, with a 200% increase in UGT1A4 activity during the first and second trimesters and a 300% increase during the third trimester.^{63,64} This change leads to lower concentrations of UGT1A4 substrates such as lamotrigine, leading directly to poorer seizure

control with advancing gestation in the absence of appropriate dose titration.

Knowledge of drug transporter function during pregnancy is necessary for a complete understanding of drug absorption, distribution, elimination, and effect.⁶³ In addition, fetal development is dependent upon the transport of nutrients by the placenta toward the fetal side, and that of products of fetal metabolism transported away from the fetus for elimination by the mother.⁶⁵ The placenta produces and secretes hormones that affect the maternal physiology and endocrine state. Compounds transported between the mother and the fetus are carried by the maternal circulation within the uterine vasculature.

The ability of the human fetus and placenta to metabolize xenobiotics is well established. Drug-metabolizing enzymes expressed in the placenta include CYP1A1, 2E1, 3A4/5, 3A7, 4B1, epoxide hydrolase, alcohol dehydrogenase, glutathione transferase, UGTs, sulfotransferases, and N-acetyltransferase. The majority of the drugs used in pregnancy readily cross the placenta, thus exposing the fetus to a large number of xenobiotic agents. The human fetus is at special risk from these substances due to the presence of the P450 system which is capable of metabolizing xenobiotics during the first part of gestation. Placentas of tobacco smokers have shown a significant increase in the rate of placental CYP1A activity. Concern for this CYP1A activity is increasing because this enzyme system is known to catalyze the formation of reactive metabolites capable of covalently binding to macromolecules, producing permanent effects (e.g., teratogenicity, hepatotoxicity, or carcinogenicity) in the fetus and newborn. A more disturbing fact is that the conjugation enzymes (i.e., glucuronosyltransferases, glutathione transferase, and sulfotransferase), which are important for the formation of Phase 2 conjugates of these reactive metabolites, are found in low to

negligible levels in the fetus and newborn, increasing the exposure of these infants to these potentially toxic metabolites.

The placenta is not a barrier protecting the fetus from xenobiotics; almost every drug present in the maternal circulation will cross the placenta and reach the fetus. For some drugs, however, the placental efflux transport protein P-gp functions as a maternofetal barrier, pumping drugs and Pgp substrates out of the fetal circulation back into the maternal circulation⁶⁵ and protecting the fetus from exposure to potentially harmful teratogenic xenobiotics/drugs and endogenous substances that have been absorbed through the placenta. The P-gp inhibitors should be carefully evaluated for their potential to increase fetal susceptibility to drug/chemical-induced teratogenesis. On the other hand, selective inhibition of P-gp could be used clinically to improve pharmacotherapy of the unborn child. Because metabolites are usually more water soluble than the parent substance, drug metabolites formed in the fetus can be trapped and accumulate on the fetal side of the placenta. Such accumulation can result in drug-induced toxicities or developmental defects. The activity of CYP3A isoenzymes in the human fetal liver is similar to that seen in adult liver microsomes. The fetal activity for CYP3A7 is unusual, as most other fetal isoenzymes of P450 exhibit activity 5%-40% of the adult isoenzymes. Fetal and neonatal drug-metabolizing enzyme activities can differ from those in the adult.

Most xenobiotics cross the placental barrier by simple diffusion. Protein binding, degree of ionization, lipid solubility, and molecular weight can affect placental transport. In fact, small, lipid-soluble, ionized, and poorly protein-bound molecules cross the placenta easily. For other substrates, the placenta facilitates maternal to fetal transport through the polarized expression of various transporters.⁶⁵ Transporters enable transport of specific endogenous substrates (such as cytokines, nucleoside analogues, and steroid hormones);

however, exogenous compounds with similar structures may also interact with them.

From the day of birth, the neonate is exposed to drugs and other foreign compounds persisting from pregnancy, as well as those transferred via breast milk. Fortunately, many of the drug-metabolizing enzymes operative in the neonate developed during the fetal period. The routine use of therapeutic agents during labor and delivery, as well as during pregnancy, is widespread, and consideration must be given to the fact that potentially harmful metabolites can be generated by the fetus and newborn. Consequently, the use of drugs capable of forming reactive metabolic intermediates should be avoided during pregnancy, delivery, and the neonatal period. The activity of Phase 1 and Phase 2 drug-metabolizing enzymes is high at birth but decreases to normal levels with increasing age. Evidence suggests increased activity of drug-metabolizing enzymes in liver microsomes of neonates resulting from treatment of the mother during the pregnancy with enzyme inducers (e.g., phenobarbital).

Genetic Polymorphism

The reality of drug therapy is that many drugs do not work in all patients.⁶⁶ By current estimates, the percentage of patients who will react favorably to a specific drug ranges from 20% to 80%. Drugs have been developed and dosage regimens prescribed under the old paradigm that “one dose fits all,” which largely ignores the fact that humans are genetically different, resulting in interindividual differences in drug metabolism and disposition.⁶⁶ It is widely accepted that genetic factors have an important impact on the oxidative metabolism and pharmacokinetics of drugs. Genotype-phenotype correlation studies (pharmacogenetics) have shown that inherited mutations in P450 genes (alleles) result in distinct phenotypic subgroups. For example, mutations in the *CYP2D6* gene result in poor (PM), intermediate (IM), extensive (EM), and ultrarapid (UM) metabolizers of *CYP2D6* substrates⁶⁷ (Table 3.8). Each of these phenotypic subgroups experiences different responses to drugs extensively metabolized by the *CYP2D6* pathway, ranging from severe toxicity to complete lack of efficacy. Genetic studies confirm that “one dose does not fit all,” leaving the question of why we would continue to develop and prescribe drugs under the old paradigm. The regulatory agencies have recognized that identifying genetic polymorphisms might allow the safe dosing, marketing, and approval of drugs that would otherwise not be approved and advised pharmaceutical companies to incorporate the knowledge of genetic polymorphisms into drug development. Importantly, pharmacogenomic testing (the study of heritable traits affecting patient response to drug treatment) can significantly increase the likelihood of developing drug regimens that benefit most patients without severe adverse events. Pharmacists play a vital role in the

development of patient-specific dosing regimens based on metabolizing phenotype.

Polymorphisms are expressed for a number of metabolizing enzymes, but the polymorphic P450 isoforms that are most important for drug metabolism include CYP2A6, CYP2A13, CYP2B6, CYP2C9, CYP2C19, and CYP2D6.⁶⁸⁻⁷¹ [1]
[5EP] These isoforms have been studied extensively and, despite their low abundance in the liver, they have been found to catalyze the metabolism of many drugs. Most gene variants are expressed into inactive, truncated proteins or they fail to express any protein. There is no clear information about CYP1A1 and CYP3A4/5 polymorphism. These polymorphic isoforms give rise to phenotypic subgroups in the population differing in their ability to perform clinically significant biotransformation reactions with obvious clinical ramifications.⁶⁶ Metabolic polymorphism can have several consequences; for example, when enzymes that metabolize drugs used either therapeutically or socially are deficient, adverse or toxic drug reactions can occur in these individuals.

The discovery of genetic polymorphism resulted from the observation of increased frequency of adverse effects or no drug effects after normal doses of drugs to some patients (e.g., hyper-CNS response from the administration of the antihistamine doxylamine or no analgesic response with codeine). Polymorphism is a difference in DNA sequence found at 1% or greater in a population and expressed as an amino acid substitution in the protein sequence of an enzyme resulting in changes in its rate of activity ($V_{\max}$) or affinity (K_m). Thus, mutant DNA sequences can lead to interindividual differences in drug metabolism. Furthermore, the polymorphisms do not occur with equivalent frequency in all racial or ethnic groups. Because of these differences, it is important to be aware of a person's race and ethnicity when giving drugs that are metabolized differently by different populations, although practitioners must guard against

stereotyping.⁶⁶ Because no other way may exist to adequately clear these drugs from the body, PMs can be at greater risk for adverse drug reactions or toxic overdoses. The signs and symptoms of these overdoses are primarily extensions of the drug's common adverse effects or pharmacological effects (Table 3.15).⁶⁶ The level of adverse reactions or overdosage depends very much on the overall contribution of the mutant isoform to the drug's metabolism. Perhaps the most interesting explanation for the various mutant isoforms is that they evolved as protective mechanisms against alkaloids and other common substances in the food chain for the different ethnicities. Although much effort has gone into finding polymorphisms of *CYP3A4* and *CYP1A2* genes, none has yet to be discovered.

TABLE 3.15
Impact of Human P450 Polymorphisms on Drug Treatment in Poor Metabolizers

Polymorphic Enzyme	Decreased Clearance	Adverse Effects (Overdosage)	Reduced Activation of Coadministered Prodrug
CYP2C9	S-warfarin	Bleeding	Losartan
	Phenytoin	Ataxia	
	Losartan		
	Tolbutamide	Hypoglycemia	
	NSAIDs	GI bleeding	
CYP2C19	Omeprazole		Proguanil
	Diazepam	Sedation	
CYP2D6	Tricyclic antidepressants	Cardiotoxicity	Tramadol
	SSRIs	Serotonin syndrome	Codeine
	Anti-arrhythmic drugs	Arrhythmias	Ethylmorphine
	Perhexiline	Neuropathy	
	Haloperidol	Parkinsonism	
	Perphenazine		
	Zuclopenthixol		
	S-Mianserin		

Polymorphic Enzyme	Decreased Clearance	Adverse Effects (Overdosage)	Reduced Activation of Coadministered Prodrug
	Tolterodine		
CYP2A6	Nicotine		

Occasionally, one derives benefit from an unusual P450 phenotype. For example, cure rates for peptic ulcer treated with omeprazole are substantially greater in individuals with defective CYP2C19 due to the sustained high plasma levels achieved.

CYP2A6

CYP2A6 is of particular importance because it activates a number of procarcinogens to carcinogens and is the major isoform metabolizing the anticoagulant warfarin and nicotine.⁶⁹ Approximately 15% of Asians express the *CYP2A6*4del* allele and 2% of Caucasians express the other *CYP2A6*2* allele. Both these alleles express either no enzyme or a nonfunctional enzyme, and individuals carrying these alleles are referred to as having the PM phenotype. A benefit from being a PM of CYP2A6 substrates might be the protection against some carcinogens, including cigarette smoke due to the high plasma levels of nicotine achieved with fewer cigarettes.

CYP2B6

CYP2B6 is of special interest due to its wide interindividual variability in expression and activity.⁷⁰ Such a large variability for an enzyme that is not highly expressed is probably because of genetic polymorphisms and exposure to drugs that are inducers or inhibitors of CYP2B6. Variant alleles resulting from splicing defects or gene deletions have been identified in PM individuals with altered metabolic activity or impaired enzyme function. At least 28 allelic variants and some subvariants of *CYP2B6* have been shown to have an effect on drug clearance and drug response. For example, HIV-infected African-American individuals with a defective variant

of *CYP2B6* are PMs of efavirenz and have plasma levels approximately 3-fold higher than individuals with the normal *CYP2B6* gene. A different variant of *CYP2B6* is associated with approximately 2-fold greater plasma levels of nevirapine in HIV-infected patients. A benefit from being a PM of *CYP2B6* antiviral drugs might be enhanced HIV protection because of the higher plasma levels of the antiviral drugs achieved with lower dosages. Smokers with another variant of *CYP2B6* may be more vulnerable to abstinence symptoms and relapse following treatment with bupropion (a *CYP2B6* substrate) as a smoking cessation agent.

CYP2C9 and CYP2C19

CYP2C9 and *CYP2C19* metabolize approximately 15% of clinically used drugs, such as phenytoin, *S*-warfarin, tolbutamide, losartan, and many NSAIDs.⁷¹ Although *CYP2C19* metabolizes fewer drugs than *CYP2D6*, the drugs that *CYP2C19* does metabolize are clinically important (Table 3.7). The deficit of *CYP2C19* found in the PM phenotype is only seen in 8%-13% of Caucasians, 20%-30% of the Asian population (11%-23% of Japanese and 5%-37% of Chinese), up to 20% of the black African-American population, 14%-15% of Saudi Arabians and Ethiopians, and up to 70% of Pacific Islanders.⁷¹ The more common mutant allele in these individuals is *CYP2C19**2, which expresses an inactive enzyme. The large interindividual variability observed in the therapeutic response to the antiseizure drug mephenytoin is attributed to *CYP2C19* polymorphism, which catalyzes the *p*-hydroxylation of the *S*-stereoisomer.⁷¹ The *R*-enantiomer is *N*-demethylated by *CYP2C8* with no difference in its metabolism between PMs and EMs.

CYP2C9 is highly polymorphic with at least 33 variants identified. *CYP2C9**2 is frequent among Caucasians with ~1% of the population being homozygous carriers and 22% heterozygous. The

corresponding homozygous/heterozygous figures for the *CYP2C9**3 allele are 0.4% and 15%, respectively. Clinical studies have addressed the importance of the *CYP2C9**2 and *3 alleles as a determining factor for drug clearance and drug response. P450 2C9 polymorphisms are relevant for the efficacy and adverse effects of numerous NSAIDs, sulfonylurea antidiabetic drugs, and, most critically, oral anticoagulants belonging to the class of vitamin K epoxide reductase inhibitors (e.g., warfarin).⁷¹ A deficiency of this isoform is seen in 8%-13% of Caucasians, 2%-3% of African-Americans, and 1% of Asians. Individuals with the PM phenotype who possess this deficient isoform variant are ineffective in clearing S-warfarin (so much so that they can be fully anticoagulated on just 0.5 mg of warfarin per day) and in the clearance of phenytoin, which has a potentially very toxic narrow therapeutic range. On the other hand, the prodrug losartan will be poorly activated and ineffective.

CYP2D6

CYP2D6 is of specific importance because it metabolizes a wide range of commonly prescribed drugs, including antidepressants, antipsychotics, β -adrenergic blockers, and antiarrhythmics (Table 3.8).^{67,68} CYP2D6 deficiency is a clinically important genetic variation of drug metabolism characterized by three phenotypes: UM, EM, and PM. About 10% of Caucasians have gene variants that result in a functionally deficient CYP2D6 enzyme, and these PMs often respond inadequately to drugs, such as codeine, that are activated by CYP2D6. The PM phenotype is inherited as an autosomal recessive trait, with 5 of 30 of the known *CYP2D6* gene mutations leading to either zero expression or the expression of a nonfunctional enzyme.⁶⁸ Approximately 12%-20% of Caucasians express the *CYP2D6**4 allele and 5% express the other *CYP2D6* alleles. Up to 34% of African-Americans express the *CYP2D6**17 allele and 5% express the other

CYP2D6 alleles. Up to 50% of Chinese express the *CYP2D6**10 allele and 5% express the other *CYP2D6* alleles (these individuals are all referred to as PM).^{67,68} Conversely, the 20%-30% of Saudi Arabians and Ethiopians who express the *CYP2D6**2XN allele are known as UMs of *CYP2D6* substrates, because they express excess enzyme because of having multicopies of the gene.⁷² Inasmuch as *CYP2D6* is not inducible, many individuals of Ethiopian and Saudi Arabian descent have developed a genetically different strategy to cope with the (presumed) high load of alkaloids and other substances in their diet, thus the high expression of *CYP2D6* using multiple copies of the gene.

Those individuals who are deficient in *CYP2D6* will be predisposed to adverse effects or drug toxicity from antidepressants or neuroleptics caused by inadequate metabolism, leading to long half-lives, but the metabolism of *CYP2D6*-dependent prodrugs in these patients will be ineffective due to lack of activation (e.g., codeine, which must be metabolized by O-demethylation to morphine). In contrast, individuals with the UM phenotype will require a dose of drugs inactivated by *CYP2D6* that is much higher than normal to attain therapeutic drug plasma concentrations (e.g., one patient required a daily dose of approximately 300 mg of nortriptyline to achieve therapeutic plasma levels; the usual dose is 25 mg) or a lower dose for prodrugs that require metabolic activation. Individuals with the PM phenotype are also characterized by loss of *CYP2D6* stereoselectivity in hydroxylation reactions. It can be anticipated that large differences in steady-state concentration for *CYP2D6* substrates will occur between individuals with the different phenotypes when they receive the same dose. Depending on the drug and reaction type, a 10- to 30-fold difference in blood concentrations can be observed in the PM-phenotype polymorphism.⁶⁸

Other Polymorphic Metabolizing Enzymes

CYP2E1 polymorphism is expressed more frequently in individuals of Chinese ancestry than in Caucasians. Those with the CYP2E1 PM phenotype exhibit tolerance to alcohol and less toxicity from halohydrocarbon solvents.

Polymorphism has been associated with serum cholinesterases, alcohol dehydrogenases, aldehyde dehydrogenases, epoxide hydrolase and xanthine oxidase. Approximately 50% of the Oriental population lack aldehyde dehydrogenase, resulting in high levels of acetaldehyde following ethanol ingestion and causing nausea and flushing. People with genetic variants of cholinesterase respond abnormally to succinylcholine, procaine, and other related choline esters. The clinical consequence of reduced enzymatic activity of cholinesterase is that succinylcholine and procaine are not hydrolyzed in the blood, resulting in prolongation of their respective pharmacological activities (e.g., skeletal muscle paralysis in the case of succinylcholine).

Increasing knowledge of genetic polymorphism has contributed a great deal to our understanding about interindividual variation in the metabolism of drugs, including how to change dose regimens accordingly to minimize drug toxicity and improve therapeutic efficacy.

Presystemic First-Pass Metabolism

Although hepatic metabolism continues to be the most important route for xenobiotics, the ability of the liver and intestine to metabolize substances to either pharmacologically inactive or bioactive metabolites before reaching the systemic circulation is called presystemic first-pass metabolism. This metabolic process results in low systemic availability for orally susceptible drugs. Sulfonation and glucuronidation are major pathways of presystemic intestinal first-pass metabolism in humans. For example, oral acetaminophen, phenylephrine, terbutaline, albuterol, fenoterol, or isoproterenol exhibits approximately 5% bioavailability because of sulfonation and glucuronidation.

The discovery that CYP3A4 is found in the mucosal enterocytes of the intestinal villi signifies its role as a key determinant in the oral bioavailability of its numerous drug substrates ([Table 3.10](#)). Drugs known to be substrates for CYP3A often have a low and/or variable oral bioavailability that can be explained by presystemic first-pass metabolism by the small intestine P450 isoforms.⁶⁸ The concentration of functional intestinal CYP3A is influenced by genetic disposition, induction, and inhibition, which to a great extent determines drug blood levels and therapeutic response. Xenobiotics when ingested orally can modify the activity of intestinal CYP3A enzymes by induction and inhibition. By modulation of the isoform pattern in the intestine, a xenobiotic could alter its own metabolism and that of others in a time- and dose-dependent manner.

The concentration of CYP3A in the intestine is comparable to that of the liver. The oral administration of dexamethasone induces the formation of CYP3A and erythromycin inhibits it. The glucocorticoid inducibility of CYP3A4 can also be a factor in differences of metabolism between males and females. Studies have suggested that

intestinal CYP3A4 C2 hydroxylation of estradiol contributed to the oxidative metabolism of endogenous estrogens circulating with the enterohepatic recycling pool.⁷² Norethisterone has a low oral bioavailability of 42% due to oxidative CYP3A first-pass metabolism, but levonorgestrel is completely available in women, having no conjugated metabolites.

Several clinically relevant drug-drug interactions between orally coadministered drugs and CYP3A4 can be explained by a modification of drug metabolism at the P450 level. If a drug has high presystemic elimination (low bioavailability) and is metabolized primarily by CYP3A4, then coadministration with a CYP3A4 inhibitor can be expected to alter the drug's pharmacokinetics by reducing its metabolism, thus increasing its plasma concentration. Drugs and some foods (e.g., grapefruit juice) that are known inhibitors, inducers, or substrates for intestinal CYP3A4 can potentially interact with the metabolism of a coadministered drug, affecting its area under the curve and rate of clearance (Tables 3.10-3.12). Inducers can reduce absorption and oral bioavailability, whereas these same factors are increased by inhibitors. For example, erythromycin can enhance the oral absorption of another drug by inhibiting its metabolism in the small intestine by CYP3A4. Because they are competitive substrates for CYP3A4, prednisone, prednisolone, and methylprednisolone (but not dexamethasone) are competitive inhibitors of synthetic glucocorticoid metabolism. This is because a major metabolic pathway for synthetic glucocorticoids involves CYP3A4. In addition to coadministered drugs, metabolic interactions with exogenous CYP3A4 substrates secreted in the bile are possible. The poor oral bioavailability for cyclosporine is attributed to a combination of intestinal metabolism by CYP3A4 and efflux by P-gp.⁷³

Because the intestinal mucosa is enriched with glucuronosyltransferases, sulfotransferases, and glutathione

transferases, presystemic first-pass metabolism for orally administered drugs susceptible to these conjugation reactions results in their low oral bioavailability. Presystemic metabolism often exceeds liver metabolism for some drugs. For example, more than 80% of intravenously administered albuterol is excreted unchanged in urine, with the balance presenting as glucuronide conjugates, whereas when albuterol is administered orally, less than 5% is systemically absorbed because of intestinal sulfonation and glucuronidation. Presystemic metabolism is a major pathway in humans for most β -adrenergic agonists, such as glucuronides or sulfate esters of terbutaline, fenoterol, albuterol and isoproterenol, morphine (3-O-glucuronide), acetaminophen (O-sulfate and O-glucuronide), and estradiol (3-O-sulfate). The bioavailability of orally administered estradiol or ethinyl estradiol in females is approximately 50% because of conjugation. Mestranol (3-methoxyethinyl estradiol) has greater oral bioavailability because, as a nonphenol, it is not significantly conjugated. Levodopa has a low oral bioavailability because of its metabolism by intestinal l-aromatic amino acid decarboxylase. Drugs subject to first-pass metabolism are included in [Table 3.16](#).

TABLE 3.16
Examples of Drugs Exhibiting Presystemic Metabolism

Acetaminophen	Isoproterenol	Oxprenolol
Albuterol	Lidocaine	Pentazocine
Alprenolol	Meperidine	Propoxyphene
Aspirin	Methyltestosterone	Propranolol
Cyclosporin	Metoprolol	Salicylamide
Desmethylinipramine	Dihydropyridines (nifedipine)	Terbutaline
Fluorouracil	Nortriptyline	Verapamil
Hydrocortisone	Organic nitrates	
Imipramine		

The extensive presystemic first-pass sulfonation of phenolic drugs, for example, can lead to increased bioavailability of other drugs by competing for the available sulfate pool, resulting in the possibility of drug toxicity. Concurrent oral administration of acetaminophen with phenolic drugs could result in an increase in drug blood levels. Ascorbic acid, which is sulfonated, also increases the bioavailability of concurrently administered phenolic drugs. Sulfonation and glucuronidation occur side by side, often competing for the same substrate, and the balance between sulfonation and glucuronidation is influenced by several factors, such as species, doses, availability of cosubstrates, inhibition, and induction of the respective transferases.

Extrahepatic Metabolism

Because the liver is the primary tissue for xenobiotic metabolism, it is not surprising that our understanding of mammalian P450 monooxygenase is based chiefly on hepatic studies. Although the tissue content of P450s is highest in the liver, P450 enzymes are found in the lung, nasal epithelium, intestinal tract, kidney, adrenal tissues, and brain. It is possible that the expression of the polymorphic genes and induction of the isoforms in the extrahepatic tissues can affect the activity of the P450 isoforms in the metabolism of endogenous steroids, drugs, and other xenobiotics. Therefore, characterization of P450, UGT, SULT, and other polymorphic drug-metabolizing enzymes in extrahepatic tissues is important to our overall understanding about the biological importance of these isoform families to improved drug therapy, design of new drugs and dosage forms, toxicity, and carcinogenicity.

The mucosal surfaces of the GIT, the nasal passages, and the lungs are major portals of entry for xenobiotics into the body and, as such, are continuously exposed to a variety of orally ingested or inhaled airborne xenobiotics, including drugs, plant toxins, environmental pollutants, and other chemical substances. As a consequence of this exposure, these tissues represent a major target for tumorigenesis and other chemically induced toxicities. Many of these toxins and chemical carcinogens are relatively inert substances that must be bioactivated to exert cytotoxicity and tumorigenicity. The epithelial cells of these tissues are capable of metabolizing a wide variety of exogenous and endogenous substances, and these cells provide the principal and initial source of biotransformation for these xenobiotics during the absorptive phase. The consequences of such presystemic biotransformation is either (1) a decrease in the amount of xenobiotics available for systemic absorption by facilitating the

elimination of polar metabolites or (2) toxification by activation to carcinogens, which can be one determinant of tissue susceptibility for the development of intestinal cancer. The risk of colon cancer can depend on dietary constituents that contain either procarcinogens or compounds modulating the response to carcinogens.

Intestinal Metabolism

As noted earlier, many of the clinically relevant aspects of P450 can, in fact, occur at the level of the intestinal mucosa and can account for differences among patients in dosing requirements. The intestinal mucosa is enriched especially with the CYP3A4 isoform, glucuronosyltransferases, sulfotransferases, and GSTs, making it particularly important for orally administered drugs susceptible to oxidation and/or Phase 2 glucuronidation, sulfonation, or glutathione conjugation pathways. The highest concentrations of P450s occur in the duodenum, with gradual tapering into the ileum. In the human intestine, CYP2E, CYP3A, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 have been identified. Therefore, intestinal P450 isoforms provide potential presystemic first-pass metabolism of ingested xenobiotics affecting their oral bioavailability (e.g., hydroxylation of naloxone) or bioactivation of carcinogens or mutagens. It is not surprising that dietary factors can affect the intestinal P450 isoforms. For example, a 2-day dietary exposure to cooked Brussels sprouts significantly decreased the 2 α -hydroxylation of testosterone, yet induced CYP1A2 activity for PAH metabolism. An 8-oz. glass of grapefruit juice inhibited the sulfoxidation metabolism of omeprazole (CYP3A4) but not its hydroxylation (CYP2C19), thus increasing its systemic blood concentrations. These types of interactions between a drug and a dietary inhibitor could result in a clinically significant drug interaction.

Intestinal UGT isoforms can glucuronidate orally administered drugs, such as morphine, acetaminophen, α - and β -adrenergic agonists, and other phenolic phenethanolamines and dietary xenobiotics, resulting in a reduction of their oral bioavailability (increasing first-pass metabolism), thus altering their pharmacokinetics and pharmacodynamics. The UGTs expressed in the intestine include UGT1A1 (bilirubin-glucuronidating isoform), UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, and UGT1A3. Substrate specificities of intestinal UGT isoforms are comparable to those in the liver. Glucuronidase hydrolysis of biliary glucuronide conjugates in the intestine can contribute to EHC of the parent drug. Likewise, the sulfotransferases in the small intestine can metabolize orally administered drugs and xenobiotics for which the primary route of conjugation is sulfonation (e.g., isoproterenol, albuterol, steroid hormones, α -methyldopa, acetaminophen, and fenoldopam), decreasing their oral bioavailability and, thus, altering their pharmacokinetics and pharmacodynamics. Competition for intestinal sulfonation between coadministered substrates can influence their bioavailability with either an enhancement or a decrease of therapeutic effects. Sulfatase hydrolysis of biliary sulfate esters in the intestine can contribute to EHC of the parent drug.

Plants contain a variety of protoxins, promutagens, and procarcinogens, and the occurrence of intestinal P450 enzymes and bacterial enzymes in the microflora allows the metabolic conversion of relatively stable environmental pollutants and food-derived xenobiotics into mutagens and carcinogens. For example, cruciferous vegetables (Brussels sprouts, cabbage, broccoli, cauliflower, and spinach) are all rich in indole compounds (e.g., indole 3-carbinol) which, with regular and chronic ingestion, are capable of inducing some intestinal P450s (CYP1A subfamily) and inhibiting others (CYP3A subfamily). It is likely that these vegetables would also alter the metabolism of food-derived mutagens and carcinogens. For

example, heterocyclic amines produced during the charbroiling of meat are P450 N-hydroxylated and become carcinogenic in a manner similar to arylamines.

Intestinal Microflora

When drugs are orally ingested, or there is considerable biliary excretion of a drug or its metabolites into the GIT, such as with a parentally administered drug (EHC or recirculation), the intestinal bacterial microflora can have a significant metabolic role. The microflora has an important role in the enterohepatic recirculation of xenobiotics via their conjugated metabolites (e.g., digoxin, the oral contraceptives norethisterone and ethinyl estradiol and chloramphenicol) and endogenous substances (steroid hormones, bile acids, folic acid, and cholesterol), which reenter the gut via the bile. Compounds eliminated in the bile are conjugated with glucuronic acid, glycine, sulfate, and glutathione and, once secreted into the small intestine, the bacterial β -glucuronidase, sulfatase, nitroreductases, and various glycosidases catalyze the hydrolysis of the conjugates. The activity of orally administered conjugated estrogens (e.g., Premarin) involves the hydrolysis of the sulfate esters by sulfatases, releasing estrogens to be reabsorbed from the intestine into the portal circulation. The clinical use of oral antibiotics (e.g., erythromycin, penicillin, clindamycin, and aminoglycosides) has a profound effect on the gut microflora and the enzymes responsible for the hydrolysis of drug conjugates undergoing EHC. Bacterial reduction includes nitro reduction of nitroimidazole, azo reduction of azides (sulfasalazine to 5-aminosalicylic acid and sulfapyridine), and reduction of the sulfoxide to its sulfide. The sulfoxide of sulindac is reduced by both gut microflora and hepatic P450s. Other ways in which bacterial flora can affect metabolism include the following: (1) production of toxic metabolites, (2) formation of carcinogens from inactive precursors, (3) detoxication,

(4) exhibition of species differences in drug metabolism, (5) exhibition of individual differences in drug metabolism, (6) production of pharmacologically active metabolites from inactive precursors, and (7) production of metabolites not formed by animal tissues. In contrast to the predominantly hepatic oxidative and conjugative metabolism of the liver, gut microflora is largely degradative, hydrolytic, and reductive, with a potential for both metabolic activation and detoxication of xenobiotics.

Lung Metabolism

Some of the hepatic xenobiotic biotransformation pathways are also operative in the lung. Because of the differences in organ size, the total content of the pulmonary xenobiotic-metabolizing enzyme systems is usually lower than in the liver, creating the impression of a minor role for the lung in xenobiotic elimination. CYP2E1 is expressed in the lung to the greatest extent. The expression of other P450s, FMO, epoxide hydrolase, and the Phase 2 conjugation pathways are comparable to those in the liver. Thus, the lungs can have a significant role in the metabolic elimination or activation of low-molecular-weight inhaled xenobiotics.

When drugs are injected intravenously, intramuscularly, or subcutaneously, or after skin absorption, the drug initially enters the pulmonary circulation, after which the lung becomes the organ of first-pass metabolism ^[1]for the drug. The blood levels and therapeutic response of the drug are influenced by genetic disposition, induction, and inhibition of the pulmonary metabolizing enzymes. By modulation of the P450 isoform pattern in the lung, a xenobiotic could alter its own metabolism and that of others in a time- and dose-dependent manner. Because of its position in the circulation, the lung provides a second-pass metabolism for xenobiotics and their metabolites exiting from the liver, but it is also

susceptible to the cytotoxicity or carcinogenicity of hepatic activated metabolites. Antihistamines, β -blockers, opioids, and tricyclic antidepressants are among the basic amines known to accumulate in the lungs because of their binding to surfactant phospholipids in lung tissue. The significance of this relationship to potential pneumotoxicity remains to be seen.

Nasal Metabolism

The nasal olfactory mucosa is recognized as a first line of defense for the lung against airborne environmental xenobiotics because the mucosa is constantly exposed to the airborne external environment.⁷⁴ Drug metabolism in the olfactory nasal epithelium represents a major metabolic pathway for protecting the CNS and brain against the entry of drugs, inhaled environmental pollutants, or other volatile chemicals. In some instances, these enzymes can biotransform a given drug or environmental or airborne xenobiotic into more reactive and potentially toxic metabolites, increasing the risk of carcinogenesis in the nasopharynx and lung (e.g., procarcinogens and nitrosamines in cigarette smoke). P450 activity is high in olfactory tissue and olfactory-specific isoforms have been identified, which include CYP1A2, CYP2A6, CYP2A13, CYP2E1, CYP3A4, and the olfactory-specific CYP2G1 isoform, as well as several isoforms of the flavin-dependent monooxygenases. Phase 2 enzymes found in the olfactory mucosa include UGTs, glutathione-S-transferases, carboxylesterases, aldehyde oxidases, and epoxide hydrolases. The most striking feature of the nasal epithelium is that the P450 catalytic activity per gram of tissue is higher than in any other extrahepatic tissue or in the liver. Nasal decongestants, essences, anesthetics, alcohols, nicotine, and cocaine have been shown to be metabolized in vitro by P450 enzymes from the nasal epithelium. Because the P450s in the nasal mucosa are active, first-

pass metabolism should be considered when delivering susceptible drugs intranasally.

The nasal mucosa is also exposed to a wide range of odorants. Odorants, which are mostly small lipophilic molecules, enter the mucosa and reach the odorant receptors on sensory neurons by a transient process requiring signal termination, which could be provided by biotransformation of the odorant in the epithelial supporting cells. Thus metabolism of odorants could be involved in both the termination and initiation of olfactory stimuli.

Brain Metabolism

The metabolism of endogenous neurochemicals and neurotransmitters by brain CYPs may help explain variations in mood, aggression, personality disorders, and other psychiatric conditions.⁷⁵⁻⁷⁷ The role of brain CYP metabolism in neurotoxicity may also contribute to our understanding of the underlying mechanisms of diseases such as Parkinson's disease. Knowledge of CYP isoforms and transporter function in the blood-brain barrier (BBB) may increase our ability to deliver active forms of drugs specifically to the brain.

The isoforms of P450s and their regulation in the brain are of interest in defining the possible involvement of P450s in CNS toxicity and carcinogenicity. The expression level of P450 in the brain is approximately 0.5%-2% of that in liver, too low to significantly influence the overall pharmacokinetics of drugs and hormones in the periphery.^{75,77} Brain CYPs can impact acute and chronic drug response and susceptibility to damage by neurotoxins and are associated with altered personality, behavior, and risk of neurological disease. The functional role for brain CYP enzyme activity can impact CNS drug activation, protection against neurotoxicity, regulate neurotransmitter metabolism, chronic (e.g.,

nicotine dependence) response to drugs, and are associated with altered personality and behavior, and risk of neurological disease. Expression on a g/tissue basis can be as high as levels in hepatocytes. However, while hepatic CYPs are expressed primarily in the endoplasmic reticulum, brain expression is found in the mitochondrial and plasma membrane fraction and other cell membrane compartments. CYP1A1, CYP2B6, CYP2D6, CYP2E1, and CYP3A, are predominantly found in neurons and glial cells. CYP3A4 and CYP2C19 are expressed in human prefrontal cortex, hippocampus, and amygdala, areas that may mediate the behavioral effects of testosterone. Human CYP1B1 is found at the blood-brain interface, where it may act in conjunction with transporters, such as ATP-binding cassette transporters, to regulate passage of xenobiotics in and/or out of the brain. The interaction of a variety of transporters, in addition to local CYP-mediated metabolism, may play an important role in regulating the levels of centrally acting drugs in the brain and, thereby, their therapeutic effects.

The expression of brain CYPs levels vary among different brain regions. For example, in the human brain, CYP2B6 protein expression varies significantly among brain regions with a 2.5-fold range.⁷⁵ Brain CYP2D6 can metabolize endogenous neurochemicals, such as tyramine to dopamine and 5-methoxytryptamine to serotonin, while the metabolism of the endogenous cannabinoid anandamide, the neurosteroid progesterone, and testosterone (to 16 α -hydroxytestosterone) is catalyzed by CYP2B6.⁷⁵ Thus these enzymes may play a functional role in the brain. CYP2C19 can metabolize the sex hormones testosterone, progesterone, and estradiol that are known to affect brain function and personality traits, such as aggression and anxiety. CYP2E1 can metabolize the fatty acid neural signaling molecule arachidonic acid, which is abundant in the brain and is required for neurologic health, and CYP19 aromatase can metabolize testosterone to estradiol, both of

which can influence personality traits, including aggression, impulsivity, and anxiety. The differences in expression of specific brain CYPs can cause shifts in the neurochemical homeostasis in the brain.

Individuals with a CYP2D6 PM genotype are at greater risk for parkinsonism, and this risk is even greater with exposure to neurotoxic pesticides.⁷⁵ CYP2D6 metabolizes and inactivates a number of compounds that can cause parkinsonian symptoms, including MPTP, a Parkinson-causing compound, and its neurotoxic metabolite MPP+. CYP2D6 is expressed in human brain regions affected by Parkinson's disease, such as the substantia nigra. The impaired ability of CYP2D6 PMs to inactivate these neurotoxic metabolites may contribute to their increased risk for Parkinson's disease. In contrast to CYP2D6 PMs, smokers are at lower risk for Parkinson's disease since nicotine is neuroprotective. In humans, CYP2D6 expression levels are higher in the brains of smokers than in those of nonsmokers, while induction of brain CYP2D6 by nicotine or smoking may reduce an individual's relative risk for the disease. These data support a contributing role for lower brain CYP2D6 in the increased risk for Parkinson's disease, likely through modulation of local neurotoxin metabolism.⁷⁵

Nicotine is the main component of cigarette smoke that causes tobacco dependence, and genetic variation in brain CYP2B6, CYP2D6, and CYP2E1 can affect smoking behaviors.^{75,76} CYP2B6 slow metabolizers (IMs) progress to tobacco dependence more quickly and have more difficulty quitting than normal (EM) metabolizers. Brain CYP2B6 metabolizes nicotine and other endogenous substrates, such as serotonin and neurosteroids. Nicotine and smoking induces the brain expression of CYP2B6, CYP2D6, and CYP2E1, all of which affect smoking behaviors. Smokers have higher levels of brain CYP2D6, but unchanged levels of hepatic CYP2D6. Individuals exposed to nicotine through

smoking or through nicotine replacement therapy are likely to have increased CYP2D6-mediated brain metabolism of centrally acting drugs, neurotoxins, and endogenous neurochemicals, increasing risk of drug interactions and neurotoxicity. Brain levels of CYP2D6 enzymes and activity may be contributing factors to the clinical observations of personality differences with chronic nicotine administration between CYP2D6 EM and PM phenotypes. Preliminary data suggest that reducing brain CYP2B and CYP2D6 activity increases the rewarding properties of nicotine by altering brain levels of nicotine and its metabolites.

Metabolism in Other Tissues

P450s in the kidney and adrenal tissues include isoforms primarily involved in the hydroxylation of steroids, arachidonic acid, and 25-hydroxycholecalciferol.

Stereochemical Aspects of Drug Metabolism

In addition to the physicochemical factors that affect xenobiotic metabolism, stereochemical factors have an important role in the biotransformation of drugs. This involvement is not unexpected, because the xenobiotic-metabolizing enzymes are also the same enzymes that metabolize certain endogenous substrates which, for the most part, are chiral molecules. Most of these CYP isoforms show stereoselectivity but not stereospecificity; in other words, one stereoisomer enters into biotransformation pathways preferentially but not exclusively. Metabolic stereochemical reactions can be categorized as follows: substrate stereoselectivity, in which two enantiomers of a chiral substrate are metabolized by CYP isoforms at different rates; product stereoselectivity, in which a new chiral center is created in a symmetric molecule and one enantiomer is metabolized preferentially; and substrate-product stereoselectivity, in which a new chiral center of a chiral molecule is metabolized preferentially to one of two possible diastereomers.

An example of substrate stereoselectivity is the preferred decarboxylation of (*S*)- α -methyldopa to (*S*)- α -methyldopamine, with almost no reaction for (*R*)- α -methyldopa. Another example is the (*S*)-ketamine enantiomer, which is preferentially N-demethylated by CYP2B6 to (*S*)-norketamine, whereas the (*R*) enantiomer is N-demethylated by CYP3A4. The reduction of ketones to stereoisomeric alcohols and the hydroxylation of enantiotropic protons or phenyl rings by P450 are examples of product stereoselectivity. For example, phenytoin undergoes aromatic *p*-hydroxylation of only one of its two phenyl rings to create a chiral center at C5 of the hydantoin ring; methadone is reduced preferentially to its α -diastereometric alcohol and naltrexone is reduced to its 6- β -alcohol. Two examples of substrate-product

stereoselectivity are the reduction of the enantiomers of warfarin, and the β -hydroxylation of (*S*)- α -methyldopamine to (1*R*,2*S*)- α -methylnorepinephrine ((*R*)- α -methyldopamine is hydroxylated only to a negligible extent). In vivo studies of this type often can be confused by the further biotransformation of one stereoisomer, giving the false impression that only one stereoisomer was formed preferentially. Moreover, some compounds show stereoselective absorption, distribution, and excretion, which proves the importance of performing in vitro studies.

Although studies regarding the stereoselective biotransformation of drug molecules are not yet extensive, those that have been done indicate that stereochemical factors have an important role in drug metabolism. In some cases, stereoselective biotransformation could account for the differences in pharmacological activity and duration of action between enantiomers.

Metabolic Bioactivation: Role in Hepatotoxicity, Idiosyncratic Reactions, and Chemical Carcinogenesis

Drug-Induced Hepatotoxicity

Drug-induced hepatotoxicity is the leading cause of hepatic injury, accounting for approximately half of all cases of acute liver failure in the U.S. Recent studies have shown that drug-induced hepatotoxicity represents a larger percentage of adverse drug reactions than reported previously, and that the incidence and severity of drug-induced liver injury is underestimated among the general population.

Acetaminophen overdose is the leading cause for calls to poison control centers (>100,000 calls/year) and accounts for more than 56,000 emergency room visits, 2600 hospitalizations, and an estimated 458 deaths from acute liver failure each year. Among the listed drugs in Table 3.17, acetaminophen is the most frequently implicated hepatotoxic agent and can cause extensive hepatic necrosis with as little as 10-12 g (30-40 tablets). Chronic alcohol intake enhances acetaminophen hepatotoxicity more than five times as compared to acute alcohol intake, yet acetaminophen is heavily marketed for its safety as compared to nonsteroidal analgesics (NSAIDs). U.S. drug manufacturers continue to market and promote extra-strength acetaminophen products (500-750 mg/tablet) and a variety of Extra-strength acetaminophen-drug combination products. Self-poisoning with acetaminophen (paracetamol) is also a common cause of hepatotoxicity in the Western [SEP] world. To reduce the number of acetaminophen poisonings in the UK, over-the-counter (nonprescription) sales of acetaminophen are limited to 16 tablets per packet.

TABLE 3.17
Some Drugs Causing Hepatic Injury^a

Acarbose	<i>Felbamate</i>	Methotrexate	Ritonavir
Acetaminophen	Fenofibrate	Methsuximide	Rosiglitazone
Allopurinol	<i>Fluconazole</i>	Methyldopa	Rosuvastatin

Amiodarone	<i>Flutamide</i>	Nabumetone	<i>Saquinavir</i>
Amprenavir	Fluvastatin	Naproxen	Simvastatin
Anagrelide	Gemfibrozil	Nefazodone (2005)	Sulindac
<i>Atomoxetine</i>	Gemtuzumab	<i>Nevirapine</i>	<i>Tacrine</i>
Atorvastatin	Griseofulvin	Niacin (SR)	Tamoxifen
Azathioprine	<i>Halothane</i>	Nitrofurantoin	Tasosartan (1998)
Bicalutamide	Imatinib	Olanzapine	<i>Terbinafine</i>
Bosentan	Indinavir	Oxaprozin	Testosterone
Bromfenac (1998)	<i>Infliximab</i>	Pemoline (2005)	Thioguanine
Carbamazepine	Isoflurane	Pentamidine	Tizanidine
Celecoxib	Isotretinoin	Pioglitazone	<i>Tolcapone</i>
Dapsone	Itraconazole	Piroxicam	Troglitazone (2000)
<i>Diclofenac</i>	<i>Ketoconazole</i>	Pravastatin	<i>Trovafloxacin</i>
Disulfiram	<i>Ketorolac</i>	<i>Pyrazinamide</i>	<i>Valproic acid</i>
<i>Duloxetine</i>	Lamivudine	Ribavirin	Voriconazole
Efavirenz	<i>Leflunomide</i>	Rifabutin	Ximelagatran (2004)
Ethotoin	Lovastatin	<i>Rifampin</i>	<i>Zileuton</i>
Ethosuximide	Meloxicam	Riluzole	<i>Zafirlukast</i>

^aDrugs in **bold** have exhibited severe drug-induced hepatotoxicity and were withdrawn either voluntarily or by a regulatory agency (year given). Drugs in *italics* have exhibited moderate-severe drug-induced hepatotoxicity requiring a black box warning restricting their use. The other drugs have exhibited mild to moderate drug-induced hepatotoxicity that can need frequent liver transaminase testing for those at risk (see [Table 3.18](#)).

Drug-induced hepatotoxicity is also the most frequent reason that new therapeutic agents are not approved by the FDA (e.g., ximelagatran in 2004) and the most common adverse drug reaction leading to withdrawal of a drug from the market ([Table 3.17](#)). Hepatotoxicity almost always involves metabolism with Phase 1 P450 enzymes rather than Phase 2 enzymes. More than 600 drugs, chemicals, and herbal remedies can cause hepatotoxicity, of which more than 30 drugs have either been withdrawn from the U.S. market because of hepatotoxicity or have carried a black box warning for hepatotoxicity since 1990. [Table 3.17](#) includes some of the more common drugs that have exhibited drug-induced hepatotoxicity ranging from severe, requiring the drug's regulatory withdrawal from the market (bold in [Table 3.17](#)); moderate to severe, requiring black box warning restrictions (*italics* in [Table 3.17](#)); or mild to moderate, requiring frequent liver function monitoring.

However, Watkins⁷⁸ reported that one-third of 106 patients taking a maximum daily acetaminophen dose of 4 g for 8 days, either alone or in

combination with hydrocodone, exhibited a 3-fold increase in liver enzymes associated with acetaminophen-induced liver injury. This 3-fold increase in transaminase levels is a signal for potential liver safety concerns in those individuals who are at risk of acetaminophen-induced liver toxicity. Drugs reported to cause hepatocellular necrosis include acetaminophen, methyldopa, valproic acid, trazodone, nefazodone, venlafaxine, and lovastatin. Drug-induced liver damage occurs after a prolonged period of drug administration.

The most commonly used indicators of hepatotoxicity (i.e., liver injury) are increased levels of the liver transaminases, aspartate aminotransferase (AST), and alanine aminotransferase (ALT).⁷⁹ Drug-induced hepatotoxicity can develop rapidly, often before abnormal laboratory tests are noticed, which are characterized by rapid elevations in ALT and AST of 8-500 times the upper normal limit, with variable elevations in bilirubin. Drugs causing acute liver injury (hepatocellular necrosis) exhibit elevations in hepatic transaminases ranging from 50 to 100 times higher than the normal level. On the other hand, the elevations of ALT and AST in alcoholic liver disease are two to three times higher than normal. Some hepatotoxins, however, do not elevate transaminases, whereas nonhepatic toxins can elevate ALT.

Most drug-induced hepatotoxicity is of an idiosyncratic nature, occurring in a small percentage of patients (1 in 5000) who ingest the drug.⁸⁰⁻⁸² These reactions tend to be of two distinct types: (1) hypersensitivity reactions that are immune mediated, occurring within the first 4-6 weeks and are associated with fever, rash, eosinophilia, and a hepatitis-like picture (e.g., phenytoin, sulindac, and allopurinol); and (2) metabolic idiosyncratic reactions that tend to occur at almost any time during the first year of treatment (e.g., troglitazone). The incidence of overt idiosyncratic liver diseases varies with the drug, ranging from approximately 1 in 1000 with phenytoin, to 1 in 10,000 or more with sulindac and troglitazone and 1 in 100,000 with diclofenac. To detect a single case of drug-induced hepatotoxicity with 95% confidence requires the number of patients studied to be 3-fold the incidence of the reaction (i.e., for one adverse drug reaction in 10,000 patients, at least 30,000 patients need to be evaluated).

Thus, many drugs are approved before liver toxicity is observed. It is the responsibility of postmarketing surveillance and monitoring of liver transaminases to identify potential cases of liver-adverse drug reactions.

Risk factors (Table 3.18) for drug-induced liver injury, such as age, gender, genetic predisposition, multiple drugs, or dietary supplements and degree of alcohol consumption, appear to increase the susceptibility to drug-induced hepatotoxicity.^{80,81} However, the drugs in Table 3.17 should be used with caution in these high-risk patients, because such patients can have altered metabolism of these drugs and, therefore, can be at increased risk for liver injury. The coadministration of drugs in Table 3.17 with enzyme inducers, such as phenobarbital, phenytoin, ethanol, and/or cigarette smoke, can induce hepatic enzymes, resulting in the enhancement of hepatotoxicity.

TABLE 3.18
Risk Factors for Drug-Induced Liver Injury

Race	Some drugs exhibit different toxicities based on race because of individual P450 polymorphism. For example, blacks and Hispanics can be more susceptible to isoniazid (INH) toxicity.
Age	Elderly persons are at increased risk of hepatic injury due to decreased clearance, drug-drug interactions, reduced hepatic blood flow, variation in drug binding, and lower hepatic volume. In addition, poor diet, infections, and multiple hospitalizations are important reasons for drug-induced hepatotoxicity. Hepatic drug reactions are rare in children (e.g., acetaminophen, halogenated general anesthetics).
Gender	Although the reasons are unknown, hepatic drug reactions are more common in females. Females are more susceptible to hepatotoxicity from acetaminophen, halothane, nitrofurantoin, diclofenac, and sulindac.
Alcohol	Alcoholics are susceptible to drug toxicity because alcohol induces liver injury and cirrhotic changes that alter drug metabolism. Alcohol causes depletion of glutathione (hepatoprotective) stores, making the person more susceptible to toxicity by drugs (e.g., acetaminophen, statins).
Liver disease	Patients with chronic liver disease are not uniformly at increased risk of hepatic injury. Although the total P450 level is reduced, some patients can be affected more than others. The modification of doses in persons with liver disease should be based on knowledge of the specific P450 isoform involved in the metabolism. Patients with HIV infection who are coinfecting with hepatitis B or C virus are at increased risk for hepatotoxic effects. Similarly, patients with cirrhosis are at increased risk to hepatotoxic drugs (e.g., methotrexate, methyl dopa, valproic acid).

Genetic factors	Genetic (polymorphic) differences in the formation of P450 isoforms (2C family and 2D6) can result in abnormal reactions to drugs, including idiosyncratic reactions
Other comorbidities	Patients with AIDS, renal disease, diabetes mellitus, persons who are malnourished, and persons who are fasting can be susceptible to drug reactions because of low glutathione stores.
Pharmacokinetics	Long-acting drugs can cause more injury than shorter-acting drugs, as well as sustained-release drug product formulation.
Drug adulterants	Contaminants are often found in noncertified herbal supplements (e.g., hepatitis C).

Most hepatic adverse effects associated with drugs occur in adults rather than children. Drug-induced liver injury occurs at a higher rate in patients older than 50 years and drug-associated jaundice occurs also more frequently in the geriatric population.^{80,81} This age-risk can be the result of increased frequency of drug exposure, multidrug therapy, and age-related changes in drug metabolism.

For reasons that are unclear, drug-induced liver injury affects females more than males. Females accounted for approximately 79% of all reactions to acetaminophen and 73% of all idiosyncratic drug-induced reactions. Females exhibit increased risk of hepatic injury from drugs such as atorvastatin, nitrofurantoin, methyldopa, and diclofenac.⁸¹

Genetic factors because of enzyme polymorphism in affected individuals can decrease the ability to metabolize or eliminate drugs, thus increasing their duration of action and drug exposure and/or decreasing the ability to modulate the immune response to drugs or metabolites. Chronic ingestion of alcohol can also predispose many patients to increased hepatotoxicity from drugs by lowering the store of glutathione (a detoxifying mechanism), which prevents trapping of the toxic metabolites as mercapturate conjugates that are excreted in the urine.

Drug-induced hepatotoxicity can be categorized as intrinsic (predictable) or idiosyncratic (unpredictable) reactions.⁸² Most drugs involved in hepatotoxicity belong to the idiosyncratic group. Intrinsic hepatotoxins produce liver injury in a dose-related manner when a toxic amount of drug is ingested without bioactivation, such as the toxins found in the *Amanita* mushroom. Idiosyncratic hepatotoxicity is the result of the toxic effects of a drug's metabolites.

The common trigger for both mild and severe forms of hepatotoxicity is bioactivation of relatively inert functional groups to reactive electrophilic intermediates, which is considered to be an obligatory event in the etiology of many drug-induced idiosyncratic hepatotoxicity.^{83,84} Research now shows that reactive metabolites are formed from drugs known to cause idiosyncratic hepatotoxicity, but how these toxic species initiate and propagate tissue damage remains poorly understood. However, the relationship between bioactivation and the occurrence of hepatic injury is not simple. For example, many drugs at therapeutic doses undergo bioactivation in the liver but are not hepatotoxic. The tight coupling of bioactivation with bioinactivation pathways can be one reason for the lack of hepatotoxicity with these drugs. Examples of bioinactivation (detoxification) pathways include glutathione conjugation of quinones by glutathione S-transferases (GSTs) and hydration of arene oxides to dihydrodiols by epoxide hydrolases. When reactive metabolites are poor substrates for such detoxifying enzymes, they can escape bioinactivation and, thereby, damage proteins and nucleic acids, prompting hepatotoxicity.

Most drugs, however, are not directly chemically reactive but, through the normal process of drug metabolism, can form electrophilic, chemically reactive metabolites. Formation of chemically reactive metabolites is mainly catalyzed by P450 enzymes (Phase 1), but products of Phase 2 metabolism (e.g., acylglucuronides, acyl CoA thioesters, or N-sulfates) can also lead to toxicity. However, if Phase 1 drug bioactivation is closely coupled with Phase 2 bioinactivation (e.g., glutathione conjugation to mercapturates), then the net chemical process is one of detoxification if the final product is rapidly cleared.

Toxicity can accrue when accumulation of a chemically reactive metabolite, if not detoxified, leads to covalent modification of biological macromolecules. The identity of the target macromolecule and the functional consequence of its modification will dictate the resulting toxicological response. P450 enzymes are present in many organs, mainly the liver but also the kidney and lung, and thus can bioactivate chemicals to cause organ-specific toxicity. Evidence for the formation of reactive

metabolites was found for 5 of the 6 drugs that have been withdrawn from the market since 1995 and for 8 of the 15 drugs that have black box warnings (Table 3.17). Evidence for reactive metabolite formation has been found for acetaminophen, bromfenac, diclofenac, clozapine and troglitazone. Acetaminophen is the most studied hepatotoxin.

The current hypotheses of how reactive metabolites lead to liver injury suggest that hepatic (target) proteins can be modified by reactive metabolites. Much more clinically important is the identification of the target proteins modified by these toxic metabolites and how these reactions alter the function of those proteins. Additionally, it is important to note that the toxicity of reactive metabolites can also be mediated by noncovalent binding mechanisms, which can have profound effects on normal liver physiology. Such information should dramatically improve our understanding of drug-induced hepatotoxic reactions. Moreover, covalent binding per se does not necessarily lead to drug hepatotoxicity. The regioisomer of acetaminophen, 3-hydroxyacetanilide, becomes covalently bound to hepatic proteins in rodents without inducing hepatotoxicity.⁸⁵

It therefore is necessary to identify targets for these reactive metabolites (i.e., covalently modified macromolecules) that are critical to the toxicological process. Unfortunately, no simple rules predict the target macromolecules for a particular chemically reactive metabolite or the biological consequences of a particular modification. Furthermore, noncovalent interactions also have a role, because covalent binding of hepatotoxins is not indiscriminate with respect to proteins. Even within a single protein, there can be selective modification of an amino acid side chain found repeatedly in the primary structure. Thus, the microenvironment (e.g., pK_a and hydrophobicity) of the amino acid in the tertiary structure appears to be the crucial determinant of selective binding and, therefore, the impact of covalent binding on protein function. In turn, the extent of binding and the biochemical role of the protein will determine the toxicological insult of drug bioactivation. The resulting pathological consequences will be a balance between the rates of protein damage and the rates of protein replacement and cellular repair.

Drug-Induced Idiosyncratic Reactions

Idiosyncratic drug reactions (IDR; type B adverse drug reactions) occur in from 1 in 1000 to 1 in 50,000 patients, are not predictable from the known pharmacology or toxicology of the drug, are not produced experimentally in vitro and in vivo, and are dose independent. The occurrence of IDRs during late clinical trials or after a drug has been released can lead to severe restriction of its use and even its withdrawal. IDRs do not as a rule result from the drug itself because most people can tolerate the drug, but rather from a unique set of patient characteristics, including gender, age, genetic predisposition, and a lack of drug-metabolizing enzymes that can increase the risk of these adverse drug reactions. Most IDRs are caused by hypersensitivity reactions and can result in hepatocellular injury. The hepatic injury occurs within 1 week to 12 months after initiation of drug therapy and is often accompanied by systemic characteristics of allergic drug reactions, such as rash and fever. Signs of hepatic injury reappear with subsequent administration of the same drug with only one or two doses. Hypersensitivity reactions can be severe and associated with fatal reactions as a multiorgan clinical syndrome characterized by the following: (1) fever; (2) rash; (3) gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea, or abdominal pain); (4) generalized malaise, fatigue, or achiness; and (5) respiratory symptoms (e.g., dyspnea, cough, or pharyngitis). Examples of drugs causing IDRs through a hypersensitivity mechanism include penicillin, methyldopa, chlorpromazine, erythromycin, azathioprine toxicity in thiopurine methyltransferase-deficient individuals, sulfonamide and acetaminophen hepatotoxicity in alcoholics and in UGT1A6-deficient cats, ivermectin neurotoxicity in Collie dogs deficient in P-gp, perhexilene hepatotoxicity in CYP2D6-deficient individuals, phenytoin toxicity in CYP2C9-deficient individuals, and valproic acid hepatotoxicity.

The clinical features of some cases of drug-induced idiosyncratic hepatotoxicity strongly suggest an involvement of the immune system.^{83,84} These clinical characteristics include the following: (1) concurrence of rash, fever, and eosinophilia; (2) delay of the initial reaction (1-8 weeks) or requirement of repeated exposure to the culprit drug; (3) rapid recurrence

of toxicity on reexposure to the drug; and (4) presence of antibodies specific for native or drug-modified hepatic proteins. Our current understanding of drug-induced adaptive immune responses is largely based on the hapten hypothesis.

Idiosyncratic drug reactions that are connected with hepatotoxicity involve the formation of reactive metabolites.^{83,84} Such reactions are not predictable, but current bioanalytical technology have enabled the in vivo identification of the formation of reactive metabolites, as evidenced by the detection of biomarkers (i.e., mercapturate or cysteine adducts) in urine, drug-specific antibodies, or antibodies to P450 isoforms.⁸⁴ As a result, some drugs known to cause hepatic injury continue to be used because the drug's benefit outweighs its risk and no alternative efficacious drug exists. Additionally, NSAIDs, including cyclooxygenase-2 inhibitors (e.g., celecoxib), commonly are associated with idiosyncratic liver injury. Most of the idiosyncratic toxins listed in [Table 3.19](#) that have been studied to date produce reactive metabolites.

TABLE 3.19
Some Examples of Idiosyncratic Toxins

Abacavir	Hypersensitivity
Acetaminophen	Hepatotoxicity
Amiodarone	Hepatotoxicity
Aromatic anticonvulsants	Hypersensitivity
Cefaclor	Hepatotoxicity
Clozapine	Agranulocytosis
Diclofenac	Hepatotoxicity
Felbamate	Aplastic anemia
Fibrates	Hepatotoxicity
Halothane	Hepatotoxicity
Indomethacin	Hepatotoxicity
Isoniazid	Hepatotoxicity
Levamisole	Hepatotoxicity
Nefazodone	Hepatotoxicity
Nevirapine	agranulocytosis
Oral contraceptives	Hepatotoxicity
Paroxetine	Hepatotoxicity
Penicillinamine ^a	Hypersensitivity
Phenytoin	Hepatotoxicity

Statins	Hepatotoxicity
Sulfonamides	Stevens-Johnson syndrome
Tamoxifen	Hepatotoxicity
Tacrine	Hepatotoxicity
Tienilic acid	Hypersensitivity
Ticlopidine	Agranulocytosis
Troglitazone	Hepatotoxicity
Valproic acid	Hepatotoxicity
Vesnarinone	Agranulocytosis

^aDoes not produce reactive metabolites.

Current hypotheses regarding IDRs suggest that metabolic activation of a drug to a reactive metabolite is a necessary step in the generation of an idiosyncratic reaction.⁸³ Evidence for this hypothesis comes from drugs that are associated with hepatotoxicity (Table 3.19) and the detection of drug-metabolite specific antibodies in affected patients.

For the other drugs that have been associated with idiosyncratic hepatotoxicity but that do not have black box warnings, either evidence for hepatotoxicity was not available or suitable studies had not been carried out. High doses increase the risk for an IDR (e.g., clozapine at 300 mg/d vs. olanzapine 20 mg/d).

The hapten hypothesis proposes that the reactive metabolites of hepatotoxic drugs act as haptens and bind covalently to endogenous proteins to form immunogenic drug-protein adducts triggering either antibody or cytotoxic T-cell responses.⁸⁴ The hapten hypothesis is supported by the detection of antibodies that recognize drug-modified hepatic proteins in the sera of drug-induced liver injury patients. For example, antibodies that recognize trifluoroacetate-altered hepatic proteins have been detected in the sera of patients with halothane-induced hepatitis. Such drug-specific antibodies that recognize native liver proteins have also been found in patients with liver injury caused by other drugs, such as diclofenac. Most drugs are small molecules and are unlikely to form haptens. Electrophilic acylators can react with the lysine ω -amino residues of the target protein or guanosine residues of DNA. Halothane is the most studied molecule for supporting this hypothesis regarding IDRs.⁸⁴ Therefore, it is not surprising that irreversible chemical modification of a protein, which has a profound effect on function, is a

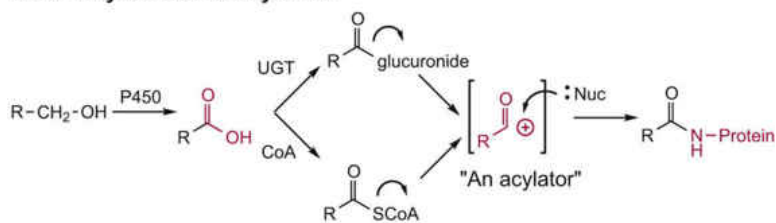
mechanism of idiosyncratic hepatotoxicity. However, it is important to note that a number of drugs (e.g., penicillins, aspirin, and omeprazole) rely on covalent binding to proteins for their efficacy; thus, prevention of their covalent binding through chemical modification of the compound can also, inadvertently, lead to loss of efficacy.

Reactive Metabolites Resulting From Bioactivation

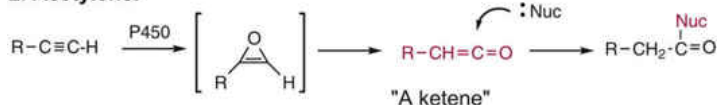
Electrophiles

The concept that small organic molecules can undergo bioactivation to electrophiles (an electron-deficient group or ion that accepts an electron pair to make a covalent bond, such as carbocations and acyl ions) and free radicals, and can elicit toxicity by chemical modification of cellular macromolecules, has its basis in chemical carcinogenicity and the pioneering work of the Millers⁸⁶ and Gillette et al.⁸⁷ Electrophiles are reactive because they possess electron-deficient centers (polarization-activated double bonds or positive-charge acylators) (Fig. 3.38) and can form covalent bonds with electron-rich biological nucleophiles such as the thiol groups in either glutathione or cysteine residues within proteins. A number of different types of reactive metabolites exist; however, they can be broadly classified as either electrophiles (Fig. 3.38) or free radicals (Fig. 3.39).⁸⁸ These reactive metabolites are short-lived, with half-lives of usually less than 1 minute, and they are not normally detectable in plasma or urine except as Phase 2 conjugates or other biomarkers.

1. Carboxylic acids as acylators:



2. Acetylene:

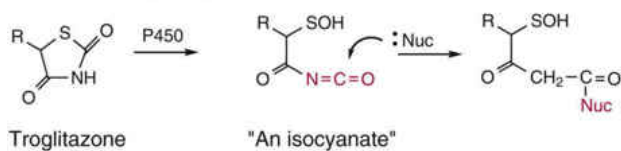


Example: Ethinylestradiol

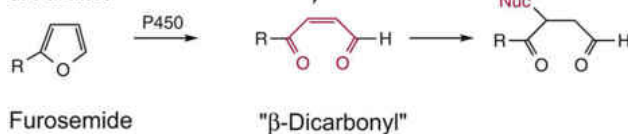
3. Halogenated hydrocarbon:



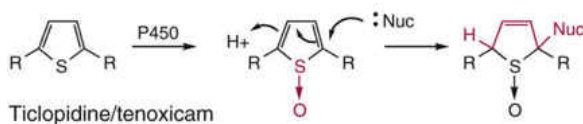
4. Thiazolidinedione:



5. Furans:



6. Thiophene:



7. Aromatics and olefins:

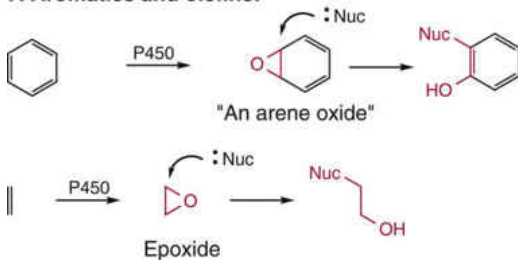
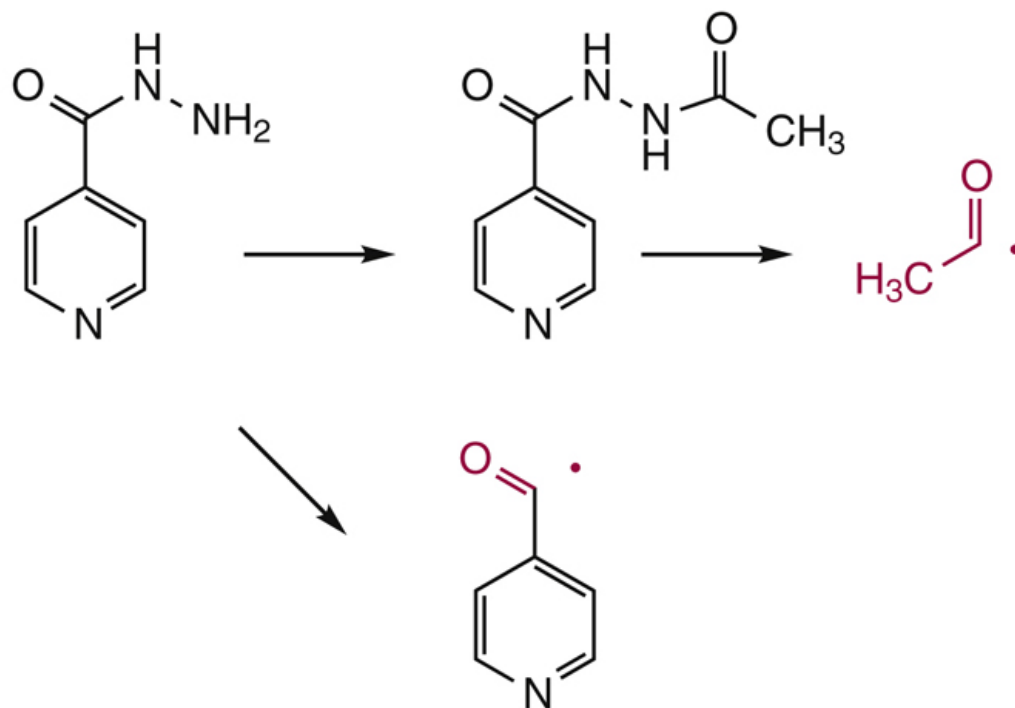


FIGURE 3.38 Some examples of electrophilic intermediates resulting from bioactivation. *Nuc*, nucleophiles.

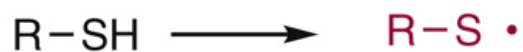
1. Isoniazid



2. Hydrazines



3. Sulfhydryl

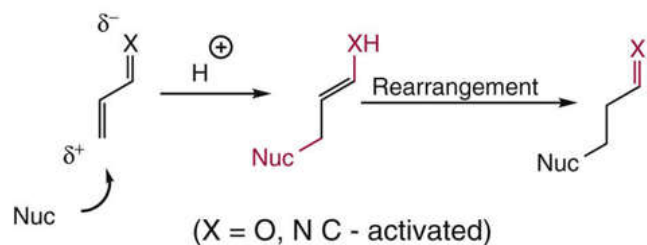


Captopril

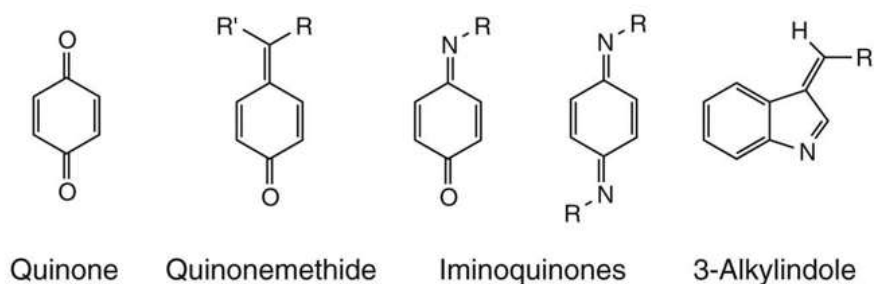
FIGURE 3.39 Drug bioactivation to free radicals.

Activated double bonds are electrophilic intermediates. Examples of activated double bond electrophiles include α,β -unsaturated carbonyl compounds, quinones, quinoneimines, quinonemethides, and diiminoquinones as shown in Figure 3.40. These electrophilic intermediates are highly polarized and can react with nucleophiles in a 1,4-Michael-type addition at the more electrophilic β -carbon of the activated double bond intermediate to generate the addition product (Fig. 3.40A). Specific examples of activated double bond electrophiles that have been proposed for the anticancer drug leflunomide, acetaminophen, the antiandrogen flutamide, the anticonvulsant felbamate, and the cytotoxic cyclophosphamide are shown in Figure 3.40C. The bioinactivation pathways for these electrophilic intermediate can involve direct addition, with or without GSH or epoxide transferases, depending upon the degree of polarization and reactivity of the electrophilic intermediate.⁸⁹

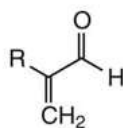
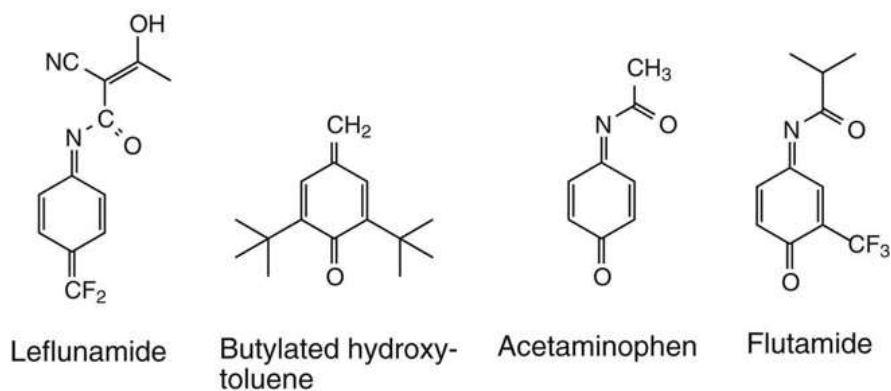
A. 1,4-Addition of a nucleophile in a Michael-type reaction:



B. Examples of activated double bonds:



C. Reactive intermediates from drugs that form activated double bonds:



R = phenyl - felbamate

R = H - acrolein from cyclophosphamide

FIGURE 3.40 Examples of activated double bonds to electrophilic reactive intermediates.

Other commonly found electrophilic intermediate for drug molecules in Figure 3.38 include ketenes from the bioactivation of acetylenic groups (e.g., ethinyl estradiol) (2 in Fig. 3.38); isocyanates from thiazolidinediones (e.g., the “glitazones”) (4 in Fig. 3.38)⁸⁸; acyloxonium ions from halogenated hydrocarbons (e.g., haloethane) (3 in Fig. 3.38)⁸⁸ and carboxylic acids; β -dicarbonyl from furans (e.g., furosemide) (5 in Fig. 3.38)⁸⁸; activated thiophene-S-oxide from thiophenes, such as ticlopidine and tenoxicam which cause an IDR, agranulocytosis (6 in Fig. 3.38)⁸⁸; and epoxides and arene oxides from olefins and aromatic compounds (7 in Fig. 3.38).⁸⁸

Drugs possessing structural features prone to metabolic epoxidations are abundant. Therefore the incidence of epoxide metabolites in mediating adverse biologic effects has aroused concern about clinically used drugs known to be metabolized to epoxides. Metabolically produced epoxides have been reported for allobarbital, secobarbital, protriptyline, carbamazepine, and cyproheptadine and are implicated with 8-methoxypsoralen and other furanocoumarins (6,7-dihydroxybergamottin in grapefruit juice), phenytoin, phenoximide, phenobarbital, mephobarbital, lorazepam, and imipramine.^{89,90} The alarming biologic effects of some epoxides, however, do not imply that all epoxides have similar effects. Epoxides vary greatly in molecular geometry, stability, electrophilic reactivity, and relative activity as substrates for epoxide-transforming enzymes (e.g., epoxide hydrolase, glutathione S-transferase, and others).

Some carboxylic acid-containing drugs have been implicated in rare IDRs, which was the basis for the market withdrawal of the NSAIDs zomiperac and benzoxaprofen.^{57,91} These drugs (e.g., NSAIDs, fibrates, “statins,” and valproic acid) can be bioactivated to acyl glucuronides or acyl CoA thioesters⁵⁷ (1 and 5 in Fig. 3.41). These products are electrophilic acylators that can acylate target proteins if they escape inactivation by S-glutathione-thioester formation.⁹² A crucial factor is the concentration of acyl glucuronides in hepatocytes due to their transport by conjugate export pumps, where acylglucuronides can selectively acylate hepatic membrane proteins. Acyl CoA esters can be either rapidly hydrolyzed or further metabolized in hepatocytes. Evidence is accumulating that acyl

glucuronides can alter cellular function by haptenation of peptides, target protein acylation or glycation, or direct stimulation of neutrophils and macrophages. The role of acyl CoA reactive metabolites is less clear. It should be noted that some noncarboxylic acid drugs can be biotransformed by oxidative metabolism in the liver to the respective carboxylic acids.⁹³

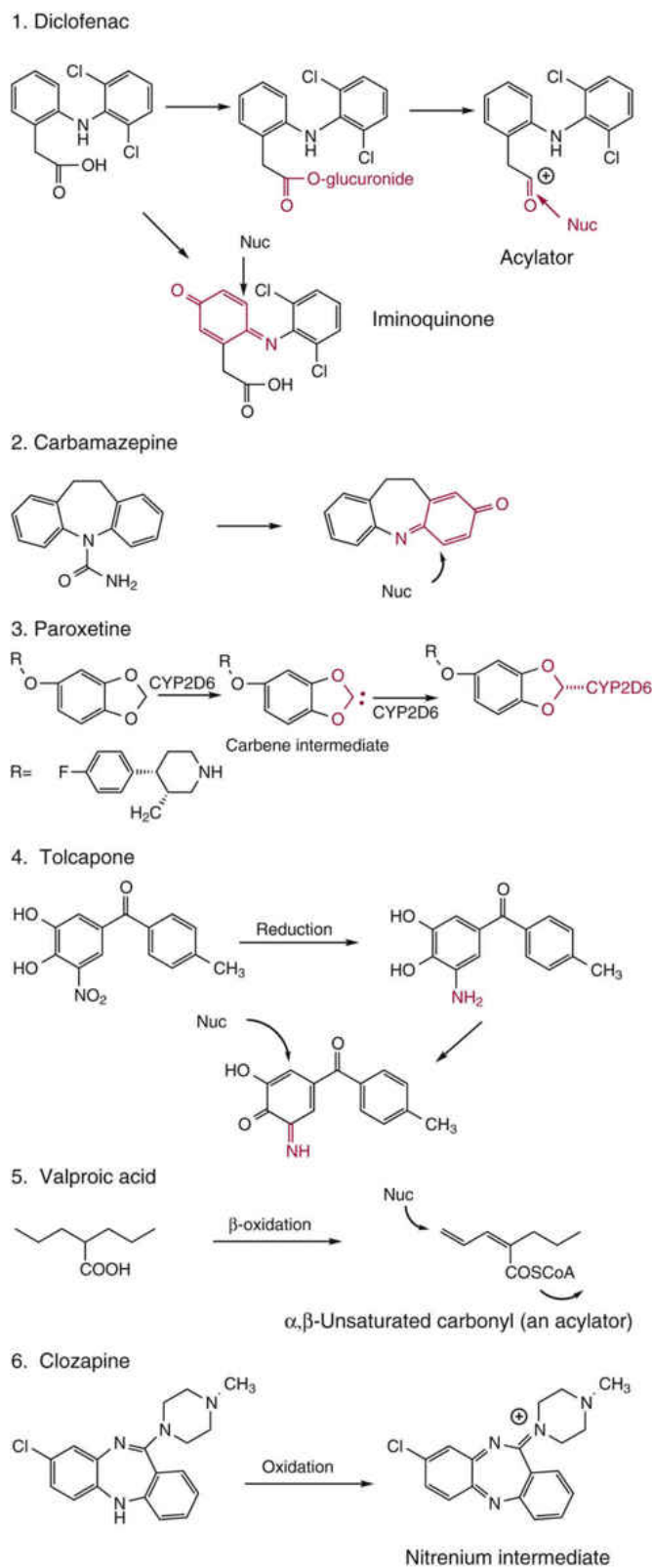


FIGURE 3.41 Examples of drug bioactivation to hepatotoxic intermediates.

Free Radicals

P450 activates molecular dioxygen to generate reactive oxygen species (ROS), such as singlet oxygen ($^1\text{O}_2$) or superoxide. Reactive metabolites that possess an odd unpaired electron are free radicals that can react with molecular oxygen (ground state triplet) to generate intracellular oxidative stress damage.⁹⁴ Free radicals abstract a hydrogen atom from other molecules rather than becoming covalently bound. Free radical reactions can be self-propagating by abstracting a hydrogen atom from the double bond of a lipid that initiates a chain reaction leading to lipid peroxidation, oxidative stress, or other types of modification of biological molecules.

Some examples of free radicals generated by the bioactivation of drug molecules are shown in [Figure 3.39](#). Isoniazid is acetylated to its major metabolite acetylisoniazid, which is hydrolyzed to acetylhydrazine and isonicotinic acid (1 in [Fig. 3.39](#)). Acetylhydrazine is further metabolized by the CYP2E1 to an N-hydroxy intermediate that hydrates into an acetyl radical, which can then initiate the process that leads to hepatic necrosis. Other carbon-centered radicals are formed from hydrazines such as the antihypertensive hydralazine and thio-radicals from the ACE inhibitor captopril (3 in [Fig. 3.39](#)).

Bioinactivation Mechanisms

Several enzyme systems exist as cellular defense (detoxification) pathways against the chemically reactive metabolites generated by P450 metabolism. These include GST, epoxide hydrolase, and quinone reductase, as well as catalase, glutathione peroxidase, and superoxide dismutase, which detoxify the peroxide and superoxide by-products of metabolism. The efficiency of the bioinactivation process is dependent on the inherent chemical reactivity of the electrophilic intermediate, its affinity and selectivity of the reactive metabolite for the bioinactivation enzymes, the tissue expression of these enzymes, and the rapid upregulation of these enzymes, and cofactors mediated by the cellular sensors of chemical stress. The reactive metabolites that can evade these defense systems can damage target proteins and nucleic acids by either oxidation or covalent modification.

The most abundant agents of cellular defense are thiols. Glutathione will only react noncatalytically with electrophiles, such as activated double bonds (Fig. 3.40). Glutathione conjugation to mercapturates is one of the most important defenses against hepatocellular injury.^{58,59} Glutathione protects cellular enzymes and membranes from toxic metabolites, and its inadequate stores can compromise efficient detoxification of the reactive metabolites. The subsequent inability to detoxify the reactive metabolites can result in hepatocellular injury. The rate-limiting factor for glutathione synthesis is the intracellular concentration of cysteine. N-acetylcysteine is often used as an alternative to glutathione to trap the iminoquinone intermediate in the treatment of acute acetaminophen toxicity and in preventing nephrotoxicity from ifosfamide-generated chloroacetaldehyde. Glutathione has a protective role in the hepatic tissue injury produced by acetaminophen, but not by furosemide.

The relationship between bioactivation, bioinactivation, and DNA adduct formation has been well established for a number of hepatocarcinogens.⁹⁴ Glutathione conjugation of carcinogens becomes more efficient when catalyzed by GSTs, an important example being the detoxification of the hepatocarcinogen aflatoxin. Aflatoxin, a hepatocarcinogen and a hepatotoxin found in mold growing on peanuts, is converted into aflatoxin B1 epoxide in rodents, which is more readily detoxified by GST enzymes than by epoxide hydrolase. The balance between these transferase reactions explains the greater DNA damage in humans compared with rodents, because human forms of GST are less able to catalyze the conjugation of aflatoxin epoxide compared with the rodent forms. Transgenic knockout mice have been used to establish the role of bioactivation by P450 and bioinactivation by GSTs for a number of carcinogenic polyaromatic hydrocarbons.

Substances that detoxify free radicals include the antioxidants vitamin C, vitamin E, and carotene, which scavenge free radicals, including reactive metabolites and reactive oxygen species generated as a consequence of chemical stress.

Specific Examples

Some examples of bioactivation to hepatotoxic or IDR electrophilic intermediates are shown in [Figure 3.41](#). Bioactivation can occur by both oxidation and conjugation reactions, such as those with diclofenac, which undergoes the formation of an acyl glucuronide to produce iminoquinones via formation of a phenol intermediary (1 in [Fig. 3.41](#)).⁹² The anticonvulsant carbamazepine is 2-hydroxylated and the elimination of the amide group yields the reactive quinoneimine intermediate (2 in [Fig. 3.41](#)). The antidepressant paroxetine and other xenobiotics with the common methylenedioxyphenyl nucleus undergo methylene oxidation to a *p*-quinoid intermediate (3 in [Fig. 3.41](#)). The nitro group of the COMT inhibitor tolcapone, used in the treatment of parkinsonism, is first reduced to an amine, then oxidized to an *o*-quinoneimine (4 in [Fig. 3.41](#)). The mitochondrial/hepatotoxicity of the anticonvulsant valproic acid results from the formation of an activated α,β -unsaturated CoA thioester via mitochondrial β -oxidation, most commonly associated with the oxidation of fatty acids (5 in [Fig. 3.41](#)). The agranulocytosis resulting from the ingestion of the antipsychotic clozapine is bioinitiated by oxidation by hypochlorous acid in neutrophils to a nitrenium intermediate (6 in [Fig. 3.41](#)).⁹³

The effect of structure modification for troglitazone that reduced its hepatotoxicity is shown in [Figure 3.42](#). The *p*-dihydroxy groups of the chroman ring nucleus (outlined in red in [Fig. 3.42](#)) of troglitazone is bioactivated to an activated double bond (*p*-quinone) was replaced with a pyridine ring that is not bioactivated, although the thiazolidone ring can be bioactivated to an isocyanate ([Fig. 3.42](#)).⁹¹

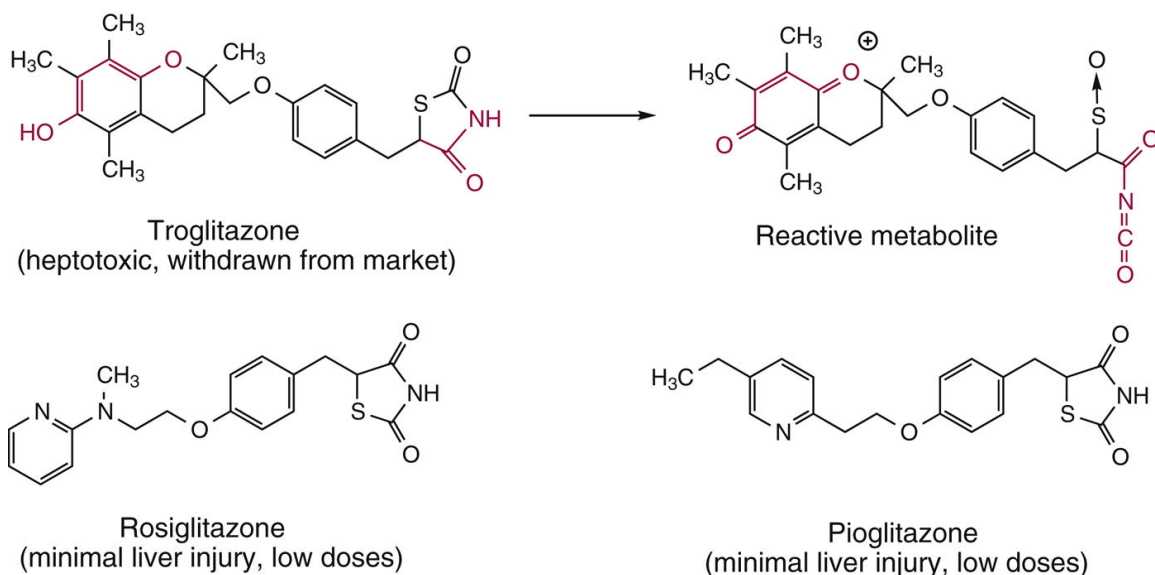


FIGURE 3.42 The effect of structure modification on drug-induced hepatotoxicity of troglitazone.

The oxidation of acetaminophen to the chemically reactive N-acetyl-p-benzoquinoneimine (Fig. 3.36) is catalyzed by the isoforms CYP1A2 and CYP2E1. The reactive quinoneimine can react covalently either with glutathione to form an inactive product or with cellular macromolecules, initiating the processes leading to hepatic necrosis (Fig. 3.36).⁹³ The usual route for acetaminophen metabolism is glucuronidation. If insufficient UDP-glucuronic acid is present, then bioactivation will dominate.

Furosemide, a frequently used diuretic drug, is reportedly a human hepatocarcinogen. The hepatic toxicity apparently results from metabolic activation of the furan ring to a β -dicarbonyl intermediate (5 in Fig. 3.38).⁹³ Ticlopidine and tenoxicam, reported to cause agranulocytosis, do so via metabolic activation of the thiophene ring to an S-oxide (6 in Fig. 3.38).⁹² The agranulocytosis resulting from the ingestion of clozapine is via its bioactivation to a nitrenium ion intermediate (6 in Fig. 3.41).^{93,94}

Drug-Induced Chemical Carcinogenesis

The mechanism whereby xenobiotics are transformed into chemical carcinogens is usually accepted as bioactivation to reactive metabolites, which are responsible for initiating carcinogenicity.^{86,87} Many carcinogens elicit their cytotoxicity through a covalent linkage to DNA. This process

can lead to mutations and, potentially, to cancer. Most chemical carcinogens of concern are chemically inert but require activation by the xenobiotic-metabolizing enzymes before they can undergo reaction with DNA or proteins (cytotoxicity). There are many ways to bioactivate procarcinogens, promutagens, plant toxins, drugs, and other xenobiotics (Fig. 3.43).

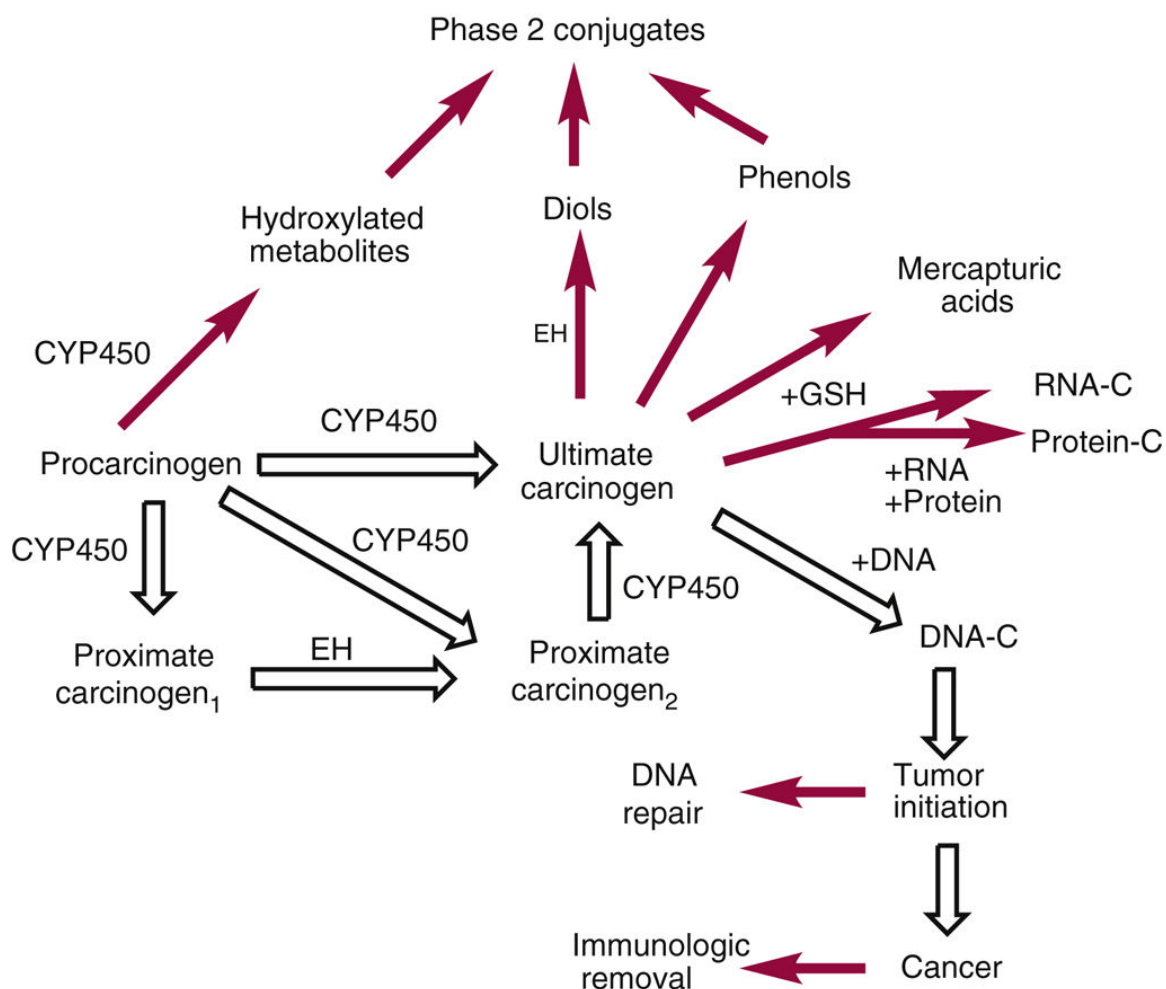


FIGURE 3.43 The bioactivation of procarcinogens and a proposed mechanism of chemical carcinogenesis.

Oxidative bioactivation reactions are by far the most studied and common. Conjugation reactions (Phase 2), however, are also capable of activating these xenobiotics to produce electrophiles, in which the conjugating derivative acts as a leaving group. These reactive metabolites are mostly

electrophiles, such as epoxides, quinones, or free radicals formed by the P450 enzymes or FMO. The reactive metabolites tend to be oxygenated in sterically hindered positions, making them poor substrates for subsequent bioinactivation enzymes, such as epoxide hydrolase and GST. Therefore, their principal fate is formation of covalent linkage to intracellular macromolecules, including enzyme proteins and DNA.^{91,92} Experimental studies indicate that the CYP1A subfamily can oxygenate aromatic hydrocarbons (e.g., PAHs) in sterically hindered positions to arene oxides. Activation by N-hydroxylation of polycyclic aromatic amines (e.g., aryl N-acetamides) appears to depend on either FMO or P450 isoforms. The formation of chemically reactive metabolites is important, because they frequently cause a number of different toxicities, including tumorigenesis, mutagenesis, tissue necrosis, and hypersensitivity reactions.

Our understanding of these reactions was advanced by the studies of the Millers⁸⁶ and Gillette et al.⁸⁷ They proposed that the proportion of the dose that binds covalently to critical macromolecules could depend on the quantity of the reactive metabolite is formed.

A scheme illustrating the complexities of drug-induced chemical carcinogenesis is shown in [Figure 3.43](#). Reactions that proceed via the open arrows eventually lead to neoplasia. Some carcinogens can form the “ultimate carcinogen” directly through P450 isoform bioactivation; others, like the PAHs (e.g., benzo[a]pyrene), appear to involve a multistep reaction sequence forming an epoxide, reduction to a diol by epoxide hydrolase, and perhaps the formation of a second epoxide group on another part of the molecule. Other procarcinogens form the N-hydroxy intermediate that requires transferase-catalyzed conjugation (e.g., O-glucuronide and O-sulfate) to form the “ultimate carcinogen.” The quantity of “ultimate carcinogen” formed should relate directly to the proportion of the dose that binds or alkylates DNA.

The solid-arrow reaction sequences in [Figure 3.43](#) are intended to show detoxification mechanisms, which involve several steps. First, the original chemical can form less active products (phenols, diols, mercapturic acids, and other conjugates). Second, the “ultimate carcinogen” can rearrange to be prevented from its reaction with DNA (or whatever the critical

macromolecule is). Third, the covalently bound DNA can be repaired. Fourth, immunologic removal of the tumor cells can occur. Several mechanisms within this scheme could regulate the quantity of covalently bound carcinogen: (1) The activity of the rate-limiting enzyme, such as epoxide hydrolase, P450 isoform, or one of the transferases, could be involved; (2) the availability of cofactors, such as glutathione, UDP-glucuronic acid, or PAPS, can be rate-limiting; (3) relative P450 activities for detoxification and activation must be considered; (4) availability of alternate reaction sites for the ultimate carcinogen (e.g., RNA and protein can be involved); (5) and possible specific transport mechanisms that deliver either the procarcinogen or its ultimate carcinogen to selected molecular or subcellular sites.

It is well established that numerous organic compounds that are essentially nontoxic as long as their structure is preserved can be converted into cytotoxic, teratogenic, mutagenic, or carcinogenic compounds by normal biotransformation pathways in both animals and humans. The reactive electrophilic intermediate involves reaction with cellular DNA constituents forming either detoxified products or covalent bonding with essential macromolecules, initiating processes that eventually lead to the toxic effect. A better understanding of the mechanisms underlying these reactions can lead to more rational approaches to the development of nontoxic therapeutic drugs. Special attention to risk factors is required for drugs that will be used for long periods in the same patient.

Drug-Drug Interactions

Drug-drug interactions represent a common clinical problem, which has been compounded by the introduction of many new drugs and the expanded use of herbal medicines. Such interactions do significant harm, prompting anywhere from 1.9 to 5 million patient visits to hospital emergency departments per year according to data from the Medication Safety Program at the Centers for Disease Control and Prevention. Approximately 50% of these serious drug-drug interactions involved P450 inhibition. The problem is likely to grow in the future as the population ages and more people take multiple medications.

Drug-drug interactions occur when the efficacy or toxicity of a medication is changed by coadministration of another substance, drug, food (i.e., grapefruit), or herbal product.^{95,96} Pharmacokinetic interactions often occur because of a change in drug metabolism. For example, CYP3A4 oxidizes more than 30% of the clinically used drugs with a broad spectrum of structural features, and its location in the small intestine and liver permits an effect on both presystemic and systemic drug disposition. Some drug-drug interactions with CYP3A4 substrates/inhibitors can also involve inhibition of P-gp. Other clinically important sequelae due to drug-drug interactions resulting from coadministration of CYP3A4 substrates or inhibitors include rhabdomyolysis with the coadministration of some 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors ("statins"), symptomatic hypotension with some dihydropyridine calcium antagonists, excessive sedation from benzodiazepine or nonbenzodiazepine hypnotics, ataxia with carbamazepine, and ergotism with ergot alkaloids.

The clinical importance of any drug-drug interaction depends on factors that are drug-, patient-, and administration-related. Drugs with low oral bioavailability or high first-pass metabolism are particularly susceptible to drug-drug interactions when inhibitors that alter absorption, distribution, and elimination are coadministered. In general, a doubling or more of the plasma drug concentration has the potential for enhanced adverse or beneficial drug response. Less pronounced pharmacokinetic drug interactions can still be clinically important for drugs with a steep concentration-response relationship or narrow therapeutic index. In most

cases, the extent of drug interaction varies markedly among individuals; this is likely to be dependent on interindividual differences in P450 content (polymorphism), preexisting medical conditions, and possibly age. Drug-drug interactions can occur under single-dose conditions or only at steady state. The pharmacodynamic consequences may or may not closely follow pharmacokinetic changes. Drug-drug interactions can be most apparent when patients are stabilized on the affected drug and the P450 substrates or inhibitors are then added to the regimen. One reason for the increased incidence of drug-drug interactions is the practice of simultaneously prescribing several potent drugs, as well as concurrently ingesting nonprescription products and herbal products that compete for, inhibit, or induce metabolizing enzymes.

Although drug-drug interactions constitute only a small proportion of adverse drug reactions, they have become an important issue in health care. Many of the drug-drug interactions can be explained by alterations in the metabolic enzymes in the liver and other extrahepatic tissues, and many of the major pharmacokinetic interactions between drugs are caused by hepatic P450 isoenzymes being affected by coadministration of other drugs. Some drugs act as potent enzyme inducers, whereas others are inhibitors. Drug-drug interactions involving enzyme inhibition, however, are much more common. Understanding these mechanisms of enzyme inhibition or induction is extremely important to appropriately administer multidrug therapies. Individuals at greatest risk for drug-drug interactions and adverse events need to be identified and treated based upon their specific pharmacogenomic-related needs.

P450s have a dominant role in the metabolism and elimination of drugs from the body, and their substrates are shown in [Tables 3.3 and 3.7-3.10](#). Drugs in ***bold italics*** have been associated with clinically relevant drug-drug interactions. Inhibitors of P450 are shown in [Table 3.11](#). Pharmacokinetic interactions can arise when the biotransformation and elimination of a drug are impaired by coadministered drugs. Thus, drugs can compete for biotransformation by a common P450. Adverse drug reactions, including toxicity, can occur if elimination is dependent on a P450 that exhibits defective gene variants. Thus, the genetic makeup of the individual (see

section Genetic Polymorphism) has a major influence on the duration of drug action, as well as on drug efficacy and safety.

P450 pharmacogenetics affects the tendency for certain drug-drug interactions to occur. Thus, the future safe use of drug combinations in patients can require genotyping and phenotyping of individuals before the commencement of therapy. Identification of subjects who metabolize drugs in a different fashion from the general population should minimize the impact of pharmacogenetic variation on drug pharmacokinetics.

Metabolism-Based Enzyme Inhibition

Many drug-drug interactions are the result of inhibition or induction of P450 enzymes. Metabolism-based enzyme inhibition involves mostly reversible competition between two drugs for the enzyme-active site. Metabolic drug-drug interactions occur when drug A (or its metabolite) alters the pharmacokinetics of a coadministered drug B by inhibiting, activating, or inducing the activity of the enzymes that metabolize drug B. Inhibitory drug-drug interactions could result in serious adverse effects, including fatalities in patients receiving multiple medications. This process is competitive and begins with the first dose of the inhibitor, with the extent of inhibition correlating with their relative affinities for the enzymes and the metabolic half-lives of the drugs involved. On the other hand, mechanism-based (irreversible) inhibition results from a metabolite that binds irreversibly to the enzyme through a covalent bond, rendering the enzyme inactive.³⁶

Enzyme-specific P450 inhibitors, including metabolism- and mechanism-based inhibitors, or drugs that are metabolized by specific P450 isoforms, are by and large excluded from further consideration for new drug development. Not only is CYP3A4 the most abundant isoform in human liver, it also metabolizes more than 30% of the drugs in clinical use, which renders CYP3A4 highly susceptible to both metabolism- (reversible) and mechanism-based inhibition. The CYP3A subfamily is involved in many clinically significant drug-drug interactions and therefore metabolism-based inhibition of CYP3A can cause clinically significant drug-drug interactions, such as those involving nonsedating antihistamines and the GI motility stimulant cisapride that can result in cardiac dysrhythmias. An excellent example of a metabolism-based inhibition drug-drug interaction that resulted in a life-

threatening ventricular arrhythmia associated with QT prolongation (torsades de pointes) occurred when CYP3A4 substrates or inhibitors were coadministered with terfenadine, astemizole, cisapride, or pimozide. This potentially lethal drug interaction led to the withdrawal of terfenadine and cisapride from clinical use and to the approved marketing of fexofenadine, the active metabolite of terfenadine, which does not have this interaction.

Inhibitors of CYP3A4 can increase the risk of toxicity from many drugs, including carbamazepine, cyclosporine, ergot alkaloids, lovastatin, protease inhibitors, rifabutin, simvastatin, tacrolimus, and vinca alkaloids. Furthermore, inhibitors of CYP1A2 can increase the risk of toxicity from clozapine or theophylline. Inhibitors of CYP2C9 can increase the risk of toxicity from phenytoin, tolbutamide, and oral anticoagulants (e.g., warfarin). Inhibitors of CYP2D6 can increase risk of toxicity of many antidepressants, opioid analgesics, and psychotherapeutic agents. Examples of enzyme inducers include barbiturates, carbamazepine, glutethimide, griseofulvin, phenytoin, primidone, rifabutin, and rifampin. Some drugs, such as ritonavir, can act as either an enzyme inhibitor or an enzyme inducer, depending on the situation. Drugs metabolized by CYP3A4 or CYP2C9 are particularly susceptible to enzyme induction.

Mechanism-Based Enzyme Inhibition

Mechanism-based inhibition differs from metabolism-based (reversible) inhibition in that the inhibitors require enzymatic activation by the target enzyme prior to exerting their inhibitory effect.³⁶ This initial activation step leads to the formation of an active inhibitor, often referred to as the metabolite-intermediate complex (MIC). The MIC can then exert its inhibitory effect by either forming a direct covalent link with the enzyme or forming a noncovalent tight binding complex. Mechanism-based inhibition is characterized by NADPH-, time-, and concentration-dependent enzyme inactivation, occurring when some drugs are converted by P450s to reactive metabolites.³⁶ Mechanism-based inactivation of CYP3A4 by drugs can be the result of chemical modification of the heme, the apoprotein, or both, because of covalent binding of the modified heme to the protein. The clinical pharmacokinetic effect of a mechanism-based CYP3A4 inhibitor is a function of its enzyme kinetics (i.e., K_m and V_{max}) and the rate of synthesis of new or

replacement enzyme. Predicting drug-drug interactions involving CYP3A4 inactivation is possible when pharmacokinetic principles are followed. Such prediction can become difficult, however, because the clinical outcomes of CYP3A4 inactivation depend on many factors associated with the enzyme, the drugs, and the patients.

Some of the clinically important drugs that have been identified to be mechanism-based CYP3A4 inhibitors include antibacterials (e.g., clarithromycin, erythromycin, isoniazid), anticancer drugs (e.g., irinotecan, tamoxifen, raloxifene), antidepressants (e.g., fluoxetine and paroxetine), anti-HIV agents (e.g., ritonavir and delavirdine), antihypertensives (e.g., dihydralazine and verapamil), steroids and their receptor modulators (e.g., ethinyl estradiol, gestodene, and raloxifene), dihydrotestosterone reductase inhibitors (e.g., finasteride), and some herbal constituents (e.g., bergamottin and glabridin). Drugs inactivating CYP3A4 often possess several common moieties such as a tertiary amine, furan ring, or an acetylene group. The chemical properties of a drug critical to CYP3A4 inactivation include formation of reactive metabolites by P450 isoenzymes, P450 inducers, P-gp substrates, or inhibitors and the occurrence of clinically significant pharmacokinetic interactions with coadministered drugs.

Compared to the more common metabolism-based (reversible) inhibition, mechanism-based inhibitors of CYP3A4 more frequently cause pharmacokinetic-pharmacodynamic drug-drug interactions and are often the cause for unfavorable drug-drug interactions because the inactivated CYP3A4 must be replaced by newly synthesized CYP3A4 protein.^{95,96} The resultant drug interactions may lead to adverse drug effects, including some fatal events. For example, raloxifene, a drug approved for the treatment of osteoporosis and chemoprevention of breast cancer in postmenopausal women at high risk for invasive breast cancer, has demonstrated the ability to act as a mechanism-based inhibitor of CYP3A4 by forming adducts with the apoprotein. It has been established that 3'-hydroxyraloxifene is produced exclusively via CYP3A4-mediated oxygenation to produce a reactive diquinone methide, excluding the alternative arene oxide pathway.

However, predicting drug-drug interactions involving CYP3A4 inactivation is difficult, since the clinical outcomes depend on a number of factors that are associated with drugs and patients. The apparent pharmacokinetic effect of a

mechanism-based inhibitor of CYP3A4 would be a function of its enzyme kinetics and the synthesis rate of new or replacement enzyme. Most CYP3A4 inhibitors are also P-gp substrates/inhibitors, confounding the in vitro to in vivo extrapolation. The clinical significance of CYP3A inhibition for drug safety and efficacy warrants an in-depth understanding of the mechanisms for each inhibitor. Furthermore, such inactivation may be exploited for therapeutic gain in certain circumstances. Clinicians should have good knowledge about these CYP3A4 inhibitors and avoid their combination.

Although CYP2D6 constitutes a relatively minor fraction of the total hepatic P450 content ([Fig. 3.2](#)), the contribution of this isoform is significant because of its role in the metabolism and clearance of many therapeutic agents that target the cardiovascular and CNS ([Fig. 3.3](#)) ([Table 3.8](#)). In addition, clinically significant polymorphisms in the CYP2D6 gene have been identified in a variety of populations with altered metabolic activity as PMs or individuals with impaired enzyme function resulting from splicing defects or gene deletions. On the other hand, EMs (individuals with normal enzyme function) are heterozygous or homozygous for the wild-type allele. In vivo clearance of CYP2D6 substrates in PMs is usually much lower than in EMs, leading to higher plasma concentrations and the potential for clinical toxicities with therapeutic doses. For example, paroxetine is a selective serotonin reuptake inhibitor that is both a substrate for and an inhibitor of CYP2D6. Paroxetine is metabolized by CYP2D6 via the formation of a carbene intermediate of the methylenedioxy group yielding an irreversible complex with CYP2D6 ([Fig. 3.41](#)).⁹⁷ Also, an in vitro study with 3,4-methylenedioxy-methamphetamine (MDMA, ecstasy) suggested that a typical recreational MDMA dose could inactivate most hepatic CYP2D6 within an hour, and the return to a basal level of CYP2D6 could take at least 10 days, impacting its pharmacokinetics.

Because of the pivotal role of P450 isoenzymes in drug metabolism, significant inactivation of these isoforms, and particularly the major human hepatic and intestinal CYP3A4 and hepatic CYP2D6, could result in drug-drug interactions and adverse drug reactions. Compared with the reversible inhibition of CYP3A4 and CYP2D6, mechanism-based inhibitors of these isoenzymes more frequently cause pharmacokinetic/pharmacodynamic drug-drug interactions, as the inactivated isoenzyme has to be replaced by newly

synthesized P450 protein. Pharmacokinetic interactions often occur because of a change in drug metabolism. For example, the macrolide antibiotics increase the plasma concentrations of therapeutic agents that are substrates of CYP3A4 (Table 3.10). Diltiazem has been shown to inhibit the metabolism of a variety of coadministered drugs including carbamazepine, quinidine, midazolam, and lovastatin (Table 3.10). Inhibition of CYP3A by ritonavir explains, in part, the remarkable elevation of blood concentrations and area under the plasma concentration-time curve (AUC) of other concomitantly administered drugs that are extensively metabolized by CYP3A4 and have significant first-pass metabolism. These drugs include rifabutin (400%), clarithromycin (77%), ketoconazole, saquinavir (5000%), amprenavir (210%), nelfinavir (152%), lopinavir (7700%), and indinavir (380%).⁹⁶ Furthermore, such inactivation can be exploited for therapeutic gain in certain circumstances, e.g., extended duration of action of the protease inhibitors ritonavir/indinavir used in the treatment of AIDS.

Beneficial Drug-Drug Interactions

By understanding the unique functions and characteristics of these isoenzymes, health care practitioners can better anticipate and manage drug-drug interactions. They can also predict or explain an individual's response to a particular therapeutic regimen. A beneficial drug interaction, for example, is the coadministration of a CYP3A4 inhibitor with cyclosporine. By inhibiting CYP3A4, the plasma concentrations of cyclosporine (a CYP3A4 substrate) are increased which allows a reduction of the cyclosporine dosage thereby improving clinical efficacy and reducing its cost. Similarly, certain HIV protease inhibitors, such as saquinavir, have a low oral bioavailability due to intestinal CYP3A4 metabolism. The oral bioavailability of saquinavir can be profoundly increased by the addition of a low dose of the mechanism-based CYP3A4 inhibitor, ritonavir. This concept of altering drug pharmacokinetics by adding a low, subtherapeutic dose of a mechanism-based CYP3A4 inhibitor (ritonavir) to increase the oral bioavailability of another protease inhibitor, lopinavir (CYP3A4 substrate), led to the marketing of Kaletra, a new drug combination of lopinavir and ritonavir. Another beneficial mechanism-based inhibition is of the P2Y₁₂ receptor (a protein found on the surface of blood platelet cells and an important

regulator in blood clotting) by the antiaggregating platelet prodrugs, clopidogrel and prasugrel. Clopidogrel is bioactivated to its reactive metabolite by CYP2C19, CYP1A2, and CYP3A4, whereas prasugrel is bioactivated by CYP3A4 and CYP2B6. The bioactivated metabolites can then react with the P2Y12 receptor protein.

Grapefruit Juice-Drug Interactions

Historical Significance of Grapefruit Juice

The discovery that grapefruit juice can markedly increase the oral bioavailability of CYP3A4 drugs was based on an unexpected observation from an interaction study between the dihydropyridine calcium channel antagonist felodipine and ethanol in which grapefruit juice was used to mask the taste of the ethanol. Subsequent investigations confirmed that grapefruit juice significantly increased the oral bioavailability of felodipine by reducing presystemic felodipine metabolism through selective inhibition of CYP3A4 expression in the intestinal wall.⁹⁸

Grapefruit juice is a beverage often consumed at breakfast for its health benefits and to mask the taste of drugs or foods. Unlike other citrus fruit juices, however, grapefruit juice can significantly increase the oral bioavailability of drugs that are metabolized primarily by intestinal CYP3A4, causing an elevation in their serum concentrations (Table 3.20). Those drugs with high oral bioavailabilities (>60%), however, are all likely safe to be taken with grapefruit juice because their high oral bioavailability leaves little room for elevation by grapefruit juice. The importance of the interaction appears to be influenced by individual patient susceptibility, type and amount of grapefruit juice, and administration-related factors.

TABLE 3.20
Some CYP3A4 Substrates and Interactions With Grapefruit Juice

Drug	Interaction ^a	Drug	Interaction ^a	Drug	Interaction ^a
Calcium channel blockers		HIV protease inhibitors		CNS drugs	
Amlodipine	Y	Indinavir	N?	Buspirone	Y
Felodipine	Y	Nelfinavir	N?	Carbamazepine	Y
Nifedipine	Y	Ritonavir	N?	Diazepam	Y
Nimodipine	Y	Saquinavir	Y	Midazolam	Y
Nisoldipine	Y	Macrolides		Triazolam	Y

Drug	Interaction ^a	Drug	Interaction ^a	Drug	Interaction ^a
Nitrendipine	Y	Clarithromycin	N	Immunosuppressants	
Pranidipine	Y	HMG-CoA reductase inhibitors		Cyclosporine	Y
Antiarrhythmics		Atorvastatin	Y	Tacrolimus	Y?
Diltiazem	N	Fluvastatin	N?	Other	
Verapamil	N	Lovastatin	Y	Methadone	Y
Quinidine	N	Pravastatin	N?	Sildenafil	Y
Antihistamines		Simvastatin	Y		
Ebastine	Y?				
Loratadine	Y?				

^a Y (yes) and N (no) indicate published evidence of the presence or absence of an interaction with grapefruit juice. Y? and N? indicate expected findings based on available data. Those drugs with Y or Y? should not be consumed with grapefruit juice in an unsupervised manner.

Grapefruit juice can alter oral drug pharmacokinetics by different mechanisms. Irreversible inactivation of intestinal CYP3A4, which can persist up to 24 hours, is produced by grapefruit juice given as a single, normal, 200- to 300-mL drink or by whole fresh fruit segments (Table 3.20). As a result, presystemic metabolism is reduced and oral drug bioavailability increased. Enhanced oral drug bioavailability can occur up to 24 hours after juice consumption, so taking CYP3A4 vulnerable drugs with water or other liquids within 24 hours of consuming grapefruit juice can still result in an interaction. Inhibition of P-gp is a possible mechanism by which grapefruit juice, and perhaps grapefruit oil, increases oral drug bioavailability by reducing intestinal and/or hepatic efflux transport. Inhibition of organic anion-transporting polypeptides by grapefruit juice and apple juice has been observed; intestinal uptake transport appeared to be decreased as oral drug bioavailability was reduced.

Numerous medications used in the prevention or treatment of coronary artery disease and its complications have been observed or predicted to interact with grapefruit juice. Such drug-drug interactions can increase the risk of rhabdomyolysis when dyslipidemia is treated with the HMG-CoA reductase inhibitors ("statins"). Such drug-drug interactions could also cause excessive vasodilatation when hypertension is managed with the dihydropyridines felodipine, nifedipine, nisoldipine, or nitrendipine. An alternative agent could be amlodipine. The therapeutic effect of the angiotensin II type I receptor antagonist losartan can be reduced

by grapefruit juice. Grapefruit juice interacting with the antidiabetic agent repaglinide can cause hypoglycemia, and interaction with the appetite suppressant sibutramine can cause elevated blood pressure and heart rate. In angina pectoris, administration of grapefruit juice could result in atrioventricular conduction disorders with verapamil or attenuated antiplatelet activity with clopidogrel. Grapefruit juice can enhance the drug toxicity for antiarrhythmic agents, such as amiodarone, quinidine, disopyramide, or propafenone and for the congestive heart failure drug carvedilol. Some drugs used for the treatment of peripheral or central vascular disease also have the potential to interact with grapefruit juice. Interaction with sildenafil, tadalafil, or vardenafil for erectile dysfunction can cause serious systemic vasodilatation, especially when combined with a nitrate. In stroke, interaction with nimodipine can cause systemic hypotension.

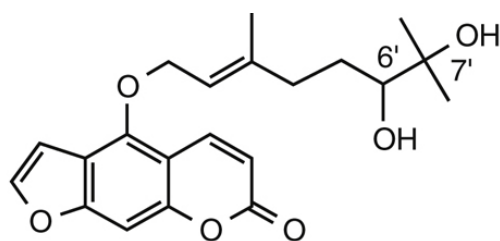
If a drug has low inherent oral bioavailability from presystemic metabolism by CYP3A4 or efflux transport by P-gp and the potential to produce serious overdose toxicity, avoidance of grapefruit juice entirely during pharmacotherapy appears mandatory. Although altered drug response is variable among individuals, the outcome is difficult to predict and avoiding the combination will guarantee that toxicity is prevented.

The mechanism by which grapefruit juice produces its effect is through inhibition of the enzymatic activity and a decrease in the intestinal expression of CYP3A4. The P-gp efflux pump also transports many CYP3A4 substrates; thus, the presence of inhibitors of P-gp in grapefruit juice (e.g., 6',7'-dihydroxybergamottin and other furanocoumarins) could be a related factor for drug-grapefruit juice interactions.⁹⁹ Numerous studies have shown that grapefruit juice consumed in normal (single glass) quantities acts on intestinal CYP3A4, not at the hepatic level.

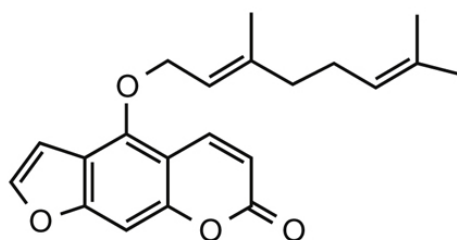
Does the quantity of juice matter? The majority of the presystemic CYP3A4 inhibition is obtained following ingestion of one glass of grapefruit juice; however, 24 hours after ingestion of a glass of grapefruit juice, 30% of its effect is still present.⁹⁹ The reduction in intestinal CYP3A4 concentration is rapid: a 47% decrease occurred in a healthy volunteer within 4 hours after consuming grapefruit juice. Daily ingestion of grapefruit juice results in a loss of CYP3A4 from the small intestinal epithelium. Consumption of very large

quantities of grapefruit juice (six to eight glasses/day) can lead to inhibition of hepatic CYP3A4.

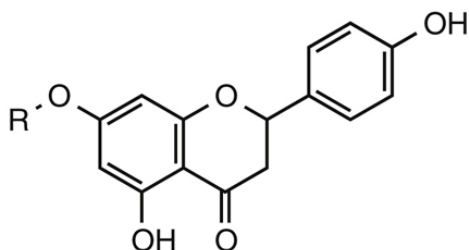
The active constituents found in grapefruit juice responsible for its effects on CYP3A4 include flavonoids (e.g., naringenin and naringin) and furanocoumarins (e.g., bergamottin and 6',7'-dihydroxybergamottin).⁹⁰



6',7'-Dihydroxybergamottin



Bergamottin



Naringin R: rhamnose-glucoside-
Naringenin R: H-

The majority of studies to date have used either freshly squeezed grapefruit juice, reconstituted frozen juice, commercial grapefruit juice, grapefruit segments, or grapefruit extract; all are capable of causing drug-drug interactions with CYP3A4 substrates (blended grapefruit juices have not yet been investigated). The active constituents in grapefruit juice are present not just in the juice but also in the pulp, peel, and core of the fruit and are responsible for its flavor. Bergamottin and 6',7'-dihydroxybergamottin are potent mechanism-based inhibitors of CYP3A4 and naringenin isomers are competitive inhibitors of CYP3A4. Higher concentrations of 6',7'-dihydroxybergamottin are present in grapefruit segments. Thus, any therapeutic concern for a drug interaction with grapefruit juice should now be extended to include whole fruit and other products derived from the grapefruit peel. The difference in the in vitro CYP3A4 inhibition between grapefruit juice and orange juice is that orange juice contained no measurable amounts of 6',7'-dihydroxybergamottin. The nutraceutical grapefruit oil

contains minor amounts of bergamottin, therefore grapefruit oil has the potential to cause drug interactions with CYP3A4 substrates.

If a patient has been taking medication with grapefruit juice for some time without ill effects, is it safe to continue to do so? Much of this unpredictability results from the inconsistency of the juice concentrations and the sporadic manner in which grapefruit juice is consumed, suggesting that this approach cannot be entirely safe. Given the unpredictability of the effect of grapefruit juice on the oral bioavailability of the drugs in [Table 3.20](#), patients should be advised to avoid this combination, thus preventing the onset of potential adverse effects. Each patient's situation should be considered, and advice should be based on consumption history and the specific medications involved. The benefits of increased and controlled drug bioavailability by grapefruit juice can, in the future, be achieved through either standardizing the constituents or coadministration of the isolated active ingredients. This would then lead to a safe, effective, and cost-saving means to enhance the absorption of many therapeutic agents.

P-Glycoprotein-Drug Interactions

P-gp-mediated transport has an important role in pharmacokinetic-mediated drug-drug interactions. The effect of P-gp inhibition is to increase the oral bioavailability of CYP3A4 substrates so that the later actions of CYP3A4 inhibition will be increased. One of the best examples is the interaction between digoxin and quinidine. Quinidine blocks P-gp in the intestinal mucosa and in the proximal renal tubule; thus, digoxin elimination into the intestine and urine is inhibited, increasing the plasma digoxin concentration to toxic levels. Another example is loperamide, which is an opioid antidiarrheal normally kept out of the brain by the P-gp pump. Inhibition of P-gp allows accumulation of loperamide in the brain, leading to respiratory depression as a result of central μ opioid receptor agonism.

The components of grapefruit juice reportedly inhibit P-gp, and this can be one of the mechanisms for the increase in bioavailability of drugs that are substrates for P-gp.

Drug-Dietary Supplement Interactions

The increasing use of dietary supplements presents a special challenge in health care; thus, there is an increasing need to predict and avoid these potential adverse drug-dietary supplement interactions.⁹⁹ The present interest and widespread use of herbal remedies has created the possibility of interaction between them and over-the-counter or prescription drugs if they are used simultaneously. As herbal medicines become more popular, herbal hepatotoxicity is being increasingly recognized. Females appear to be predisposed to hepatotoxicity, and coadministered agents that induce P450 enzymes (e.g., St. John's wort) can also increase individual susceptibility to some dietary supplements. Nearly one in

five adults taking prescription medicines is also taking at least one dietary supplement. The mechanisms for drug-dietary supplement interactions are similar to those for drug-drug interactions affecting the pharmacokinetics of the respective drug. Little is known regarding the pharmacokinetic properties of many of the substances in dietary supplements. Therefore, the potential for drug-dietary supplement interactions has greatly increased.

A commonly reported drug-dietary supplement interaction is between St. John's wort and HIV protease inhibitors, leading to drug resistance and treatment failure. ^{[1][SEP]}St. John's wort is a popular dietary supplement often used for depression. Of the two substances found in St. John's wort, hypericin and hyperforin, hyperforin appears to be the main constituent, with in vitro selective serotonin reuptake inhibitor (SSRI) activity. Hyperforin appears also to be the more potent inducer of CYP3A enzymes based on in vitro and in vivo studies.

Other Dietary Supplements Exhibiting Drug-Induced Hepatotoxicity

DHEA and androstenedione are dietary testosterone precursors that have been associated with hepatic toxicity and should not be taken by those with hepatic disease. Co-ingestion with other potentially hepatotoxic products or enzyme inducers that might increase the risk of liver damage should also be avoided.

Black cohosh, commonly used by women for menopausal symptoms, including hot flashes and sleep disorders, forms quinone metabolites in vitro, but no mercapturate conjugates were detected in urine samples from women who consumed multiple oral doses of up to 256 mg of a standardized black cohosh extract. At moderate doses of black cohosh, the risk of liver injury is minimal.

Silybum marianum (milk thistle) is used in the treatment of chronic or acute liver disease, as well as in protecting the liver against toxicity. *Silybum* is cited as one of the oldest known herbal medicines. The active constituents of milk thistle are flavonolignans, which are known collectively as silymarin.

Miscellaneous Drug-Drug Interactions

The ability of drugs and other foreign substances to induce metabolism of other drugs has already been discussed. Phenobarbital, for example, stimulates metabolism of a variety of drugs (e.g., phenytoin and coumarin anticoagulants). Stimulation of bishydroxycoumarin metabolism can create a problem in patients undergoing anticoagulant therapy. If phenobarbital administration is stopped, the rate of metabolism of the anticoagulant decreases, resulting in greater plasma concentrations of bishydroxycoumarin and enhanced anticoagulant activity, increasing the possibility of hemorrhage. Serious side effects have resulted from this type of interaction. These observations indicate that combined therapy of a potent drug (e.g., bishydroxycoumarin) and an inducer of drug metabolism (e.g., phenobarbital) can create a hazardous situation if the enzyme inducer is withdrawn and therapy with the potent drug is continued without an appropriate decrease in dose.

Serious reactions have been reported in patients treated with an MAO inhibitor such as tranylcypromine because they are, as a rule, sensitive to a subsequent dose of a sympathomimetic amine (e.g., amphetamine) or a tricyclic antidepressant (e.g., amitriptyline), which is metabolized by MAO.

Allopurinol, a xanthine oxidase inhibitor used for the treatment of gout, inhibits metabolism of 6-mercaptopurine and other drugs metabolized by this enzyme. A serious drug interaction results from the concurrent use of allopurinol for gout and 6-mercaptopurine to

block the immune response from a tissue transplant or as antimetabolite in neoplastic diseases.

Gender Differences in Drug Metabolism

Although numerous gender differences have been described in humans, so far most clinical research has been carried out with the questionable view that the male can fulfill the function of the true representative of the human species. However, in spite of the increasing evidence pointing out physiological and pathological differences between the sexes, beyond those related to reproduction, women differ from men in gene expression and regulation, in the susceptibility to and risk for many medical conditions, and in the response to numerous drugs.^{100,101} Gender difference in drug response may explain, at least in part, the interindividual variations occurring in therapeutic response and toxicity, especially considering that female sex has been shown to be a risk factor for the development of adverse drug reactions (ADRs). Nonetheless, current studies indicate that gender differences in pharmacological response are more widespread than originally believed and involve pharmacodynamics and pharmacokinetics, with pharmacokinetic being the most investigated.

The role of pharmacokinetics versus pharmacodynamics is not yet completely appreciated, and only a few contributions have evaluated the impact of genetics, hormonal variations, and their relative interactions.¹⁰⁰ The role of gender as a contributor to variability in xenobiotic metabolism and ADRs, which are more common in women than in men, is not clear, but increasing numbers of reports show differences in metabolism between men and women, raising the intriguing possibility that endogenous sex hormones, hydrocortisone, or their synthetic equivalents can influence the activity of inducible CYP3A. N-demethylation of erythromycin was significantly higher in females than males, which was persistent

throughout adulthood. In contrast, males exhibited unchanged N-demethylation values.

Gender-dependent differences of metabolic rates have been detected for some drugs. Side-chain oxidation of propranolol was 50% faster in males than in females, but no differences between genders were noted in aromatic ring hydroxylation. N-demethylation of meperidine was depressed during pregnancy and for women taking oral contraceptives. CYP1A2 shows a higher activity in men, and therefore, clearance of antidepressant, antipsychotic drugs, and theophylline is faster in men than in women. CYP1A2 activity is decreased during pregnancy. Other examples of drugs cleared by CYP3A4 more rapidly in men than in women include chlordiazepoxide and lidocaine. Diazepam, prednisolone, caffeine, and acetaminophen are metabolized slightly faster by women than by men. No gender differences have been observed in the clearance of phenytoin, nitrazepam, and trazodone, which interestingly are not substrates for the CYP3A subfamily. CYP3A4 activity is higher in women than in men. Drugs such as cyclosporine, erythromycin, nimodipine, and cortisol are substrates of CYP3A4, showing faster clearance among women. Although CYP3A4 is also responsible for about 60% of CYP-mediated metabolism of zolpidem, its overall clearance is actually slower in women than in men, requiring a FDA-recommended 50% dose reduction. Gender differences in the rate of glucuronidation have been noted. Men show a faster clearance of drugs that are primarily eliminated by glucuronidation. Thus, oxazepam, metabolized mainly by UGT2B15, has a longer half-life in women than in men. Gender differences are also found in other pharmacokinetic parameters such as drug absorption, drug distribution, and excretion.

Historically, women were less enrolled in clinical trials because both the pharmacokinetics and pharmacodynamics of a drug can be influenced by menstrual cycle phases, hormonal fluctuations, use of

oral contraceptives, and hormonal therapy. There was also concern about life events such as pregnancy and lactation, including the potential negative impact of investigational drugs on the fetus or nursing neonate.¹⁰⁰ The number of trials enrolling women has increased after an FDA request to include a fair representation of both genders as participants, but overall, women are still underrepresented. Women are generally treated with doses that essentially reflect the results obtained by trials carried out mainly in men.

Despite the differences in drug pharmacokinetics between men and women, sex-specific recommendations in dosage do not exist for most drugs.¹⁰¹ Pharmacists need to recognize the underrepresentation of women in clinical trials and take the responsibility to inform consumers and emphasize to clinicians that women can differ significantly from men with respect to metabolism, absorption, distribution, and excretion of drugs. Pharmacists also need to be aware that pregnancy, oral contraceptive use, and hormonal replacement therapy can significantly change drug metabolism and drug clearance. If a woman consistently experiences more adverse drug events or less therapeutic effect from a particular drug, it may be necessary to discuss with her physician the possibility of changing the dosing regimen or switching to a different medication. Even in postmenopausal women, CYP3A function can be altered and influenced by the lack of estrogen or the presence of androgens.

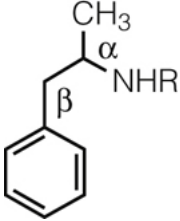
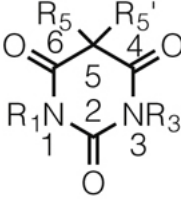
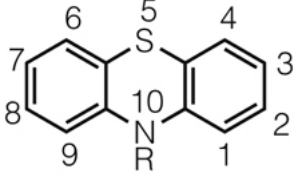
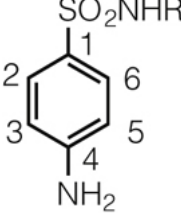
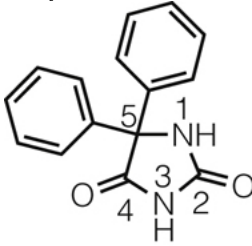
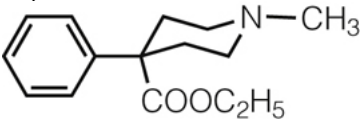
Compared to men, there are more women in the population, more women with chronic diseases, and more women visiting physicians. It is, therefore, obvious that there needs to be more interest in knowing how women react to drugs. Ultimately, a better understanding of sex-related influences on drug responses will help to improve drug safety and efficacy and will also permit to

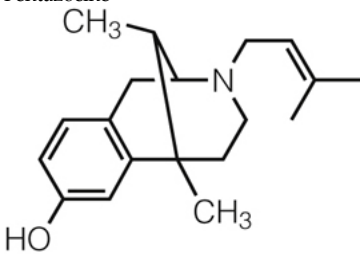
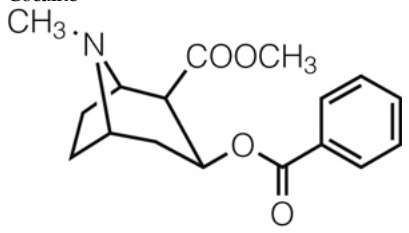
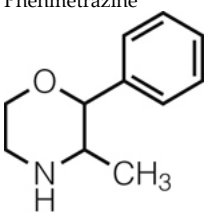
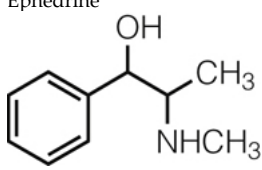
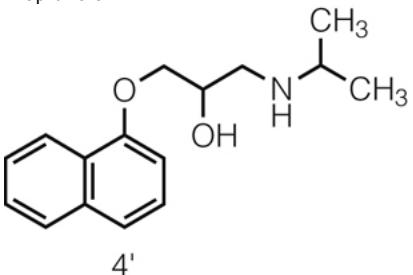
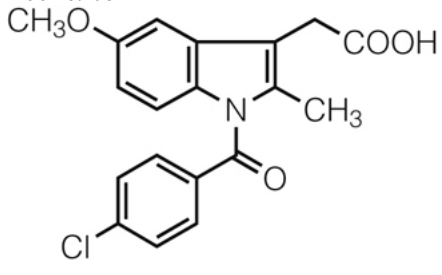
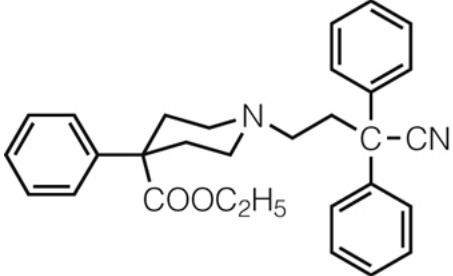
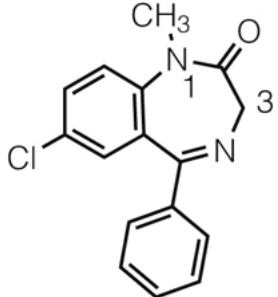
practitioners to “tailor” pharmacological treatments both in men and women.

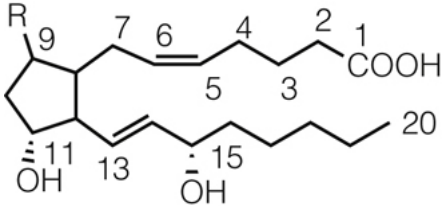
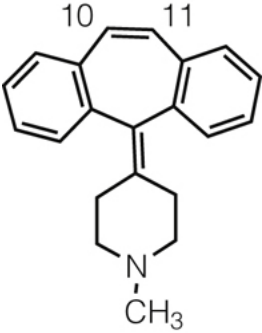
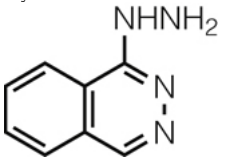
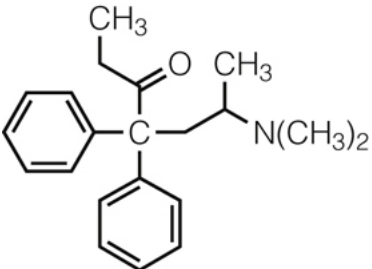
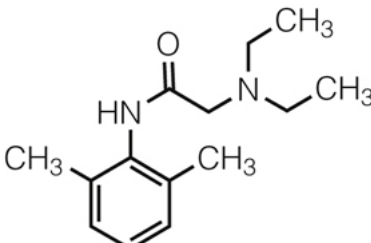
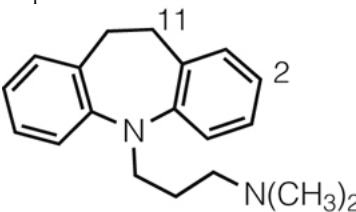
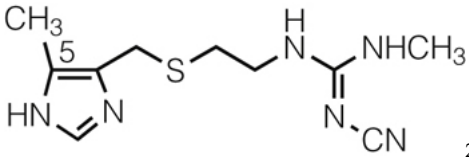
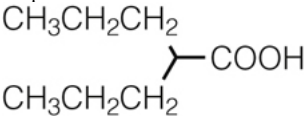
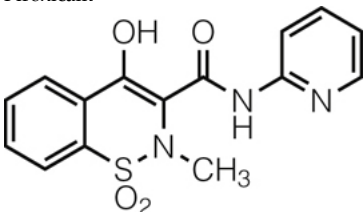
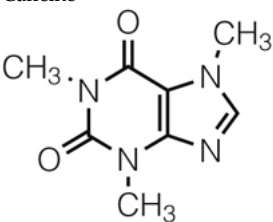
Major Pathways of Metabolism

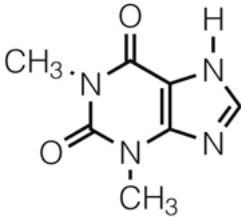
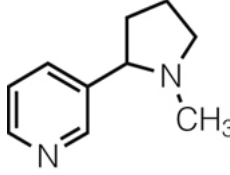
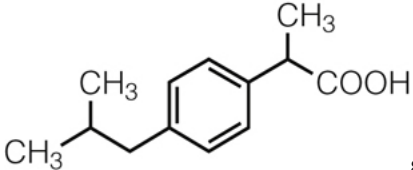
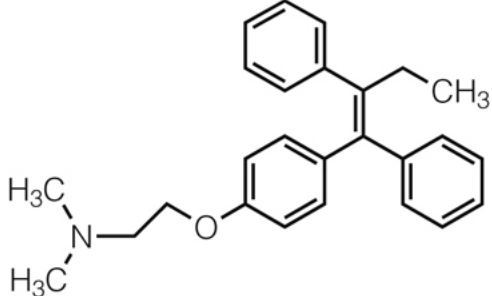
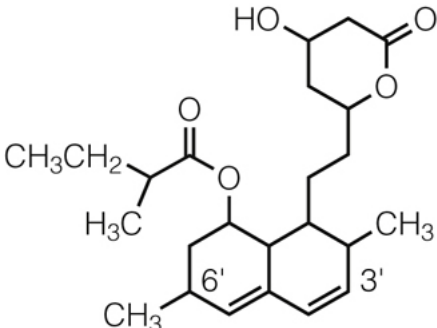
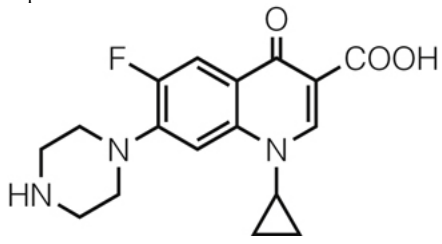
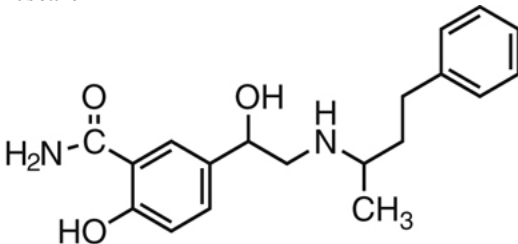
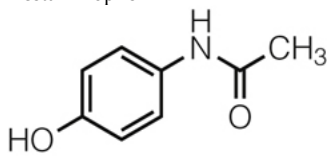
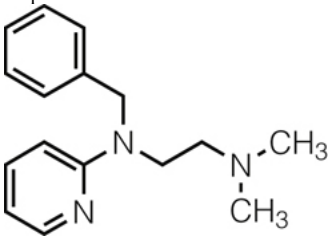
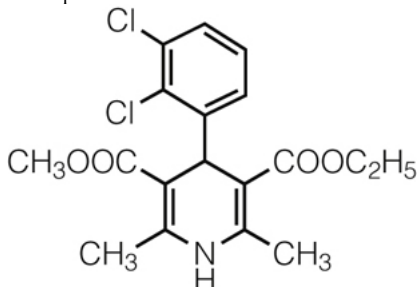
Table 3.21 contains an extensive list of commonly used drugs and the P450 isoforms that catalyze their metabolism. In addition, Phase 1 and Phase 2 metabolic pathways for some common drugs are listed in Table 3.21.

TABLE 3.21
Metabolic Pathways of Common Drugs

Drug	Pathway	Drug
Amphetamines 	Deamination (followed by oxidation and reduction of the ketone formed) N-oxidation N-dealkylation Hydroxylation of the aromatic ring Hydroxylation of the β-carbon atom Conjugation with glucuronic acid of the acid and alcohol products from the ketone formed by deamination	Barbiturates 
Phenothiazines 	N-dealkylation in the N10 side chain N-oxidation in the N10 side chain Oxidation of the heterocyclic S atom to sulfoxide or sulfone Hydroxylation of one or both aromatic rings Conjugation of phenolic metabolites with glucuronic acid or sulfate Scission of the N10 side chain	Sulfonamides 
Phenytoin 	Hydroxylation of one aromatic ring Conjugation of phenolic products with glucuronic acid or sulfate Hydrolytic scission of the hydantoin ring at the bond between carbons 3 and 4 to give 5,5-diphenylhydantoic acid	Meperidine 

Drug	Pathway	Drug
Pentazocine 	Hydroxylation of terminal methyl groups of the alkenyl side chain to give <i>cis</i> and <i>trans</i> (major) alcohols Oxidation of hydroxymethyl product of the alkenyl side chain to carboxylic acids Reduction of alkenyl side chain and oxidation of terminal methyl group	Cocaine 
Phenmetrazine 	Oxidation to lactam Aromatic hydroxylation N-oxidation Conjugation of phenolic products	Ephedrine 
Propranolol 	Aromatic hydroxylation at C4' N-dealkylation Oxidative deamination Oxidation of deaminated product to naphthoxylactic acid Conjugation with glucuronic acid O-dealkylation	Indomethacin 
Diphenoxylate 	Hydrolysis of ester to acid Hydroxylation of one aromatic ring attached to the N-alkyl side chain	Diazepam 

Drug	Pathway	Drug
Prostaglandins 	Reduction of double bonds at carbons 5 and 6 and 13 and 14 Oxidation of 15-hydroxyl to ketone β -Oxidation of carbons 3, 5 and 7 ω -Oxidation of carbon 20 to acid	Cyproheptadine 
Hydralazine 	N-acetylation with cyclization to a methyl-S-triazolophthalazine N-formylation with cyclization to an S-triazolophthalazine Aromatic hydroxylation of benzene ring Oxidative loss of hydrazinyl group to 1-hydroxy Hydroxylation of methyl of methyl-S-triazolophthalazine Conjugation with glucuronic acid	Methadone 
Lidocaine 	N-dealkylation Oxidative cyclization to a 4-imidazolidone N-oxidation of amide N Aromatic hydroxylation ortho to methyl Hydrolysis of amide	Imipramine 
Cimetidine 	S-oxidation Hydroxylation of 5-methyl	Valproic acid 
Piroxicam 	Pyridine 3'-hydroxylation Hydrolysis of amide Decarboxylation	Caffeine 

Drug Theophylline 	Pathway N3-demethylation N1-demethylation C8 oxidation to uric acids Imidazole ring opened 1-Me xanthine to 1-Me uric acid – xanthine oxidase	Drug Nicotine 
Ibuprofen 	CoA thioester and epimerization of R- to S+ enantiomer Methyl hydroxylation to CH₂OH CH₂OH to COOH Acylglucuronide	Tamoxifen 
Lovastatin 	6'-Hydroxylation 3'-Side chain hydroxylation 3'-Hydroxylation β-oxidation of lactone O-glucuronides	Ciprofloxacin 
Labetalol 	O-sulfate (major) O-glucuronide	Acetaminophen 
Tripelennamine 	<i>p</i>-Hydroxylation Benzylic C-hydroxylation N-depyridinylation N-debenzylation	Felodipine 

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Acetylation

Acetylation is principally a reaction of amino groups involving the transfer of acetyl CoA to primary aliphatic and aromatic amines, amino acids, hydrazines, or sulfonamide groups. The liver is the primary site of acetylation, although extrahepatic sites have been identified. Antimicrobial sulfonamides (e.g., sulfisoxazole), being difunctional, can form either N1 or N4 acetyl derivatives. Secondary amines are not acetylated. Acetylation can produce conjugates that retain the pharmacological activity of the parent drug (e.g., N-acetylprocainamide) ([Fig. 3.32](#)).

Acetylation, a nonmicrosomal form of metabolism, also exhibits polymorphisms and was first demonstrated in the acetylation of isoniazid. Several forms of N-acetyltransferase (NAT1 and NAT2) occur in humans. NAT1 (arylamine N-acetyltransferase) is widely distributed and catalyzes the N-acetylation of acidic arylamines, such as *p*-aminobenzoic acid and *p*-aminosalicylic acid; therefore its expression levels in the body have toxicological importance with regard to drug toxicity and cancer risk (e.g., bladder cancer and colorectal cancer).

NAT2 is found predominately in the liver. Clinically used drugs undergoing NAT2-catalyzed N-acetylation include isoniazid, procainamide, hydralazine, phenelzine, dapsone, caffeine, and the carcinogenic secondary N-alkylarylamines (2-aminofluorene, benzidine, and 4-aminobiphenyl). Substituents ortho to the amino group sterically block acetylation. Polymorphisms in the NAT2 gene in human populations can be segregated into fast, intermediate, and slow acetylator phenotypes. Polymorphisms in NAT2 are also associated with higher incidences of cancer and drug toxicity. Intestinal N-acetyltransferase appears not to be polymorphic (i.e., 5-aminosalicylic acid). The proportion of the fast acetylation phenotype is approximately 30%-45% in Caucasians, 89%-90% in the Oriental population, and 100% in Canadian Eskimos. Drug-induced systemic lupus erythematosus from chronic procainamide therapy is more likely to appear with slow acetylators.

Acetylation polymorphism has been associated with differences in human drug toxicity between slow and fast acetylator phenotypes. Slow

acetylators are more prone to drug-induced toxicities and accumulate higher blood concentrations of the unacetylated drug than do fast acetylators. Examples of toxicities that can result include hydralazine- and procainamide-induced lupus erythematosus, isoniazid-induced peripheral nerve damage, and sulfasalazine-induced hematologic disorders. Fast acetylators eliminate the drug more rapidly by conversion to its relatively nontoxic N-acetyl metabolite. However, for some drug substances, fast acetylators can pose a greater risk of liver toxicity than slow acetylators because fast acetylators produce toxic metabolites more rapidly. The possibility arises that genetic differences in acetylating capacity can confer differences in susceptibility to chemical carcinogenicity from arylamines. The tumorigenic activity of arylamines (1 in Fig. 3.33) can be the result of a complex series of sequential metabolic reactions commencing with N-acetylation (2 in Fig. 3.33), subsequent oxidation to arylhydroxamic acids (3 in Fig. 3.33), and metabolic transformation to acetoxarylamine by N,O-acyltransferase (4 in Fig. 3.33).⁵⁴ The acetoxarylamine can eliminate the acetox group to form the reactive arylnitrenium ion (5 in Fig. 3.33), which is capable of covalently binding to nucleic acids and proteins, thus increasing the risk for development of bladder and liver tumors.⁵⁴ The rapid acetylator phenotype is expected to form the acetoxarylamine metabolite at a greater rate than the slow acetylator and thereby, to present a greater risk for development of tumors compared with the slow acetylator.

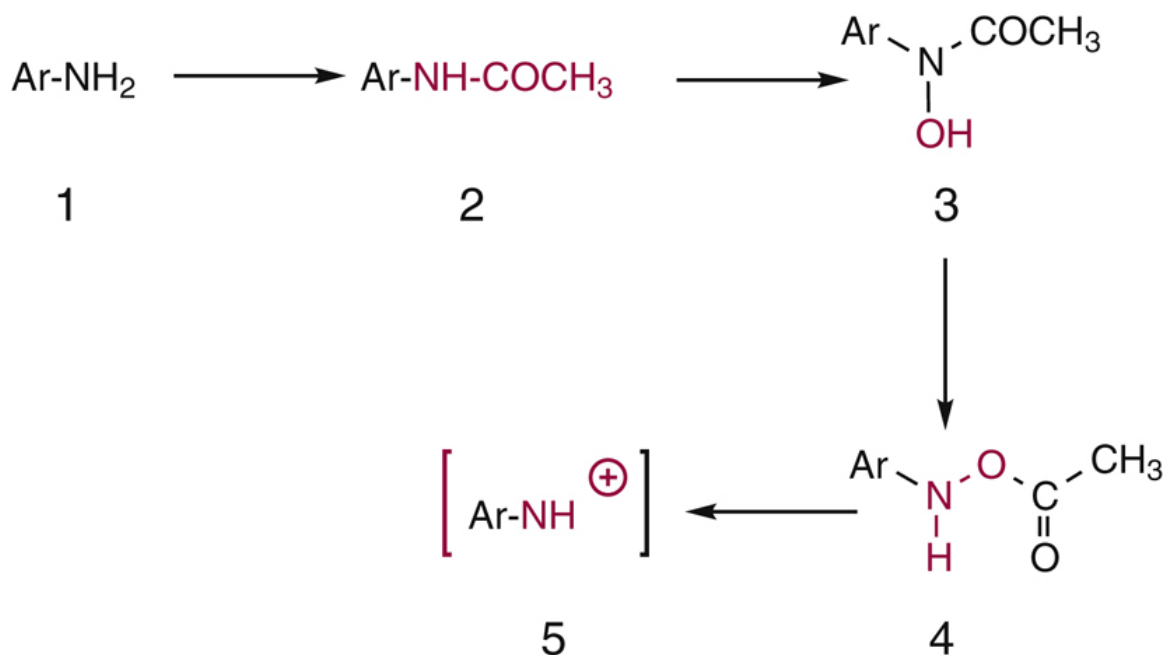


FIGURE 3.33 Bioactivation of acetylated arylamines.

Glutathione Conjugation and Mercapturic Acid Synthesis

Mercapturic acids are S-derivatives of N-acetylcysteine synthesized from glutathione (GSH).^{5,58,59} The mercapturic acid pathway appears to have evolved as a protective (scavenger) mechanism against xenobiotic-induced hepatotoxicity or carcinogenicity, serving to detoxify a large number of noxious substances that we inhale or ingest or that are produced daily in the human body. Most xenobiotics that are metabolized to mercapturic acids first undergo conjugation with GSH catalyzed by the enzyme glutathione S-transferase (GST), a multigene isoenzyme family that is abundant in the soluble supernatant liver fractions. In humans, the GST family consists of cytosolic dimeric isoenzymes of 45-55 kDa size that have been assigned to at least seven classes: alpha (five members), kappa (one member), mu (six members), omega (two members), pi (one member), theta (two members), zeta (one member), and microsomal (three members). The principal drug substrates for the mu family are the nitrosourea and mustard-type anticancer drugs. The theta isoform metabolizes small organic molecules, such as solvents, halocarbons, and electrophilic compounds (e.g., α,β -unsaturated carbonyl compounds). The

GSH conjugation reaction to mercapturic acid metabolites is depicted in Figure 3.34.

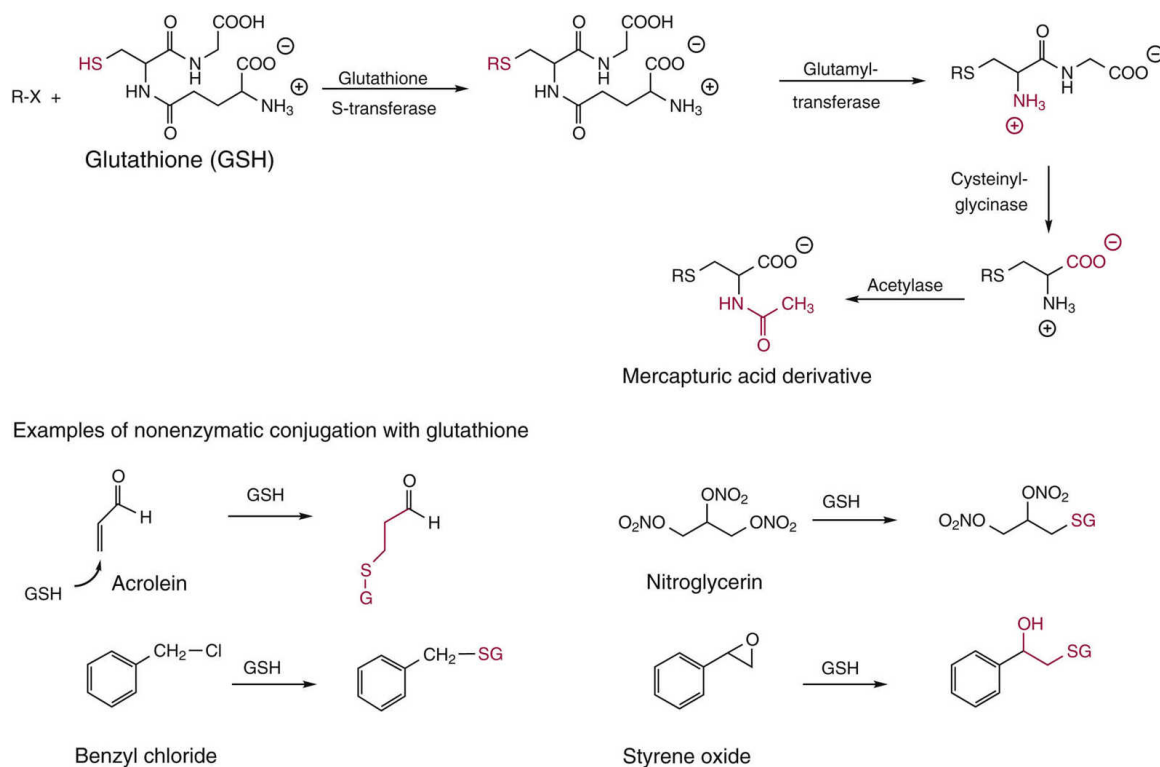


FIGURE 3.34 Glutathione and mercapturic acid conjugation pathways.

GST increases the ionization of the thiol group of GSH, increasing its nucleophilicity toward electrophiles (a group or ion that accepts an electron pair to make a covalent bond such as a carbocation or acyl ion) and thereby increasing the rate of conjugation with these potentially harmful electrophiles. In this way, GSH conjugation protects other vital nucleophilic centers in the cell, such as nucleic acids and proteins, from these electrophiles. Glutathione is also capable of reacting nonenzymatically with nucleophilic sites on neighboring macromolecules (Fig. 3.34) (see also section Metabolic Activation). Once conjugated with GSH, the electrophiles are excreted in the bile and urine.

A range of functional groups yields thioether conjugates of GSH, as well as products other than thioethers (Fig. 3.34). The nucleophilic attack by GSH occurs on electrophilic carbons with leaving groups (e.g., halogen [alkyl, alkenyl, aryl, or aralkyl halides], sulfonates [alkylmethanesulfonates] and

nitro [alkyl nitrates] groups), ring opening of small ring ethers (epoxides and β -lactones, e.g., β -propiolactone), and the Michael-type addition to the activated β -carbon of an α,β -unsaturated carbonyl compound (e.g., acrolein) (see section Reactive Metabolites Resulting from Bioactivation). The lack of substrate specificity gives argument to the fact that glutathione transferase has undergone adaptive changes to accommodate the variety of xenobiotics to which it is exposed. The conjugation of an electrophilic compound with GSH is usually a reaction of detoxication, but in some cases carcinogens have been activated through conjugation with GSH.^{58,59} The enzymatic conjugation of GSH with epoxides provides a mechanism for protecting the liver from injury caused by certain bioactivated intermediates (see section Reactive Metabolites Resulting from Bioactivation). Not all epoxides are substrates for this enzyme, but the more chemically reactive epoxides appear to be better substrates. Important among the epoxides that are substrates for this enzyme are those produced from halobenzenes and polycyclic aromatic hydrocarbons through the action of a P450 monooxygenase. Epoxide formation represents bioactivation because the epoxides are reactive and potentially toxic, whereas their GSH conjugates are inactive. Conjugation of GSH with the epoxides of aryl hydrocarbons eventually results in the formation of hydroxymercapturic acids (premercapturic acids) which undergo acid-catalyzed dehydration to the mercapturic acids. The halobenzenes are typically conjugated in the *p*-position.

Monohalogenated, *gem*-dihalogenated, and vicinal dihalogenated alkanes undergo glutathione transferase-catalyzed conjugation reactions to produce S-substituted glutathione derivatives that are metabolically transformed into the more stable and less toxic mercapturic acids. This common route of metabolism occurs through nucleophilic displacement of a halide ion by the thiolate anion of glutathione. The mutagenicity of the 1,2-dihaloethanes (e.g., the pesticide and fumigant ethylene dibromide) has been attributed to GSH displacing bromide with the formation of the S-(2-haloethyl) glutathione, which subsequently rearranges to a reactive episulfonium ion electrophile that, in turn, alkylates DNA. Many of the

halogenated hydrocarbons exhibiting nephrotoxicity undergo the formation of similar S-substituted cysteine derivatives.

A correlation exists between the hepatotoxicity of acetaminophen and levels of GSH in the liver. The probable mechanism of toxicity that has emerged from animal studies is that acetaminophen is oxidized by CYP1A2 and CYP2E1 to the N-acetyl-p-benzoquinonimine intermediate, which conjugates with and depletes hepatic GSH levels (Fig. 3.35). This action allows the benzoquinonimine to bind covalently to tissue macromolecules. The mercapturic acid derivative of acetaminophen represents approximately 2% of the administered dose of acetaminophen. Thus, the possibility exists that those toxic metabolites that are mostly detoxified by conjugating with GSH exhibit their hepatotoxicity (or, perhaps, carcinogenicity) because the liver has been depleted of GSH and is incapable of inactivating them. Pretreatment of animals with phenobarbital often hastens the depletion of GSH by increasing the formation of epoxides or other reactive intermediates.

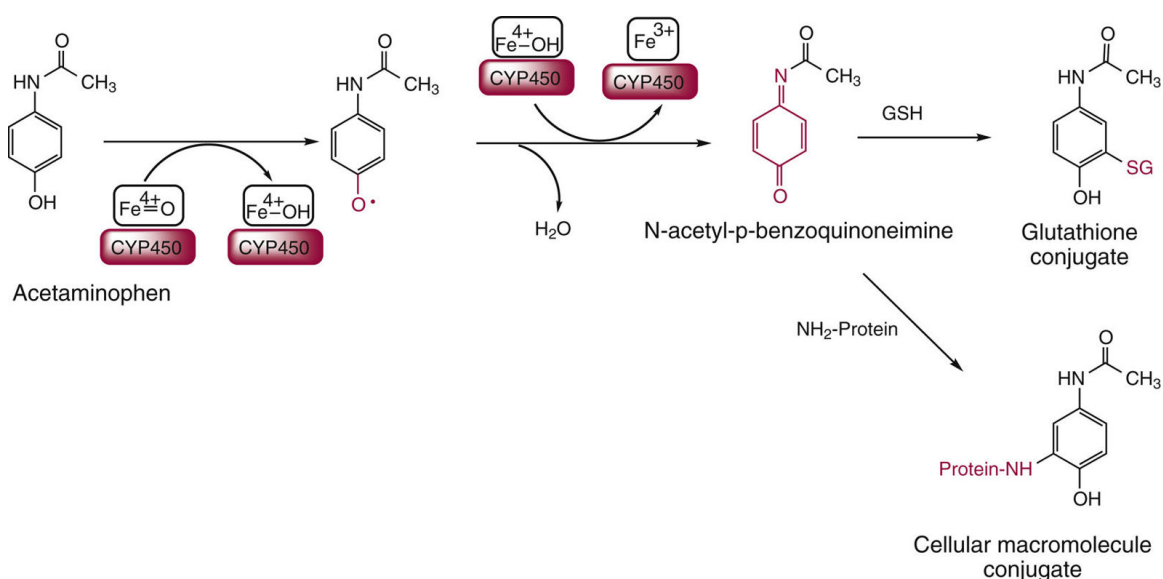


FIGURE 3.35 Proposed mechanism for the CYP450-catalyzed oxidation of acetaminophen to its N-acetyl-p-benzoquinoneimine intermediate, which can further react with either glutathione (GSH) or cellular macromolecules (NH₂-protein).

Methylation

Methylation is a common biochemical reaction but appears to be of greater significance in the metabolism of endogenous compounds than for drugs and other xenobiotics. Methylation differs from other conjugation processes in that the O-methyl metabolites formed can, in some cases, have as great or greater pharmacological activity and lipophilicity than the parent molecule (e.g., the conversion of norepinephrine to epinephrine). Methionine is involved in the methylation of endogenous and exogenous substrates, because it transfers its methyl group via the activated intermediate S-adenosylmethionine to the substrate under the influence of methyltransferases (Fig. 3.36). Methylation results principally in the formation of O-methylated, N-methylated, and S-methylated products.

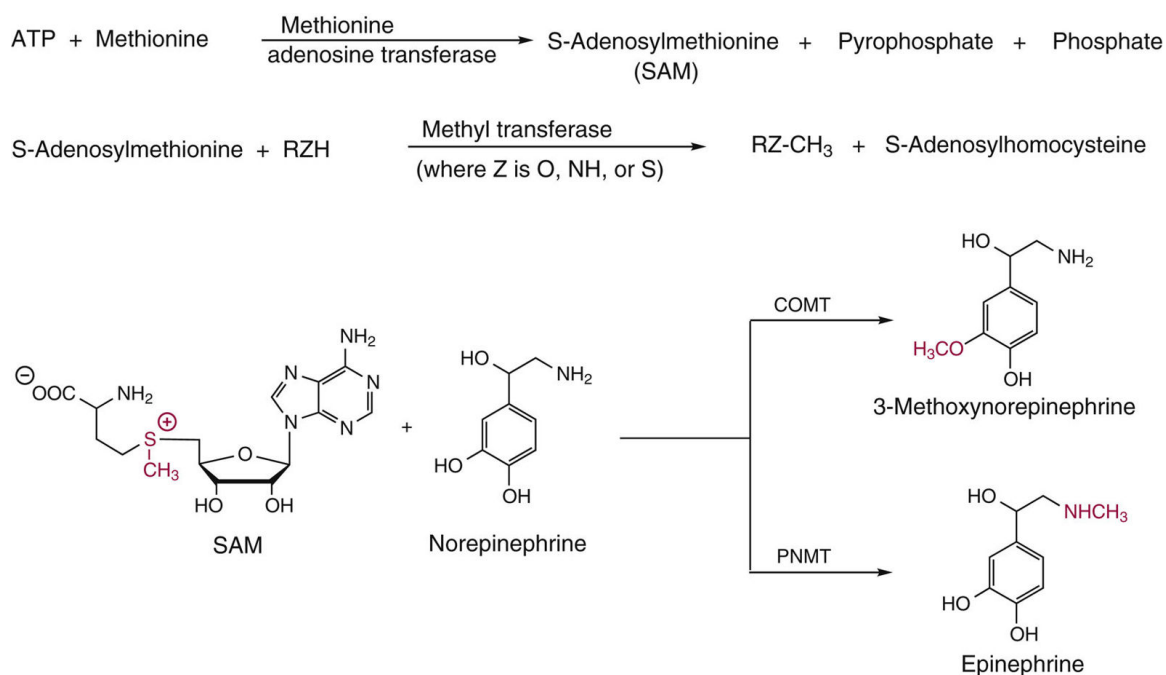


FIGURE 3.36 Methylation pathways.

O-Methylation

O-methylation is catalyzed by the magnesium-dependent enzyme catechol-O-methyltransferase (COMT), which transfers a methyl group primarily to the *m*- or 3-hydroxy group of the catechol moiety (3,4-dihydroxylphenyl moiety) of norepinephrine, epinephrine, or dopamine, resulting in inactivation. Less frequently, the *p*- or 4-hydroxy group of

these catecholamines is methylated (regioselectivity), along with their deaminated metabolites. COMT does not methylate monophenols or other dihydroxy non-catechol phenols. The *m:p* product ratio depends greatly on the type of substituent attached to the catechol ring. Substrates specific for COMT include the aforementioned catecholamines norepinephrine, epinephrine, and dopamine; the catechol amino acids L-DOPA and α -methyl-L-DOPA; and 2- and 4-hydroxyestradiol (catechol-like) metabolites of estradiol. It is found in liver, kidneys, nervous tissue, and other tissues. Hydroxyindole-O-methyltransferase, which O-methylates N-acetylserotonin, serotonin, and other hydroxyindoles, is found in the pineal gland and is involved in the formation of melatonin, a hormone associated with the dark-light diurnal cycle in humans. This enzyme differs from COMT in that it does not methylate catecholamines and has no requirement for magnesium ion.

N-Methylation

N-Methylation is among several conjugation pathways for metabolizing amines. Specific N-methyltransferases catalyze the transfer of active methyl groups from S-adenosylmethionine to the acceptor substance. Phenylethanolamine-N-methyltransferase methylates a number of endogenous and exogenous phenylethanolamines (e.g., normetanephrine, norepinephrine, and norephedrine) but does not methylate phenylethylamines. Histamine-N-methyltransferase methylates specifically histamine, producing the inactive metabolite N¹-methylhistamine. Amine-N-methyltransferase will N-methylate a variety of primary and secondary amines from a number of sources, including endogenous biogenic amines (serotonin, tryptamine, tyramine, and dopamine) and drugs (desmethylinipramine, amphetamine, and normorphine). Amine-N-methyltransferases seem to have a role in recycling N-demethylated drugs.

S-Methylation

As a rule, thiols are toxic, and the role of thiol S-methyltransferases is a nonoxidative detoxification pathway of these compounds. S-methylation

of sulfhydryl compounds also involves a microsomal enzyme requiring S-adenosylmethionine. Although a wide range of exogenous sulfhydryl compounds are S-methylated by this microsomal enzyme, none of the endogenous sulfhydryl compounds (e.g., cysteine and GSH) can function as substrates. S-methylation represents a detoxication step for thiols. Dialkyldithiocarbamates (e.g., disulfiram) and the antithyroid drugs (e.g., 6-propyl-2-thiouracil), mercaptans, and hydrogen sulfide (from thioglycosides as natural constituents of foods, mineral sulfides in water, fermented beverages, and bacterial digestion) are S-methylated. Other drugs undergoing S-methylation include captopril, thiopurine, azaprime, penicillamine, and 6-mercaptopurine.

Thiopurine methyltransferase or thiopurine S-methyl-transferase (TPMT) catalyzes the S-methylation of thiopurine drugs, such as azathioprine, 6-mercaptopurine, and 6-thioguanine. Interindividual variations in sensitivity and toxicity to thiopurine are correlated with TPMT genetic polymorphisms. Defects in the TPMT gene result in decreased methylation and decreased inactivation of the thiopurine drugs, leading to enhanced bone marrow toxicity. The clinical result may be myelosuppression, anemia, bleeding tendency, leukopenia, and infection. Approximately, 5% of all thiopurine therapies will fail due to toxicity related to TPMT polymorphism.

Elimination Pathways

Most xenobiotics are lipid-soluble and are altered chemically by the metabolizing enzymes, usually into less toxic and more water-soluble substances, before being excreted into the urine or, in some cases, bile. The formation of conjugates with sulfonate, amino acids, and glucuronic acid is particularly effective in increasing the hydrophilicity of drug molecules. The principal route of excretion of drugs and their metabolites is via the urine. If drugs and other compounds foreign to the body are not metabolized in this manner, substances with a high lipid-water partition coefficient could be reabsorbed readily from the urine through the renal tubular membranes and into the plasma. Therefore, such substances would continue to be recirculated and their pharmacological or toxic effects would be prolonged. Very polar or highly ionized drug molecules often are excreted in the urine unchanged. Urine is not the only route for excreting drugs and their metabolites from the body. Other routes include bile, saliva, lungs, sweat, and milk. The bile and biliary efflux transporters have been recognized as major routes of excretion for many endogenous and exogenous compounds.

Enterohepatic Cycling of Drugs

The liver is the principal organ for the metabolism and eventual elimination of xenobiotics from the human body in either the urine or the bile. When eliminated in the bile, steroid hormones, bile acids, drugs, and their respective conjugated metabolites are available for reabsorption from the duodenal-intestinal tract into the portal circulation, undergoing the process of enterohepatic cycling (EHC) ([Fig. 3.37](#)).⁶⁰ Nearly all drugs are excreted in the bile, but only a few are concentrated in the bile. For example, the bile salts are efficiently concentrated in the bile and reabsorbed from the GIT, such that the entire body pool of bile acids/salts are recycled multiple times per day. Therefore, EHC is responsible for the conservation of bile acids, steroid hormones, thyroid hormones, and other endogenous substances. In humans, compounds excreted into the bile typically have a molecular weight greater than 500 Da. In humans,

metabolites with a molecular weight between 300 and 500 Da are excreted in both urine and bile. Compounds excreted into bile are, as a rule, hydrophilic substances that can be either charged (anionic) or uncharged (e.g., cardiac glycosides and steroid hormones). Biotransformation of these compounds by means of Phase 1 and Phase 2 reactions would produce a conjugated metabolite, which is anionic and hydrophilic with a molecular weight greater than that of the parent compound. The conjugated metabolites are most often glucuronides, because glucuronidation adds 176 Da to the molecular weight of the parent compound. Unchanged drug in the bile is either excreted with the feces, metabolized by the bacterial flora in the intestinal tract, or reabsorbed into the portal circulation via EHC.

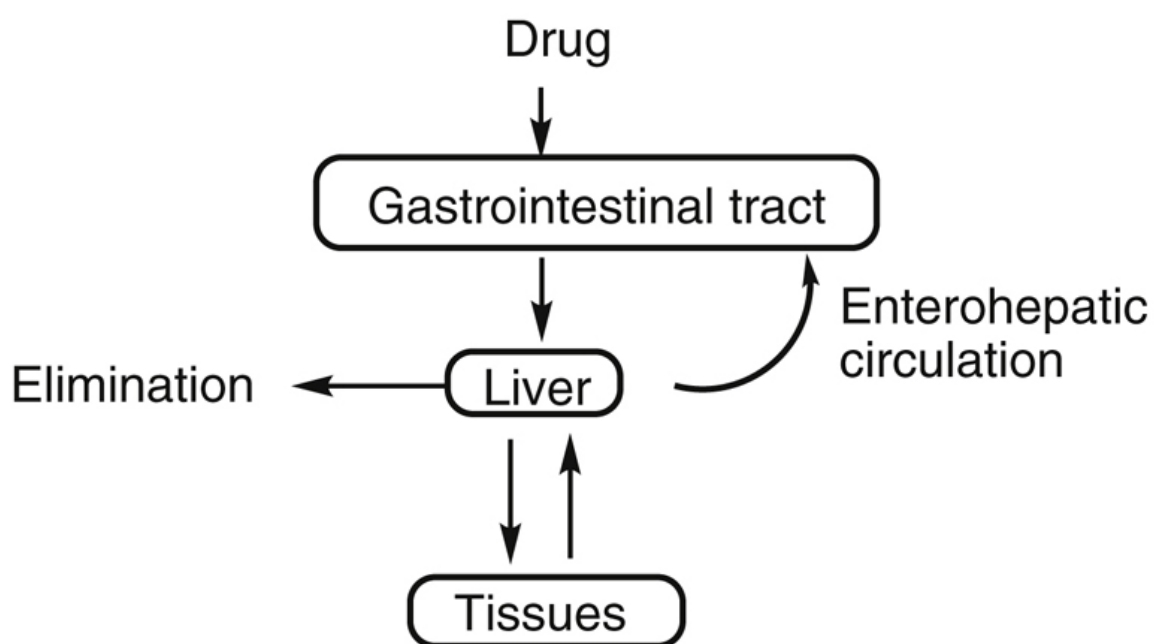


FIGURE 3.37 Enterohepatic cycling.

The bacterial intestinal flora is directly involved in EHC and the recycling of drugs through the portal circulation.⁶¹ A conjugated drug and metabolites excreted via the bile can be hydrolyzed by enzymes of the bacterial flora, releasing the parent drug or its Phase 1 metabolite for reabsorption into the portal circulation. Among the numerous compounds metabolized in the enterohepatic circulation are the estrogenic and

progestational steroids, digitoxin, indomethacin, diazepam, pentaerythritol tetranitrate, mercurials, arsenicals, and morphine. The oral ingestion of xenobiotics inhibiting the gut flora (i.e., nonabsorbable antibiotics) can affect the pharmacokinetics of drugs.

The impact of EHC on the pharmacokinetics and pharmacodynamics of a drug depends on the importance of biliary excretion relative to renal clearance and on the efficiency of gastrointestinal absorption. The EHC becomes dominant when biliary excretion is the major clearance mechanism for the drug. Because the majority of the bile is stored in the gallbladder and released on the ingestion of food, intermittent spikes in the plasma drug concentration is observed following reentry of the drug from the bile via EHC. From a pharmacodynamic point of view, the net effect of EHC is to increase the duration of a drug in the body and to prolong its pharmacological action.

Drug Metabolism and Age

Metabolism in the Elderly

The widespread use of medications in the elderly increases the potential for an increased incidence of drug-related interactions, which can be related to changes in drug metabolism and clearance from the body (Table 3.14).^{62,63} The interpretation of the age-related alteration in drug response must consider the contributions of absorption, distribution, metabolism, and excretion. Drug therapy in the elderly is expected to become one of the more significant problems for clinical medicine. It has been well documented that the metabolism of many drugs and their elimination is impaired in the elderly.

TABLE 3.14
Effect of Age on the Clearance of Some Drugs

No Change	Decrease
Acetaminophen ^a	Alprazolam
Aspirin	Amitriptyline
Diclofenac	Chlordiazepoxide
Diphenhydramine	Chlormethiazole
Ethanol	Diazepam
Flunitrazepam	Labetalol
Midazolam	Lidocaine
Nitrazepam	Lorazepam
Oxazepam	Morphine
Phenytoin ^a	Meperidine
Prazosin	Nifedipine and other dihydropyridines
Propylthiouracil	Norepinephrine
Temazepam	Nortriptyline
Thiopental ^a	Phenytoin
Tolbutamide ^a	Piroxicam
Warfarin	Propranolol
	Quinidine

No Change	Decrease
	Quinine
	Theophylline
	Verapamil

^aDrugs for which clearance is disputable but can be increased

The decline in drug metabolism due to advancing age is associated with physiological changes that have pharmacokinetic implications affecting the steady-state plasma concentrations and renal clearance for the parent drug and its metabolites. Those changes relevant to the bioavailability of drugs in the elderly are decreases in hepatic blood flow, glomerular filtration rate, hepatic P450 activity, plasma protein binding, and body mass. Because the rate of a drug's elimination from the blood through hepatic metabolism is determined by hepatic blood flow, protein binding, and intrinsic clearance, a reduction in hepatic blood flow can lead to an increase in drug bioavailability and decreased clearance, with the symptoms of drug overdose and toxicity as the outcome (e.g., warfarin in patients with congestive heart failure^{63,64}). Those orally administered drugs exhibiting a reduction in first-pass metabolism in the elderly include antihypertensives, diuretics, and antidepressants.

Age-related changes in drug metabolism are a complicated interplay between the age-related physiological changes, genetics, environmental influences (diet and nutritional status, smoking, and enzyme induction), concomitant diseases states, and drug intake. In most studies, the elderly appear to be just as responsive to drug-metabolizing enzyme activity (Phase 1 and Phase 2) as young individuals. Given the number of factors that determine the rate of drug metabolism, it is not surprising that the effects of aging on drug elimination by metabolism have yielded variable results, even for the same drug. The fact that drug elimination can be altered in old age suggests that doses of metabolized drugs should be initially reduced in older patients and then adjusted according to the clinical

response. A decrease in hepatic drug metabolism coupled with age-related alterations in clearance, volume of distribution, and receptor sensitivity can lead to prolonged plasma half-life and increased drug toxicity.

Those P450 substrates reported to cause drug-drug interactions are shown in [Tables 3.3-3.10](#) in bold italics.

Drug Metabolism in the Fetus and During Pregnancy

Over two-thirds of women receive prescription drugs while pregnant, with treatment and dosing strategies based on data from healthy male volunteers and nonpregnant women, and with little adjustment for the complex physiology of pregnancy and its unique disease states. Pregnancy influences hepatic metabolism of drugs in a CYP-dependent manner. Hormone levels in the plasma which rise during pregnancy are capable of regulating expression of hepatic drug metabolism enzymes. These hormones include estrogens, progesterone, cortisol, and prolactin. Studies are needed to examine the role of pregnancy hormones in altered drug metabolism during pregnancy.

The activities of CYP3A4, CYP2A6, CYP2D6, and CYP2C9 are all increased during pregnancy. Changes in CYP3A4 activity lead to increased metabolism of drugs ([Table 3.10](#)). By contrast, CYP1A2 and CYP2C19 appear to undergo a gradual decrease in activity with advancing gestation,^{63,64} though with uncertain effects on drug therapy. The activity of Phase 2 enzymes, including uridine 5'-diphosphate glucuronosyltransferases (UGTs), is also altered during pregnancy, with a 200% increase in UGT1A4 activity during the first and second trimesters and a 300% increase during the third trimester.^{63,64} This change leads to lower concentrations of UGT1A4 substrates such as lamotrigine, leading directly to poorer seizure

control with advancing gestation in the absence of appropriate dose titration.

Knowledge of drug transporter function during pregnancy is necessary for a complete understanding of drug absorption, distribution, elimination, and effect.⁶³ In addition, fetal development is dependent upon the transport of nutrients by the placenta toward the fetal side, and that of products of fetal metabolism transported away from the fetus for elimination by the mother.⁶⁵ The placenta produces and secretes hormones that affect the maternal physiology and endocrine state. Compounds transported between the mother and the fetus are carried by the maternal circulation within the uterine vasculature.

The ability of the human fetus and placenta to metabolize xenobiotics is well established. Drug-metabolizing enzymes expressed in the placenta include CYP1A1, 2E1, 3A4/5, 3A7, 4B1, epoxide hydrolase, alcohol dehydrogenase, glutathione transferase, UGTs, sulfotransferases, and N-acetyltransferase. The majority of the drugs used in pregnancy readily cross the placenta, thus exposing the fetus to a large number of xenobiotic agents. The human fetus is at special risk from these substances due to the presence of the P450 system which is capable of metabolizing xenobiotics during the first part of gestation. Placentas of tobacco smokers have shown a significant increase in the rate of placental CYP1A activity. Concern for this CYP1A activity is increasing because this enzyme system is known to catalyze the formation of reactive metabolites capable of covalently binding to macromolecules, producing permanent effects (e.g., teratogenicity, hepatotoxicity, or carcinogenicity) in the fetus and newborn. A more disturbing fact is that the conjugation enzymes (i.e., glucuronosyltransferases, glutathione transferase, and sulfotransferase), which are important for the formation of Phase 2 conjugates of these reactive metabolites, are found in low to

negligible levels in the fetus and newborn, increasing the exposure of these infants to these potentially toxic metabolites.

The placenta is not a barrier protecting the fetus from xenobiotics; almost every drug present in the maternal circulation will cross the placenta and reach the fetus. For some drugs, however, the placental efflux transport protein P-gp functions as a maternofetal barrier, pumping drugs and Pgp substrates out of the fetal circulation back into the maternal circulation⁶⁵ and protecting the fetus from exposure to potentially harmful teratogenic xenobiotics/drugs and endogenous substances that have been absorbed through the placenta. The P-gp inhibitors should be carefully evaluated for their potential to increase fetal susceptibility to drug/chemical-induced teratogenesis. On the other hand, selective inhibition of P-gp could be used clinically to improve pharmacotherapy of the unborn child. Because metabolites are usually more water soluble than the parent substance, drug metabolites formed in the fetus can be trapped and accumulate on the fetal side of the placenta. Such accumulation can result in drug-induced toxicities or developmental defects. The activity of CYP3A isoenzymes in the human fetal liver is similar to that seen in adult liver microsomes. The fetal activity for CYP3A7 is unusual, as most other fetal isoenzymes of P450 exhibit activity 5%-40% of the adult isoenzymes. Fetal and neonatal drug-metabolizing enzyme activities can differ from those in the adult.

Most xenobiotics cross the placental barrier by simple diffusion. Protein binding, degree of ionization, lipid solubility, and molecular weight can affect placental transport. In fact, small, lipid-soluble, ionized, and poorly protein-bound molecules cross the placenta easily. For other substrates, the placenta facilitates maternal to fetal transport through the polarized expression of various transporters.⁶⁵ Transporters enable transport of specific endogenous substrates (such as cytokines, nucleoside analogues, and steroid hormones);

however, exogenous compounds with similar structures may also interact with them.

From the day of birth, the neonate is exposed to drugs and other foreign compounds persisting from pregnancy, as well as those transferred via breast milk. Fortunately, many of the drug-metabolizing enzymes operative in the neonate developed during the fetal period. The routine use of therapeutic agents during labor and delivery, as well as during pregnancy, is widespread, and consideration must be given to the fact that potentially harmful metabolites can be generated by the fetus and newborn. Consequently, the use of drugs capable of forming reactive metabolic intermediates should be avoided during pregnancy, delivery, and the neonatal period. The activity of Phase 1 and Phase 2 drug-metabolizing enzymes is high at birth but decreases to normal levels with increasing age. Evidence suggests increased activity of drug-metabolizing enzymes in liver microsomes of neonates resulting from treatment of the mother during the pregnancy with enzyme inducers (e.g., phenobarbital).

Genetic Polymorphism

The reality of drug therapy is that many drugs do not work in all patients.⁶⁶ By current estimates, the percentage of patients who will react favorably to a specific drug ranges from 20% to 80%. Drugs have been developed and dosage regimens prescribed under the old paradigm that “one dose fits all,” which largely ignores the fact that humans are genetically different, resulting in interindividual differences in drug metabolism and disposition.⁶⁶ It is widely accepted that genetic factors have an important impact on the oxidative metabolism and pharmacokinetics of drugs. Genotype-phenotype correlation studies (pharmacogenetics) have shown that inherited mutations in P450 genes (alleles) result in distinct phenotypic subgroups. For example, mutations in the *CYP2D6* gene result in poor (PM), intermediate (IM), extensive (EM), and ultrarapid (UM) metabolizers of *CYP2D6* substrates⁶⁷ (Table 3.8). Each of these phenotypic subgroups experiences different responses to drugs extensively metabolized by the *CYP2D6* pathway, ranging from severe toxicity to complete lack of efficacy. Genetic studies confirm that “one dose does not fit all,” leaving the question of why we would continue to develop and prescribe drugs under the old paradigm. The regulatory agencies have recognized that identifying genetic polymorphisms might allow the safe dosing, marketing, and approval of drugs that would otherwise not be approved and advised pharmaceutical companies to incorporate the knowledge of genetic polymorphisms into drug development. Importantly, pharmacogenomic testing (the study of heritable traits affecting patient response to drug treatment) can significantly increase the likelihood of developing drug regimens that benefit most patients without severe adverse events. Pharmacists play a vital role in the

development of patient-specific dosing regimens based on metabolizing phenotype.

Polymorphisms are expressed for a number of metabolizing enzymes, but the polymorphic P450 isoforms that are most important for drug metabolism include CYP2A6, CYP2A13, CYP2B6, CYP2C9, CYP2C19, and CYP2D6.⁶⁸⁻⁷¹ [1]
[SEP] These isoforms have been studied extensively and, despite their low abundance in the liver, they have been found to catalyze the metabolism of many drugs. Most gene variants are expressed into inactive, truncated proteins or they fail to express any protein. There is no clear information about CYP1A1 and CYP3A4/5 polymorphism. These polymorphic isoforms give rise to phenotypic subgroups in the population differing in their ability to perform clinically significant biotransformation reactions with obvious clinical ramifications.⁶⁶ Metabolic polymorphism can have several consequences; for example, when enzymes that metabolize drugs used either therapeutically or socially are deficient, adverse or toxic drug reactions can occur in these individuals.

The discovery of genetic polymorphism resulted from the observation of increased frequency of adverse effects or no drug effects after normal doses of drugs to some patients (e.g., hyper-CNS response from the administration of the antihistamine doxylamine or no analgesic response with codeine). Polymorphism is a difference in DNA sequence found at 1% or greater in a population and expressed as an amino acid substitution in the protein sequence of an enzyme resulting in changes in its rate of activity ($V_{\max}$) or affinity (K_m). Thus, mutant DNA sequences can lead to interindividual differences in drug metabolism. Furthermore, the polymorphisms do not occur with equivalent frequency in all racial or ethnic groups. Because of these differences, it is important to be aware of a person's race and ethnicity when giving drugs that are metabolized differently by different populations, although practitioners must guard against

stereotyping.⁶⁶ Because no other way may exist to adequately clear these drugs from the body, PMs can be at greater risk for adverse drug reactions or toxic overdoses. The signs and symptoms of these overdoses are primarily extensions of the drug's common adverse effects or pharmacological effects (Table 3.15).⁶⁶ The level of adverse reactions or overdosage depends very much on the overall contribution of the mutant isoform to the drug's metabolism. Perhaps the most interesting explanation for the various mutant isoforms is that they evolved as protective mechanisms against alkaloids and other common substances in the food chain for the different ethnicities. Although much effort has gone into finding polymorphisms of *CYP3A4* and *CYP1A2* genes, none has yet to be discovered.

TABLE 3.15

Impact of Human P450 Polymorphisms on Drug Treatment in Poor Metabolizers

Polymorphic Enzyme	Decreased Clearance	Adverse Effects (Overdosage)	Reduced Activation of Coadministered Prodrug
CYP2C9	S-warfarin	Bleeding	Losartan
	Phenytoin	Ataxia	
	Losartan		
	Tolbutamide	Hypoglycemia	
	NSAIDs	GI bleeding	
CYP2C19	Omeprazole		Proguanil
	Diazepam	Sedation	
CYP2D6	Tricyclic antidepressants	Cardiotoxicity	Tramadol
	SSRIs	Serotonin syndrome	Codeine
	Anti-arrhythmic drugs	Arrhythmias	Ethylmorphine
	Perhexiline	Neuropathy	
	Haloperidol	Parkinsonism	
	Perphenazine		
	Zuclopenthixol		
	S-Mianserin		

Polymorphic Enzyme	Decreased Clearance	Adverse Effects (Overdosage)	Reduced Activation of Coadministered Prodrug
	Tolterodine		
CYP2A6	Nicotine		

Occasionally, one derives benefit from an unusual P450 phenotype. For example, cure rates for peptic ulcer treated with omeprazole are substantially greater in individuals with defective CYP2C19 due to the sustained high plasma levels achieved.

CYP2A6

CYP2A6 is of particular importance because it activates a number of procarcinogens to carcinogens and is the major isoform metabolizing the anticoagulant warfarin and nicotine.⁶⁹ Approximately 15% of Asians express the *CYP2A6*4del* allele and 2% of Caucasians express the other *CYP2A6*2* allele. Both these alleles express either no enzyme or a nonfunctional enzyme, and individuals carrying these alleles are referred to as having the PM phenotype. A benefit from being a PM of CYP2A6 substrates might be the protection against some carcinogens, including cigarette smoke due to the high plasma levels of nicotine achieved with fewer cigarettes.

CYP2B6

CYP2B6 is of special interest due to its wide interindividual variability in expression and activity.⁷⁰ Such a large variability for an enzyme that is not highly expressed is probably because of genetic polymorphisms and exposure to drugs that are inducers or inhibitors of CYP2B6. Variant alleles resulting from splicing defects or gene deletions have been identified in PM individuals with altered metabolic activity or impaired enzyme function. At least 28 allelic variants and some subvariants of *CYP2B6* have been shown to have an effect on drug clearance and drug response. For example, HIV-infected African-American individuals with a defective variant

of *CYP2B6* are PMs of efavirenz and have plasma levels approximately 3-fold higher than individuals with the normal *CYP2B6* gene. A different variant of *CYP2B6* is associated with approximately 2-fold greater plasma levels of nevirapine in HIV-infected patients. A benefit from being a PM of *CYP2B6* antiviral drugs might be enhanced HIV protection because of the higher plasma levels of the antiviral drugs achieved with lower dosages. Smokers with another variant of *CYP2B6* may be more vulnerable to abstinence symptoms and relapse following treatment with bupropion (a *CYP2B6* substrate) as a smoking cessation agent.

CYP2C9 and CYP2C19

CYP2C9 and *CYP2C19* metabolize approximately 15% of clinically used drugs, such as phenytoin, *S*-warfarin, tolbutamide, losartan, and many NSAIDs.⁷¹ Although *CYP2C19* metabolizes fewer drugs than *CYP2D6*, the drugs that *CYP2C19* does metabolize are clinically important (Table 3.7). The deficit of *CYP2C19* found in the PM phenotype is only seen in 8%-13% of Caucasians, 20%-30% of the Asian population (11%-23% of Japanese and 5%-37% of Chinese), up to 20% of the black African-American population, 14%-15% of Saudi Arabians and Ethiopians, and up to 70% of Pacific Islanders.⁷¹ The more common mutant allele in these individuals is *CYP2C19**2, which expresses an inactive enzyme. The large interindividual variability observed in the therapeutic response to the antiseizure drug mephenytoin is attributed to *CYP2C19* polymorphism, which catalyzes the *p*-hydroxylation of the *S*-stereoisomer.⁷¹ The *R*-enantiomer is *N*-demethylated by *CYP2C8* with no difference in its metabolism between PMs and EMs.

CYP2C9 is highly polymorphic with at least 33 variants identified. *CYP2C9**2 is frequent among Caucasians with ~1% of the population being homozygous carriers and 22% heterozygous. The

corresponding homozygous/heterozygous figures for the *CYP2C9**3 allele are 0.4% and 15%, respectively. Clinical studies have addressed the importance of the *CYP2C9**2 and *3 alleles as a determining factor for drug clearance and drug response. P450 2C9 polymorphisms are relevant for the efficacy and adverse effects of numerous NSAIDs, sulfonylurea antidiabetic drugs, and, most critically, oral anticoagulants belonging to the class of vitamin K epoxide reductase inhibitors (e.g., warfarin).⁷¹ A deficiency of this isoform is seen in 8%-13% of Caucasians, 2%-3% of African-Americans, and 1% of Asians. Individuals with the PM phenotype who possess this deficient isoform variant are ineffective in clearing S-warfarin (so much so that they can be fully anticoagulated on just 0.5 mg of warfarin per day) and in the clearance of phenytoin, which has a potentially very toxic narrow therapeutic range. On the other hand, the prodrug losartan will be poorly activated and ineffective.

CYP2D6

CYP2D6 is of specific importance because it metabolizes a wide range of commonly prescribed drugs, including antidepressants, antipsychotics, β -adrenergic blockers, and antiarrhythmics (Table 3.8).^{67,68} CYP2D6 deficiency is a clinically important genetic variation of drug metabolism characterized by three phenotypes: UM, EM, and PM. About 10% of Caucasians have gene variants that result in a functionally deficient CYP2D6 enzyme, and these PMs often respond inadequately to drugs, such as codeine, that are activated by CYP2D6. The PM phenotype is inherited as an autosomal recessive trait, with 5 of 30 of the known *CYP2D6* gene mutations leading to either zero expression or the expression of a nonfunctional enzyme.⁶⁸ Approximately 12%-20% of Caucasians express the *CYP2D6**4 allele and 5% express the other *CYP2D6* alleles. Up to 34% of African-Americans express the *CYP2D6**17 allele and 5% express the other

CYP2D6 alleles. Up to 50% of Chinese express the *CYP2D6**10 allele and 5% express the other *CYP2D6* alleles (these individuals are all referred to as PM).^{67,68} Conversely, the 20%-30% of Saudi Arabians and Ethiopians who express the *CYP2D6**2XN allele are known as UMs of *CYP2D6* substrates, because they express excess enzyme because of having multicopies of the gene.⁷² Inasmuch as *CYP2D6* is not inducible, many individuals of Ethiopian and Saudi Arabian descent have developed a genetically different strategy to cope with the (presumed) high load of alkaloids and other substances in their diet, thus the high expression of *CYP2D6* using multiple copies of the gene.

Those individuals who are deficient in *CYP2D6* will be predisposed to adverse effects or drug toxicity from antidepressants or neuroleptics caused by inadequate metabolism, leading to long half-lives, but the metabolism of *CYP2D6*-dependent prodrugs in these patients will be ineffective due to lack of activation (e.g., codeine, which must be metabolized by O-demethylation to morphine). In contrast, individuals with the UM phenotype will require a dose of drugs inactivated by *CYP2D6* that is much higher than normal to attain therapeutic drug plasma concentrations (e.g., one patient required a daily dose of approximately 300 mg of nortriptyline to achieve therapeutic plasma levels; the usual dose is 25 mg) or a lower dose for prodrugs that require metabolic activation. Individuals with the PM phenotype are also characterized by loss of *CYP2D6* stereoselectivity in hydroxylation reactions. It can be anticipated that large differences in steady-state concentration for *CYP2D6* substrates will occur between individuals with the different phenotypes when they receive the same dose. Depending on the drug and reaction type, a 10- to 30-fold difference in blood concentrations can be observed in the PM-phenotype polymorphism.⁶⁸

Other Polymorphic Metabolizing Enzymes

CYP2E1 polymorphism is expressed more frequently in individuals of Chinese ancestry than in Caucasians. Those with the CYP2E1 PM phenotype exhibit tolerance to alcohol and less toxicity from halohydrocarbon solvents.

Polymorphism has been associated with serum cholinesterases, alcohol dehydrogenases, aldehyde dehydrogenases, epoxide hydrolase and xanthine oxidase. Approximately 50% of the Oriental population lack aldehyde dehydrogenase, resulting in high levels of acetaldehyde following ethanol ingestion and causing nausea and flushing. People with genetic variants of cholinesterase respond abnormally to succinylcholine, procaine, and other related choline esters. The clinical consequence of reduced enzymatic activity of cholinesterase is that succinylcholine and procaine are not hydrolyzed in the blood, resulting in prolongation of their respective pharmacological activities (e.g., skeletal muscle paralysis in the case of succinylcholine).

Increasing knowledge of genetic polymorphism has contributed a great deal to our understanding about interindividual variation in the metabolism of drugs, including how to change dose regimens accordingly to minimize drug toxicity and improve therapeutic efficacy.

Presystemic First-Pass Metabolism

Although hepatic metabolism continues to be the most important route for xenobiotics, the ability of the liver and intestine to metabolize substances to either pharmacologically inactive or bioactive metabolites before reaching the systemic circulation is called presystemic first-pass metabolism. This metabolic process results in low systemic availability for orally susceptible drugs. Sulfonation and glucuronidation are major pathways of presystemic intestinal first-pass metabolism in humans. For example, oral acetaminophen, phenylephrine, terbutaline, albuterol, fenoterol, or isoproterenol exhibits approximately 5% bioavailability because of sulfonation and glucuronidation.

The discovery that CYP3A4 is found in the mucosal enterocytes of the intestinal villi signifies its role as a key determinant in the oral bioavailability of its numerous drug substrates ([Table 3.10](#)). Drugs known to be substrates for CYP3A often have a low and/or variable oral bioavailability that can be explained by presystemic first-pass metabolism by the small intestine P450 isoforms.⁶⁸ The concentration of functional intestinal CYP3A is influenced by genetic disposition, induction, and inhibition, which to a great extent determines drug blood levels and therapeutic response. Xenobiotics when ingested orally can modify the activity of intestinal CYP3A enzymes by induction and inhibition. By modulation of the isoform pattern in the intestine, a xenobiotic could alter its own metabolism and that of others in a time- and dose-dependent manner.

The concentration of CYP3A in the intestine is comparable to that of the liver. The oral administration of dexamethasone induces the formation of CYP3A and erythromycin inhibits it. The glucocorticoid inducibility of CYP3A4 can also be a factor in differences of metabolism between males and females. Studies have suggested that

intestinal CYP3A4 C2 hydroxylation of estradiol contributed to the oxidative metabolism of endogenous estrogens circulating with the enterohepatic recycling pool.⁷² Norethisterone has a low oral bioavailability of 42% due to oxidative CYP3A first-pass metabolism, but levonorgestrel is completely available in women, having no conjugated metabolites.

Several clinically relevant drug-drug interactions between orally coadministered drugs and CYP3A4 can be explained by a modification of drug metabolism at the P450 level. If a drug has high presystemic elimination (low bioavailability) and is metabolized primarily by CYP3A4, then coadministration with a CYP3A4 inhibitor can be expected to alter the drug's pharmacokinetics by reducing its metabolism, thus increasing its plasma concentration. Drugs and some foods (e.g., grapefruit juice) that are known inhibitors, inducers, or substrates for intestinal CYP3A4 can potentially interact with the metabolism of a coadministered drug, affecting its area under the curve and rate of clearance (Tables 3.10-3.12). Inducers can reduce absorption and oral bioavailability, whereas these same factors are increased by inhibitors. For example, erythromycin can enhance the oral absorption of another drug by inhibiting its metabolism in the small intestine by CYP3A4. Because they are competitive substrates for CYP3A4, prednisone, prednisolone, and methylprednisolone (but not dexamethasone) are competitive inhibitors of synthetic glucocorticoid metabolism. This is because a major metabolic pathway for synthetic glucocorticoids involves CYP3A4. In addition to coadministered drugs, metabolic interactions with exogenous CYP3A4 substrates secreted in the bile are possible. The poor oral bioavailability for cyclosporine is attributed to a combination of intestinal metabolism by CYP3A4 and efflux by P-gp.⁷³

Because the intestinal mucosa is enriched with glucuronosyltransferases, sulfotransferases, and glutathione

transferases, presystemic first-pass metabolism for orally administered drugs susceptible to these conjugation reactions results in their low oral bioavailability. Presystemic metabolism often exceeds liver metabolism for some drugs. For example, more than 80% of intravenously administered albuterol is excreted unchanged in urine, with the balance presenting as glucuronide conjugates, whereas when albuterol is administered orally, less than 5% is systemically absorbed because of intestinal sulfonation and glucuronidation. Presystemic metabolism is a major pathway in humans for most β -adrenergic agonists, such as glucuronides or sulfate esters of terbutaline, fenoterol, albuterol and isoproterenol, morphine (3-O-glucuronide), acetaminophen (O-sulfate and O-glucuronide), and estradiol (3-O-sulfate). The bioavailability of orally administered estradiol or ethinyl estradiol in females is approximately 50% because of conjugation. Mestranol (3-methoxyethinyl estradiol) has greater oral bioavailability because, as a nonphenol, it is not significantly conjugated. Levodopa has a low oral bioavailability because of its metabolism by intestinal l-aromatic amino acid decarboxylase. Drugs subject to first-pass metabolism are included in [Table 3.16](#).

TABLE 3.16
Examples of Drugs Exhibiting Presystemic Metabolism

Acetaminophen	Isoproterenol	Oxprenolol
Albuterol	Lidocaine	Pentazocine
Alprenolol	Meperidine	Propoxyphene
Aspirin	Methyltestosterone	Propranolol
Cyclosporin	Metoprolol	Salicylamide
Desmethylinipramine	Dihydropyridines (nifedipine)	Terbutaline
Fluorouracil	Nortriptyline	Verapamil
Hydrocortisone	Organic nitrates	
Imipramine		

The extensive presystemic first-pass sulfonation of phenolic drugs, for example, can lead to increased bioavailability of other drugs by competing for the available sulfate pool, resulting in the possibility of drug toxicity. Concurrent oral administration of acetaminophen with phenolic drugs could result in an increase in drug blood levels. Ascorbic acid, which is sulfonated, also increases the bioavailability of concurrently administered phenolic drugs. Sulfonation and glucuronidation occur side by side, often competing for the same substrate, and the balance between sulfonation and glucuronidation is influenced by several factors, such as species, doses, availability of cosubstrates, inhibition, and induction of the respective transferases.

Extrahepatic Metabolism

Because the liver is the primary tissue for xenobiotic metabolism, it is not surprising that our understanding of mammalian P450 monooxygenase is based chiefly on hepatic studies. Although the tissue content of P450s is highest in the liver, P450 enzymes are found in the lung, nasal epithelium, intestinal tract, kidney, adrenal tissues, and brain. It is possible that the expression of the polymorphic genes and induction of the isoforms in the extrahepatic tissues can affect the activity of the P450 isoforms in the metabolism of endogenous steroids, drugs, and other xenobiotics. Therefore, characterization of P450, UGT, SULT, and other polymorphic drug-metabolizing enzymes in extrahepatic tissues is important to our overall understanding about the biological importance of these isoform families to improved drug therapy, design of new drugs and dosages forms, toxicity, and carcinogenicity.

The mucosal surfaces of the GIT, the nasal passages, and the lungs are major portals of entry for xenobiotics into the body and, as such, are continuously exposed to a variety of orally ingested or inhaled airborne xenobiotics, including drugs, plant toxins, environmental pollutants, and other chemical substances. As a consequence of this exposure, these tissues represent a major target for tumorigenesis and other chemically induced toxicities. Many of these toxins and chemical carcinogens are relatively inert substances that must be bioactivated to exert cytotoxicity and tumorigenicity. The epithelial cells of these tissues are capable of metabolizing a wide variety of exogenous and endogenous substances, and these cells provide the principal and initial source of biotransformation for these xenobiotics during the absorptive phase. The consequences of such presystemic biotransformation is either (1) a decrease in the amount of xenobiotics available for systemic absorption by facilitating the

elimination of polar metabolites or (2) toxification by activation to carcinogens, which can be one determinant of tissue susceptibility for the development of intestinal cancer. The risk of colon cancer can depend on dietary constituents that contain either procarcinogens or compounds modulating the response to carcinogens.

Intestinal Metabolism

As noted earlier, many of the clinically relevant aspects of P450 can, in fact, occur at the level of the intestinal mucosa and can account for differences among patients in dosing requirements. The intestinal mucosa is enriched especially with the CYP3A4 isoform, glucuronosyltransferases, sulfotransferases, and GSTs, making it particularly important for orally administered drugs susceptible to oxidation and/or Phase 2 glucuronidation, sulfonation, or glutathione conjugation pathways. The highest concentrations of P450s occur in the duodenum, with gradual tapering into the ileum. In the human intestine, CYP2E, CYP3A, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 have been identified. Therefore, intestinal P450 isoforms provide potential presystemic first-pass metabolism of ingested xenobiotics affecting their oral bioavailability (e.g., hydroxylation of naloxone) or bioactivation of carcinogens or mutagens. It is not surprising that dietary factors can affect the intestinal P450 isoforms. For example, a 2-day dietary exposure to cooked Brussels sprouts significantly decreased the 2 α -hydroxylation of testosterone, yet induced CYP1A2 activity for PAH metabolism. An 8-oz. glass of grapefruit juice inhibited the sulfoxidation metabolism of omeprazole (CYP3A4) but not its hydroxylation (CYP2C19), thus increasing its systemic blood concentrations. These types of interactions between a drug and a dietary inhibitor could result in a clinically significant drug interaction.

Intestinal UGT isoforms can glucuronidate orally administered drugs, such as morphine, acetaminophen, α - and β -adrenergic agonists, and other phenolic phenethanolamines and dietary xenobiotics, resulting in a reduction of their oral bioavailability (increasing first-pass metabolism), thus altering their pharmacokinetics and pharmacodynamics. The UGTs expressed in the intestine include UGT1A1 (bilirubin-glucuronidating isoform), UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, and UGT1A3. Substrate specificities of intestinal UGT isoforms are comparable to those in the liver. Glucuronidase hydrolysis of biliary glucuronide conjugates in the intestine can contribute to EHC of the parent drug. Likewise, the sulfotransferases in the small intestine can metabolize orally administered drugs and xenobiotics for which the primary route of conjugation is sulfonation (e.g., isoproterenol, albuterol, steroid hormones, α -methyldopa, acetaminophen, and fenoldopam), decreasing their oral bioavailability and, thus, altering their pharmacokinetics and pharmacodynamics. Competition for intestinal sulfonation between coadministered substrates can influence their bioavailability with either an enhancement or a decrease of therapeutic effects. Sulfatase hydrolysis of biliary sulfate esters in the intestine can contribute to EHC of the parent drug.

Plants contain a variety of protoxins, promutagens, and procarcinogens, and the occurrence of intestinal P450 enzymes and bacterial enzymes in the microflora allows the metabolic conversion of relatively stable environmental pollutants and food-derived xenobiotics into mutagens and carcinogens. For example, cruciferous vegetables (Brussels sprouts, cabbage, broccoli, cauliflower, and spinach) are all rich in indole compounds (e.g., indole 3-carbinol) which, with regular and chronic ingestion, are capable of inducing some intestinal P450s (CYP1A subfamily) and inhibiting others (CYP3A subfamily). It is likely that these vegetables would also alter the metabolism of food-derived mutagens and carcinogens. For

example, heterocyclic amines produced during the charbroiling of meat are P450 N-hydroxylated and become carcinogenic in a manner similar to arylamines.

Intestinal Microflora

When drugs are orally ingested, or there is considerable biliary excretion of a drug or its metabolites into the GIT, such as with a parentally administered drug (EHC or recirculation), the intestinal bacterial microflora can have a significant metabolic role. The microflora has an important role in the enterohepatic recirculation of xenobiotics via their conjugated metabolites (e.g., digoxin, the oral contraceptives norethisterone and ethinyl estradiol and chloramphenicol) and endogenous substances (steroid hormones, bile acids, folic acid, and cholesterol), which reenter the gut via the bile. Compounds eliminated in the bile are conjugated with glucuronic acid, glycine, sulfate, and glutathione and, once secreted into the small intestine, the bacterial β -glucuronidase, sulfatase, nitroreductases, and various glycosidases catalyze the hydrolysis of the conjugates. The activity of orally administered conjugated estrogens (e.g., Premarin) involves the hydrolysis of the sulfate esters by sulfatases, releasing estrogens to be reabsorbed from the intestine into the portal circulation. The clinical use of oral antibiotics (e.g., erythromycin, penicillin, clindamycin, and aminoglycosides) has a profound effect on the gut microflora and the enzymes responsible for the hydrolysis of drug conjugates undergoing EHC. Bacterial reduction includes nitro reduction of nitroimidazole, azo reduction of azides (sulfasalazine to 5-aminosalicylic acid and sulfapyridine), and reduction of the sulfoxide to its sulfide. The sulfoxide of sulindac is reduced by both gut microflora and hepatic P450s. Other ways in which bacterial flora can affect metabolism include the following: (1) production of toxic metabolites, (2) formation of carcinogens from inactive precursors, (3) detoxication,

(4) exhibition of species differences in drug metabolism, (5) exhibition of individual differences in drug metabolism, (6) production of pharmacologically active metabolites from inactive precursors, and (7) production of metabolites not formed by animal tissues. In contrast to the predominantly hepatic oxidative and conjugative metabolism of the liver, gut microflora is largely degradative, hydrolytic, and reductive, with a potential for both metabolic activation and detoxication of xenobiotics.

Lung Metabolism

Some of the hepatic xenobiotic biotransformation pathways are also operative in the lung. Because of the differences in organ size, the total content of the pulmonary xenobiotic-metabolizing enzyme systems is usually lower than in the liver, creating the impression of a minor role for the lung in xenobiotic elimination. CYP2E1 is expressed in the lung to the greatest extent. The expression of other P450s, FMO, epoxide hydrolase, and the Phase 2 conjugation pathways are comparable to those in the liver. Thus, the lungs can have a significant role in the metabolic elimination or activation of low-molecular-weight inhaled xenobiotics.

When drugs are injected intravenously, intramuscularly, or subcutaneously, or after skin absorption, the drug initially enters the pulmonary circulation, after which the lung becomes the organ of first-pass metabolism ^[1]for the drug. The blood levels and therapeutic response of the drug are influenced by genetic disposition, induction, and inhibition of the pulmonary metabolizing enzymes. By modulation of the P450 isoform pattern in the lung, a xenobiotic could alter its own metabolism and that of others in a time- and dose-dependent manner. Because of its position in the circulation, the lung provides a second-pass metabolism for xenobiotics and their metabolites exiting from the liver, but it is also

susceptible to the cytotoxicity or carcinogenicity of hepatic activated metabolites. Antihistamines, β -blockers, opioids, and tricyclic antidepressants are among the basic amines known to accumulate in the lungs because of their binding to surfactant phospholipids in lung tissue. The significance of this relationship to potential pneumotoxicity remains to be seen.

Nasal Metabolism

The nasal olfactory mucosa is recognized as a first line of defense for the lung against airborne environmental xenobiotics because the mucosa is constantly exposed to the airborne external environment.⁷⁴ Drug metabolism in the olfactory nasal epithelium represents a major metabolic pathway for protecting the CNS and brain against the entry of drugs, inhaled environmental pollutants, or other volatile chemicals. In some instances, these enzymes can biotransform a given drug or environmental or airborne xenobiotic into more reactive and potentially toxic metabolites, increasing the risk of carcinogenesis in the nasopharynx and lung (e.g., procarcinogens and nitrosamines in cigarette smoke). P450 activity is high in olfactory tissue and olfactory-specific isoforms have been identified, which include CYP1A2, CYP2A6, CYP2A13, CYP2E1, CYP3A4, and the olfactory-specific CYP2G1 isoform, as well as several isoforms of the flavin-dependent monooxygenases. Phase 2 enzymes found in the olfactory mucosa include UGTs, glutathione-S-transferases, carboxylesterases, aldehyde oxidases, and epoxide hydrolases. The most striking feature of the nasal epithelium is that the P450 catalytic activity per gram of tissue is higher than in any other extrahepatic tissue or in the liver. Nasal decongestants, essences, anesthetics, alcohols, nicotine, and cocaine have been shown to be metabolized in vitro by P450 enzymes from the nasal epithelium. Because the P450s in the nasal mucosa are active, first-

pass metabolism should be considered when delivering susceptible drugs intranasally.

The nasal mucosa is also exposed to a wide range of odorants. Odorants, which are mostly small lipophilic molecules, enter the mucosa and reach the odorant receptors on sensory neurons by a transient process requiring signal termination, which could be provided by biotransformation of the odorant in the epithelial supporting cells. Thus metabolism of odorants could be involved in both the termination and initiation of olfactory stimuli.

Brain Metabolism

The metabolism of endogenous neurochemicals and neurotransmitters by brain CYPs may help explain variations in mood, aggression, personality disorders, and other psychiatric conditions.⁷⁵⁻⁷⁷ The role of brain CYP metabolism in neurotoxicity may also contribute to our understanding of the underlying mechanisms of diseases such as Parkinson's disease. Knowledge of CYP isoforms and transporter function in the blood-brain barrier (BBB) may increase our ability to deliver active forms of drugs specifically to the brain.

The isoforms of P450s and their regulation in the brain are of interest in defining the possible involvement of P450s in CNS toxicity and carcinogenicity. The expression level of P450 in the brain is approximately 0.5%-2% of that in liver, too low to significantly influence the overall pharmacokinetics of drugs and hormones in the periphery.^{75,77} Brain CYPs can impact acute and chronic drug response and susceptibility to damage by neurotoxins and are associated with altered personality, behavior, and risk of neurological disease. The functional role for brain CYP enzyme activity can impact CNS drug activation, protection against neurotoxicity, regulate neurotransmitter metabolism, chronic (e.g.,

nicotine dependence) response to drugs, and are associated with altered personality and behavior, and risk of neurological disease. Expression on a g/tissue basis can be as high as levels in hepatocytes. However, while hepatic CYPs are expressed primarily in the endoplasmic reticulum, brain expression is found in the mitochondrial and plasma membrane fraction and other cell membrane compartments. CYP1A1, CYP2B6, CYP2D6, CYP2E1, and CYP3A, are predominantly found in neurons and glial cells. CYP3A4 and CYP2C19 are expressed in human prefrontal cortex, hippocampus, and amygdala, areas that may mediate the behavioral effects of testosterone. Human CYP1B1 is found at the blood-brain interface, where it may act in conjunction with transporters, such as ATP-binding cassette transporters, to regulate passage of xenobiotics in and/or out of the brain. The interaction of a variety of transporters, in addition to local CYP-mediated metabolism, may play an important role in regulating the levels of centrally acting drugs in the brain and, thereby, their therapeutic effects.

The expression of brain CYPs levels vary among different brain regions. For example, in the human brain, CYP2B6 protein expression varies significantly among brain regions with a 2.5-fold range.⁷⁵ Brain CYP2D6 can metabolize endogenous neurochemicals, such as tyramine to dopamine and 5-methoxytryptamine to serotonin, while the metabolism of the endogenous cannabinoid anandamide, the neurosteroid progesterone, and testosterone (to 16 α -hydroxytestosterone) is catalyzed by CYP2B6.⁷⁵ Thus these enzymes may play a functional role in the brain. CYP2C19 can metabolize the sex hormones testosterone, progesterone, and estradiol that are known to affect brain function and personality traits, such as aggression and anxiety. CYP2E1 can metabolize the fatty acid neural signaling molecule arachidonic acid, which is abundant in the brain and is required for neurologic health, and CYP19 aromatase can metabolize testosterone to estradiol, both of

which can influence personality traits, including aggression, impulsivity, and anxiety. The differences in expression of specific brain CYPs can cause shifts in the neurochemical homeostasis in the brain.

Individuals with a CYP2D6 PM genotype are at greater risk for parkinsonism, and this risk is even greater with exposure to neurotoxic pesticides.⁷⁵ CYP2D6 metabolizes and inactivates a number of compounds that can cause parkinsonian symptoms, including MPTP, a Parkinson-causing compound, and its neurotoxic metabolite MPP+. CYP2D6 is expressed in human brain regions affected by Parkinson's disease, such as the substantia nigra. The impaired ability of CYP2D6 PMs to inactivate these neurotoxic metabolites may contribute to their increased risk for Parkinson's disease. In contrast to CYP2D6 PMs, smokers are at lower risk for Parkinson's disease since nicotine is neuroprotective. In humans, CYP2D6 expression levels are higher in the brains of smokers than in those of nonsmokers, while induction of brain CYP2D6 by nicotine or smoking may reduce an individual's relative risk for the disease. These data support a contributing role for lower brain CYP2D6 in the increased risk for Parkinson's disease, likely through modulation of local neurotoxin metabolism.⁷⁵

Nicotine is the main component of cigarette smoke that causes tobacco dependence, and genetic variation in brain CYP2B6, CYP2D6, and CYP2E1 can affect smoking behaviors.^{75,76} CYP2B6 slow metabolizers (IMs) progress to tobacco dependence more quickly and have more difficulty quitting than normal (EM) metabolizers. Brain CYP2B6 metabolizes nicotine and other endogenous substrates, such as serotonin and neurosteroids. Nicotine and smoking induces the brain expression of CYP2B6, CYP2D6, and CYP2E1, all of which affect smoking behaviors. Smokers have higher levels of brain CYP2D6, but unchanged levels of hepatic CYP2D6. Individuals exposed to nicotine through

smoking or through nicotine replacement therapy are likely to have increased CYP2D6-mediated brain metabolism of centrally acting drugs, neurotoxins, and endogenous neurochemicals, increasing risk of drug interactions and neurotoxicity. Brain levels of CYP2D6 enzymes and activity may be contributing factors to the clinical observations of personality differences with chronic nicotine administration between CYP2D6 EM and PM phenotypes. Preliminary data suggest that reducing brain CYP2B and CYP2D6 activity increases the rewarding properties of nicotine by altering brain levels of nicotine and its metabolites.

Metabolism in Other Tissues

P450s in the kidney and adrenal tissues include isoforms primarily involved in the hydroxylation of steroids, arachidonic acid, and 25-hydroxycholecalciferol.

Stereochemical Aspects of Drug Metabolism

In addition to the physicochemical factors that affect xenobiotic metabolism, stereochemical factors have an important role in the biotransformation of drugs. This involvement is not unexpected, because the xenobiotic-metabolizing enzymes are also the same enzymes that metabolize certain endogenous substrates which, for the most part, are chiral molecules. Most of these CYP isoforms show stereoselectivity but not stereospecificity; in other words, one stereoisomer enters into biotransformation pathways preferentially but not exclusively. Metabolic stereochemical reactions can be categorized as follows: substrate stereoselectivity, in which two enantiomers of a chiral substrate are metabolized by CYP isoforms at different rates; product stereoselectivity, in which a new chiral center is created in a symmetric molecule and one enantiomer is metabolized preferentially; and substrate-product stereoselectivity, in which a new chiral center of a chiral molecule is metabolized preferentially to one of two possible diastereomers.

An example of substrate stereoselectivity is the preferred decarboxylation of (*S*)- α -methyldopa to (*S*)- α -methyldopamine, with almost no reaction for (*R*)- α -methyldopa. Another example is the (*S*)-ketamine enantiomer, which is preferentially N-demethylated by CYP2B6 to (*S*)-norketamine, whereas the (*R*) enantiomer is N-demethylated by CYP3A4. The reduction of ketones to stereoisomeric alcohols and the hydroxylation of enantiotropic protons or phenyl rings by P450 are examples of product stereoselectivity. For example, phenytoin undergoes aromatic *p*-hydroxylation of only one of its two phenyl rings to create a chiral center at C5 of the hydantoin ring; methadone is reduced preferentially to its α -diastereometric alcohol and naltrexone is reduced to its 6- β -alcohol. Two examples of substrate-product

stereoselectivity are the reduction of the enantiomers of warfarin, and the β -hydroxylation of (*S*)- α -methyldopamine to (1*R*,2*S*)- α -methylnorepinephrine ((*R*)- α -methyldopamine is hydroxylated only to a negligible extent). In vivo studies of this type often can be confused by the further biotransformation of one stereoisomer, giving the false impression that only one stereoisomer was formed preferentially. Moreover, some compounds show stereoselective absorption, distribution, and excretion, which proves the importance of performing in vitro studies.

Although studies regarding the stereoselective biotransformation of drug molecules are not yet extensive, those that have been done indicate that stereochemical factors have an important role in drug metabolism. In some cases, stereoselective biotransformation could account for the differences in pharmacological activity and duration of action between enantiomers.

Metabolic Bioactivation: Role in Hepatotoxicity, Idiosyncratic Reactions, and Chemical Carcinogenesis

Drug-Induced Hepatotoxicity

Drug-induced hepatotoxicity is the leading cause of hepatic injury, accounting for approximately half of all cases of acute liver failure in the U.S. Recent studies have shown that drug-induced hepatotoxicity represents a larger percentage of adverse drug reactions than reported previously, and that the incidence and severity of drug-induced liver injury is underestimated among the general population.

Acetaminophen overdose is the leading cause for calls to poison control centers (>100,000 calls/year) and accounts for more than 56,000 emergency room visits, 2600 hospitalizations, and an estimated 458 deaths from acute liver failure each year. Among the listed drugs in Table 3.17, acetaminophen is the most frequently implicated hepatotoxic agent and can cause extensive hepatic necrosis with as little as 10-12 g (30-40 tablets). Chronic alcohol intake enhances acetaminophen hepatotoxicity more than five times as compared to acute alcohol intake, yet acetaminophen is heavily marketed for its safety as compared to nonsteroidal analgesics (NSAIDs). U.S. drug manufacturers continue to market and promote extra-strength acetaminophen products (500-750 mg/tablet) and a variety of Extra-strength acetaminophen-drug combination products. Self-poisoning with acetaminophen (paracetamol) is also a common cause of hepatotoxicity in the Western [SEP] world. To reduce the number of acetaminophen poisonings in the UK, over-the-counter (nonprescription) sales of acetaminophen are limited to 16 tablets per packet.

TABLE 3.17

Some Drugs Causing Hepatic Injury^a

Acarbose	<i>Felbamate</i>	Methotrexate	Ritonavir
Acetaminophen	Fenofibrate	Methsuximide	Rosiglitazone
Allopurinol	<i>Fluconazole</i>	Methyldopa	Rosuvastatin

Amiodarone	<i>Flutamide</i>	Nabumetone	<i>Saquinavir</i>
Amprenavir	Fluvastatin	Naproxen	Simvastatin
Anagrelide	Gemfibrozil	Nefazodone (2005)	Sulindac
<i>Atomoxetine</i>	Gemtuzumab	<i>Nevirapine</i>	<i>Tacrine</i>
Atorvastatin	Griseofulvin	Niacin (SR)	Tamoxifen
Azathioprine	<i>Halothane</i>	Nitrofurantoin	Tasosartan (1998)
Bicalutamide	Imatinib	Olanzapine	<i>Terbinafine</i>
Bosentan	Indinavir	Oxaprozin	Testosterone
Bromfenac (1998)	<i>Infliximab</i>	Pemoline (2005)	Thioguanine
Carbamazepine	Isoflurane	Pentamidine	Tizanidine
Celecoxib	Isotretinoin	Pioglitazone	<i>Tolcapone</i>
Dapsone	Itraconazole	Piroxicam	Troglitazone (2000)
<i>Diclofenac</i>	<i>Ketoconazole</i>	Pravastatin	<i>Trovafloxacin</i>
Disulfiram	<i>Ketorolac</i>	<i>Pyrazinamide</i>	<i>Valproic acid</i>
<i>Duloxetine</i>	Lamivudine	Ribavirin	Voriconazole
Efavirenz	<i>Leflunomide</i>	Rifabutin	Ximelagatran (2004)
Ethotoin	Lovastatin	<i>Rifampin</i>	<i>Zileuton</i>
Ethosuximide	Meloxicam	Riluzole	<i>Zafirlukast</i>

^aDrugs in **bold** have exhibited severe drug-induced hepatotoxicity and were withdrawn either voluntarily or by a regulatory agency (year given). Drugs in *italics* have exhibited moderate-severe drug-induced hepatotoxicity requiring a black box warning restricting their use. The other drugs have exhibited mild to moderate drug-induced hepatotoxicity that can need frequent liver transaminase testing for those at risk (see [Table 3.18](#)).

Drug-induced hepatotoxicity is also the most frequent reason that new therapeutic agents are not approved by the FDA (e.g., ximelagatran in 2004) and the most common adverse drug reaction leading to withdrawal of a drug from the market ([Table 3.17](#)). Hepatotoxicity almost always involves metabolism with Phase 1 P450 enzymes rather than Phase 2 enzymes. More than 600 drugs, chemicals, and herbal remedies can cause hepatotoxicity, of which more than 30 drugs have either been withdrawn from the U.S. market because of hepatotoxicity or have carried a black box warning for hepatotoxicity since 1990. [Table 3.17](#) includes some of the more common drugs that have exhibited drug-induced hepatotoxicity ranging from severe, requiring the drug's regulatory withdrawal from the market (bold in [Table 3.17](#)); moderate to severe, requiring black box warning restrictions (*italics* in [Table 3.17](#)); or mild to moderate, requiring frequent liver function monitoring.

However, Watkins⁷⁸ reported that one-third of 106 patients taking a maximum daily acetaminophen dose of 4 g for 8 days, either alone or in

combination with hydrocodone, exhibited a 3-fold increase in liver enzymes associated with acetaminophen-induced liver injury. This 3-fold increase in transaminase levels is a signal for potential liver safety concerns in those individuals who are at risk of acetaminophen-induced liver toxicity. Drugs reported to cause hepatocellular necrosis include acetaminophen, methyldopa, valproic acid, trazodone, nefazodone, venlafaxine, and lovastatin. Drug-induced liver damage occurs after a prolonged period of drug administration.

The most commonly used indicators of hepatotoxicity (i.e., liver injury) are increased levels of the liver transaminases, aspartate aminotransferase (AST), and alanine aminotransferase (ALT).⁷⁹ Drug-induced hepatotoxicity can develop rapidly, often before abnormal laboratory tests are noticed, which are characterized by rapid elevations in ALT and AST of 8-500 times the upper normal limit, with variable elevations in bilirubin. Drugs causing acute liver injury (hepatocellular necrosis) exhibit elevations in hepatic transaminases ranging from 50 to 100 times higher than the normal level. On the other hand, the elevations of ALT and AST in alcoholic liver disease are two to three times higher than normal. Some hepatotoxins, however, do not elevate transaminases, whereas nonhepatic toxins can elevate ALT.

Most drug-induced hepatotoxicity is of an idiosyncratic nature, occurring in a small percentage of patients (1 in 5000) who ingest the drug.⁸⁰⁻⁸² These reactions tend to be of two distinct types: (1) hypersensitivity reactions that are immune mediated, occurring within the first 4-6 weeks and are associated with fever, rash, eosinophilia, and a hepatitis-like picture (e.g., phenytoin, sulindac, and allopurinol); and (2) metabolic idiosyncratic reactions that tend to occur at almost any time during the first year of treatment (e.g., troglitazone). The incidence of overt idiosyncratic liver diseases varies with the drug, ranging from approximately 1 in 1000 with phenytoin, to 1 in 10,000 or more with sulindac and troglitazone and 1 in 100,000 with diclofenac. To detect a single case of drug-induced hepatotoxicity with 95% confidence requires the number of patients studied to be 3-fold the incidence of the reaction (i.e., for one adverse drug reaction in 10,000 patients, at least 30,000 patients need to be evaluated).

Thus, many drugs are approved before liver toxicity is observed. It is the responsibility of postmarketing surveillance and monitoring of liver transaminases to identify potential cases of liver-adverse drug reactions.

Risk factors (Table 3.18) for drug-induced liver injury, such as age, gender, genetic predisposition, multiple drugs, or dietary supplements and degree of alcohol consumption, appear to increase the susceptibility to drug-induced hepatotoxicity.^{80,81} However, the drugs in Table 3.17 should be used with caution in these high-risk patients, because such patients can have altered metabolism of these drugs and, therefore, can be at increased risk for liver injury. The coadministration of drugs in Table 3.17 with enzyme inducers, such as phenobarbital, phenytoin, ethanol, and/or cigarette smoke, can induce hepatic enzymes, resulting in the enhancement of hepatotoxicity.

TABLE 3.18
Risk Factors for Drug-Induced Liver Injury

Race	Some drugs exhibit different toxicities based on race because of individual P450 polymorphism. For example, blacks and Hispanics can be more susceptible to isoniazid (INH) toxicity.
Age	Elderly persons are at increased risk of hepatic injury due to decreased clearance, drug-drug interactions, reduced hepatic blood flow, variation in drug binding, and lower hepatic volume. In addition, poor diet, infections, and multiple hospitalizations are important reasons for drug-induced hepatotoxicity. Hepatic drug reactions are rare in children (e.g., acetaminophen, halogenated general anesthetics).
Gender	Although the reasons are unknown, hepatic drug reactions are more common in females. Females are more susceptible to hepatotoxicity from acetaminophen, halothane, nitrofurantoin, diclofenac, and sulindac.
Alcohol	Alcoholics are susceptible to drug toxicity because alcohol induces liver injury and cirrhotic changes that alter drug metabolism. Alcohol causes depletion of glutathione (hepatoprotective) stores, making the person more susceptible to toxicity by drugs (e.g., acetaminophen, statins).
Liver disease	Patients with chronic liver disease are not uniformly at increased risk of hepatic injury. Although the total P450 level is reduced, some patients can be affected more than others. The modification of doses in persons with liver disease should be based on knowledge of the specific P450 isoform involved in the metabolism. Patients with HIV infection who are coinfectd with hepatitis B or C virus are at increased risk for hepatotoxic effects. Similarly, patients with cirrhosis are at increased risk to hepatotoxic drugs (e.g., methotrexate, methyl dopa, valproic acid).

Genetic factors	Genetic (polymorphic) differences in the formation of P450 isoforms (2C family and 2D6) can result in abnormal reactions to drugs, including idiosyncratic reactions
Other comorbidities	Patients with AIDS, renal disease, diabetes mellitus, persons who are malnourished, and persons who are fasting can be susceptible to drug reactions because of low glutathione stores.
Pharmacokinetics	Long-acting drugs can cause more injury than shorter-acting drugs, as well as sustained-release drug product formulation.
Drug adulterants	Contaminants are often found in noncertified herbal supplements (e.g., hepatitis C).

Most hepatic adverse effects associated with drugs occur in adults rather than children. Drug-induced liver injury occurs at a higher rate in patients older than 50 years and drug-associated jaundice occurs also more frequently in the geriatric population.^{80,81} This age-risk can be the result of increased frequency of drug exposure, multidrug therapy, and age-related changes in drug metabolism.

For reasons that are unclear, drug-induced liver injury affects females more than males. Females accounted for approximately 79% of all reactions to acetaminophen and 73% of all idiosyncratic drug-induced reactions. Females exhibit increased risk of hepatic injury from drugs such as atorvastatin, nitrofurantoin, methyldopa, and diclofenac.⁸¹

Genetic factors because of enzyme polymorphism in affected individuals can decrease the ability to metabolize or eliminate drugs, thus increasing their duration of action and drug exposure and/or decreasing the ability to modulate the immune response to drugs or metabolites. Chronic ingestion of alcohol can also predispose many patients to increased hepatotoxicity from drugs by lowering the store of glutathione (a detoxifying mechanism), which prevents trapping of the toxic metabolites as mercapturate conjugates that are excreted in the urine.

Drug-induced hepatotoxicity can be categorized as intrinsic (predictable) or idiosyncratic (unpredictable) reactions.⁸² Most drugs involved in hepatotoxicity belong to the idiosyncratic group. Intrinsic hepatotoxins produce liver injury in a dose-related manner when a toxic amount of drug is ingested without bioactivation, such as the toxins found in the *Amanita* mushroom. Idiosyncratic hepatotoxicity is the result of the toxic effects of a drug's metabolites.

The common trigger for both mild and severe forms of hepatotoxicity is bioactivation of relatively inert functional groups to reactive electrophilic intermediates, which is considered to be an obligatory event in the etiology of many drug-induced idiosyncratic hepatotoxicity.^{83,84} Research now shows that reactive metabolites are formed from drugs known to cause idiosyncratic hepatotoxicity, but how these toxic species initiate and propagate tissue damage remains poorly understood. However, the relationship between bioactivation and the occurrence of hepatic injury is not simple. For example, many drugs at therapeutic doses undergo bioactivation in the liver but are not hepatotoxic. The tight coupling of bioactivation with bioinactivation pathways can be one reason for the lack of hepatotoxicity with these drugs. Examples of bioinactivation (detoxification) pathways include glutathione conjugation of quinones by glutathione S-transferases (GSTs) and hydration of arene oxides to dihydrodiols by epoxide hydrolases. When reactive metabolites are poor substrates for such detoxifying enzymes, they can escape bioinactivation and, thereby, damage proteins and nucleic acids, prompting hepatotoxicity.

Most drugs, however, are not directly chemically reactive but, through the normal process of drug metabolism, can form electrophilic, chemically reactive metabolites. Formation of chemically reactive metabolites is mainly catalyzed by P450 enzymes (Phase 1), but products of Phase 2 metabolism (e.g., acylglucuronides, acyl CoA thioesters, or N-sulfates) can also lead to toxicity. However, if Phase 1 drug bioactivation is closely coupled with Phase 2 bioinactivation (e.g., glutathione conjugation to mercapturates), then the net chemical process is one of detoxification if the final product is rapidly cleared.

Toxicity can accrue when accumulation of a chemically reactive metabolite, if not detoxified, leads to covalent modification of biological macromolecules. The identity of the target macromolecule and the functional consequence of its modification will dictate the resulting toxicological response. P450 enzymes are present in many organs, mainly the liver but also the kidney and lung, and thus can bioactivate chemicals to cause organ-specific toxicity. Evidence for the formation of reactive

metabolites was found for 5 of the 6 drugs that have been withdrawn from the market since 1995 and for 8 of the 15 drugs that have black box warnings (Table 3.17). Evidence for reactive metabolite formation has been found for acetaminophen, bromfenac, diclofenac, clozapine and troglitazone. Acetaminophen is the most studied hepatotoxin.

The current hypotheses of how reactive metabolites lead to liver injury suggest that hepatic (target) proteins can be modified by reactive metabolites. Much more clinically important is the identification of the target proteins modified by these toxic metabolites and how these reactions alter the function of those proteins. Additionally, it is important to note that the toxicity of reactive metabolites can also be mediated by noncovalent binding mechanisms, which can have profound effects on normal liver physiology. Such information should dramatically improve our understanding of drug-induced hepatotoxic reactions. Moreover, covalent binding per se does not necessarily lead to drug hepatotoxicity. The regioisomer of acetaminophen, 3-hydroxyacetanilide, becomes covalently bound to hepatic proteins in rodents without inducing hepatotoxicity.⁸⁵

It therefore is necessary to identify targets for these reactive metabolites (i.e., covalently modified macromolecules) that are critical to the toxicological process. Unfortunately, no simple rules predict the target macromolecules for a particular chemically reactive metabolite or the biological consequences of a particular modification. Furthermore, noncovalent interactions also have a role, because covalent binding of hepatotoxins is not indiscriminate with respect to proteins. Even within a single protein, there can be selective modification of an amino acid side chain found repeatedly in the primary structure. Thus, the microenvironment (e.g., pK_a and hydrophobicity) of the amino acid in the tertiary structure appears to be the crucial determinant of selective binding and, therefore, the impact of covalent binding on protein function. In turn, the extent of binding and the biochemical role of the protein will determine the toxicological insult of drug bioactivation. The resulting pathological consequences will be a balance between the rates of protein damage and the rates of protein replacement and cellular repair.

Drug-Induced Idiosyncratic Reactions

Idiosyncratic drug reactions (IDR; type B adverse drug reactions) occur in from 1 in 1000 to 1 in 50,000 patients, are not predictable from the known pharmacology or toxicology of the drug, are not produced experimentally in vitro and in vivo, and are dose independent. The occurrence of IDRs during late clinical trials or after a drug has been released can lead to severe restriction of its use and even its withdrawal. IDRs do not as a rule result from the drug itself because most people can tolerate the drug, but rather from a unique set of patient characteristics, including gender, age, genetic predisposition, and a lack of drug-metabolizing enzymes that can increase the risk of these adverse drug reactions. Most IDRs are caused by hypersensitivity reactions and can result in hepatocellular injury. The hepatic injury occurs within 1 week to 12 months after initiation of drug therapy and is often accompanied by systemic characteristics of allergic drug reactions, such as rash and fever. Signs of hepatic injury reappear with subsequent administration of the same drug with only one or two doses. Hypersensitivity reactions can be severe and associated with fatal reactions as a multiorgan clinical syndrome characterized by the following: (1) fever; (2) rash; (3) gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea, or abdominal pain); (4) generalized malaise, fatigue, or achiness; and (5) respiratory symptoms (e.g., dyspnea, cough, or pharyngitis). Examples of drugs causing IDRs through a hypersensitivity mechanism include penicillin, methyldopa, chlorpromazine, erythromycin, azathioprine toxicity in thiopurine methyltransferase-deficient individuals, sulfonamide and acetaminophen hepatotoxicity in alcoholics and in UGT1A6-deficient cats, ivermectin neurotoxicity in Collie dogs deficient in P-gp, perhexilene hepatotoxicity in CYP2D6-deficient individuals, phenytoin toxicity in CYP2C9-deficient individuals, and valproic acid hepatotoxicity.

The clinical features of some cases of drug-induced idiosyncratic hepatotoxicity strongly suggest an involvement of the immune system.^{83,84} These clinical characteristics include the following: (1) concurrence of rash, fever, and eosinophilia; (2) delay of the initial reaction (1-8 weeks) or requirement of repeated exposure to the culprit drug; (3) rapid recurrence

of toxicity on reexposure to the drug; and (4) presence of antibodies specific for native or drug-modified hepatic proteins. Our current understanding of drug-induced adaptive immune responses is largely based on the hapten hypothesis.

Idiosyncratic drug reactions that are connected with hepatotoxicity involve the formation of reactive metabolites.^{83,84} Such reactions are not predictable, but current bioanalytical technology have enabled the in vivo identification of the formation of reactive metabolites, as evidenced by the detection of biomarkers (i.e., mercapturate or cysteine adducts) in urine, drug-specific antibodies, or antibodies to P450 isoforms.⁸⁴ As a result, some drugs known to cause hepatic injury continue to be used because the drug's benefit outweighs its risk and no alternative efficacious drug exists. Additionally, NSAIDs, including cyclooxygenase-2 inhibitors (e.g., celecoxib), commonly are associated with idiosyncratic liver injury. Most of the idiosyncratic toxins listed in Table 3.19 that have been studied to date produce reactive metabolites.

TABLE 3.19
Some Examples of Idiosyncratic Toxins

Abacavir	Hypersensitivity
Acetaminophen	Hepatotoxicity
Amiodarone	Hepatotoxicity
Aromatic anticonvulsants	Hypersensitivity
Cefaclor	Hepatotoxicity
Clozapine	Agranulocytosis
Diclofenac	Hepatotoxicity
Felbamate	Aplastic anemia
Fibrates	Hepatotoxicity
Halothane	Hepatotoxicity
Indomethacin	Hepatotoxicity
Isoniazid	Hepatotoxicity
Levamisole	Hepatotoxicity
Nefazodone	Hepatotoxicity
Nevirapine	agranulocytosis
Oral contraceptives	Hepatotoxicity
Paroxetine	Hepatotoxicity
Penicillinamine ^a	Hypersensitivity
Phenytoin	Hepatotoxicity

Statins	Hepatotoxicity
Sulfonamides	Stevens-Johnson syndrome
Tamoxifen	Hepatotoxicity
Tacrine	Hepatotoxicity
Tienilic acid	Hypersensitivity
Ticlopidine	Agranulocytosis
Troglitazone	Hepatotoxicity
Valproic acid	Hepatotoxicity
Vesnarinone	Agranulocytosis

^aDoes not produce reactive metabolites.

Current hypotheses regarding IDRs suggest that metabolic activation of a drug to a reactive metabolite is a necessary step in the generation of an idiosyncratic reaction.⁸³ Evidence for this hypothesis comes from drugs that are associated with hepatotoxicity (Table 3.19) and the detection of drug-metabolite specific antibodies in affected patients.

For the other drugs that have been associated with idiosyncratic hepatotoxicity but that do not have black box warnings, either evidence for hepatotoxicity was not available or suitable studies had not been carried out. High doses increase the risk for an IDR (e.g., clozapine at 300 mg/d vs. olanzapine 20 mg/d).

The hapten hypothesis proposes that the reactive metabolites of hepatotoxic drugs act as haptens and bind covalently to endogenous proteins to form immunogenic drug-protein adducts triggering either antibody or cytotoxic T-cell responses.⁸⁴ The hapten hypothesis is supported by the detection of antibodies that recognize drug-modified hepatic proteins in the sera of drug-induced liver injury patients. For example, antibodies that recognize trifluoroacetate-altered hepatic proteins have been detected in the sera of patients with halothane-induced hepatitis. Such drug-specific antibodies that recognize native liver proteins have also been found in patients with liver injury caused by other drugs, such as diclofenac. Most drugs are small molecules and are unlikely to form haptens. Electrophilic acylators can react with the lysine ω -amino residues of the target protein or guanosine residues of DNA. Halothane is the most studied molecule for supporting this hypothesis regarding IDRs.⁸⁴ Therefore, it is not surprising that irreversible chemical modification of a protein, which has a profound effect on function, is a

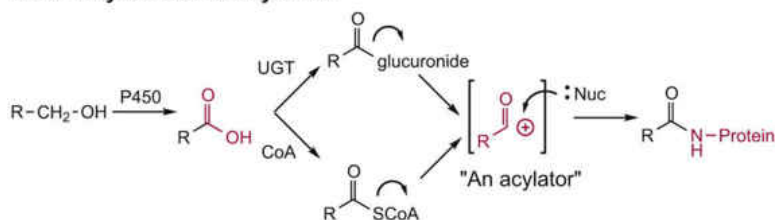
mechanism of idiosyncratic hepatotoxicity. However, it is important to note that a number of drugs (e.g., penicillins, aspirin, and omeprazole) rely on covalent binding to proteins for their efficacy; thus, prevention of their covalent binding through chemical modification of the compound can also, inadvertently, lead to loss of efficacy.

Reactive Metabolites Resulting From Bioactivation

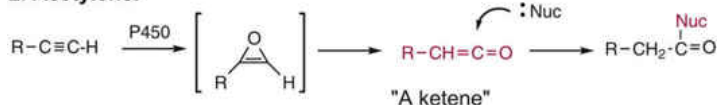
Electrophiles

The concept that small organic molecules can undergo bioactivation to electrophiles (an electron-deficient group or ion that accepts an electron pair to make a covalent bond, such as carbocations and acyl ions) and free radicals, and can elicit toxicity by chemical modification of cellular macromolecules, has its basis in chemical carcinogenicity and the pioneering work of the Millers⁸⁶ and Gillette et al.⁸⁷ Electrophiles are reactive because they possess electron-deficient centers (polarization-activated double bonds or positive-charge acylators) (Fig. 3.38) and can form covalent bonds with electron-rich biological nucleophiles such as the thiol groups in either glutathione or cysteine residues within proteins. A number of different types of reactive metabolites exist; however, they can be broadly classified as either electrophiles (Fig. 3.38) or free radicals (Fig. 3.39).⁸⁸ These reactive metabolites are short-lived, with half-lives of usually less than 1 minute, and they are not normally detectable in plasma or urine except as Phase 2 conjugates or other biomarkers.

1. Carboxylic acids as acylators:



2. Acetylene:



Example: Ethinylestradiol

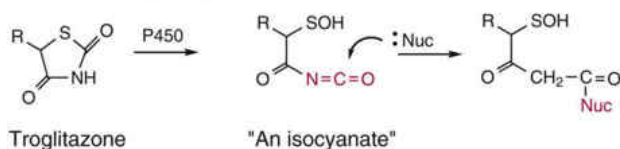
3. Halogenated hydrocarbon:



Haloethane

Trifluoroacetyl halide
(An acylator)

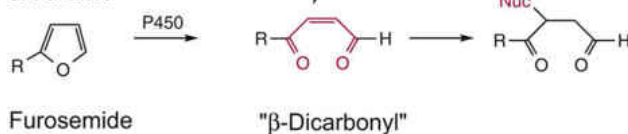
4. Thiazolidinedione:



Troglitazone

"An isocyanate"

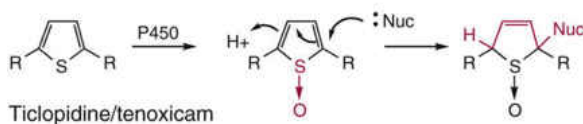
5. Furans:



Furosemide

" β -Dicarbonyl"

6. Thiophene:



Ticlopidine/tenoxicam

7. Aromatics and olefins:

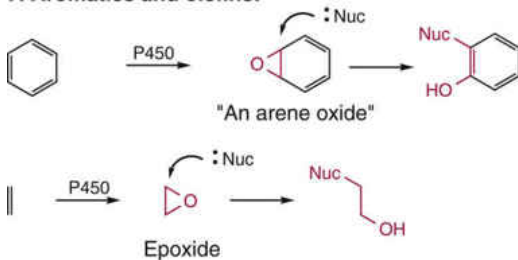
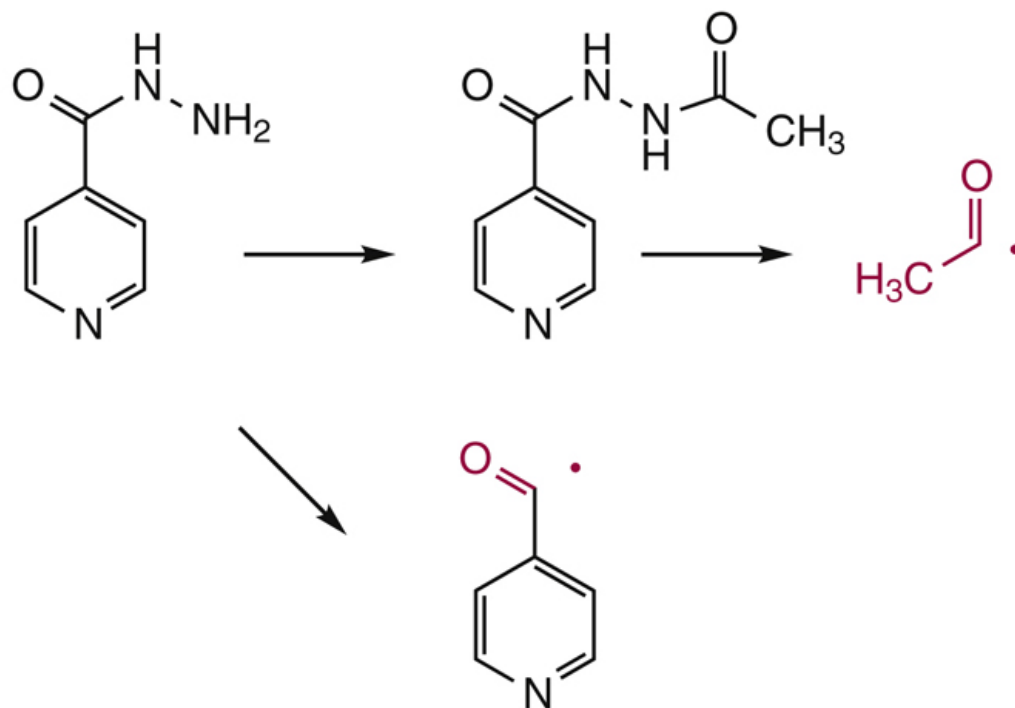


FIGURE 3.38 Some examples of electrophilic intermediates resulting from bioactivation. *NUC*, nucleophiles.

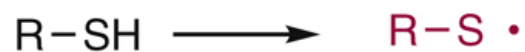
1. Isoniazid



2. Hydrazines



3. Sulfhydryl

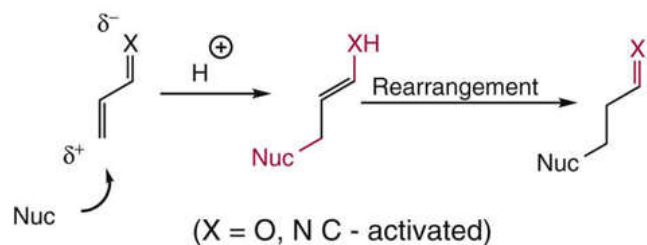


Captopril

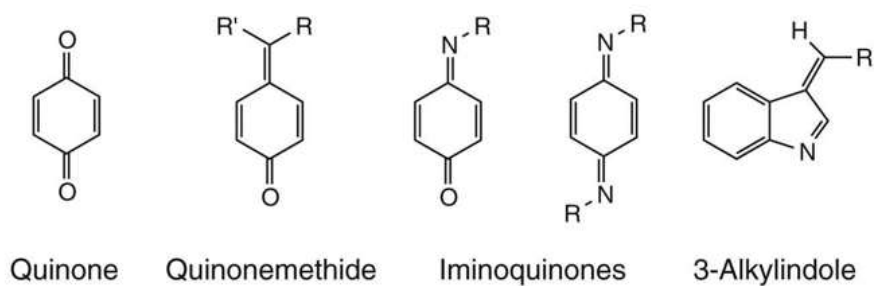
FIGURE 3.39 Drug bioactivation to free radicals.

Activated double bonds are electrophilic intermediates. Examples of activated double bond electrophiles include α,β -unsaturated carbonyl compounds, quinones, quinoneimines, quinonemethides, and diiminoquinones as shown in Figure 3.40. These electrophilic intermediates are highly polarized and can react with nucleophiles in a 1,4-Michael-type addition at the more electrophilic β -carbon of the activated double bond intermediate to generate the addition product (Fig. 3.40A). Specific examples of activated double bond electrophiles that have been proposed for the anticancer drug leflunomide, acetaminophen, the antiandrogen flutamide, the anticonvulsant felbamate, and the cytotoxic cyclophosphamide are shown in Figure 3.40C. The bioinactivation pathways for these electrophilic intermediate can involve direct addition, with or without GSH or epoxide transferases, depending upon the degree of polarization and reactivity of the electrophilic intermediate.⁸⁹

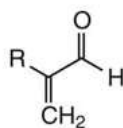
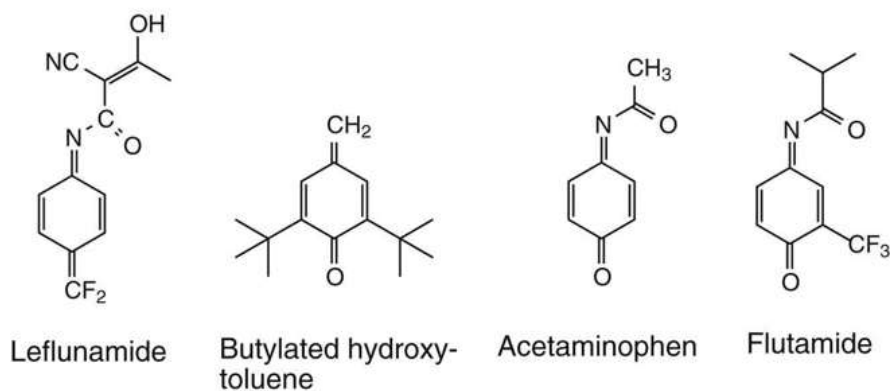
A. 1,4-Addition of a nucleophile in a Michael-type reaction:



B. Examples of activated double bonds:



C. Reactive intermediates from drugs that form activated double bonds:



R = phenyl - felbamate

R = H - acrolein from cyclophosphamide

FIGURE 3.40 Examples of activated double bonds to electrophilic reactive intermediates.

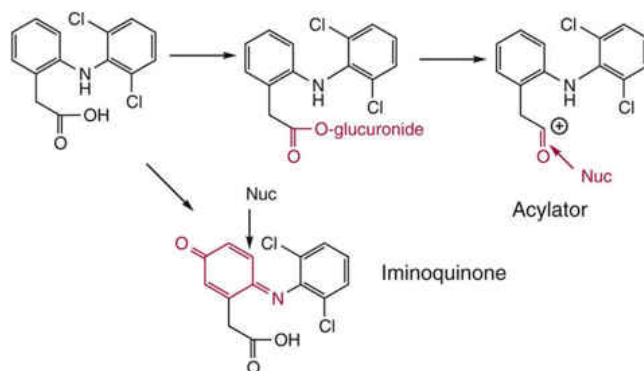
Other commonly found electrophilic intermediate for drug molecules in Figure 3.38 include ketenes from the bioactivation of acetylenic groups (e.g., ethinyl estradiol) (2 in Fig. 3.38); isocyanates from thiazolidinediones (e.g., the “glitazones”) (4 in Fig. 3.38)⁸⁸; acyloxonium ions from halogenated hydrocarbons (e.g., haloethane) (3 in Fig. 3.38)⁸⁸ and carboxylic acids; β -dicarbonyl from furans (e.g., furosemide) (5 in Fig. 3.38)⁸⁸; activated thiophene-S-oxide from thiophenes, such as ticlopidine and tenoxicam which cause an IDR, agranulocytosis (6 in Fig. 3.38)⁸⁸; and epoxides and arene oxides from olefins and aromatic compounds (7 in Fig. 3.38).⁸⁸

Drugs possessing structural features prone to metabolic epoxidations are abundant. Therefore the incidence of epoxide metabolites in mediating adverse biologic effects has aroused concern about clinically used drugs known to be metabolized to epoxides. Metabolically produced epoxides have been reported for allobarbital, secobarbital, protriptyline, carbamazepine, and cyproheptadine and are implicated with 8-methoxypsoralen and other furanocoumarins (6,7-dihydroxybergamottin in grapefruit juice), phenytoin, phenoximide, phenobarbital, mephobarbital, lorazepam, and imipramine.^{89,90} The alarming biologic effects of some epoxides, however, do not imply that all epoxides have similar effects. Epoxides vary greatly in molecular geometry, stability, electrophilic reactivity, and relative activity as substrates for epoxide-transforming enzymes (e.g., epoxide hydrolase, glutathione S-transferase, and others).

Some carboxylic acid-containing drugs have been implicated in rare IDRs, which was the basis for the market withdrawal of the NSAIDs zomiperac and benzoxaprofen.^{57,91} These drugs (e.g., NSAIDs, fibrates, “statins,” and valproic acid) can be bioactivated to acyl glucuronides or acyl CoA thioesters⁵⁷ (1 and 5 in Fig. 3.41). These products are electrophilic acylators that can acylate target proteins if they escape inactivation by S-glutathione-thioester formation.⁹² A crucial factor is the concentration of acyl glucuronides in hepatocytes due to their transport by conjugate export pumps, where acylglucuronides can selectively acylate hepatic membrane proteins. Acyl CoA esters can be either rapidly hydrolyzed or further metabolized in hepatocytes. Evidence is accumulating that acyl

glucuronides can alter cellular function by haptening of peptides, target protein acylation or glycation, or direct stimulation of neutrophils and macrophages. The role of acyl CoA reactive metabolites is less clear. It should be noted that some noncarboxylic acid drugs can be biotransformed by oxidative metabolism in the liver to the respective carboxylic acids.⁹³

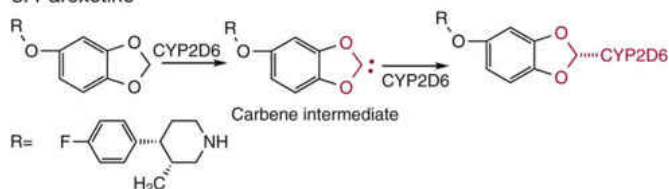
1. Diclofenac



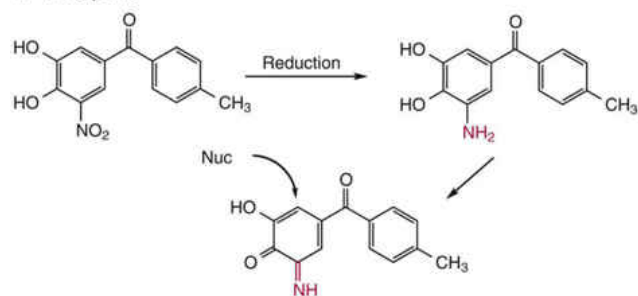
2. Carbamazepine



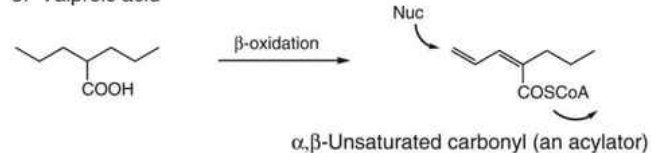
3. Paroxetine



4. Tolcapone



5. Valproic acid



6. Clozapine

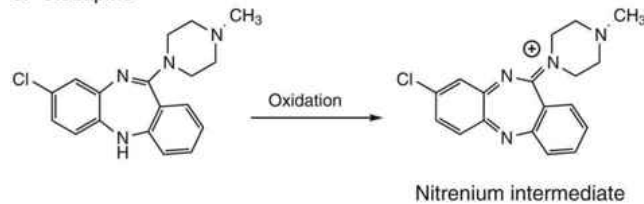


FIGURE 3.41 Examples of drug bioactivation to hepatotoxic intermediates.

Free Radicals

P450 activates molecular dioxygen to generate reactive oxygen species (ROS), such as singlet oxygen ($^1\text{O}_2$) or superoxide. Reactive metabolites that possess an odd unpaired electron are free radicals that can react with molecular oxygen (ground state triplet) to generate intracellular oxidative stress damage.⁹⁴ Free radicals abstract a hydrogen atom from other molecules rather than becoming covalently bound. Free radical reactions can be self-propagating by abstracting a hydrogen atom from the double bond of a lipid that initiates a chain reaction leading to lipid peroxidation, oxidative stress, or other types of modification of biological molecules.

Some examples of free radicals generated by the bioactivation of drug molecules are shown in Figure 3.39. Isoniazid is acetylated to its major metabolite acetylisoniazid, which is hydrolyzed to acetylhydrazine and isonicotinic acid (1 in Fig. 3.39). Acetylhydrazine is further metabolized by the CYP2E1 to an N-hydroxy intermediate that hydrates into an acetyl radical, which can then initiate the process that leads to hepatic necrosis. Other carbon-centered radicals are formed from hydrazines such as the antihypertensive hydralazine and thio-radicals from the ACE inhibitor captopril (3 in Fig. 3.39).

Bioinactivation Mechanisms

Several enzyme systems exist as cellular defense (detoxification) pathways against the chemically reactive metabolites generated by P450 metabolism. These include GST, epoxide hydrolase, and quinone reductase, as well as catalase, glutathione peroxidase, and superoxide dismutase, which detoxify the peroxide and superoxide by-products of metabolism. The efficiency of the bioinactivation process is dependent on the inherent chemical reactivity of the electrophilic intermediate, its affinity and selectivity of the reactive metabolite for the bioinactivation enzymes, the tissue expression of these enzymes, and the rapid upregulation of these enzymes, and cofactors mediated by the cellular sensors of chemical stress. The reactive metabolites that can evade these defense systems can damage target proteins and nucleic acids by either oxidation or covalent modification.

The most abundant agents of cellular defense are thiols. Glutathione will only react noncatalytically with electrophiles, such as activated double bonds (Fig. 3.40). Glutathione conjugation to mercapturates is one of the most important defenses against hepatocellular injury.^{58,59} Glutathione protects cellular enzymes and membranes from toxic metabolites, and its inadequate stores can compromise efficient detoxification of the reactive metabolites. The subsequent inability to detoxify the reactive metabolites can result in hepatocellular injury. The rate-limiting factor for glutathione synthesis is the intracellular concentration of cysteine. N-acetylcysteine is often used as an alternative to glutathione to trap the iminoquinone intermediate in the treatment of acute acetaminophen toxicity and in preventing nephrotoxicity from ifosfamide-generated chloroacetaldehyde. Glutathione has a protective role in the hepatic tissue injury produced by acetaminophen, but not by furosemide.

The relationship between bioactivation, bioinactivation, and DNA adduct formation has been well established for a number of hepatocarcinogens.⁹⁴ Glutathione conjugation of carcinogens becomes more efficient when catalyzed by GSTs, an important example being the detoxification of the hepatocarcinogen aflatoxin. Aflatoxin, a hepatocarcinogen and a hepatotoxin found in mold growing on peanuts, is converted into aflatoxin B1 epoxide in rodents, which is more readily detoxified by GST enzymes than by epoxide hydrolase. The balance between these transferase reactions explains the greater DNA damage in humans compared with rodents, because human forms of GST are less able to catalyze the conjugation of aflatoxin epoxide compared with the rodent forms. Transgenic knockout mice have been used to establish the role of bioactivation by P450 and bioinactivation by GSTs for a number of carcinogenic polyaromatic hydrocarbons.

Substances that detoxify free radicals include the antioxidants vitamin C, vitamin E, and carotene, which scavenge free radicals, including reactive metabolites and reactive oxygen species generated as a consequence of chemical stress.

Specific Examples

Some examples of bioactivation to hepatotoxic or IDR electrophilic intermediates are shown in Figure 3.41. Bioactivation can occur by both oxidation and conjugation reactions, such as those with diclofenac, which undergoes the formation of an acyl glucuronide to produce iminoquinones via formation of a phenol intermediary (1 in Fig. 3.41).⁹² The anticonvulsant carbamazepine is 2-hydroxylated and the elimination of the amide group yields the reactive quinoneimine intermediate (2 in Fig. 3.41). The antidepressant paroxetine and other xenobiotics with the common methylenedioxyphenyl nucleus undergo methylene oxidation to a *p*-quinoid intermediate (3 in Fig. 3.41). The nitro group of the COMT inhibitor tolcapone, used in the treatment of parkinsonism, is first reduced to an amine, then oxidized to an *o*-quinoneimine (4 in Fig. 3.41). The mitochondrial/hepatotoxicity of the anticonvulsant valproic acid results from the formation of an activated α,β -unsaturated CoA thioester via mitochondrial β -oxidation, most commonly associated with the oxidation of fatty acids (5 in Fig. 3.41). The agranulocytosis resulting from the ingestion of the antipsychotic clozapine is bioinitiated by oxidation by hypochlorous acid in neutrophils to a nitrenium intermediate (6 in Fig. 3.41).⁹³

The effect of structure modification for troglitazone that reduced its hepatotoxicity is shown in Figure 3.42. The *p*-dihydroxy groups of the chroman ring nucleus (outlined in red in Fig. 3.42) of troglitazone is bioactivated to an activated double bond (*p*-quinone) was replaced with a pyridine ring that is not bioactivated, although the thiazolidone ring can be bioactivated to an isocyanate (Fig. 3.42).⁹¹

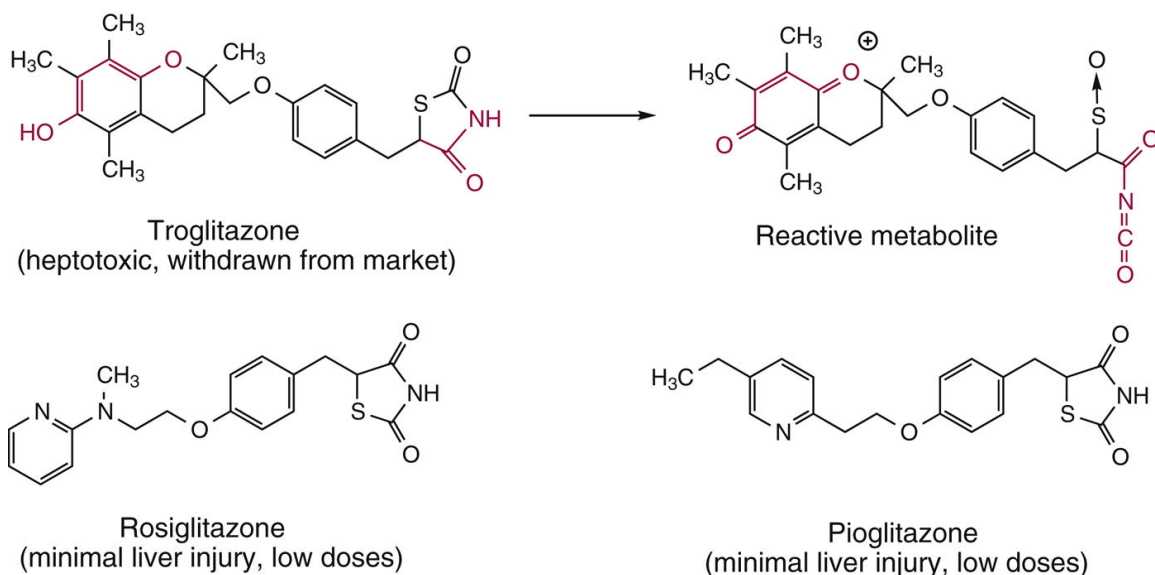


FIGURE 3.42 The effect of structure modification on drug-induced hepatotoxicity of troglitazone.

The oxidation of acetaminophen to the chemically reactive N-acetyl-p-benzoquinoneimine (Fig. 3.36) is catalyzed by the isoforms CYP1A2 and CYP2E1. The reactive quinoneimine can react covalently either with glutathione to form an inactive product or with cellular macromolecules, initiating the processes leading to hepatic necrosis (Fig. 3.36).⁹³ The usual route for acetaminophen metabolism is glucuronidation. If insufficient UDP-glucuronic acid is present, then bioactivation will dominate.

Furosemide, a frequently used diuretic drug, is reportedly a human hepatocarcinogen. The hepatic toxicity apparently results from metabolic activation of the furan ring to a β -dicarbonyl intermediate (5 in Fig. 3.38).⁹³ Ticlopidine and tenoxicam, reported to cause agranulocytosis, do so via metabolic activation of the thiophene ring to an S-oxide (6 in Fig. 3.38).⁹² The agranulocytosis resulting from the ingestion of clozapine is via its bioactivation to a nitrenium ion intermediate (6 in Fig. 3.41).^{93,94}

Drug-Induced Chemical Carcinogenesis

The mechanism whereby xenobiotics are transformed into chemical carcinogens is usually accepted as bioactivation to reactive metabolites, which are responsible for initiating carcinogenicity.^{86,87} Many carcinogens elicit their cytotoxicity through a covalent linkage to DNA. This process

can lead to mutations and, potentially, to cancer. Most chemical carcinogens of concern are chemically inert but require activation by the xenobiotic-metabolizing enzymes before they can undergo reaction with DNA or proteins (cytotoxicity). There are many ways to bioactivate procarcinogens, promutagens, plant toxins, drugs, and other xenobiotics (Fig. 3.43).

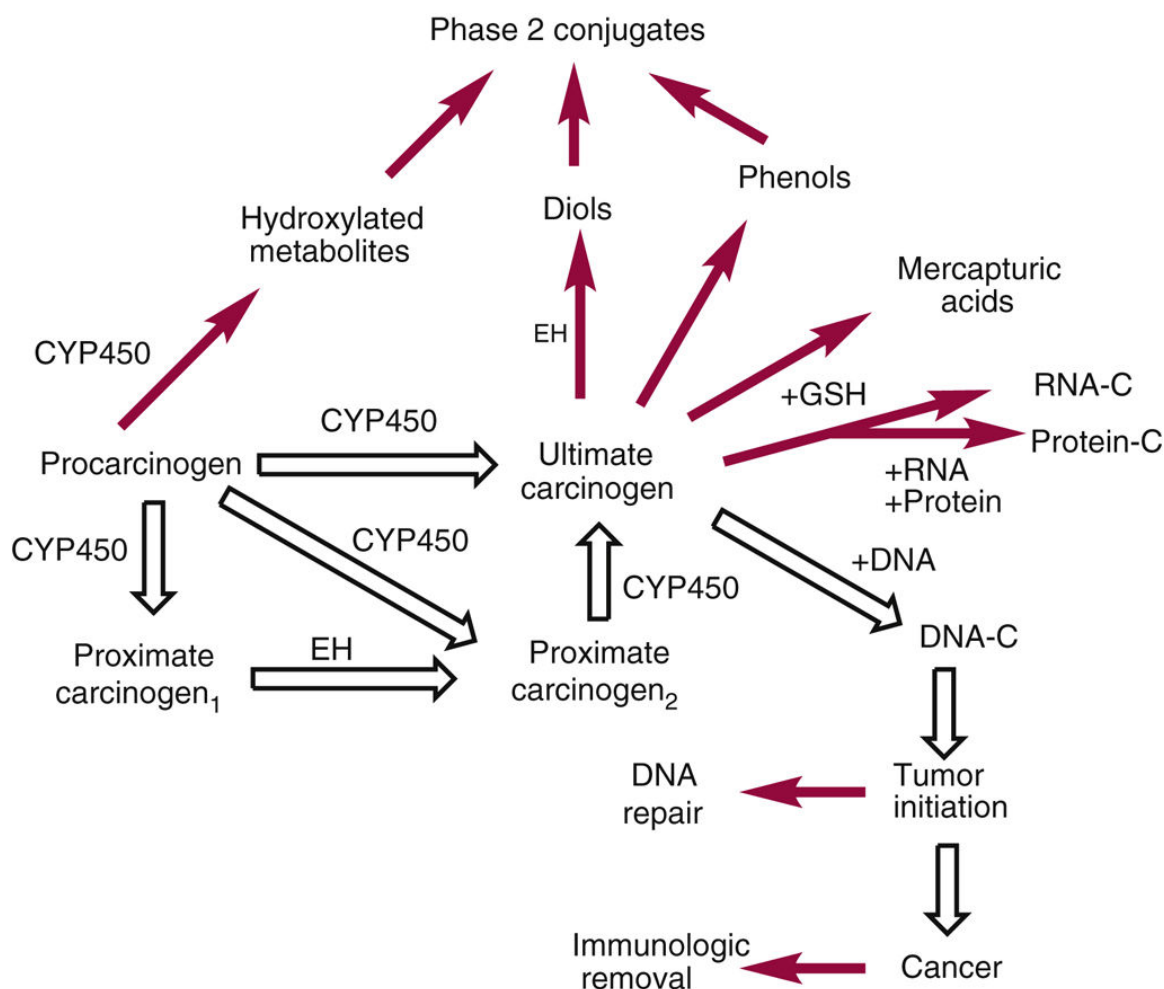


FIGURE 3.43 The bioactivation of procarcinogens and a proposed mechanism of chemical carcinogenesis.

Oxidative bioactivation reactions are by far the most studied and common. Conjugation reactions (Phase 2), however, are also capable of activating these xenobiotics to produce electrophiles, in which the conjugating derivative acts as a leaving group. These reactive metabolites are mostly

electrophiles, such as epoxides, quinones, or free radicals formed by the P450 enzymes or FMO. The reactive metabolites tend to be oxygenated in sterically hindered positions, making them poor substrates for subsequent bioinactivation enzymes, such as epoxide hydrolase and GST. Therefore, their principal fate is formation of covalent linkage to intracellular macromolecules, including enzyme proteins and DNA.^{91,92} Experimental studies indicate that the CYP1A subfamily can oxygenate aromatic hydrocarbons (e.g., PAHs) in sterically hindered positions to arene oxides. Activation by N-hydroxylation of polycyclic aromatic amines (e.g., aryl N-acetamides) appears to depend on either FMO or P450 isoforms. The formation of chemically reactive metabolites is important, because they frequently cause a number of different toxicities, including tumorigenesis, mutagenesis, tissue necrosis, and hypersensitivity reactions.

Our understanding of these reactions was advanced by the studies of the Millers⁸⁶ and Gillette et al.⁸⁷ They proposed that the proportion of the dose that binds covalently to critical macromolecules could depend on the quantity of the reactive metabolite is formed.

A scheme illustrating the complexities of drug-induced chemical carcinogenesis is shown in [Figure 3.43](#). Reactions that proceed via the open arrows eventually lead to neoplasia. Some carcinogens can form the “ultimate carcinogen” directly through P450 isoform bioactivation; others, like the PAHs (e.g., benzo[a]pyrene), appear to involve a multistep reaction sequence forming an epoxide, reduction to a diol by epoxide hydrolase, and perhaps the formation of a second epoxide group on another part of the molecule. Other procarcinogens form the N-hydroxy intermediate that requires transferase-catalyzed conjugation (e.g., O-glucuronide and O-sulfate) to form the “ultimate carcinogen.” The quantity of “ultimate carcinogen” formed should relate directly to the proportion of the dose that binds or alkylates DNA.

The solid-arrow reaction sequences in [Figure 3.43](#) are intended to show detoxification mechanisms, which involve several steps. First, the original chemical can form less active products (phenols, diols, mercapturic acids, and other conjugates). Second, the “ultimate carcinogen” can rearrange to be prevented from its reaction with DNA (or whatever the critical

macromolecule is). Third, the covalently bound DNA can be repaired. Fourth, immunologic removal of the tumor cells can occur. Several mechanisms within this scheme could regulate the quantity of covalently bound carcinogen: (1) The activity of the rate-limiting enzyme, such as epoxide hydrolase, P450 isoform, or one of the transferases, could be involved; (2) the availability of cofactors, such as glutathione, UDP-glucuronic acid, or PAPS, can be rate-limiting; (3) relative P450 activities for detoxification and activation must be considered; (4) availability of alternate reaction sites for the ultimate carcinogen (e.g., RNA and protein can be involved); (5) and possible specific transport mechanisms that deliver either the procarcinogen or its ultimate carcinogen to selected molecular or subcellular sites.

It is well established that numerous organic compounds that are essentially nontoxic as long as their structure is preserved can be converted into cytotoxic, teratogenic, mutagenic, or carcinogenic compounds by normal biotransformation pathways in both animals and humans. The reactive electrophilic intermediate involves reaction with cellular DNA constituents forming either detoxified products or covalent bonding with essential macromolecules, initiating processes that eventually lead to the toxic effect. A better understanding of the mechanisms underlying these reactions can lead to more rational approaches to the development of nontoxic therapeutic drugs. Special attention to risk factors is required for drugs that will be used for long periods in the same patient.

Drug-Drug Interactions

Drug-drug interactions represent a common clinical problem, which has been compounded by the introduction of many new drugs and the expanded use of herbal medicines. Such interactions do significant harm, prompting anywhere from 1.9 to 5 million patient visits to hospital emergency departments per year according to data from the Medication Safety Program at the Centers for Disease Control and Prevention. Approximately 50% of these serious drug-drug interactions involved P450 inhibition. The problem is likely to grow in the future as the population ages and more people take multiple medications.

Drug-drug interactions occur when the efficacy or toxicity of a medication is changed by coadministration of another substance, drug, food (i.e., grapefruit), or herbal product.^{95,96} Pharmacokinetic interactions often occur because of a change in drug metabolism. For example, CYP3A4 oxidizes more than 30% of the clinically used drugs with a broad spectrum of structural features, and its location in the small intestine and liver permits an effect on both presystemic and systemic drug disposition. Some drug-drug interactions with CYP3A4 substrates/inhibitors can also involve inhibition of P-gp. Other clinically important sequelae due to drug-drug interactions resulting from coadministration of CYP3A4 substrates or inhibitors include rhabdomyolysis with the coadministration of some 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors ("statins"), symptomatic hypotension with some dihydropyridine calcium antagonists, excessive sedation from benzodiazepine or nonbenzodiazepine hypnotics, ataxia with carbamazepine, and ergotism with ergot alkaloids.

The clinical importance of any drug-drug interaction depends on factors that are drug-, patient-, and administration-related. Drugs with low oral bioavailability or high first-pass metabolism are particularly susceptible to drug-drug interactions when inhibitors that alter absorption, distribution, and elimination are coadministered. In general, a doubling or more of the plasma drug concentration has the potential for enhanced adverse or beneficial drug response. Less pronounced pharmacokinetic drug interactions can still be clinically important for drugs with a steep concentration-response relationship or narrow therapeutic index. In most

cases, the extent of drug interaction varies markedly among individuals; this is likely to be dependent on interindividual differences in P450 content (polymorphism), preexisting medical conditions, and possibly age. Drug-drug interactions can occur under single-dose conditions or only at steady state. The pharmacodynamic consequences may or may not closely follow pharmacokinetic changes. Drug-drug interactions can be most apparent when patients are stabilized on the affected drug and the P450 substrates or inhibitors are then added to the regimen. One reason for the increased incidence of drug-drug interactions is the practice of simultaneously prescribing several potent drugs, as well as concurrently ingesting nonprescription products and herbal products that compete for, inhibit, or induce metabolizing enzymes.

Although drug-drug interactions constitute only a small proportion of adverse drug reactions, they have become an important issue in health care. Many of the drug-drug interactions can be explained by alterations in the metabolic enzymes in the liver and other extrahepatic tissues, and many of the major pharmacokinetic interactions between drugs are caused by hepatic P450 isoenzymes being affected by coadministration of other drugs. Some drugs act as potent enzyme inducers, whereas others are inhibitors. Drug-drug interactions involving enzyme inhibition, however, are much more common. Understanding these mechanisms of enzyme inhibition or induction is extremely important to appropriately administer multidrug therapies. Individuals at greatest risk for drug-drug interactions and adverse events need to be identified and treated based upon their specific pharmacogenomic-related needs.

P450s have a dominant role in the metabolism and elimination of drugs from the body, and their substrates are shown in [Tables 3.3 and 3.7-3.10](#). Drugs in ***bold italics*** have been associated with clinically relevant drug-drug interactions. Inhibitors of P450 are shown in [Table 3.11](#). Pharmacokinetic interactions can arise when the biotransformation and elimination of a drug are impaired by coadministered drugs. Thus, drugs can compete for biotransformation by a common P450. Adverse drug reactions, including toxicity, can occur if elimination is dependent on a P450 that exhibits defective gene variants. Thus, the genetic makeup of the individual (see

section Genetic Polymorphism) has a major influence on the duration of drug action, as well as on drug efficacy and safety.

P450 pharmacogenetics affects the tendency for certain drug-drug interactions to occur. Thus, the future safe use of drug combinations in patients can require genotyping and phenotyping of individuals before the commencement of therapy. Identification of subjects who metabolize drugs in a different fashion from the general population should minimize the impact of pharmacogenetic variation on drug pharmacokinetics.

Metabolism-Based Enzyme Inhibition

Many drug-drug interactions are the result of inhibition or induction of P450 enzymes. Metabolism-based enzyme inhibition involves mostly reversible competition between two drugs for the enzyme-active site. Metabolic drug-drug interactions occur when drug A (or its metabolite) alters the pharmacokinetics of a coadministered drug B by inhibiting, activating, or inducing the activity of the enzymes that metabolize drug B. Inhibitory drug-drug interactions could result in serious adverse effects, including fatalities in patients receiving multiple medications. This process is competitive and begins with the first dose of the inhibitor, with the extent of inhibition correlating with their relative affinities for the enzymes and the metabolic half-lives of the drugs involved. On the other hand, mechanism-based (irreversible) inhibition results from a metabolite that binds irreversibly to the enzyme through a covalent bond, rendering the enzyme inactive.³⁶

Enzyme-specific P450 inhibitors, including metabolism- and mechanism-based inhibitors, or drugs that are metabolized by specific P450 isoforms, are by and large excluded from further consideration for new drug development. Not only is CYP3A4 the most abundant isoform in human liver, it also metabolizes more than 30% of the drugs in clinical use, which renders CYP3A4 highly susceptible to both metabolism- (reversible) and mechanism-based inhibition. The CYP3A subfamily is involved in many clinically significant drug-drug interactions and therefore metabolism-based inhibition of CYP3A can cause clinically significant drug-drug interactions, such as those involving nonsedating antihistamines and the GI motility stimulant cisapride that can result in cardiac dysrhythmias. An excellent example of a metabolism-based inhibition drug-drug interaction that resulted in a life-

threatening ventricular arrhythmia associated with QT prolongation (torsades de pointes) occurred when CYP3A4 substrates or inhibitors were coadministered with terfenadine, astemizole, cisapride, or pimozide. This potentially lethal drug interaction led to the withdrawal of terfenadine and cisapride from clinical use and to the approved marketing of fexofenadine, the active metabolite of terfenadine, which does not have this interaction.

Inhibitors of CYP3A4 can increase the risk of toxicity from many drugs, including carbamazepine, cyclosporine, ergot alkaloids, lovastatin, protease inhibitors, rifabutin, simvastatin, tacrolimus, and vinca alkaloids. Furthermore, inhibitors of CYP1A2 can increase the risk of toxicity from clozapine or theophylline. Inhibitors of CYP2C9 can increase the risk of toxicity from phenytoin, tolbutamide, and oral anticoagulants (e.g., warfarin). Inhibitors of CYP2D6 can increase risk of toxicity of many antidepressants, opioid analgesics, and psychotherapeutic agents. Examples of enzyme inducers include barbiturates, carbamazepine, glutethimide, griseofulvin, phenytoin, primidone, rifabutin, and rifampin. Some drugs, such as ritonavir, can act as either an enzyme inhibitor or an enzyme inducer, depending on the situation. Drugs metabolized by CYP3A4 or CYP2C9 are particularly susceptible to enzyme induction.

Mechanism-Based Enzyme Inhibition

Mechanism-based inhibition differs from metabolism-based (reversible) inhibition in that the inhibitors require enzymatic activation by the target enzyme prior to exerting their inhibitory effect.³⁶ This initial activation step leads to the formation of an active inhibitor, often referred to as the metabolite-intermediate complex (MIC). The MIC can then exert its inhibitory effect by either forming a direct covalent link with the enzyme or forming a noncovalent tight binding complex. Mechanism-based inhibition is characterized by NADPH-, time-, and concentration-dependent enzyme inactivation, occurring when some drugs are converted by P450s to reactive metabolites.³⁶ Mechanism-based inactivation of CYP3A4 by drugs can be the result of chemical modification of the heme, the apoprotein, or both, because of covalent binding of the modified heme to the protein. The clinical pharmacokinetic effect of a mechanism-based CYP3A4 inhibitor is a function of its enzyme kinetics (i.e., K_m and V_{max}) and the rate of synthesis of new or

replacement enzyme. Predicting drug-drug interactions involving CYP3A4 inactivation is possible when pharmacokinetic principles are followed. Such prediction can become difficult, however, because the clinical outcomes of CYP3A4 inactivation depend on many factors associated with the enzyme, the drugs, and the patients.

Some of the clinically important drugs that have been identified to be mechanism-based CYP3A4 inhibitors include antibacterials (e.g., clarithromycin, erythromycin, isoniazid), anticancer drugs (e.g., irinotecan, tamoxifen, raloxifene), antidepressants (e.g., fluoxetine and paroxetine), anti-HIV agents (e.g., ritonavir and delavirdine), antihypertensives (e.g., dihydralazine and verapamil), steroids and their receptor modulators (e.g., ethinyl estradiol, gestodene, and raloxifene), dihydrotestosterone reductase inhibitors (e.g., finasteride), and some herbal constituents (e.g., bergamottin and glabridin). Drugs inactivating CYP3A4 often possess several common moieties such as a tertiary amine, furan ring, or an acetylene group. The chemical properties of a drug critical to CYP3A4 inactivation include formation of reactive metabolites by P450 isoenzymes, P450 inducers, P-gp substrates, or inhibitors and the occurrence of clinically significant pharmacokinetic interactions with coadministered drugs.

Compared to the more common metabolism-based (reversible) inhibition, mechanism-based inhibitors of CYP3A4 more frequently cause pharmacokinetic-pharmacodynamic drug-drug interactions and are often the cause for unfavorable drug-drug interactions because the inactivated CYP3A4 must be replaced by newly synthesized CYP3A4 protein.^{95,96} The resultant drug interactions may lead to adverse drug effects, including some fatal events. For example, raloxifene, a drug approved for the treatment of osteoporosis and chemoprevention of breast cancer in postmenopausal women at high risk for invasive breast cancer, has demonstrated the ability to act as a mechanism-based inhibitor of CYP3A4 by forming adducts with the apoprotein. It has been established that 3'-hydroxyraloxifene is produced exclusively via CYP3A4-mediated oxygenation to produce a reactive diquinone methide, excluding the alternative arene oxide pathway.

However, predicting drug-drug interactions involving CYP3A4 inactivation is difficult, since the clinical outcomes depend on a number of factors that are associated with drugs and patients. The apparent pharmacokinetic effect of a

mechanism-based inhibitor of CYP3A4 would be a function of its enzyme kinetics and the synthesis rate of new or replacement enzyme. Most CYP3A4 inhibitors are also P-gp substrates/inhibitors, confounding the in vitro to in vivo extrapolation. The clinical significance of CYP3A inhibition for drug safety and efficacy warrants an in-depth understanding of the mechanisms for each inhibitor. Furthermore, such inactivation may be exploited for therapeutic gain in certain circumstances. Clinicians should have good knowledge about these CYP3A4 inhibitors and avoid their combination.

Although CYP2D6 constitutes a relatively minor fraction of the total hepatic P450 content (Fig. 3.2), the contribution of this isoform is significant because of its role in the metabolism and clearance of many therapeutic agents that target the cardiovascular and CNS (Fig. 3.3) (Table 3.8). In addition, clinically significant polymorphisms in the CYP2D6 gene have been identified in a variety of populations with altered metabolic activity as PMs or individuals with impaired enzyme function resulting from splicing defects or gene deletions. On the other hand, EMs (individuals with normal enzyme function) are heterozygous or homozygous for the wild-type allele. In vivo clearance of CYP2D6 substrates in PMs is usually much lower than in EMs, leading to higher plasma concentrations and the potential for clinical toxicities with therapeutic doses. For example, paroxetine is a selective serotonin reuptake inhibitor that is both a substrate for and an inhibitor of CYP2D6. Paroxetine is metabolized by CYP2D6 via the formation of a carbene intermediate of the methylenedioxy group yielding an irreversible complex with CYP2D6 (Fig. 3.41).⁹⁷ Also, an in vitro study with 3,4-methylenedioxy-methamphetamine (MDMA, ecstasy) suggested that a typical recreational MDMA dose could inactivate most hepatic CYP2D6 within an hour, and the return to a basal level of CYP2D6 could take at least 10 days, impacting its pharmacokinetics.

Because of the pivotal role of P450 isoenzymes in drug metabolism, significant inactivation of these isoforms, and particularly the major human hepatic and intestinal CYP3A4 and hepatic CYP2D6, could result in drug-drug interactions and adverse drug reactions. Compared with the reversible inhibition of CYP3A4 and CYP2D6, mechanism-based inhibitors of these isoenzymes more frequently cause pharmacokinetic/pharmacodynamic drug-drug interactions, as the inactivated isoenzyme has to be replaced by newly

synthesized P450 protein. Pharmacokinetic interactions often occur because of a change in drug metabolism. For example, the macrolide antibiotics increase the plasma concentrations of therapeutic agents that are substrates of CYP3A4 (Table 3.10). Diltiazem has been shown to inhibit the metabolism of a variety of coadministered drugs including carbamazepine, quinidine, midazolam, and lovastatin (Table 3.10). Inhibition of CYP3A by ritonavir explains, in part, the remarkable elevation of blood concentrations and area under the plasma concentration-time curve (AUC) of other concomitantly administered drugs that are extensively metabolized by CYP3A4 and have significant first-pass metabolism. These drugs include rifabutin (400%), clarithromycin (77%), ketoconazole, saquinavir (5000%), amprenavir (210%), nelfinavir (152%), lopinavir (7700%), and indinavir (380%).⁹⁶ Furthermore, such inactivation can be exploited for therapeutic gain in certain circumstances, e.g., extended duration of action of the protease inhibitors ritonavir/indinavir used in the treatment of AIDS.

Beneficial Drug-Drug Interactions

By understanding the unique functions and characteristics of these isoenzymes, health care practitioners can better anticipate and manage drug-drug interactions. They can also predict or explain an individual's response to a particular therapeutic regimen. A beneficial drug interaction, for example, is the coadministration of a CYP3A4 inhibitor with cyclosporine. By inhibiting CYP3A4, the plasma concentrations of cyclosporine (a CYP3A4 substrate) are increased which allows a reduction of the cyclosporine dosage thereby improving clinical efficacy and reducing its cost. Similarly, certain HIV protease inhibitors, such as saquinavir, have a low oral bioavailability due to intestinal CYP3A4 metabolism. The oral bioavailability of saquinavir can be profoundly increased by the addition of a low dose of the mechanism-based CYP3A4 inhibitor, ritonavir. This concept of altering drug pharmacokinetics by adding a low, subtherapeutic dose of a mechanism-based CYP3A4 inhibitor (ritonavir) to increase the oral bioavailability of another protease inhibitor, lopinavir (CYP3A4 substrate), led to the marketing of Kaletra, a new drug combination of lopinavir and ritonavir. Another beneficial mechanism-based inhibition is of the P2Y₁₂ receptor (a protein found on the surface of blood platelet cells and an important

regulator in blood clotting) by the antiaggregating platelet prodrugs, clopidogrel and prasugrel. Clopidogrel is bioactivated to its reactive metabolite by CYP2C19, CYP1A2, and CYP3A4, whereas prasugrel is bioactivated by CYP3A4 and CYP2B6. The bioactivated metabolites can then react with the P2Y₁₂ receptor protein.

Grapefruit Juice-Drug Interactions

Historical Significance of Grapefruit Juice

The discovery that grapefruit juice can markedly increase the oral bioavailability of CYP3A4 drugs was based on an unexpected observation from an interaction study between the dihydropyridine calcium channel antagonist felodipine and ethanol in which grapefruit juice was used to mask the taste of the ethanol. Subsequent investigations confirmed that grapefruit juice significantly increased the oral bioavailability of felodipine by reducing presystemic felodipine metabolism through selective inhibition of CYP3A4 expression in the intestinal wall.⁹⁸

Grapefruit juice is a beverage often consumed at breakfast for its health benefits and to mask the taste of drugs or foods. Unlike other citrus fruit juices, however, grapefruit juice can significantly increase the oral bioavailability of drugs that are metabolized primarily by intestinal CYP3A4, causing an elevation in their serum concentrations (Table 3.20). Those drugs with high oral bioavailabilities (>60%), however, are all likely safe to be taken with grapefruit juice because their high oral bioavailability leaves little room for elevation by grapefruit juice. The importance of the interaction appears to be influenced by individual patient susceptibility, type and amount of grapefruit juice, and administration-related factors.

TABLE 3.20

Some CYP3A4 Substrates and Interactions With Grapefruit Juice

Drug	Interaction ^a	Drug	Interaction ^a	Drug	Interaction ^a
Calcium channel blockers		HIV protease inhibitors		CNS drugs	
Amlodipine	Y	Indinavir	N?	Buspirone	Y
Felodipine	Y	Nelfinavir	N?	Carbamazepine	Y
Nifedipine	Y	Ritonavir	N?	Diazepam	Y
Nimodipine	Y	Saquinavir	Y	Midazolam	Y
Nisoldipine	Y	Macrolides		Triazolam	Y

Drug	Interaction ^a	Drug	Interaction ^a	Drug	Interaction ^a
Nitrendipine	Y	Clarithromycin	N	Immunosuppressants	
Pranidipine	Y	HMG-CoA reductase inhibitors		Cyclosporine	Y
Antiarrhythmics		Atorvastatin	Y	Tacrolimus	Y?
Diltiazem	N	Fluvastatin	N?	Other	
Verapamil	N	Lovastatin	Y	Methadone	Y
Quinidine	N	Pravastatin	N?	Sildenafil	Y
Antihistamines		Simvastatin	Y		
Ebastine	Y?				
Loratadine	Y?				

^a Y (yes) and N (no) indicate published evidence of the presence or absence of an interaction with grapefruit juice. Y? and N? indicate expected findings based on available data. Those drugs with Y or Y? should not be consumed with grapefruit juice in an unsupervised manner.

Grapefruit juice can alter oral drug pharmacokinetics by different mechanisms. Irreversible inactivation of intestinal CYP3A4, which can persist up to 24 hours, is produced by grapefruit juice given as a single, normal, 200- to 300-mL drink or by whole fresh fruit segments ([Table 3.20](#)). As a result, presystemic metabolism is reduced and oral drug bioavailability increased. Enhanced oral drug bioavailability can occur up to 24 hours after juice consumption, so taking CYP3A4 vulnerable drugs with water or other liquids within 24 hours of consuming grapefruit juice can still result in an interaction. Inhibition of P-gp is a possible mechanism by which grapefruit juice, and perhaps grapefruit oil, increases oral drug bioavailability by reducing intestinal and/or hepatic efflux transport. Inhibition of organic anion-transporting polypeptides by grapefruit juice and apple juice has been observed; intestinal uptake transport appeared to be decreased as oral drug bioavailability was reduced.

Numerous medications used in the prevention or treatment of coronary artery disease and its complications have been observed or predicted to interact with grapefruit juice. Such drug-drug interactions can increase the risk of rhabdomyolysis when dyslipidemia is treated with the HMG-CoA reductase inhibitors (“statins”). Such drug-drug interactions could also cause excessive vasodilatation when hypertension is managed with the dihydropyridines felodipine, nifedipine, nisoldipine, or nitrendipine. An alternative agent could be amlodipine. The therapeutic effect of the angiotensin II type I receptor antagonist losartan can be reduced

by grapefruit juice. Grapefruit juice interacting with the antidiabetic agent repaglinide can cause hypoglycemia, and interaction with the appetite suppressant sibutramine can cause elevated blood pressure and heart rate. In angina pectoris, administration of grapefruit juice could result in atrioventricular conduction disorders with verapamil or attenuated antiplatelet activity with clopidogrel. Grapefruit juice can enhance the drug toxicity for antiarrhythmic agents, such as amiodarone, quinidine, disopyramide, or propafenone and for the congestive heart failure drug carvedilol. Some drugs used for the treatment of peripheral or central vascular disease also have the potential to interact with grapefruit juice. Interaction with sildenafil, tadalafil, or vardenafil for erectile dysfunction can cause serious systemic vasodilatation, especially when combined with a nitrate. In stroke, interaction with nimodipine can cause systemic hypotension.

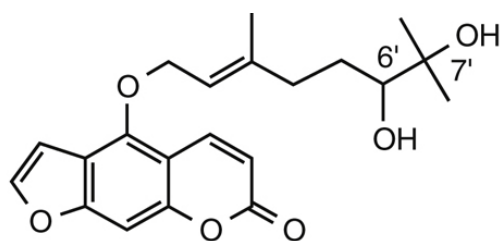
If a drug has low inherent oral bioavailability from presystemic metabolism by CYP3A4 or efflux transport by P-gp and the potential to produce serious overdose toxicity, avoidance of grapefruit juice entirely during pharmacotherapy appears mandatory. Although altered drug response is variable among individuals, the outcome is difficult to predict and avoiding the combination will guarantee that toxicity is prevented.

The mechanism by which grapefruit juice produces its effect is through inhibition of the enzymatic activity and a decrease in the intestinal expression of CYP3A4. The P-gp efflux pump also transports many CYP3A4 substrates; thus, the presence of inhibitors of P-gp in grapefruit juice (e.g., 6',7'-dihydroxybergamottin and other furanocoumarins) could be a related factor for drug-grapefruit juice interactions.⁹⁹ Numerous studies have shown that grapefruit juice consumed in normal (single glass) quantities acts on intestinal CYP3A4, not at the hepatic level.

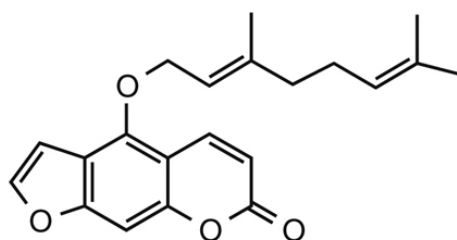
Does the quantity of juice matter? The majority of the presystemic CYP3A4 inhibition is obtained following ingestion of one glass of grapefruit juice; however, 24 hours after ingestion of a glass of grapefruit juice, 30% of its effect is still present.⁹⁹ The reduction in intestinal CYP3A4 concentration is rapid: a 47% decrease occurred in a healthy volunteer within 4 hours after consuming grapefruit juice. Daily ingestion of grapefruit juice results in a loss of CYP3A4 from the small intestinal epithelium. Consumption of very large

quantities of grapefruit juice (six to eight glasses/day) can lead to inhibition of hepatic CYP3A4.

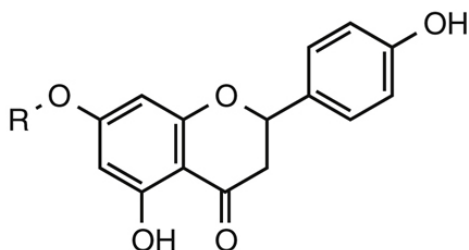
The active constituents found in grapefruit juice responsible for its effects on CYP3A4 include flavonoids (e.g., naringenin and naringin) and furanocoumarins (e.g., bergamottin and 6',7'-dihydroxybergamottin).⁹⁰



6',7'-Dihydroxybergamottin



Bergamottin



Naringin R: rhamnose-glucoside-
Naringenin R: H-

The majority of studies to date have used either freshly squeezed grapefruit juice, reconstituted frozen juice, commercial grapefruit juice, grapefruit segments, or grapefruit extract; all are capable of causing drug-drug interactions with CYP3A4 substrates (blended grapefruit juices have not yet been investigated). The active constituents in grapefruit juice are present not just in the juice but also in the pulp, peel, and core of the fruit and are responsible for its flavor. Bergamottin and 6',7'-dihydroxybergamottin are potent mechanism-based inhibitors of CYP3A4 and naringenin isomers are competitive inhibitors of CYP3A4. Higher concentrations of 6',7'-dihydroxybergamottin are present in grapefruit segments. Thus, any therapeutic concern for a drug interaction with grapefruit juice should now be extended to include whole fruit and other products derived from the grapefruit peel. The difference in the in vitro CYP3A4 inhibition between grapefruit juice and orange juice is that orange juice contained no measurable amounts of 6',7'-dihydroxybergamottin. The nutraceutical grapefruit oil

contains minor amounts of bergamottin, therefore grapefruit oil has the potential to cause drug interactions with CYP3A4 substrates.

If a patient has been taking medication with grapefruit juice for some time without ill effects, is it safe to continue to do so? Much of this unpredictability results from the inconsistency of the juice concentrations and the sporadic manner in which grapefruit juice is consumed, suggesting that this approach cannot be entirely safe. Given the unpredictability of the effect of grapefruit juice on the oral bioavailability of the drugs in [Table 3.20](#), patients should be advised to avoid this combination, thus preventing the onset of potential adverse effects. Each patient's situation should be considered, and advice should be based on consumption history and the specific medications involved. The benefits of increased and controlled drug bioavailability by grapefruit juice can, in the future, be achieved through either standardizing the constituents or coadministration of the isolated active ingredients. This would then lead to a safe, effective, and cost-saving means to enhance the absorption of many therapeutic agents.

P-Glycoprotein-Drug Interactions

P-gp-mediated transport has an important role in pharmacokinetic-mediated drug-drug interactions. The effect of P-gp inhibition is to increase the oral bioavailability of CYP3A4 substrates so that the later actions of CYP3A4 inhibition will be increased. One of the best examples is the interaction between digoxin and quinidine. Quinidine blocks P-gp in the intestinal mucosa and in the proximal renal tubule; thus, digoxin elimination into the intestine and urine is inhibited, increasing the plasma digoxin concentration to toxic levels. Another example is loperamide, which is an opioid antidiarrheal normally kept out of the brain by the P-gp pump. Inhibition of P-gp allows accumulation of loperamide in the brain, leading to respiratory depression as a result of central μ opioid receptor agonism.

The components of grapefruit juice reportedly inhibit P-gp, and this can be one of the mechanisms for the increase in bioavailability of drugs that are substrates for P-gp.

Drug-Dietary Supplement Interactions

The increasing use of dietary supplements presents a special challenge in health care; thus, there is an increasing need to predict and avoid these potential adverse drug-dietary supplement interactions.⁹⁹ The present interest and widespread use of herbal remedies has created the possibility of interaction between them and over-the-counter or prescription drugs if they are used simultaneously. As herbal medicines become more popular, herbal hepatotoxicity is being increasingly recognized. Females appear to be predisposed to hepatotoxicity, and coadministered agents that induce P450 enzymes (e.g., St. John's wort) can also increase individual susceptibility to some dietary supplements. Nearly one in

five adults taking prescription medicines is also taking at least one dietary supplement. The mechanisms for drug-dietary supplement interactions are similar to those for drug-drug interactions affecting the pharmacokinetics of the respective drug. Little is known regarding the pharmacokinetic properties of many of the substances in dietary supplements. Therefore, the potential for drug-dietary supplement interactions has greatly increased.

A commonly reported drug-dietary supplement interaction is between St. John's wort and HIV protease inhibitors, leading to drug resistance and treatment failure. [SEP]St. John's wort is a popular dietary supplement often used for depression. Of the two substances found in St. John's wort, hypericin and hyperforin, hyperforin appears to be the main constituent, with in vitro selective serotonin reuptake inhibitor (SSRI) activity. Hyperforin appears also to be the more potent inducer of CYP3A enzymes based on in vitro and in vivo studies.

Other Dietary Supplements Exhibiting Drug-Induced Hepatotoxicity

DHEA and androstenedione are dietary testosterone precursors that have been associated with hepatic toxicity and should not be taken by those with hepatic disease. Co-ingestion with other potentially hepatotoxic products or enzyme inducers that might increase the risk of liver damage should also be avoided.

Black cohosh, commonly used by women for menopausal symptoms, including hot flashes and sleep disorders, forms quinone metabolites in vitro, but no mercapturate conjugates were detected in urine samples from women who consumed multiple oral doses of up to 256 mg of a standardized black cohosh extract. At moderate doses of black cohosh, the risk of liver injury is minimal.

Silybum marianum (milk thistle) is used in the treatment of chronic or acute liver disease, as well as in protecting the liver against toxicity. Silybum is cited as one of the oldest known herbal medicines. The active constituents of milk thistle are flavonolignans, which are known collectively as silymarin.

Miscellaneous Drug-Drug Interactions

The ability of drugs and other foreign substances to induce metabolism of other drugs has already been discussed. Phenobarbital, for example, stimulates metabolism of a variety of drugs (e.g., phenytoin and coumarin anticoagulants). Stimulation of bishydroxycoumarin metabolism can create a problem in patients undergoing anticoagulant therapy. If phenobarbital administration is stopped, the rate of metabolism of the anticoagulant decreases, resulting in greater plasma concentrations of bishydroxycoumarin and enhanced anticoagulant activity, increasing the possibility of hemorrhage. Serious side effects have resulted from this type of interaction. These observations indicate that combined therapy of a potent drug (e.g., bishydroxycoumarin) and an inducer of drug metabolism (e.g., phenobarbital) can create a hazardous situation if the enzyme inducer is withdrawn and therapy with the potent drug is continued without an appropriate decrease in dose.

Serious reactions have been reported in patients treated with an MAO inhibitor such as tranylcypromine because they are, as a rule, sensitive to a subsequent dose of a sympathomimetic amine (e.g., amphetamine) or a tricyclic antidepressant (e.g., amitriptyline), which is metabolized by MAO.

Allopurinol, a xanthine oxidase inhibitor used for the treatment of gout, inhibits metabolism of 6-mercaptopurine and other drugs metabolized by this enzyme. A serious drug interaction results from the concurrent use of allopurinol for gout and 6-mercaptopurine to

block the immune response from a tissue transplant or as antimetabolite in neoplastic diseases.

Gender Differences in Drug Metabolism

Although numerous gender differences have been described in humans, so far most clinical research has been carried out with the questionable view that the male can fulfill the function of the true representative of the human species. However, in spite of the increasing evidence pointing out physiological and pathological differences between the sexes, beyond those related to reproduction, women differ from men in gene expression and regulation, in the susceptibility to and risk for many medical conditions, and in the response to numerous drugs.^{100,101} Gender difference in drug response may explain, at least in part, the interindividual variations occurring in therapeutic response and toxicity, especially considering that female sex has been shown to be a risk factor for the development of adverse drug reactions (ADRs). Nonetheless, current studies indicate that gender differences in pharmacological response are more widespread than originally believed and involve pharmacodynamics and pharmacokinetics, with pharmacokinetic being the most investigated.

The role of pharmacokinetics versus pharmacodynamics is not yet completely appreciated, and only a few contributions have evaluated the impact of genetics, hormonal variations, and their relative interactions.¹⁰⁰ The role of gender as a contributor to variability in xenobiotic metabolism and ADRs, which are more common in women than in men, is not clear, but increasing numbers of reports show differences in metabolism between men and women, raising the intriguing possibility that endogenous sex hormones, hydrocortisone, or their synthetic equivalents can influence the activity of inducible CYP3A. N-demethylation of erythromycin was significantly higher in females than males, which was persistent

throughout adulthood. In contrast, males exhibited unchanged N-demethylation values.

Gender-dependent differences of metabolic rates have been detected for some drugs. Side-chain oxidation of propranolol was 50% faster in males than in females, but no differences between genders were noted in aromatic ring hydroxylation. N-demethylation of meperidine was depressed during pregnancy and for women taking oral contraceptives. CYP1A2 shows a higher activity in men, and therefore, clearance of antidepressant, antipsychotic drugs, and theophylline is faster in men than in women. CYP1A2 activity is decreased during pregnancy. Other examples of drugs cleared by CYP3A4 more rapidly in men than in women include chlordiazepoxide and lidocaine. Diazepam, prednisolone, caffeine, and acetaminophen are metabolized slightly faster by women than by men. No gender differences have been observed in the clearance of phenytoin, nitrazepam, and trazodone, which interestingly are not substrates for the CYP3A subfamily. CYP3A4 activity is higher in women than in men. Drugs such as cyclosporine, erythromycin, nimodipine, and cortisol are substrates of CYP3A4, showing faster clearance among women. Although CYP3A4 is also responsible for about 60% of CYP-mediated metabolism of zolpidem, its overall clearance is actually slower in women than in men, requiring a FDA-recommended 50% dose reduction. Gender differences in the rate of glucuronidation have been noted. Men show a faster clearance of drugs that are primarily eliminated by glucuronidation. Thus, oxazepam, metabolized mainly by UGT2B15, has a longer half-life in women than in men. Gender differences are also found in other pharmacokinetic parameters such as drug absorption, drug distribution, and excretion.

Historically, women were less enrolled in clinical trials because both the pharmacokinetics and pharmacodynamics of a drug can be influenced by menstrual cycle phases, hormonal fluctuations, use of

oral contraceptives, and hormonal therapy. There was also concern about life events such as pregnancy and lactation, including the potential negative impact of investigational drugs on the fetus or nursing neonate.¹⁰⁰ The number of trials enrolling women has increased after an FDA request to include a fair representation of both genders as participants, but overall, women are still underrepresented. Women are generally treated with doses that essentially reflect the results obtained by trials carried out mainly in men.

Despite the differences in drug pharmacokinetics between men and women, sex-specific recommendations in dosage do not exist for most drugs.¹⁰¹ Pharmacists need to recognize the underrepresentation of women in clinical trials and take the responsibility to inform consumers and emphasize to clinicians that women can differ significantly from men with respect to metabolism, absorption, distribution, and excretion of drugs. Pharmacists also need to be aware that pregnancy, oral contraceptive use, and hormonal replacement therapy can significantly change drug metabolism and drug clearance. If a woman consistently experiences more adverse drug events or less therapeutic effect from a particular drug, it may be necessary to discuss with her physician the possibility of changing the dosing regimen or switching to a different medication. Even in postmenopausal women, CYP3A function can be altered and influenced by the lack of estrogen or the presence of androgens.

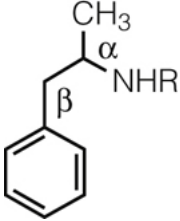
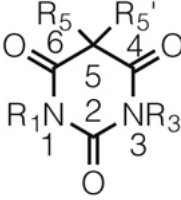
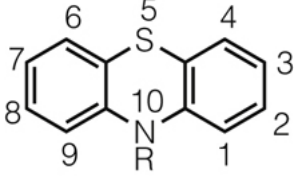
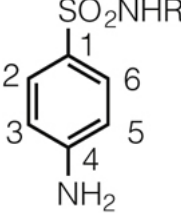
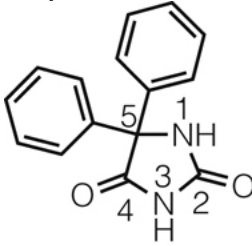
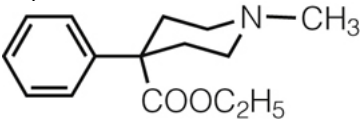
Compared to men, there are more women in the population, more women with chronic diseases, and more women visiting physicians. It is, therefore, obvious that there needs to be more interest in knowing how women react to drugs. Ultimately, a better understanding of sex-related influences on drug responses will help to improve drug safety and efficacy and will also permit to

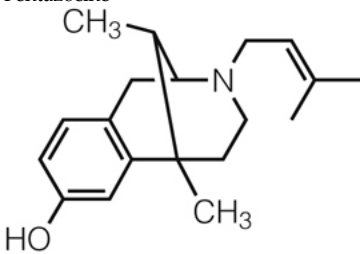
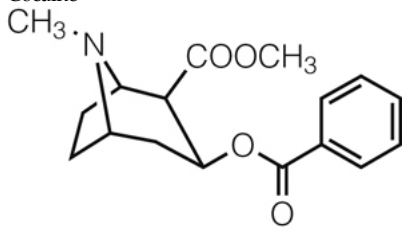
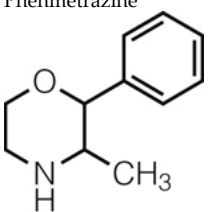
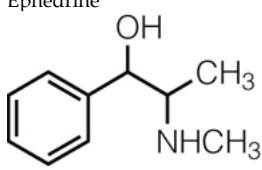
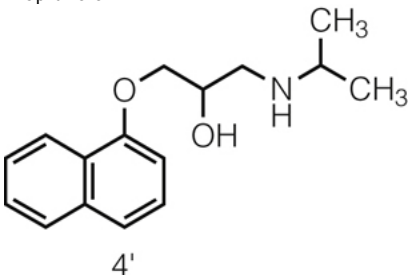
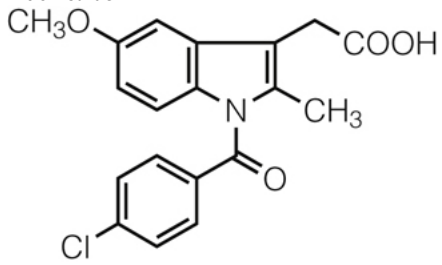
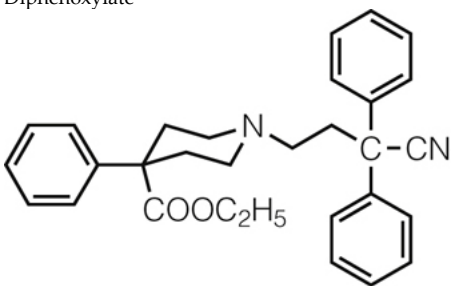
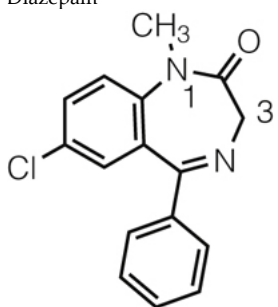
practitioners to “tailor” pharmacological treatments both in men and women.

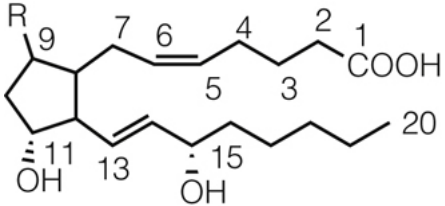
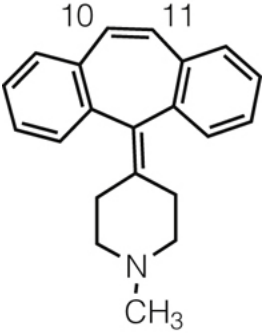
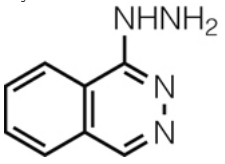
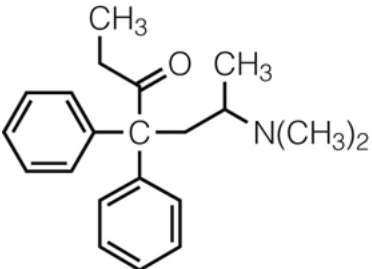
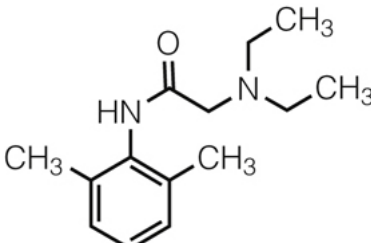
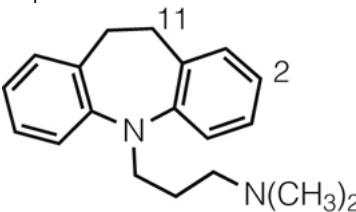
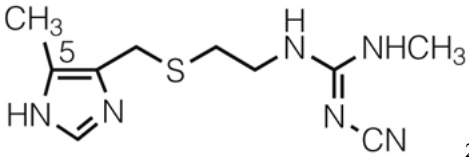
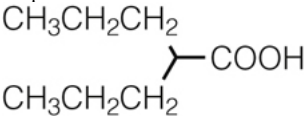
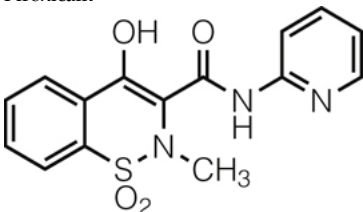
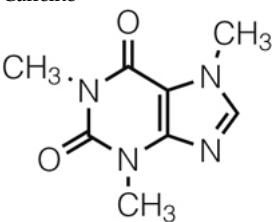
Major Pathways of Metabolism

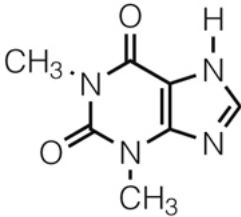
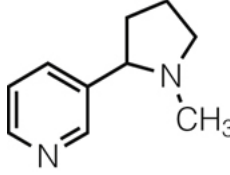
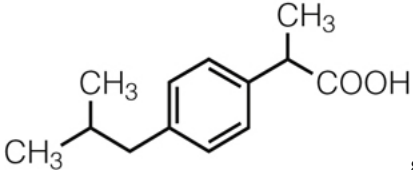
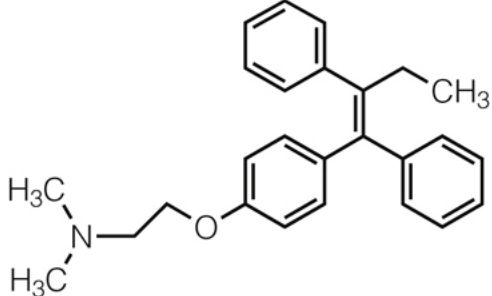
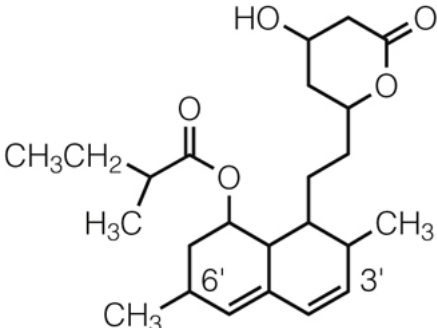
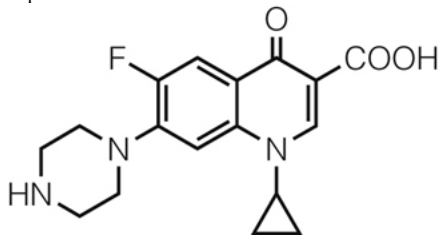
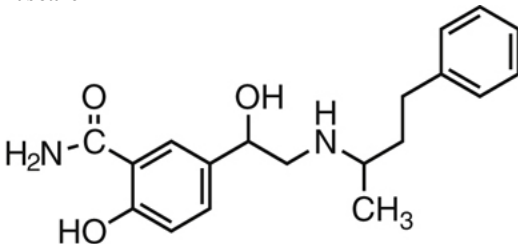
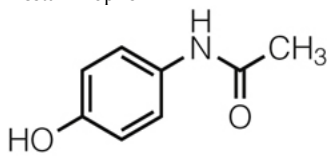
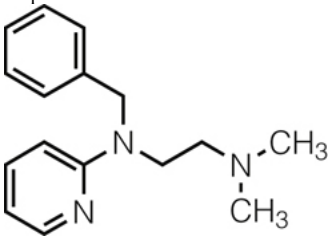
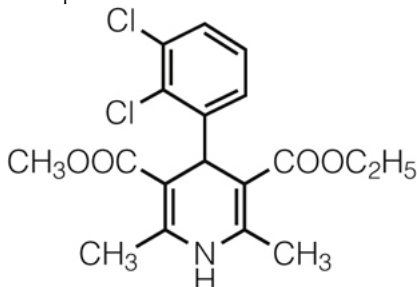
Table 3.21 contains an extensive list of commonly used drugs and the P450 isoforms that catalyze their metabolism. In addition, Phase 1 and Phase 2 metabolic pathways for some common drugs are listed in Table 3.21.

TABLE 3.21
Metabolic Pathways of Common Drugs

Drug	Pathway	Drug
Amphetamines 	Deamination (followed by oxidation and reduction of the ketone formed) N-oxidation N-dealkylation Hydroxylation of the aromatic ring Hydroxylation of the β-carbon atom Conjugation with glucuronic acid of the acid and alcohol products from the ketone formed by deamination	Barbiturates 
Phenothiazines 	N-dealkylation in the N10 side chain N-oxidation in the N10 side chain Oxidation of the heterocyclic S atom to sulfoxide or sulfone Hydroxylation of one or both aromatic rings Conjugation of phenolic metabolites with glucuronic acid or sulfate Scission of the N10 side chain	Sulfonamides 
Phenytoin 	Hydroxylation of one aromatic ring Conjugation of phenolic products with glucuronic acid or sulfate Hydrolytic scission of the hydantoin ring at the bond between carbons 3 and 4 to give 5,5- diphenylhydantoic acid	Meperidine 

Drug	Pathway	Drug
Pentazocine 	Hydroxylation of terminal methyl groups of the alkenyl side chain to give <i>cis</i> and <i>trans</i> (major) alcohols Oxidation of hydroxymethyl product of the alkenyl side chain to carboxylic acids Reduction of alkenyl side chain and oxidation of terminal methyl group	Cocaine 
Phenmetrazine 	Oxidation to lactam Aromatic hydroxylation N-oxidation Conjugation of phenolic products	Ephedrine 
Propranolol 	Aromatic hydroxylation at C4' N-dealkylation Oxidative deamination Oxidation of deaminated product to naphthoxylactic acid Conjugation with glucuronic acid O-dealkylation	Indomethacin 
Diphenoxylate 	Hydrolysis of ester to acid Hydroxylation of one aromatic ring attached to the N-alkyl side chain	Diazepam 

Drug	Pathway	Drug
Prostaglandins 	Reduction of double bonds at carbons 5 and 6 and 13 and 14 Oxidation of 15-hydroxyl to ketone β -Oxidation of carbons 3, 5 and 7 ω -Oxidation of carbon 20 to acid	Cyproheptadine 
Hydralazine 	N-acetylation with cyclization to a methyl-S-triazolophthalazine N-formylation with cyclization to an S-triazolophthalazine Aromatic hydroxylation of benzene ring Oxidative loss of hydrazinyl group to 1-hydroxy Hydroxylation of methyl of methyl-S-triazolophthalazine Conjugation with glucuronic acid	Methadone 
Lidocaine 	N-dealkylation Oxidative cyclization to a 4-imidazolidone N-oxidation of amide N Aromatic hydroxylation ortho to methyl Hydrolysis of amide	Imipramine 
Cimetidine 	S-oxidation Hydroxylation of 5-methyl	Valproic acid 
Piroxicam 	Pyridine 3'-hydroxylation Hydrolysis of amide Decarboxylation	Caffeine 

Drug Theophylline 	Pathway N3-demethylation N1-demethylation C8 oxidation to uric acids Imidazole ring opened 1-Me xanthine to 1-Me uric acid – xanthine oxidase	Drug Nicotine 
Ibuprofen 	CoA thioester and epimerization of R- to S+ enantiomer Methyl hydroxylation to CH₂OH CH₂OH to COOH Acylglucuronide	Tamoxifen 
Lovastatin 	6'-Hydroxylation 3'-Side chain hydroxylation 3'-Hydroxylation β-oxidation of lactone O-glucuronides	Ciprofloxacin 
Labetalol 	O-sulfate (major) O-glucuronide	Acetaminophen 
Tripelennamine 	<i>p</i>-Hydroxylation Benzylic C-hydroxylation N-depyridinylation N-debenzylation	Felodipine 

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CHAPTER 4

Membrane Drug Transporters

Marilyn E. Morris and Bridget L. Morse

Drugs covered in this chapter:

- Acyclovir
- Adefovir
- Atorvastatin
- Cerivastatin
- Cidofovir
- Cisplatin
- Cyclosporine
- Dabigatran
- Digoxin
- Dofetilide
- Doxorubicin
- Erythromycin
- Fexofenadine
- Furosemide
- Gabapentin
- Gabapentin enacarbil
- Gemfibrozil
- Lamivudine
- Metformin
- Methotrexate
- Morphine
- Mycophenolate mofetil
- Nitrofurantoin
- Olmesartan

- Oxaliplatin
- Paclitaxel
- Penicillin
- Pravastatin
- Probenecid
- Repaglinide
- Rifampin
- Rosuvastatin
- Simvastatin
- Sumatriptan
- Tenofovir
- Topotecan
- Valacyclovir
- Valsartan
- Verapamil

Abbreviations

- ABC** ATP-binding cassette
- ACE** angiotensin-converting enzyme
- ATP** adenosine triphosphate
- AUC** area under the curve
- BBB** blood-brain barrier
- BCRP** breast cancer resistance protein
- BSEP** bile salt export pump
- CNT** concentrative nucleoside transporter
- ENT** equilibrative nucleoside transporter
- FXR** farnesoid X receptor
- GI** gastrointestinal
- GHB** γ -hydroxybutyrate
- HMG-CoA** 3-hydroxy-3-methyl-^{[[SEP]]}glutaryl-coenzyme A
- LAT** l-type amino acid transporter

MATE multidrug and toxin extrusion protein
MCT monocarboxylate transporter
MDR multidrug resistance
MRP multidrug resistance-associated protein
OAT organic anion transporter
OATP organic anion transporting polypeptide
OCT organic cation transporter
OCTN organic cation/carnitine transporter
P-gp P-glycoprotein
PEPT peptide transporter
PHT peptide/histidine transporter
PXR pregnane X receptor
SLC solute carrier
SMCT sodium-coupled monocarboxylate transporter
SNP single nucleotide polymorphism

Introduction



The Objectives of the Chapter are:

- Emphasize the significance of transport in pharmacokinetics and pharmacodynamics
- Provide an overview of transport mechanisms and classification of drug transporters
- Summarize transporters relevant to drug disposition and their drug substrates
- Provide evidence and mechanisms for transport interactions clinically relevant in therapeutics
- Provide an understanding of mechanisms underlying interindividual variability in the pharmacokinetics of drug transporter substrates

Transporters have fundamental roles in cell homeostasis and physiologic function by facilitating the movement of molecules across biological membranes. Transporters are responsible for maintaining ionic and osmotic gradients necessary for normal cell activity. Transporters facilitate the oxygen binding and release in red blood cells, and they are necessary for the transport of nutrients to vital organs. The primary function of transporters is to transport endogenous substances, such as hormones, glucose, and amino acids; however, many of these transporters also transport xenobiotics. It is these drug transporters that are of importance when considering drug disposition and drug response.

Drug transporters are localized to barrier membranes of the body responsible for xenobiotic entry and exit. They are expressed in organs of absorption, such as the intestine, and clearance organs including the liver and kidney. Transporters are also expressed on membranes that separate particularly susceptible organs from the rest of the body, including the blood-brain and blood-placenta barriers, where they facilitate the influx of nutrients and efflux of potentially harmful xenobiotics. Because of their location on barrier membranes, transporters have an important role in drug pharmacokinetics and pharmacodynamics. Transporters can have a role in drug absorption and can facilitate or prevent drug entry into the body. Transporters also have a role in drug distribution, as they

facilitate the movement of drugs between the blood and peripheral tissues. The role of transporters in drug distribution can also affect drug response by allowing or preventing drug access to the site of action. One of the most interesting roles of drug transporters is their indirect effects on drug metabolism. Transporters can restrict or allow a drug's distribution into organs that contain drug-metabolizing enzymes, particularly the liver and the intestine. In this regard, there can be extensive transport-metabolism interplay, and transport or metabolism can be the rate-limiting process controlling drug elimination. Transporters are also responsible for transport and removal of drug metabolites. Finally, transporters have a significant role in drug excretion, as they are present in the kidney and on the canalicular membrane of the liver where they can facilitate drug elimination into the urine or bile, respectively.

Because transporters can have a significant effect on drug absorption, distribution, and clearance, changes in the function of these transporters can significantly alter drug pharmacokinetics and pharmacodynamics. Factors leading to changes in transporter function include interactions with other drugs, disease states, and genetic variability in expression. Understanding transporter effects and the effects of changes in transporter function is important for effective therapeutic use of drugs that interact with transporters.

Mechanisms of Membrane Transport

Membrane transport is essential for almost every drug to be therapeutically effective. This can be achieved by different mechanisms of transport (Fig. 4.1). Passive diffusion is the simplest way for a drug to pass through a membrane and depends only on the existence of a concentration gradient for a molecule across the membrane. Because of the lipophilicity of biologic membranes, diffusion is energetically unfavorable for drugs that are relatively hydrophilic, particularly for drugs that are predominantly ionized at physiologic pH, and these molecules require facilitated transport. Facilitated transport refers to any transport aided by a facilitating protein. Similar to diffusion, passive facilitated transport depends only on the existence of a concentration gradient and involves movement of molecules down this gradient. Active transport uses a separate energy source to move molecules against their concentration gradient. Drug transporters can use either passive or active transport mechanisms. Paracellular transport (transfer of substances between cells of an epithelial cell layer) and transcytosis (vesicular transfer of substances across the interior of a cell) are less commonly used mechanisms of membrane transport. Mannitol can pass through membranes via paracellular transport and can be used as a probe for this type of transport. Transcytosis is often receptor mediated and is a mechanism of transport for endogenous substances such as insulin and transferrin.

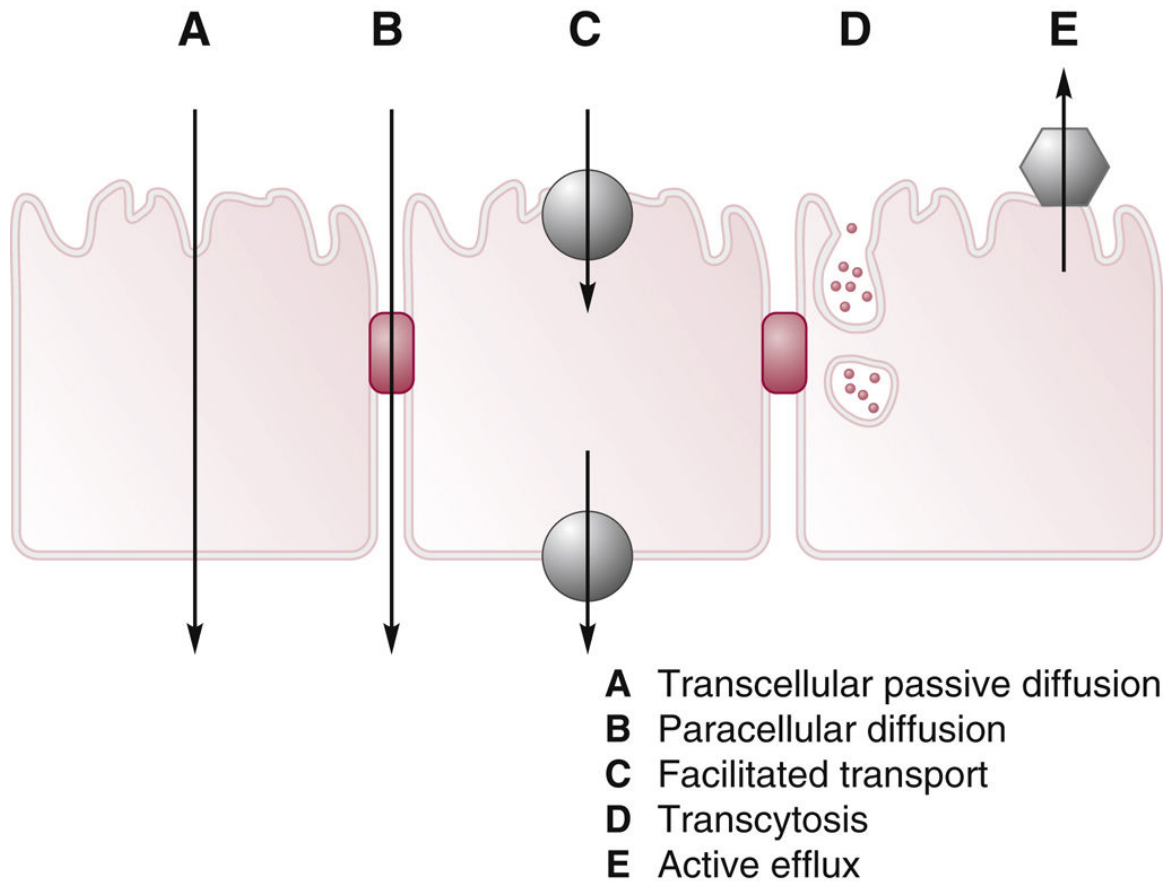


FIGURE 4.1 Mechanisms of membrane transport. Transport mechanisms most commonly affecting therapeutic agents include passive diffusion, facilitated transport and active efflux.

Molecules can use one or more of these mechanisms to cross biological membranes. Transporter processes are saturable and substrates are often lipophilic and capable of passive diffusion; therefore, drug transport can

$$Cl_{int} = Cl_{met}$$

often be described with the following equation:

where J is the rate of total drug transport, V_{max} is the maximum rate of the saturable transporter system, K_m reflects the affinity of a drug substrate for the transporter system, P is the rate of nonsaturable transport or passive diffusion, and C is the drug concentration. The importance of a transporter depends on to what extent transporter-mediated transport (V_{max}/K_m) is responsible for membrane passage of a drug compared to other mechanisms like passive diffusion (P). Transporter-mediated transport

becomes particularly important when the need is present to move a molecule against its concentration gradient, as this type of transport can only be achieved by active transporters.

When considering drug transport, it is important to consider the polarization of biological membranes, in that different membranes can have different properties relating to their function as a barrier, including the expression of different transporters. The membrane facing the systemic circulation is referred to as the basolateral membrane, or the sinusoidal membrane in the liver. The membrane facing the exterior, such as the gut lumen, is referred to as the apical membrane. The apical membrane is also called the brush border membrane in the kidney or intestine and the canalicular membrane in the liver. Transport across membranes is actually a two-step process in which molecules must be transported across the apical then basolateral membrane or vice versa. Drug transport is a concerted action between transporters expressed on both membranes.

Classification of Transporters

Facilitative Versus Active Transporters

Although all transporters are facilitating proteins, the terms facilitative and active transporters refer to transporters that transport substrates down their concentration gradients and against their concentration gradients, respectively. Transporters that are classified as facilitative transporters only move molecules down their concentration gradient, without the use of a separate energy source. The transporters of this type should not be confused with channels. Channels also facilitate the movement of ions and hydrophilic molecules across membranes and down their concentration gradient; however, channels control transport or flow of substrates by gating mechanisms, whereas transporters bind to their substrates and undergo a conformational change to transport the substrate across a membrane.

Primary Versus Secondary Active Transporters

Most drug transporters are active transporters that use an energy source other than the drug's concentration gradient to transport substrates across membranes from a region of lower concentration to a region of higher concentration (i.e., against its concentration gradient). Active transporters are further classified as primary or secondary active transporters. Primary active transporters most commonly use adenosine triphosphate (ATP) as an energy source for substrate transport. Secondary active transporters use the concentration gradient of another substance, such as protons or sodium ions, but also other ionic endogenous substances as energy sources to drive transport. The concentration gradients that drive secondary active transport are generally created by primary active transporters; an example of interplay between the two types of transporters is depicted in [Figure 4.2](#). As shown, primary active transport by the Na^+/K^+ ATPase results in a concentration gradient of Na^+ ions. This concentration gradient drives secondary active transport by the Na^+/H^+ exchanger, and the resulting proton gradient drives transport of the drug substrate against its

concentration gradient. The Na^+/K^+ ATPase and Na^+/H^+ exchanger do not transport drug substrate but are involved in drug transport by producing the sodium and proton gradients used as driving forces. Molecules used to drive secondary active transport are also substrates for the secondary active transporter and are simultaneously transported across the membrane. This simultaneous transport may be in the same direction, referred to as *symport*, or in opposite directions, referred to as *antiport*.

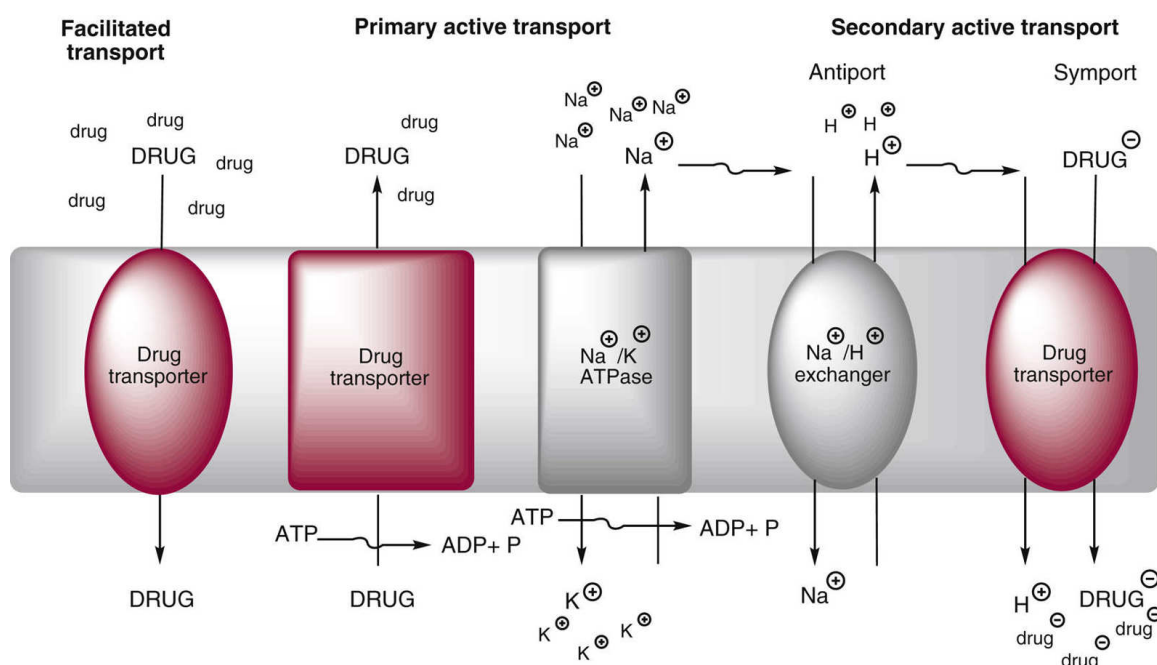


FIGURE 4.2 Types of drug transporters. Primary active transporters use ATP and have the ability to transport substrates against their concentration gradient. Secondary active transporters use gradients created by primary active transport to transport drug substrates. Facilitated transporters only transport substrates down their concentration gradient. Transporters that transport drug substrates are shown in *red*, whereas those shown in *gray* provide driving forces for drug transport.

Influx Versus Efflux Transporters

Influx transporters transport substrates from extracellular spaces into cells. Efflux transporters transport substrates out of cells. Transporters are usually responsible for either drug influx or drug efflux, but in some cases facilitate both types of transport.

Secretory Versus Absorptive Transporters

Secretory transporters facilitate drug clearance and are responsible for transport of drugs from the blood, such as excretion into the urine. Absorptive transporters allow a drug access into the blood, such as those facilitating absorption in the gut or reabsorption in the kidney. A transporter can be both secretory and absorptive depending on its physiologic locations. The same transporter is often expressed at both the site responsible for drug absorption and that responsible for drug clearance; hence, the transporter would serve both secretory and absorptive functions. When considering transport across membranes of the brain and placenta, the terms secretion and absorption are applied differently. With respect to these sites, absorptive transporters allow access into the sites and out of the circulation, and secretory transporters efflux drugs out of these sites and back into the blood circulation.

ABC Versus SLC Transporters

Drug transporters have been classified into two families, namely the ABC (ATP-binding cassette) and SLC (solute carrier) families. Members of the ABC family are primary active transporters that use ATP as an energy source. Most SLC transporters are secondary active transporters, using the concentration gradients of several different molecules as a driving force for transport. The SLC family also includes a handful of facilitative transporters. Many members of each family have a role in drug disposition, and these transporters are summarized in [Table 4.1](#).

TABLE 4.1
Drug Transporters

Transporter Family	Family Member	Gene Name	Location	Role in Drug Disposition

Transporter Family	Family Member	Gene Name	Location	Role in Drug Disposition
MDR	MDR1	<i>ABCB1</i>	Intestine, liver, kidneys, brain, heart, placenta	• Role in oral absorption, biliary clearance, renal secretion, and drug penetration of blood-brain barrier • Drug-drug interactions • Known polymorphisms • Role in multidrug resistance
MRP	MRP1	<i>ABCC1</i>	Intestine, brain	• Role in multidrug resistance • Facilitates basolateral drug efflux
	MRP2	<i>ABCC2</i>	Intestine, liver, kidney	• Role in oral absorption, biliary clearance, and renal secretion of drugs and drug conjugates • Known polymorphisms that affect drug disposition
	MRP3	<i>ABCC3</i>	Liver, intestine, brain	• Facilitates sinusoidal efflux of bile acids, drugs and drug conjugates
	MRP4	<i>ABCC4</i>	Kidney, liver, brain	• Facilitates renal secretion and sinusoidal efflux of bile acids, drugs and drug conjugates
BCRP	BCRP1	<i>ABCG2</i>	Intestine, liver, kidney, brain, heart, placenta	• Role in oral absorption, biliary secretion, and drug penetration of blood-brain barrier • Drug-drug interactions • Known polymorphisms that affect drug disposition • Role in multidrug resistance
OCT	OCT1	<i>SLC22A1</i>	Liver	• Facilitates sinusoidal uptake • Drug-drug interactions • Known polymorphisms that affect drug disposition

Transporter Family	Family Member	Gene Name	Location	Role in Drug Disposition
	OCT2	<i>SLC22A2</i>	Kidney	- Facilitates renal secretion - Drug-drug interactions - Known polymorphisms that affect drug disposition
OCTN	OCTN1	<i>SLC22A4</i>	Kidney, intestine	Role in oral absorption and renal reabsorption
	OCTN2	<i>SLC22A5</i>	Kidney, intestine, liver, brain, muscle, lung	- Role in oral absorption and renal reabsorption - Known polymorphisms
OAT	OAT1	<i>SLC22A6</i>	Kidney	- Facilitates renal secretion - Drug-drug interactions
	OAT2	<i>SLC22A7</i>	Liver	Facilitates sinusoidal uptake and renal secretion
	OAT3	<i>SLC22A8</i>	Kidney	- Facilitates renal secretion - Drug-drug interactions
	OAT4	<i>SLC22A11</i>	Kidney	Facilitates renal reabsorption
OATP	OATP1A2	<i>SLCO1A2</i>	Brain	Role in brain uptake
	OATP1B1	<i>SLCO1B1</i>	Liver	- Facilitates sinusoidal uptake - Drug-drug interactions - Known polymorphisms that affect in vivo drug disposition
	OATP1B3	<i>SLCO1B3</i>	Liver	- Facilitates sinusoidal uptake - Known polymorphisms
	OATP2B1	<i>SLCO2B1</i>	Intestine, liver	Role in oral absorption and sinusoidal uptake
	OATP4C1	<i>SLCO4C1</i>	Kidney	Facilitates renal secretion

Transporter Family	Family Member	Gene Name	Location	Role in Drug Disposition
MATE	MATE1	<i>SLC47A1</i>	Kidney, liver	• Role in renal and biliary secretion • Drug-drug interactions • Known polymorphisms that affect in vivo drug disposition
	MATE2-K	<i>SLC47A2</i>	Kidney	• Facilitates renal secretion
MCT	MCT1	<i>SLC16A7</i>	Ubiquitous	• Role in oral absorption and renal reabsorption • Overexpression in tumor cells
PEPT	PEPT1	<i>SLC15A1</i>	Intestine, kidney	• Facilitates oral absorption • Drug-drug interactions
	PEPT2	<i>SLC15A2</i>	Kidney	• Facilitates renal reabsorption
CNT	CNT1	<i>SLC28A1</i>	Intestine, kidney, liver	• Role in absorption and disposition of nucleoside analogues
	CNT2	<i>SLC28A2</i>	Intestine, kidney, liver	• Role in absorption and disposition of nucleoside analogues
	CNT3	<i>SLC28A3</i>	Kidney, brain, placenta	• Role in distribution and renal reabsorption of nucleoside analogues
ENT	ENT1	<i>SLC29A1</i>	Ubiquitous	• Role in absorption and disposition of nucleoside analogues
	ENT2	<i>SLC29A2</i>	Ubiquitous	• Role in absorption and disposition of nucleoside analogues

Transporter Family	Family Member	Gene Name	Location	Role in Drug Disposition
Bile acid	BSEP	<i>ABCB11</i>	Liver	• Role in biliary secretion of bile acids • Known polymorphisms
	NTCP	<i>SLC10A1</i>	Liver	• Sinusoidal uptake of bile acids
	OST $\alpha\beta$	<i>OSTA/B</i>	Liver	• Compensatory efflux of bile acids on sinusoidal membrane during cholestasis
	ASBT	<i>SLC10A2</i>	Intestine	• Role in absorption of bile acids

ABC Transporters

ABC transporters are efflux transporters, many of which are located on the apical side of biological membranes, facilitating drug secretion. ABC transporters are primary active transporters and use ATP as an energy source. The widespread physiologic expression of ABC transporters and their extensive range of substrates make it inevitable for these transporters to have effects on drug pharmacokinetics and elicit clinically significant drug interactions. Substrates and inhibitors for ABC transporters are given in [Table 4.2](#).

TABLE 4.2
Relevant Drug Substrates and Inhibitors of ABC Transporters

Transporter	Substrates	Inhibitors
MDR1	Atorvastatin, cyclosporine, dabigatran, daunorubicin, dexamethasone, digoxin, diltiazem, docetaxel, doxorubicin, erythromycin, etoposide, fexofenadine, ketoconazole, loperamide, methadone, mitoxantrone, morphine, paclitaxel, phenytoin, rifampin, ritonavir, saquinavir, simvastatin, tacrolimus, talinolol, verapamil, vinblastine, vincristine	Clarithromycin, cyclosporine, elacridar, quinidine, verapamil

Transporter	Substrates	Inhibitors
MRP2	Ampicillin, ceftriaxone, cisplatin, daunomycin, doxorubicin, etoposide, fluorouracil, glucuronide conjugates, glutathione conjugates, irinotecan, methotrexate, mitoxantrone, olmesartan, pravastatin, ritonavir, rosuvastatin, saquinavir, SN-38-glucuronide, valsartan, vinblastine, vincristine	Cyclosporine, probenecid, efavirenz, emtricitabine
MRP3	Bile acids, fexofenadine, glucuronide conjugates, methotrexate	Efavirenz, emtricitabine
MRP4	Adefovir, furosemide, methotrexate, rosuvastatin, tenofovir, topotecan	Diclofenac
BCRP	Atorvastatin, ciprofloxacin, imatinib, irinotecan, methotrexate, mitoxantrone, nitrofurantoin, rosuvastatin, sorafenib, topotecan	Elacridar

Multidrug Resistance Proteins (ABCB)

MDR1 (ABCB1).

MDR1, more commonly known as P-glycoprotein (P-gp), is the most extensively characterized of all drug transporters. The structure of P-gp consists of two homologous halves each with six transmembrane-spanning domains and one ATP binding site (Fig. 4.3). Transport by P-gp is notably complicated, with at least two binding sites and three different proposed mechanisms by which it transports substrates.^{1,2}

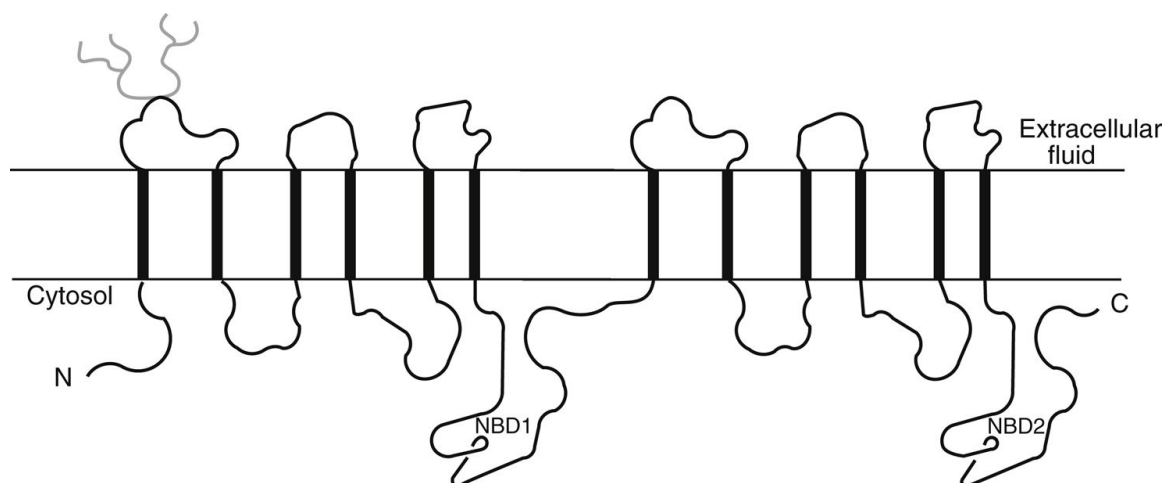
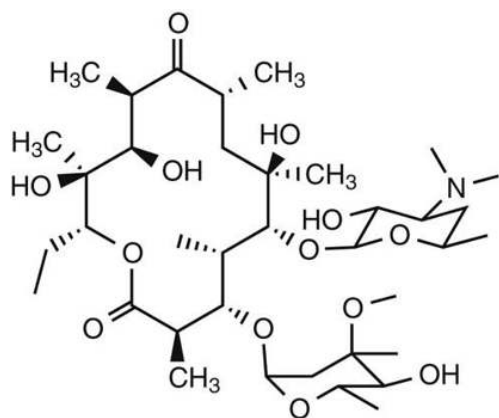


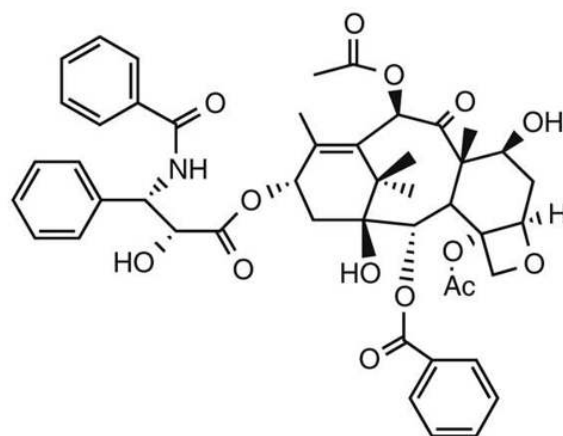
FIGURE 4.3 Structure of P-glycoprotein including 12 transmembrane-spanning domains and two nucleotide-binding domains (NBDs).

P-gp is expressed in most tissues and is involved in drug transport in the intestine, liver, kidney, brain, and placenta, as well as in tumor cells. This is one reason P-gp has been, and remains, a transporter of clinical interest.

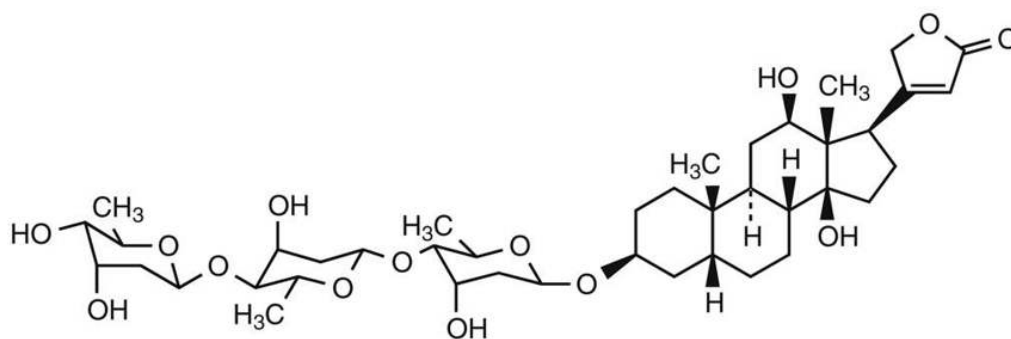
The ability of P-gp to transport a wide range of substrates is another remarkable feature. These substrates span many therapeutic areas and drug classes, making it difficult to determine the structure-activity relationship for P-gp-mediated transport, though much progress has been made in this area in recent years.³ Digoxin serves as a probe for P-gp in both in vitro and in vivo studies.⁴ Other substrates include many chemotherapeutic drugs, other cardiovascular agents, and HIV protease inhibitors. One common feature of P-gp substrates is that they are generally lipophilic (Fig. 4.4). The relative lipophilicity of these molecules implies that most P-gp substrates may also have high rates of passive diffusion. This means that P-gp transport must be efficient to have an effect on the passage of its substrates across membranes, and P-gp transport does not always result in clinically relevant effects. P-gp substrates are rarely specific and are also often substrates of other transporters and drug-metabolizing enzymes, particularly CYP3A4, making it difficult to attribute in vivo pharmacokinetic effects or interactions to P-gp alone. Mouse models of P-gp knockout indicate intestinal efflux by P-gp contributes to the low bioavailability of many compounds.⁵ Accordingly, significant drug-drug interactions have also been demonstrated involving P-gp in humans at the intestine, as well as in the kidneys.^{6,7} P-gp is also likely important in pregnancy in that it protects the fetus from xenobiotics by effluxing them back into the maternal blood circulation.⁸ In addition, as the original name “multidrug resistance protein” (MDR) implies, P-gp is also recognized as a potential factor for chemotherapeutic drug resistance due to its overexpression in tumor cells. While clinical studies evaluating P-gp inhibition to overcome resistance have had varying results, this continues to be considered as potential concurrent treatment with chemotherapy.⁹ Similarly while single nucleotide polymorphisms (SNPs) in the *MDR1* gene have been identified, the impact of these on drug disposition remains controversial.¹⁰



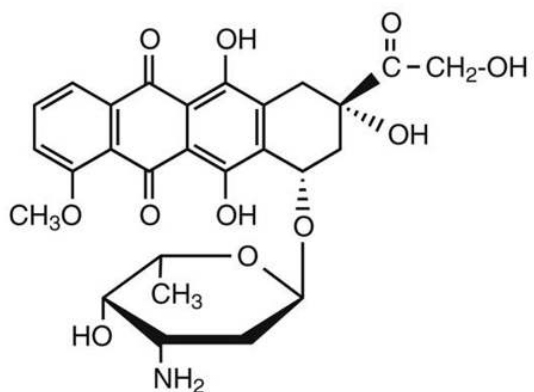
Erythromycin



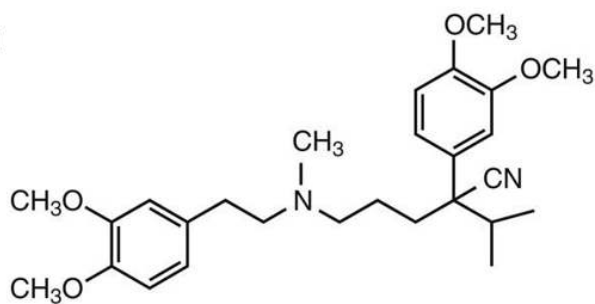
Paclitaxel



Digoxin



Doxorubicin



Verapamil

FIGURE 4.4 Examples of P-gp substrates/inhibitors.

Clinical Significance

P-gp and Digoxin

Digoxin undergoes little metabolism in humans, and transport by P-gp is the primary determinant of digoxin pharmacokinetics. One of the first drug interactions noted with digoxin was that with cyclosporine, leading to decreased digoxin clearance and digoxin-associated arrhythmias.¹¹ It was later discovered that cyclosporine and other therapeutic agents decrease digoxin clearance due to inhibition of P-gp-mediated renal secretion.¹² Nonrenal clearance of digoxin is minimal; however, P-gp inhibitors have similarly been demonstrated to decrease digoxin biliary excretion.¹³ P-gp function also affects digoxin absorption, and concomitant oral administration of clarithromycin was demonstrated to increase digoxin bioavailability along with decreasing digoxin renal clearance.⁶ Because in vivo P-gp inhibition can affect both absorption and clearance, there exists substantial risk of elevated digoxin plasma concentrations and digoxin-associated toxicity with concomitant inhibitor administration.

P-gp induction can also affect digoxin pharmacokinetics. In a study of human volunteers, chronic rifampin administration resulted in a 3.5-fold increase in duodenal P-gp expression leading to a decrease in digoxin bioavailability.¹⁴ P-gp expression correlated well with digoxin area under the curve (AUC) in this study, emphasizing the significance of intestinal P-gp on digoxin exposure.

Multidrug Resistance–Associated Proteins (ABCC)

MRP2 (ABCC2).

Multidrug resistance–associated protein 2 (MRP2) is primarily localized to three apical membrane barriers, the liver canalicular membrane, the brush border membrane in the kidney, and the apical membrane of the gut, where it serves to excrete substrates into the bile, urine, and back into the gut lumen, respectively. The major substrates of MRP2 are conjugates, including glucuronide, glutathione, and sulfate conjugates, and the primary endogenous function of MRP2 is the secretion of bilirubin and bile acid conjugates across the canalicular membrane into the bile. In addition to phase 2 drug metabolites, MRP2 substrates also include many unconjugated therapeutic drugs including vinca alkaloids. Glutathione

symport is often required for transport of unconjugated substances.¹⁵ MRP2 can contribute to the renal secretion of some drug substrates; however, the main role of MRP2 in drug disposition appears to be hepatobiliary transport.^{1,16} Due to transport of drug conjugates, MRP2 has an important role in enterohepatic cycling. MRP2 transports conjugates into the bile, which empties into the gut lumen. Bacteria in the gut metabolize the conjugate back to the parent drug, which can then be reabsorbed back into the systemic blood circulation. In this way, MRP2 can be responsible for maintaining plasma concentrations of drugs that are not substrates, due to transport of their conjugated metabolites. P-gp is also expressed on the canalicular membrane, although it does not appear to have a role in biliary secretion of drug conjugates. Because of overlapping specificity of MRP2 substrates and inhibitors with P-gp, it can sometimes be difficult to attribute in vivo pharmacokinetic effects to MRP2 alone. SNPs in MRP2 have been identified, resulting in a condition called Dubin-Johnson syndrome. While not leading to severe pathology, subjects with this condition display increased plasma levels of endogenous MRP2 substrates such as conjugated bilirubin.¹⁷

Other MRPs.

In contrast to the apical expression of MRP2, MRPs 1, 3, 4, 5, and 6 are basolateral efflux transporters. MRP1 is another ABC transporter that can be overexpressed in cancer cells, resulting in resistance to chemotherapy.¹⁵ MRP3 has an important role in the transport of conjugated drug metabolites in the liver, particularly glucuronide conjugates. Located on the sinusoidal membrane, MRP3 serves to efflux metabolites out of hepatocytes into the plasma, resulting in conjugate excretion into the urine. Although the bile acid transporters discussed below play a primary adaptive role in response to cholestasis, MRP3 also transports conjugated bile acids and can be induced in cholestatic conditions.¹⁸ MRP3 protein has also been detected in the intestine.¹⁹ MRP4 is expressed on the brush border membrane of the kidney and has a role in anionic drug secretion into the urine.¹⁶ Similar to MRP3, it has a postulated compensatory role in effluxing substrates from the liver during cholestasis or other forms of liver injury.²⁰ MRP4 and MRP5 also transport cGMP and cAMP and

therefore play a role in maintaining the intracellular concentrations of these molecules.²¹

Breast Cancer Resistance Protein

BCRP (ABCG2).

Breast cancer resistance protein (BCRP) is actually a “half” transporter, in that it consists of only six transmembrane-spanning domains and one ATP binding site (Fig. 4.5). Like P-gp, BCRP expression is widespread. Its highest expression is in the placenta, and it is present in the intestine, liver, kidney, brain, heart, testes, and ovaries.²² Also similar to P-gp, multiple binding sites have been proposed for BCRP. As its name implies, BCRP has been attributed with causing chemotherapeutic drug resistance due to overexpression in tumor cells, and among its substrates are several chemotherapeutic agents. BCRP shares many substrates with P-gp and is commonly localized with P-gp, making it difficult to attribute drug transport or inhibition to BCRP. However, the use of BCRP inhibitors, Bcrp knockout animals, and identification of SNPs in the *ABCG2* gene have demonstrated the importance of BCRP in drug disposition. Significant effects of BCRP transport have been demonstrated on drug absorption, biliary clearance, and interestingly on drug transport in the mammary gland, mediating the transport of some drugs into breast milk.²³⁻²⁵ BCRP has a similar role as P-gp in pregnancy, as it is highly expressed at the blood-placenta barrier, where it also serves a protective function to efflux potentially harmful xenobiotics.⁸ Likewise, given their co-localization and shared substrate specificity, synergism between P-gp and BCRP has been demonstrated at other particularly vulnerable physiologic barriers, such as those in the brain and testes in P-gp/Bcrp knockout animal models.²⁶

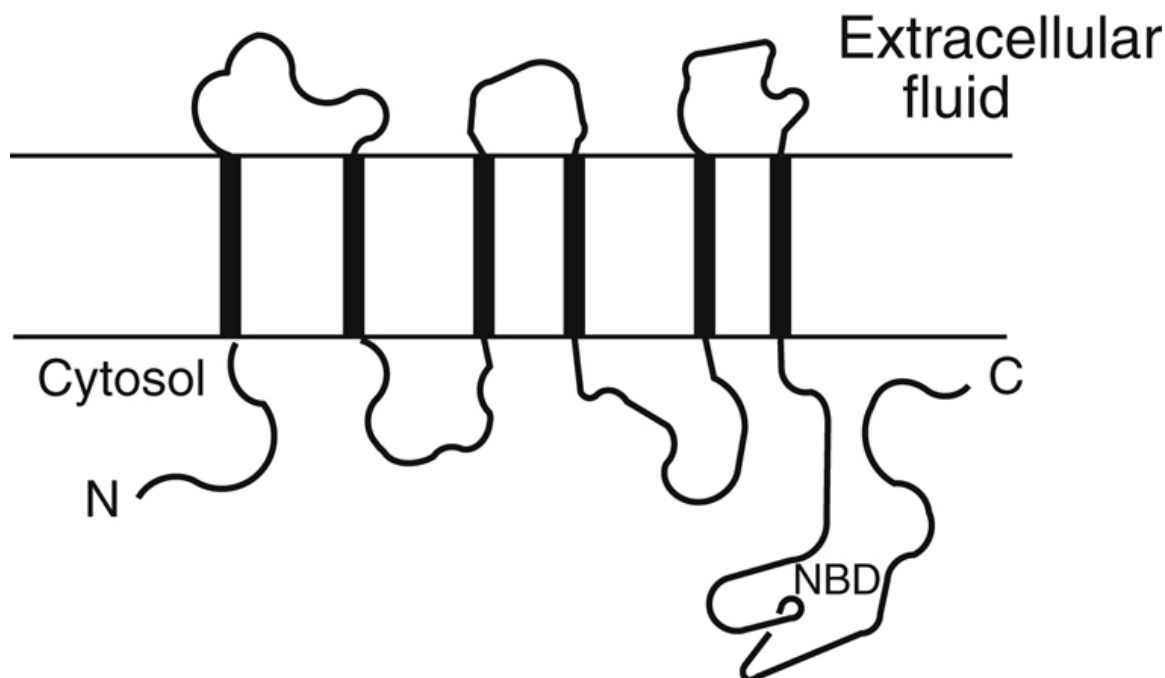


FIGURE 4.5 Structure of BCRP. BCRP is regarded as a half transporter due to its structure including only six transmembrane-spanning domains and one nucleotide-binding domain (NBD).

SLC Transporters

Members of the SLC transporter family are secondary active transporters and, as such, use the concentration gradients of many other substances to transport drug substrates. The concentration gradients of these other substances generally facilitate drug influx, although in some physiologic locations they can facilitate efflux, and some transporters can be capable of transport in both directions. Like the ABC transporters, SLC transporters are physiologically expressed throughout the body. They transport many different xenobiotics along with many endogenous substances, leading to effects on drug disposition and drug interactions. Substrates and inhibitors of SLC transporters are given in [Table 4.3](#).

TABLE 4.3

Relevant Drug Substrates and Inhibitors of SLC Transporters

Transporter	Substrates	Inhibitors

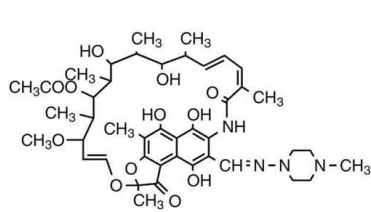
Transporter	Substrates	Inhibitors
OATP1B1	Atorvastatin, bosentan, caspofungin, cerivastatin, enalapril, erythromycin, fexofenadine, fluvastatin, methotrexate, olmesartan, pravastatin, repaglinide, rifampin, rosuvastatin, simvastatin, SN-38, valsartan	Cyclosporine, erythromycin, gemfibrozil, rifampin, ritonavir, saquinavir, tacrolimus
OATP1B3	Digoxin, docetaxel, fexofenadine, olmesartan, paclitaxel, rifampin, telmisartan	Cyclosporine, erythromycin, rifampin, ritonavir
OATP1A2	Digoxin, erythromycin, fexofenadine, imatinib, levofloxacin, methotrexate, pitavastatin, rocuronium, rosuvastatin, saquinavir, sumatriptan	Grapefruit juice, rifampin, ritonavir, saquinavir, verapamil
OATP2B1	Atorvastatin, bosentan, fexofenadine, pravastatin, rosuvastatin	Grapefruit juice, cyclosporine, gemfibrozil, rifampin
OAT1	Acyclovir, adefovir, cidofovir, ciprofloxacin, lamivudine, methotrexate, penicillins, tenofovir, zidovudine	Probenecid, NSAIDs
OAT2	Ketoprofen, theophylline	
OAT3	Bumetanide, cefaclor, ceftizoxime, furosemide, methotrexate, NSAIDs, penicillins	Probenecid, NSAIDs
OCT1	Metformin, morphine, ondansetron, oxaliplatin, sumatriptan	Erlotinib, propranolol, quinidine, verapamil
OCT2	Cisplatin, lamivudine, metformin, oxaliplatin, procainamide	Cimetidine, pyrimethamine, vandetanib, dolutegravir
MATE1	Cisplatin, metformin, oxaliplatin	Cimetidine, cobicistat, famotidine, quinidine, pyrimethamine, trimethoprim, vandetanib
MCT1	Atorvastatin, salicylic acid, valproic acid, γ -hydroxybutyric acid	Dietary flavonoids
PEPT1	Captopril, cefadroxil, cephalexin, enalapril, valacyclovir	Glycyl-proline, zinc
PEPT2	Captopril, cefadroxil, cephalexin, enalapril, valacyclovir	Fosinopril
CNT/ENT	Clofarabine, ribavirin, other nucleoside analogues	Dipyridamole
NTCP	Atorvastatin, pravastatin, rosuvastatin	Bosentan, cyclosporine, gemfibrozil

NSAIDs, nonsteroidal anti-inflammatory drugs.

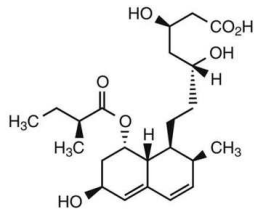
Organic Anion Transporting Polypeptides (SLCO)

OATP1B1 (SLCO1B1).

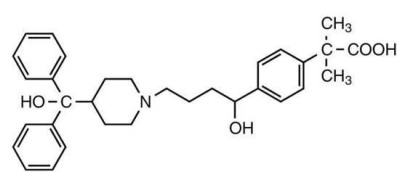
This transporter is one of the most clinically relevant in that its substrates include drugs such as the 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors, or statins, which are widely prescribed. This transporter is localized specifically on the sinusoidal membrane of the liver, where it serves to influx substrates into hepatocytes, where they can be metabolized or transported into bile. The driving force of organic anion transporter polypeptide (OATP) 1B1 and other OATPs is unclear, but it is proposed that they are antiporters, potentially using the high intracellular concentration of glutathione as a driving force. Although the name implies anion transport, OATPs are also capable of transporting cationic and unionized compounds, with OATP1B1 displaying the broadest substrate specificity overall. OATP1B1 transports many endogenous substrates including conjugates of bilirubin and estrogen. Common drug substrates of OATP1B1 and other OATPs are given in [Figure 4.6](#). Inhibitors of this transporter are also commonly used in the clinic for similar or concomitant disease states. Rifampin and cyclosporine potently inhibit OATP1B1 in vivo and are used in the clinic for assessment of interactions with potential OATP substrates. Interactions with these inhibitors can result in very large increases in substrate exposure. Recent findings may help explain the large magnitude of these interactions in that long-lasting inhibition has been demonstrated in vitro and in vivo, at least in rats, particularly for cyclosporine.^{27,28} This transporter has also gained attention due to the recognition that it is highly polymorphic, with a large number of SNPs having been identified in the *SLCO1B1* gene. Many of these SNPs translate into decreased hepatic uptake in vivo, resulting in decreased clearance of OATP1B1 substrates.²⁹



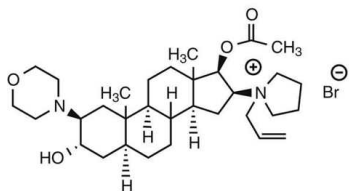
Rifampin
OATP1B1
OATP1B3
Clinical inhibitor



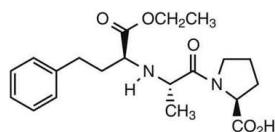
Pravastatin
OATP1B1
OATP2B1



Fexofenadine
OATP1A2
OATP1B1
OATP1B3
OATP2B1



Rocuronium bromide
OATP1B1



Enalapril
OATP1B1

FIGURE 4.6 Common OATP substrates/inhibitors.

Transporter Polymorphisms: Role in Statin Disposition

The effect of OATP1B1 polymorphisms on statin pharmacokinetics and response further emphasizes the significance of OATP-mediated uptake for the hepatic clearance of these compounds. Studies of subjects with the common SLCO1B1 c.521CC genotype have reported increases in plasma AUC from 65% with rosuvastatin to 221% with simvastatin, compared to subjects carrying the wild-type allele.²⁹ Likely the most prominent finding with regard to statin myopathy was the genome-wide association study which found this polymorphism to be the strongest predictor of the toxicity of simvastatin.³⁰ A recent study also found the SLCO2B1 c.1475CT polymorphism had an effect on rosuvastatin lipid-lowering effect, although this has yet to be replicated.³¹ Given that many statins are also substrates for BCRP and there are known polymorphisms in ABCG2, studies have also evaluated the effect of the ABCG2 c.388GA polymorphism on statin exposure. The greatest effect has been demonstrated for rosuvastatin; the increased plasma exposure associated with this SNP may involve increased bioavailability and/or decreased biliary secretion by BCRP.³² Increased effect of rosuvastatin was also demonstrated in this particular study. Alterations in the function of both OATPs and BCRP can significantly affect the safe and effective use of statins. Algorithms for statin dosing incorporating SLCO1B1 and ABCG2 genotyping are currently proposed for individualizing statin therapy.³³

Other OATPs.

Like OATP1B1, OATP1B3 is expressed exclusively on the sinusoidal membrane of the liver. The substrates for OATP1B3 are generally shared by OATP1B1, although there are compounds, such as telmisartan, to which OATP1B3 transport is attributed to be predominant.³⁴ Therefore, OATP1B3 may act as a compensatory transport mechanism when transport by OATP1B1 is deficient due to inhibition or genetic polymorphisms. The other OATPs with identified drug substrates and effects on drug disposition include OATP1A2, 2B1, and 4C1. OATP1A2 is expressed in the brain and may be responsible for blood-brain barrier transport of drugs and endogenous substances. OATP2B1 is also expressed on the sinusoidal membrane of the liver with OATP1B1 and 1B3; however, unlike these transporters, it is also expressed in other tissues including the intestine.¹⁹ OATP4C1 is expressed on the basolateral membrane of the kidney. Among its substrates are digoxin and the antidiabetic drug sitagliptin; however, the role of this transporter in

the in vivo disposition or drug interactions of these drugs is as of yet unclear.³⁵

Organic Anion Transporters (SLC22A)

The organic anion transporters (OATs) are highly expressed in the kidney where they have a significant role in the renal clearance of many anionic drugs. The driving forces and polarized renal expression of OATs support renal secretion of drug substrates from the blood circulation into the urine. OAT1 is expressed primarily in the kidney, on the basolateral membrane of renal tubule cells, along with OAT3. These transporters facilitate anion transport into renal tubule cells through antiport with intracellular dicarboxylates like α -ketoglutarate. Located on the apical membrane, OAT4 can transport drugs from the tubule cells into the urine, but it is likely primarily responsible for renal reabsorption.³⁶ OATs transport an expansive range of drugs, and the importance of OAT expression in the kidney has been demonstrated with numerous clinically relevant drug interactions. Coadministration of OAT inhibitors can lead to decreased renal secretion of OAT substrates including penicillin antibiotics and diuretic agents (Tables 4.3 and 4.4).¹⁶ Along with renal transport, OATs can also mediate hepatic anion transport. The primary location of OAT2 is on the liver sinusoidal membrane, where it facilitates anion uptake into hepatocytes. OAT2 expression has also recently been demonstrated in the kidney and has been implicated to play a role in the renal excretion of creatinine.³⁷ OATs are also present on the choroid plexus (a structure in the brain where cerebrospinal fluid [CSF] is produced), where they can have a role in transfer of drugs between the blood and CSF.³⁸

TABLE 4.4
Select Clinically Relevant Drug Interactions Involving Transporters

Transporter	Substrate	Inhibitor(s)	Effect on Substrate Pharmacokinetics
MDR1	Digoxin	Clarithromycin, cyclosporine, ritonavir, verapamil	↑ plasma AUC (↓ Cl_r , ↓ Cl_{bile} , and/or ↑ F)
Rifampin, St. John's wort		↓ plasma AUC, ↓ F (induction)	
Fexofenadine		↑ F	
Dabigatran	Clarithromycin, verapamil	↑ plasma AUC, ↑ F	
BCRP	Topotecan	Elacridar	↑ plasma AUC, ↑ F
	Rosuvastatin	Atazanavir/ritonavir, cyclosporine	↑ plasma AUC, ↑ F and/or ↓ Cl_{bile}
MRP2	Mycophenolic acid (glucuronide conjugates)	Cyclosporine	↓ Cl_{bile} of conjugates leading to ↓ enterohepatic circulation
OATP1B1/1B3	Statins	Cyclosporine, gemfibrozil, lopinavir/ritonavir, rifampin	↑ plasma AUC, ↓ Cl
	Bosentan	Cyclosporine, lopinavir/ritonavir, rifampin	↑ plasma AUC, ↓ Cl
	Repaglinide	Cyclosporine, gemfibrozil	↑ plasma AUC, ↓ Cl
OATP2B1	Rosuvastatin	Ronacaleret	↓ plasma AUC, ↓ F
OAT1/3	Acyclovir, cephalosporins, cidofovir, ciprofloxacin, furosemide, methotrexate, penicillins	Probenecid	↑ plasma AUC, ↓ Cl_r
OCT2/MATE1	Dofetilide, metformin	Cimetidine, dolutegravir, pyrimethamine, vandetanib	↑ plasma AUC, ↓ Cl_r

AUC, area under the curve; Cl , clearance; Cl_{bile} , biliary clearance; Cl_r , renal clearance; F , bioavailability.

Organic Cation/Carnitine Transporters (SLC22A)

Organic cation transporters (OCTs) are electrogenic uniporters, which use only the negative membrane potential as a driving force and require no co-substrate. OCT1 is expressed primarily in the liver and OCT2 in the kidney, both on the basolateral membrane. Although they are named cation transporters, these transporters are also capable of transporting some anionic and unionized compounds. SNPs in OCT1 have demonstrated effects on the plasma exposure of multiple OCT1 substrates.³⁹ Drug interactions and drug toxicity have been attributed to OCT2-mediated transport, due to a substrate range that includes commonly prescribed therapeutic agents, such as metformin (Table 4.3). Coadministration of OCT2 inhibitors and genetic variation in OCT2 has been demonstrated to increase plasma concentrations of metformin due to its decreased renal secretion (Table 4.4).⁴⁰⁻⁴² Renal transport of cisplatin by OCT2 has also been identified as a significant factor in cisplatin-induced nephrotoxicity.⁴³ OCT3 transports monoamines and is located in the brain and placenta, where its primary role can be to eliminate catecholamines from the fetal blood circulation.⁴⁴ Decreased OCT3 transport has also been suggested to be related to cases of preeclampsia.⁴⁵

Organic cation/carnitine transporter (OCTN) 1 and OCTN2 are proton antiporters expressed in the kidney, where they are localized on the apical membrane. Expression of human OCTN1/2 mRNA has also been detected in the intestine.⁴⁶ OCTN2 is responsible for carnitine transport into tissues and is widely expressed, including in the skeletal muscle, liver, brain, and lung.⁴⁷ These transporters share substrates with OCTs, and their apical expression may allow for the concerted movement of these substrates across membranes in which they are co-expressed. These transporters are of particular importance in the placenta, as they are responsible for transporting

carnitine to the fetus.⁴⁴ Some antiepileptics, like valproate, inhibit OCTNs, and it has been suggested that inhibition of carnitine transport can be partly responsible for teratogenicity and other adverse effects associated with valproate use.⁴⁸ Mutations in OCTN2 lead to systemic carnitine deficiency syndrome, causing early-onset cardiomyopathy, including congestive heart failure.⁴⁹ The anticholinergic drugs ipratropium and tiotropium are transported primarily by OCTN2 and, to a lesser extent, by OCTN1, by bronchial epithelial cells.⁵⁰ These findings are consistent with the pharmacologic activity of the drugs after administration via inhalation.

Multidrug and Toxin Extrusion Transporters (*SLC47A*)

Multidrug and toxin extrusion transporter (MATE) 1 and MATE2-K are cation transporters that use proton antiport as a driving force. MATE1 is located primarily on the apical membrane of the kidney and canalicular membrane of the liver. MATE2-K is kidney specific, and recently it was found that expression even in human proximal tubule cells of kidney is low, leading to ambiguity of its in vivo relevance given that substrates are often shared with MATE1.⁵¹ The significance of MATE1 transport in metformin pharmacokinetics was characterized in MATE1 knockout mice, in which the renal clearance of metformin was decreased to less than 20% that of controls, indicating the importance of this transporter for metformin renal secretion.⁵² Drug interactions with metformin and other substrates in humans involve MATE inhibition in kidney (Table 4.4).⁵³ Inhibition of MATE1 in mice increased the liver accumulation of metformin, and SNPs with decreased function in the *SLC47A* gene have been associated with increased metformin response.^{54,55} MATE1 has a role in the renal secretion and nephrotoxicity of platinum agents, where MATE1 secretion appears protective due to the prevention of renal

cell accumulation.^{56,57} Many drug substrates and inhibitors of these transporters have been identified and can be cationic, unionized, or anionic at physiologic pH.⁵³ MATE substrates and inhibitors are often shared with those of OCT2, and sometimes OAT1 or OAT3, which can lead to drug-drug interactions involving both uptake and efflux into proximal tubule cells, discussed in further detail below.⁵⁸

Monocarboxylate Transporters (*SLC16A* and *SLC5A*)

The first discovered monocarboxylate transporters (MCTs) were proton coupled, transporting monocarboxylates via symport. Because the transport of substrates by MCTs is driven by the proton gradient, these transporters can influx or efflux substrates depending on this gradient. The MCTs are ubiquitously expressed, and their role in the transport of endogenous substances, including lactate and pyruvate, has been extensively characterized.⁵⁹ Many therapeutic agents have also been identified as substrates; however, the clinical relevance of MCT transport of these agents is unclear (Table 4.3). A relevant therapeutic aspect of MCT1 is its high expression in the intestine, making this transporter a target as a means for facilitating oral drug absorption.⁶⁰ MCT1 has also been identified to be overexpressed in tumor cells, and inhibition of this transporter represents a possible therapeutic strategy for some cancers.⁶¹ The drug of abuse γ -hydroxybutyrate (GHB) has been identified as a MCT substrate, and the relevance of MCT transport of this drug has been established. Inhibition of the MCT-mediated renal reabsorption of GHB has been demonstrated to increase its renal and total clearance, making MCT inhibition a possible strategy for the treatment of GHB overdose.^{62,63} Other MCTs have been less well studied, but both MCT8 and MCT10 have demonstrated affinity for thyroid hormones.⁶⁴

MCT1 and Oral Drug Delivery

The antiepileptic agent, gabapentin, has unfavorable oral pharmacokinetics, with low, dose-dependent bioavailability and poor rates of clinical response. In an effort to improve the oral delivery of gabapentin, a prodrug, gabapentin enacarbil, was designed to target absorption by MCT1. In a pilot study comparing the pharmacokinetics of gabapentin to the prodrug, gabapentin displayed dose-dependent bioavailability ranging from 27%-65%, whereas gabapentin enacarbil displayed bioavailability above 68% for all doses and dose-proportional oral exposure.⁶⁵ Gabapentin enacarbil resulted in higher plasma concentrations of gabapentin at similar doses compared to gabapentin itself. This case emphasizes the utility of high-capacity transporters like MCT1 for facilitating oral drug delivery and achieving clinically desirable drug pharmacokinetics.

Another subset of MCTs has been identified that use sodium for monocarboxylate symport and are referred to as sodium-coupled MCTs (SMCTs). Expression of SMCTs is also widely distributed, including the apical membranes of the intestine and kidney, where they likely act in conjunction with proton-coupled MCTs to transport monocarboxylates across membranes.

Peptide Transporters (SLC15A)

Peptide transporters (PEPTs) mediate the transport of di- and tripeptides via proton symport. PEPT1 has very high expression on the apical membrane of the intestine and, as such, is used for drug delivery.¹⁹ Transport mediated by PEPT1 facilitates sufficient oral absorption to allow oral administration for many therapeutic drugs and prodrugs structurally designed to target this transporter (Fig. 4.7 and Table 4.3).⁶⁰ PEPT1 is also present at lower levels on the brush border membrane of the kidney; however, PEPT2 is the major peptide transporter at this site. PEPT2 is also localized to the apical membrane where it serves mainly to reabsorb peptides from the urine in renal tubule cells. It also facilitates transport of drug substrates similar to PEPT1, including β -lactam antibiotics and angiotensin-converting enzyme (ACE) inhibitors (Table 4.3).⁶⁶ Two peptide/histidine transporters (PHTs) were recently identified, PHT1 and PHT2. Expression of these transporters has been demonstrated in the intestine, and they likely have a role in peptide absorption, along with PEPT1. Expression of mRNA for these transporters has also been

identified in several other human tissues.⁶⁷ The role of the PHTs in drug disposition has yet to be elucidated.

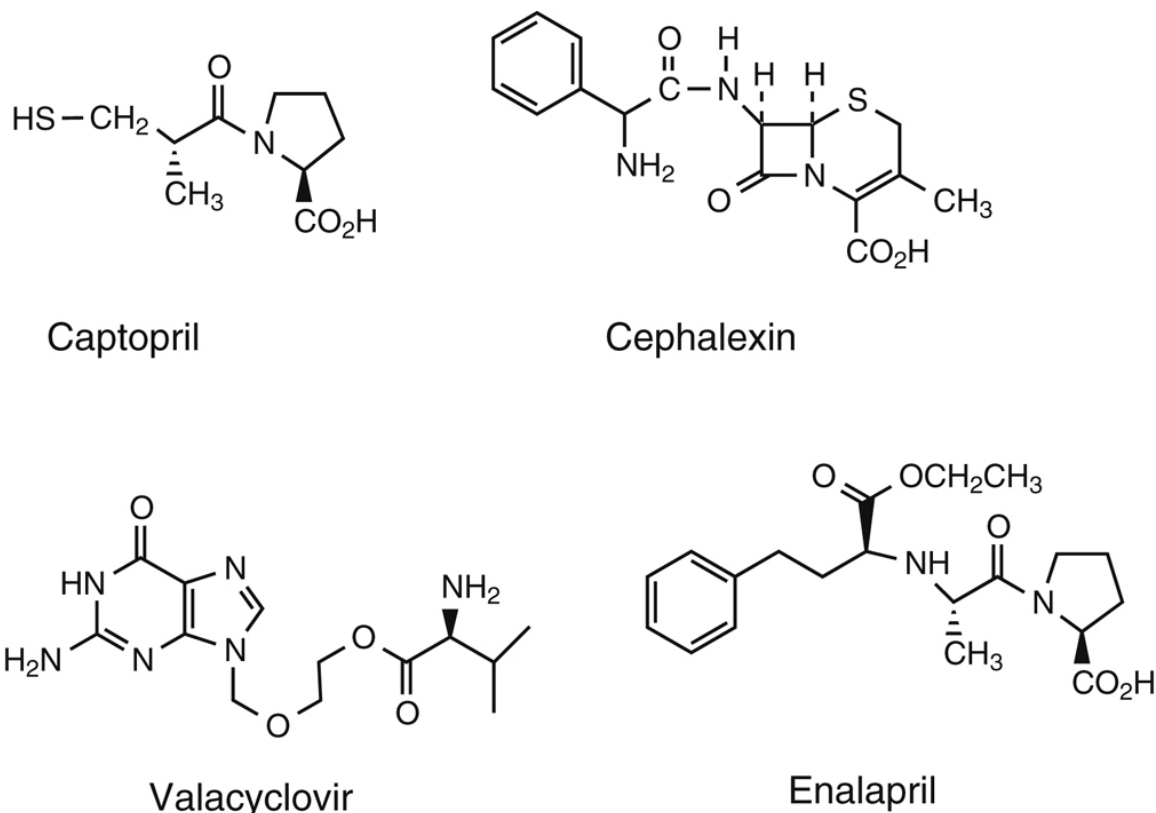


FIGURE 4.7 Drug substrates of peptide transporters.

Nucleoside Transporters (*SLC28* and *SLC29*)

Two nucleoside transporter groups exist, the concentrative nucleoside transporters (CNTs) and the equilibrative nucleoside transporters (ENTs). CNTs are present on the apical membrane of cells and are sodium dependent, whereas ENTs are facilitative and located primarily on basolateral membranes.⁶⁸ These two groups of transporters work together to achieve vectorial transport of purines and pyrimidines. The expression of both groups of transporters is widespread, and although their primary function is to facilitate cellular uptake of nucleosides, they also transport many nucleoside analogues (Fig. 4.8), and these transporters are associated with toxicity and therapeutic response to these drugs (Table 4.3).^{68,69}

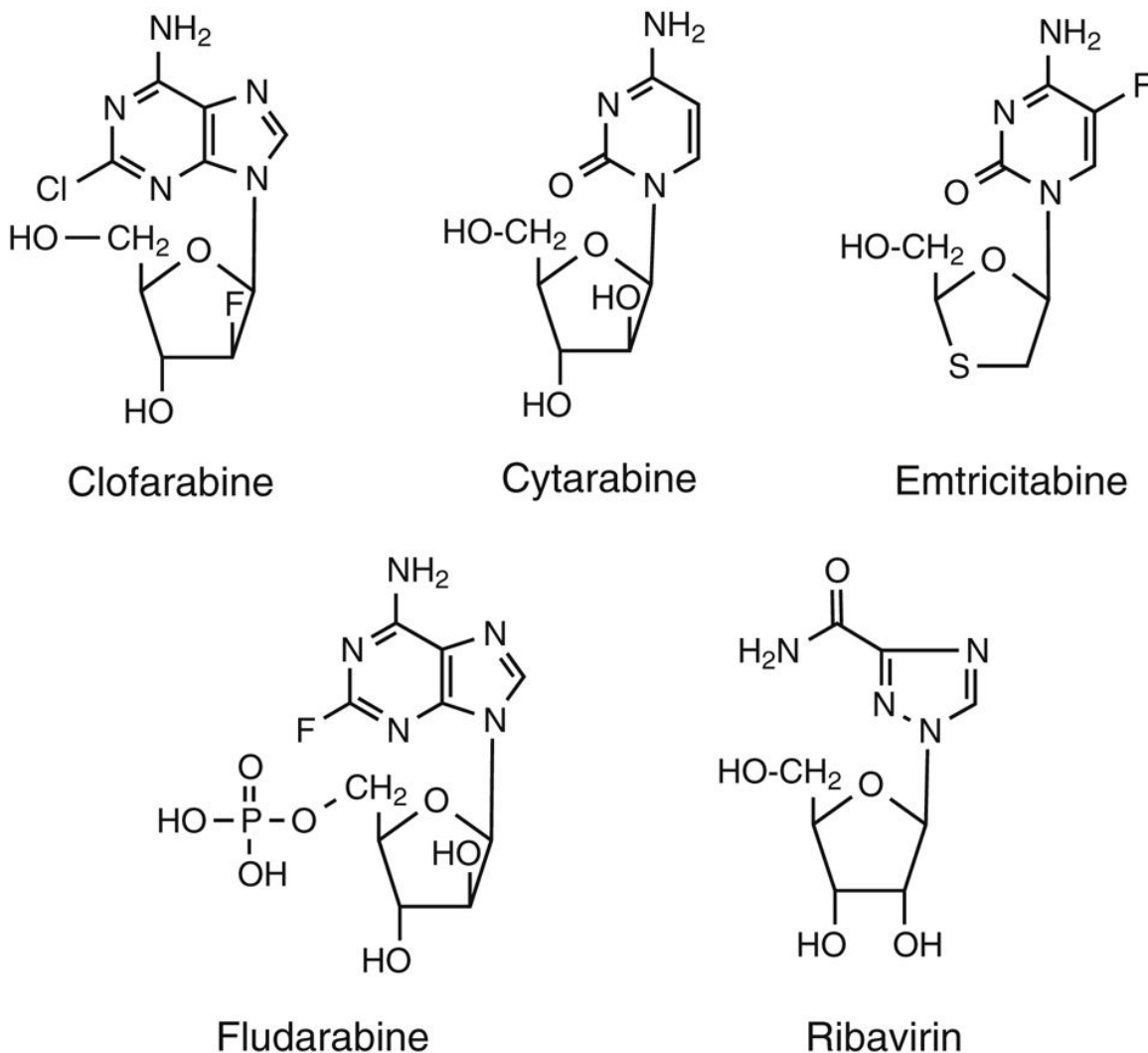


FIGURE 4.8 Drug substrates of nucleoside transporters.

Bile Acid Transporters (*ABCB11*, *SLC10A*, and *OSTA/B*)

The human in vivo bile acid pool is highly conserved due to enterohepatic recirculation; therefore, the bile acid transporters involved in this process have been well characterized.⁷⁰ It has been recently demonstrated, however, that these transporters can also transport xenobiotics. NTCP (sodium-taurocholate cotransporting polypeptide) is primarily responsible for the hepatic uptake of bile acids, and this transporter has been demonstrated to transport some of the statins. Many drugs are also known to inhibit NTCP.⁷¹ The bile salt export pump (BSEP) is likely the most notable bile acid transporter that interacts with drugs, as it has been

shown that inhibitors of this transporter often lead to hepatotoxicity.⁷² Recently, other transporters in the liver have also been recognized to be involved in bile acid-induced hepatotoxicity such as MRP3/4 and Ost α /Ost β . These transporters are expressed on the basolateral membrane of the liver and their role is compensatory to BSEP inhibition in that bile acid accumulation/liver injury leads to their upregulation.^{18,73} Many inhibitors of BSEP also inhibit these compensatory transporters, which further explains the resulting hepatotoxicity observed with these compounds.⁷⁴ There are also bile acid transporters in the intestine which are responsible for reabsorption of bile acids in the gut following their export by BSEP into the bile, and drugs can also interact with these transporters, both as substrates and inhibitors. Inhibition of the apical sodium-dependent bile acid transporter (ASBT) in the gut in particular has been postulated as potential therapy for hypercholesterolemia and diabetes by preventing the conservation of bile acids.⁷⁵

Evaluating Transporter Effects In Vivo

Many transporters share substrates with other transporters and with drug-metabolizing enzymes, making it difficult to attribute in vivo effects to a specific transporter. The use of probe substrates and specific inhibitors makes this identification easier, but these are not available for most transporters or may not be suitable for in vivo use. Knockout animals can be used to assess the effects of a specific transporter in vivo, whereas transfected cell lines or oocytes and specific silencing of a transporter using small interfering double-stranded RNA can be useful to assess effects in vitro. Assessment of the effects of transporter polymorphisms can be useful to allow some determination of a transporter's role in the disposition of a drug in humans. When evaluating the effects of drug transport on bioavailability, it can be difficult to attribute effects to transport in the intestine alone, as changes in both bioavailability and clearance can affect plasma concentrations after oral administration. It is necessary to assess administration of substrates and inhibitors both orally and intravenously to determine if transport primarily affects substrate bioavailability or systemic clearance. Often both parameters are affected by changes in transport because the same transporters are responsible for both processes. Significant effects on absorption cannot always be demonstrated with efflux transporter substrates in vivo. This can be due to the very high luminal concentrations of substrate causing transporter saturation and to the passive membrane permeability of these compounds. A lack of effect can also be due to shared transporter substrates, allowing compensatory transport by one transporter when another is inhibited.

Effect of Transport on Drug Pharmacokinetics and Drug-Drug Interactions

Intestinal Transport

The importance of transporter-mediated influx or efflux in the intestine depends primarily on two factors. The first is the contribution of transporter-mediated transport compared to the other mechanisms of drug passage across the gut wall. The second is drug concentration. Drug concentrations are generally much higher in the gut lumen compared to the systemic blood circulation due to a lower volume of distribution. This causes the concentration gradient driving diffusion to be very large but, more importantly, can lead to transporter saturation. This can result in low bioavailability due to saturation of influx transporters and nonlinearity in drug absorption. Transporter saturation can also aid in drug absorption in that high drug concentrations in the enterocyte can saturate efflux transporters. High drug concentrations in the gut also increase the risk of drug interactions affecting oral absorption of other drugs. The U.S. Food and Drug Administration–recommended Biopharmaceutics Classification System names solubility and permeability as determining factors of oral absorption,⁷⁶ factors which can be translated into effects on drug concentration (C) and nonsaturable transport (P) in the intestine. Considering these factors, the predicted effects of drug transporters on oral absorption are given in [Figure 4.9](#).⁷⁷

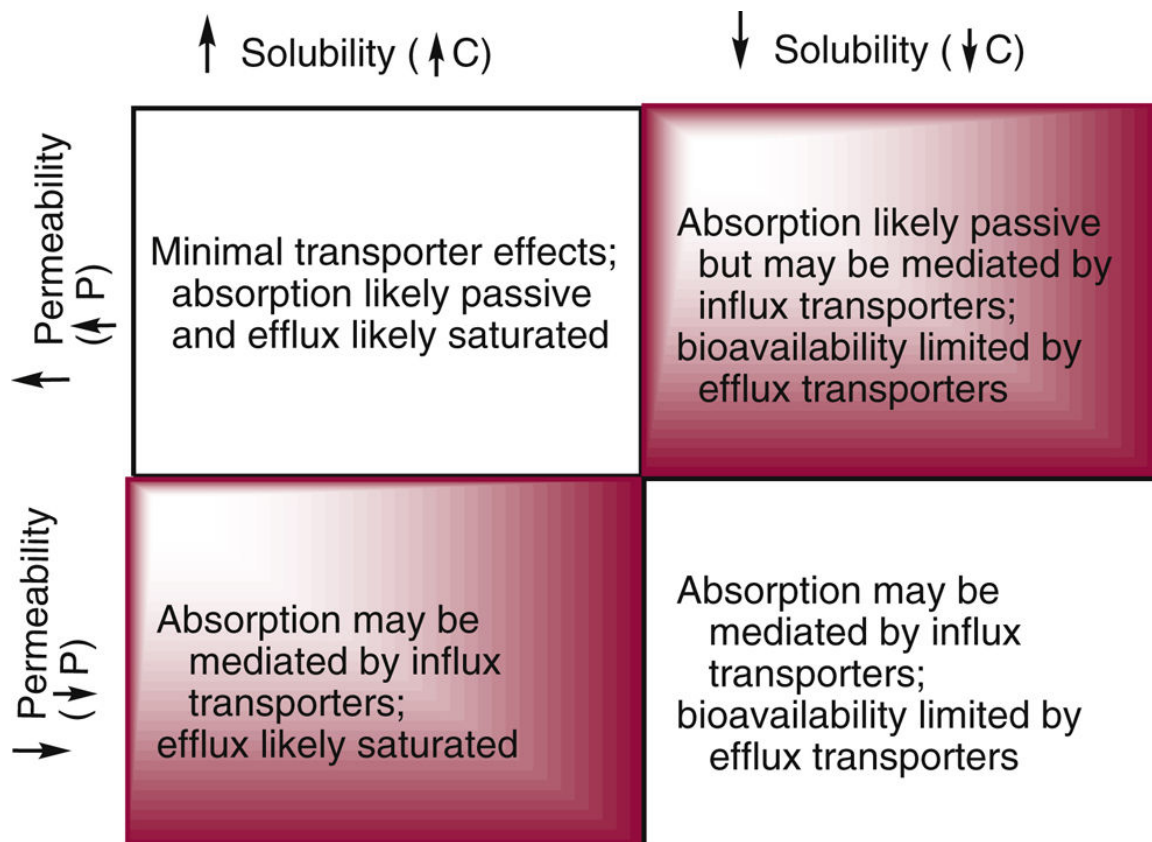


FIGURE 4.9 Role of transporters in drug absorption. High aqueous drug solubility will result in high luminal drug concentrations, allowing for possible transporter saturation. High permeability results in high passive diffusion, allowing these drugs to be orally absorbed without dependence on transporters.

Adapted from Shugarts S, Benet LZ. The role of transporters in the pharmacokinetics of orally administered drugs. *Pharm Res.* 2009;26:2039-2054.

The polarized expression of relevant drug transporters in the intestine is illustrated in [Figure 4.10](#). Clinical relevance of drug transport is determined not only by the presence of transporters on a biologic membrane but also by their relative expression. The transporters with highest intestinal expression include BCRP, PEPT1, P-gp, and MCT1.^{19,78} High expression of influx transporters, such as PEPT1 and MCT1, allows the use of these high-capacity transporters as a drug delivery mechanism. High expression of efflux transporters makes these transporters important in limiting drug bioavailability.

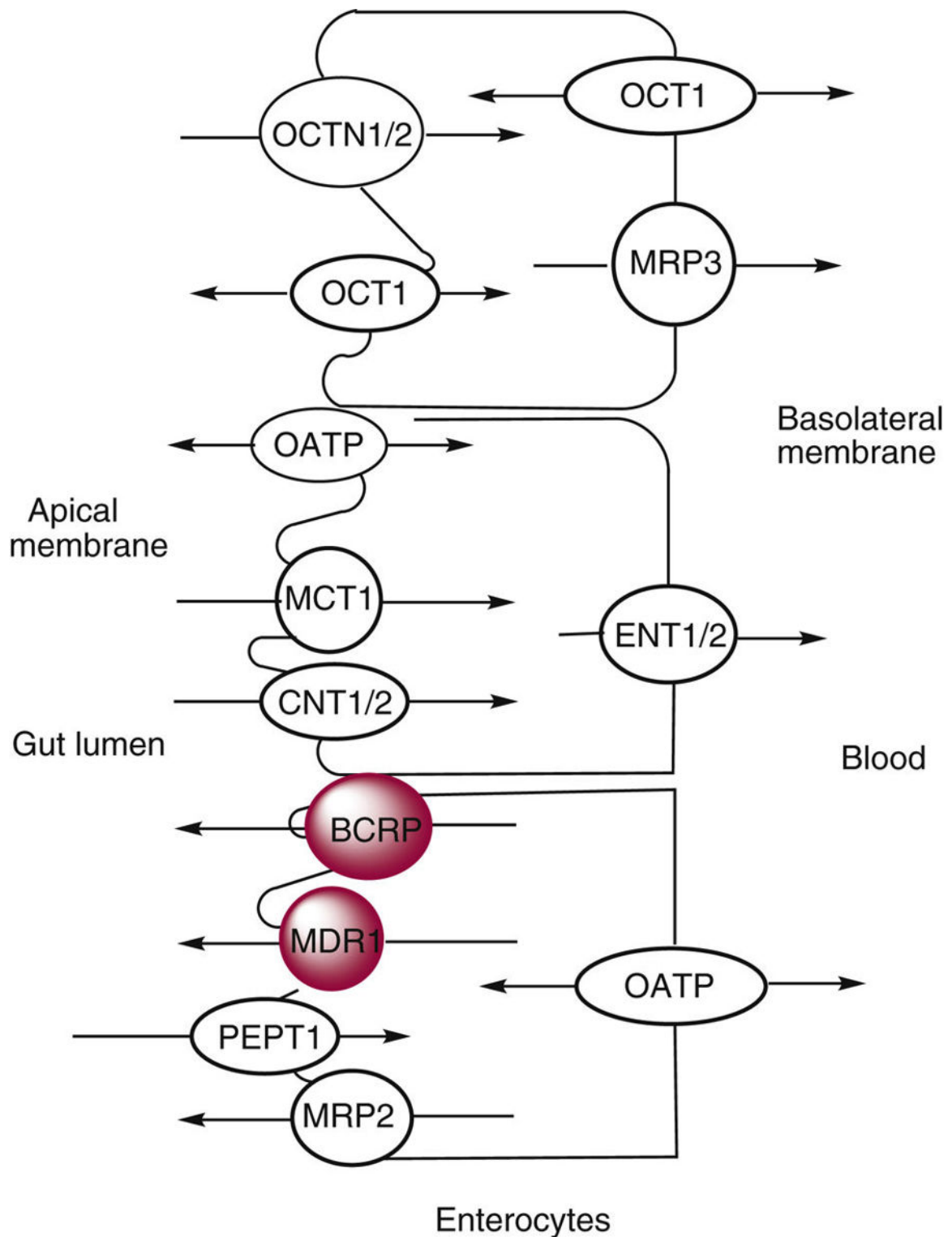


FIGURE 4.10 Polarized expression of drug transporters in the intestine. Transporters most relevant to drug disposition are shown in *red*.

Along with drug transporters, drug-metabolizing enzymes, including phase 1 and phase 2 enzymes, are present in the enterocytes forming the gut wall. First-pass metabolism in the gut can be a reason for low oral bioavailability. Transporters often share substrates with drug-metabolizing enzymes, and the intestine is one site in which substantial transport-metabolism interplay has been recognized. In particular, it has been noted that P-gp shares many substrates with the enzyme CYP3A4, and it is proposed that P-gp can prevent oral absorption through drug efflux and by facilitating drug metabolism by CYP3A4.⁷⁹ Effects on metabolism likely occur by multiple mechanisms, including decreasing intracellular substrate concentrations preventing saturation of metabolism, and by the cycling of substrates through the gut lumen and enterocytes, allowing enzymes such as CYP3A4 multiple opportunities to metabolize their substrates.⁸⁰ This interplay is depicted in [Figure 4.11](#).

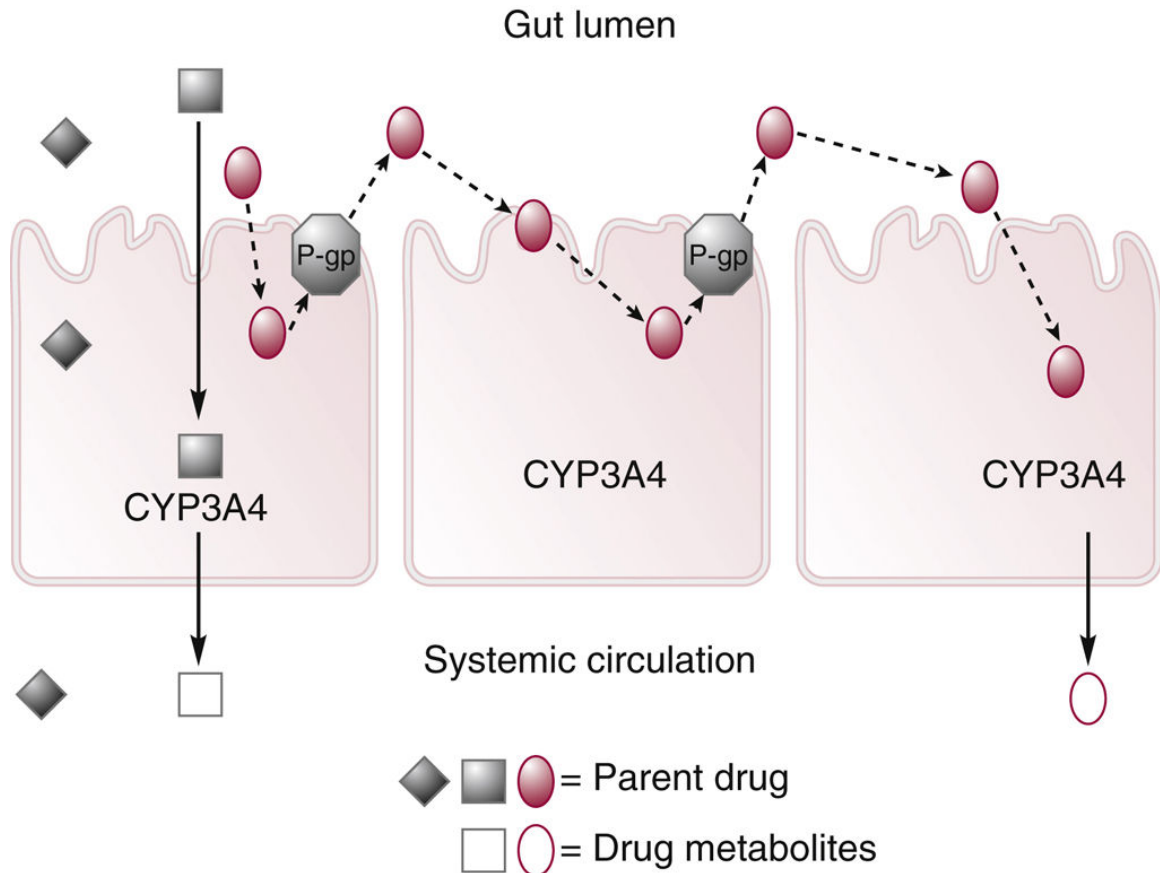


FIGURE 4.11 Transport-metabolism interplay in the intestine. Drugs may be orally absorbed without undergoing metabolic conversion. Drugs may undergo metabolism in the gut, and this may occur upon initial entry into the enterocyte or after cycling between the gut lumen and the enterocyte. P-gp facilitates CYP3A4 metabolism by decreasing intracellular substrate concentrations preventing enzyme saturation and by allowing the enzyme multiple opportunities for drug metabolism, as demonstrated by the molecule shown in red.

Adapted from Benet LZ. The drug transporter-metabolism alliance: uncovering and defining the interplay. *Mol Pharm.* 2009;6:1631-1643.

Efflux Transporters and Drug Bioavailability

Studies using specific P-gp inhibitors and Mdr1 knockout animals have demonstrated the importance of P-gp in the oral bioavailability of drugs, including HIV protease inhibitors, β -receptor antagonists, morphine, and many other therapeutic agents.⁸¹ Many of the drug interactions in the intestine with P-gp or other efflux transporters involve inhibition of transport, resulting in increased bioavailability of the transporter substrate. Efflux transporters, specifically P-gp, may also be induced,

resulting in decreased bioavailability (Table 4.5). Administration of P-gp inhibitors and inducers has demonstrated clinically significant effects on the bioavailability of P-gp substrates, including digoxin, paclitaxel, and dabigatran.^{12,14}

TABLE 4.5
Nuclear Receptors Involved in Transporter Regulation

Nuclear Receptor or Transcription Factor	Agents That Act on Receptor	Transporters Affected
Pregnane X receptor (PXR)	Rifampin, cytokines	MDR1, MRP2, MRP3, OATP1B1
Constitutive androstane receptor (CAR)	Phenobarbital	MDR1, MRP2, MRP3, MRP4
Farnesoid X receptor (FXR)	Bile acids	MRP2, MRP1, OATP1B1, OATP1B3, NTCP, Ostαβ

Drug bioavailability can also be limited by BCRP. Administration of the P-gp/BCRP inhibitor elacridar increased the bioavailability of topotecan sixfold in Mdr1 knockout mice, and oral coadministration with elacridar has been shown to increase the bioavailability of topotecan in humans to almost 100%.^{23,24} Similarly, oral coadministration of the statin rosuvastatin, a BCRP substrate, and the antivirals atazanavir/ritonavir, known to inhibit BCRP, significantly increased rosuvastatin exposure in humans, most likely due to inhibition of BCRP-mediated intestinal efflux and biliary secretion.⁸² Polymorphisms in the *ABCG2* gene can also affect the plasma exposure of rosuvastatin and other BCRP substrates.

Influx Transporters and Drug Bioavailability

Select influx transporters in the intestine can be used to facilitate oral absorption. The high expression of PEPT1 has made it a target for oral drug delivery, and among its substrates are ACE inhibitors, β-lactam antibiotics, and the antiviral drug valacyclovir.⁶⁶ Nonlinearity in the oral pharmacokinetics of valacyclovir can be attributed to saturable PEPT1-mediated absorption.⁸³ Other transporters involved in oral drug delivery include OATPs and MCT1.⁶⁰

Influx transporters can also be responsible for drug interactions in the intestine, although fewer drug interactions have been noted compared with efflux transporters. Specifically interactions have been attributed to

OATP transport in the intestine. Originally, it was thought that OATP1A2 was the primary OATP expressed in the intestine; however, recent data indicate measurable expression of OATP2B1 with concurrent negligible OATP1A2 expression.¹⁹ Decreased oral exposure of rosuvastatin was recently attributed to inhibition of OATP2B1 in the intestine by ronacaleret.⁸⁴ While this interaction was supported by inhibition of OATP2B1 by ronacaleret in vitro, recent data in CaCo-2 cells, a human colon carcinoma epithelial cell line, indicate OATP2B1 expression on the basolateral rather than apical membrane, leaving a potential for interaction with another transporter prior to rosuvastatin reaching the basolateral membrane.⁸⁵ Plasma concentrations of fexofenadine, another OATP substrate, are decreased with concomitant ingestion of grapefruit juice.⁸⁶ This is most likely due to transporter inhibition by the naringin and other flavonoids in grapefruit juice, to which drug interactions with intestinal P-gp have also been attributed.⁸⁷

Hepatic Transport

As a clearance organ, the liver can eliminate drugs through two pathways, metabolism and biliary excretion. Uptake into the liver is necessary for both of these processes, and for hydrophilic drugs that are primarily ionized at blood pH, this is often a transporter-mediated process. Active transport is also necessary for biliary excretion, as diffusion of drugs into the bile is unlikely due to the low volume of the bile canaliculus resulting in high drug concentrations on the biliary side of the canalicular membrane. As with the intestine, there exists transporter-metabolism interplay in the liver because transporters regulate the distribution of drug substrates into the liver and therefore their access to drug-metabolizing enzymes. Just as the significance of a transporter on a drug's membrane permeability is determined by the contribution of that process to overall transport, uptake by transporters or by limited diffusion needs to be rate-limiting for uptake to be a significant factor in drug elimination. The intrinsic clearance of drugs in the liver has traditionally been thought of as simply the rate of metabolism, therefore:

$$Cl_{int} = Cl_{met/bile} \cdot \frac{Cl_{active} + Cl_{passive}}{Cl_{passive} + Cl_{met/bile}} \text{ i.e.,}$$

the hepatic intrinsic clearance (Cl_{int}) can be defined as solely the intrinsic ability of the liver to metabolize a drug (metabolic clearance, Cl_{met}). It is now recognized that for drugs which undergo active uptake by drug transporters, multiple processes need to be incorporated to define its intrinsic clearance, namely those in the “extended clearance” equation:

$$J = \frac{V_{max} \times C}{K_m + C} + P \times C$$

where $Cl_{met/bile}$ is the combined metabolic and biliary clearances and Cl_{active} and $Cl_{passive}$ represent the active transporter-mediated and passive diffusion clearances in the liver, respectively.⁸⁸ Cl_{int} can then be incorporated into a hepatic clearance model incorporating other physiologic parameters to derive the in vivo hepatic clearance from Cl_{int} .⁸⁹ The transporters facilitating both drug uptake and biliary excretion in the liver are depicted in [Figure 4.12](#). As shown in the figure, while it is known that efflux transporters are present not only on the canalicular membrane but also on the sinusoidal membrane of hepatocytes, there is as of yet limited knowledge of the role or clinical relevance of these transporters in drug disposition or drug-drug interactions.

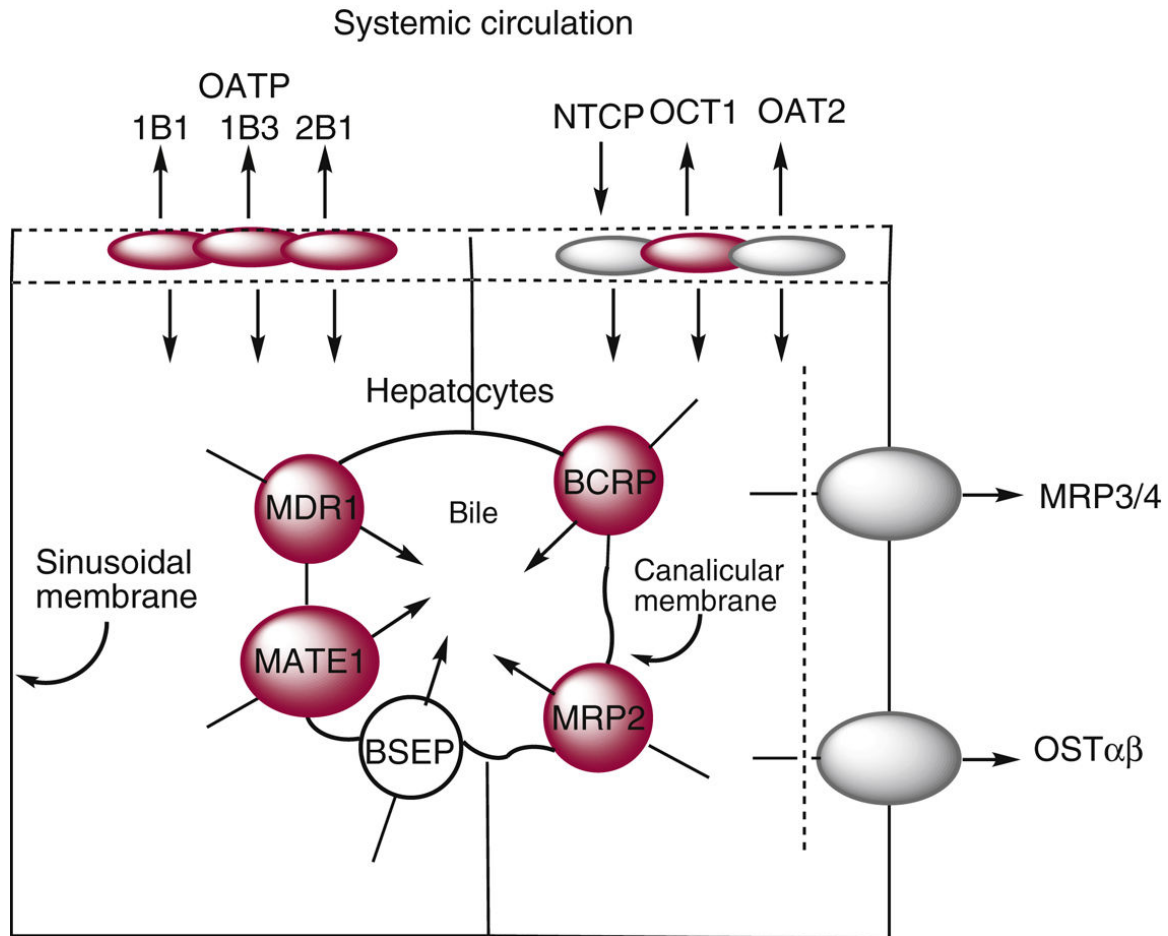


FIGURE 4.12 Drug transporter expression in hepatocytes. Transporters most relevant to drug disposition are shown in red.

Role of Transporters in Hepatic Clearance and Drug Interactions at the Sinusoidal Membrane

Of the transporters present on the sinusoidal membrane, the greatest number of drug-drug interactions has been demonstrated with the OATPs. One of the first interactions noted was the severalfold increase in cerivastatin plasma concentrations following gemfibrozil administration, resulting in severe cases of rhabdomyolysis.^{90,91} This drug interaction was determined to be due to inhibition of OATP1B1 and CYP2C8 by gemfibrozil and its glucuronide metabolite and eventually contributed to cerivastatin being withdrawn from market.⁹¹ Gemfibrozil increases the plasma AUC of other statins which undergo little metabolism (e.g., rosuvastatin), indicating the significance of OATP-mediated transport.^{29,92} Similar effects on statin pharmacokinetics have been demonstrated with other OATP1B1 inhibitors, including cyclosporine and rifampin.⁹³⁻⁹⁵ OATP-

mediated hepatic uptake may not only relate to the pharmacokinetics of drugs, it can impact pharmacodynamics as well, given they allow the statins access to their site of action, the liver. Drug interactions resulting in OATP1B1 inhibition, and numerous polymorphisms in the *SLCO1B1* gene, demonstrate decreased clearance of statins as well as other OATP substrates, including repaglinide, methotrexate, and the active metabolite of irinotecan, SN-38.^{29,96} While OATP-mediated transport put the concept of uptake rate-limited hepatic clearance on the map, more recently clinical relevance of other hepatic transporters has surfaced, specifically that of OCT1. Polymorphisms have been identified in the *SLC22A1* gene that have reduced uptake of OCT1 substrates.³⁹ Clinical studies have shown homozygous carriers of OCT1 variants with reduced function to affect the plasma exposure of sumatriptan, morphine, and 5HT-3 antagonists.⁹⁷⁻⁹⁹ While metformin is not eliminated by metabolism or biliary clearance and therefore is cleared completely by the kidneys, OCT1-mediated uptake of metformin is another example of hepatic transporters having a role in pharmacodynamics. Carriers of reduced function *SLC22A1* variants have reduced response to metformin; a similar reduction has been observed with inhibition of OCT1 by verapamil.^{100,101}

Role of Transporters in Biliary Drug Excretion and the Effect of Drugs on Canalicular Membrane Transport

It can be difficult to attribute clearance to biliary excretion in vivo, given that sampling bile is very technically challenging and, in the absence of this, drug excreted into bile ends up in the feces and is therefore indistinguishable from unabsorbed drug following oral absorption. Fecal drug amounts following IV administration can be evaluated and other methods for sampling drug in the duodenum and/or via noninvasive imaging methods are becoming useful. Accordingly, bile duct-cannulated mice, including transporter knockout mice, are often relied upon to identify this route and mechanism of elimination for drugs. Some of the first drug interactions discovered to affect biliary clearance of drugs in humans involved the ABC transporter P-gp. It was demonstrated that quinidine and verapamil, known P-gp inhibitors, significantly decreased the biliary clearance of digoxin, resulting in increased digoxin plasma

concentrations.^{13,102} P-gp efflux also has a role in the biliary excretion of doxorubicin, as demonstrated by an 80%-90% decrease in doxorubicin biliary clearance in Mdr1 knockout mice.¹⁰³ Fecal excretion is also a significant elimination pathway for doxorubicin in humans suggesting biliary clearance. MRP2 also mediates biliary excretion of doxorubicin and other therapeutic agents, including pravastatin and valsartan (Table 4.3).¹⁰⁴⁻¹⁰⁶ MRP2 has a significant role in the biliary excretion of drug conjugates, resulting in their enterohepatic cycling. There is a clear in vivo drug-drug interaction between cyclosporine and mycophenolic acid, which has been attributed to inhibition of MRP2-mediated biliary secretion of the glucuronide conjugate leading to decreased hydrolysis of the conjugate in the intestinal tract and reabsorption of mycophenolic acid itself.¹⁰⁷ BCRP has a more significant role in the excretion of sulfate conjugates, along with its role in secreting unconjugated drugs. Decreased biliary excretion of acetaminophen sulfate and other sulfate conjugates has been reported in mice lacking Bcrp but not in those lacking Mdr2.^{108,109} BCRP knockout or inhibition results in decreased biliary clearance of therapeutic agents in mice, including topotecan, nitrofurantoin, and ciprofloxacin.^{24,25,110} In addition, polymorphisms in the *ABCG2* gene have been shown to correlate with increased response to rosuvastatin, which may be explained by increased concentrations in the liver, the site of action, due to decreased biliary excretion by BCRP, along with increased absorption.³² As mentioned above efflux transporters MRP2 and BSEP are also primarily responsible for the biliary secretion of endogenous substances, and drug interactions may also interfere with these processes through inhibition of these transporters (Table 4.3).

Renal Transport

Renal clearance is mediated by three primary pathways: glomerular filtration, tubular secretion, and renal reabsorption. Small molecules that are not protein bound will be filtered at the glomerulus. Drugs that undergo glomerular filtration can have negligible renal clearance if they are sufficiently lipophilic to be passively reabsorbed from the tubular lumen back into systemic circulation. Drugs that undergo filtration and are

hydrophilic or ionized will be excreted into the urine unless they are actively reabsorbed by transporters. Drugs can have extensive renal clearance (Cl_r), sometimes much greater than the glomerular filtration rate, if they undergo renal secretion. The following equation can be used to determine renal clearance considering the possibility of all three processes:

$$Cl_r = (GFR * f_{up} + Cl_{rs}) (1 - FR) \quad \text{where}$$

GFR is the glomerular filtration rate; f_{up} is the fraction unbound in the plasma; Cl_{rs} is the clearance by renal secretion; and FR is the fraction of unchanged drug that is filtered and secreted in the urine, which is reabsorbed. Given that urine is a biological sample easily collected, renal clearance and the net contribution of active secretion or reabsorption are readily calculable; transporters are involved whenever active secretion or active reabsorption occur. Secretion and reabsorption require transport of drug across both apical and basolateral membranes, and the polarized expression of organic anion and organic cation transporters on both membranes allows directional transport of ionized substrates. The transporters responsible for active secretion and active reabsorption and their polarized expression in the kidney are illustrated in [Figure 4.13](#).

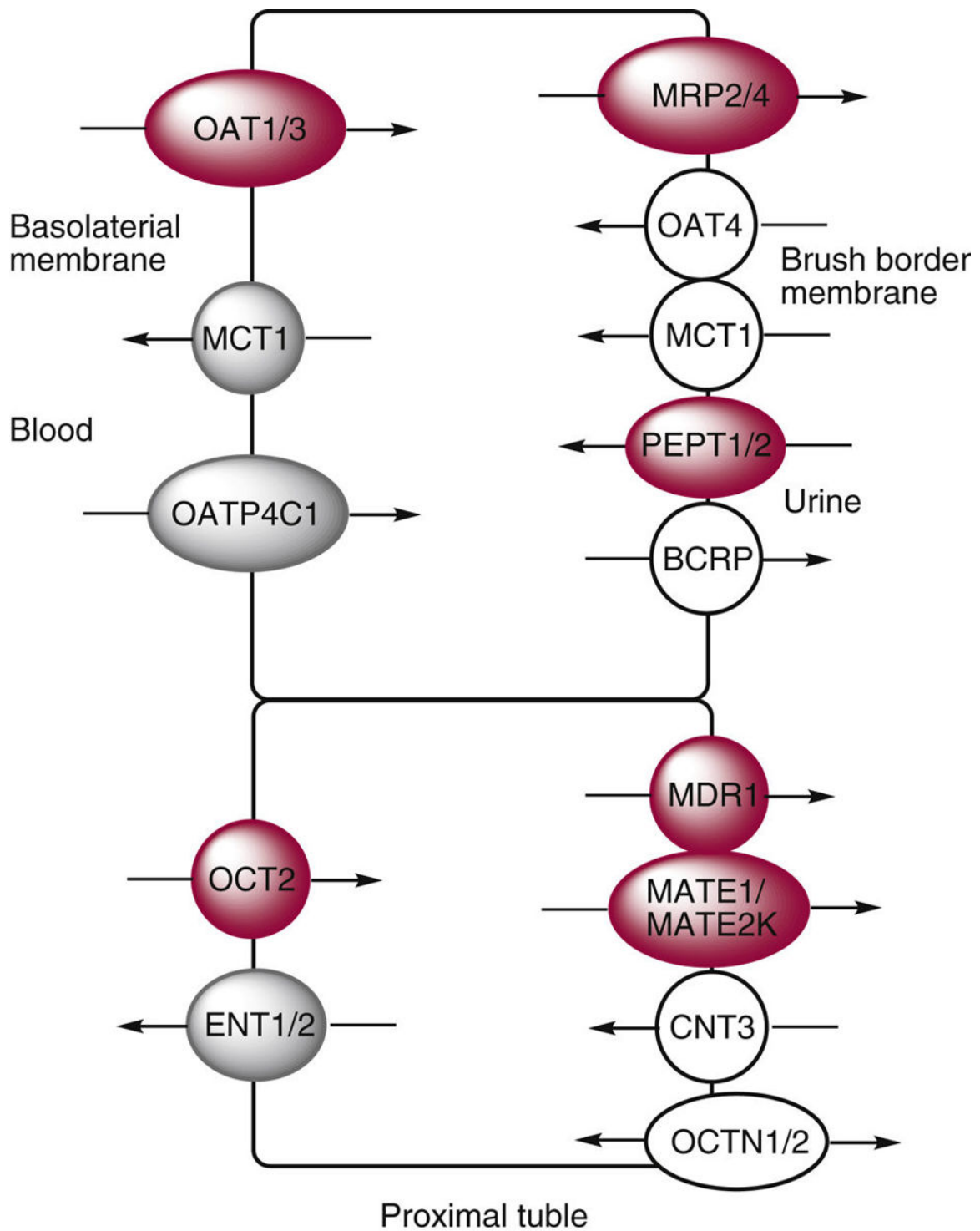


FIGURE 4.13 Drug transporter expression in the renal proximal tubule. Transporters most relevant to drug disposition are shown in *red*.

Renal Clearance of Anions

Tubular secretion of anions across the basolateral membrane is mediated primarily by OAT1 and OAT3. Substrates of these transporters include β -lactam antibiotics, nonsteroidal anti-inflammatory drugs, and many other anionic therapeutic agents (Table 4.3). Probenecid is a known inhibitor of OATs, and coadministration with OAT substrates leads to decreased renal clearance of these substrates. One of the most highly recognized transporter interactions is that between probenecid and penicillin derivatives, and coadministration of probenecid has been demonstrated to decrease the renal clearance of numerous drugs of this class, including piperacillin, nafcillin, ticarcillin, and others.¹⁶ Inhibition of OAT transport by probenecid also causes decreased renal clearance of diuretics like furosemide and results in diminished diuretic effect due to decreased tubular drug concentrations.^{111,112} OAT-mediated drug interactions affect renal secretion of many other OAT substrates including methotrexate, acyclovir, and zidovudine.¹⁶ Secretion of anions across the renal brush border membrane is primarily mediated by MRP2 and MRP4. Probenecid is also an MRP2 inhibitor, and some of its effects on renal clearance can be due to combined inhibition of OATs and MRP2. MRP4 is present at similar levels to MRP2 in human kidney, and Mrp4 knockout mice display significant renal cell accumulation of the nucleotide phosphonates adefovir and tenofovir.^{51,113} Additionally, case reports exist in which administration of MRP4 inhibitors have been associated with nephrotoxicity of tenofovir.¹¹⁴ Interestingly, cidofovir does not appear to be transported by MRP4, and this may be a reason for the significant nephrotoxicity associated with this drug. These drugs are also OAT substrates, and OAT inhibition may have therapeutic potential for preventing renal accumulation and toxicity with these agents.¹¹³

Renal Clearance by Oct2/Mate: Cisplatin Toxicity

The platinum agents are used clinically for treatment of a variety of cancers. Treatment with cisplatin has been limited by its adverse effects, including nephrotoxicity, ototoxicity, and neurotoxicity.¹¹⁵ Transport by OCT2 has been attributed with facilitating cisplatin toxicity in the kidney, demonstrated by decreased nephrotoxicity with coadministration of OCT2 inhibitors and in Oct2 knockout animals.¹¹⁶ Genetic polymorphism in OCT2 has also been correlated with decreased serum creatinine concentrations in patients undergoing cisplatin treatment, suggesting decreased nephrotoxicity.⁴³ Oxaliplatin, another OCT2 substrate, is not associated with severe nephrotoxicity. Oxaliplatin is efficiently transported by MATE1 and MATE2-K, allowing for secretion of this drug from tubule cells into the urine.⁵⁷ Cisplatin, a poor MATE substrate, has been demonstrated to accumulate in renal tubule cells.¹¹⁷ Carboplatin, another platinum-based agent, is a substrate for neither OCT2 nor MATE transporters and, similarly to oxaliplatin, does not display nephrotoxicity.⁵⁷ It is apparent that the effects of cisplatin in the kidney can be attributed to OCT2-mediated influx followed by inefficient apical efflux, resulting in renal cell accumulation and toxicity. Recently, OCT2 was also found to be present in the cochlea, and Oct2 knockout prevented cisplatin-induced ototoxicity, along with reducing nephrotoxicity.¹¹⁸ OCT2 inhibition represents a potentially clinically relevant therapy for the prevention of both toxicities during cisplatin therapy; however, selective inhibition of OCT2 over MATE1 is essential.

Renal Clearance of Cations

While not always true, renal secretion of cations generally involves transport across the basolateral membrane by OCT2 followed by transport across the brush border membrane by MATE1 or possibly P-gp. Cations can be substrates of OATs as well, and MATE1 can also be involved in the secretion of anions.⁵³ Metformin is a substrate of both OCT2 and MATE1, and drug interactions involving either or both of these transporters result in significant decreases in metformin renal clearance ([Table 4.4](#)).^{40,119} As mentioned previously, inhibitor specificity is often, but not always, shared between OCT2 and MATEs. Genetic variants of both OCT2 and MATE1 have also been correlated with decreased metformin clearance and/or increased glucose-lowering effects with metformin treatment.^{42,55,120} Platinum-based chemotherapeutic agents also use the OCT2/MATE transport system, and this transport is a factor in toxicity of these agents. A topic of great recent interest in drug development is the

inhibition of OCT2 or MATEs by compounds leading to increased serum creatinine, due to its decreased active secretion.

Renal Transporters and Creatinine Clearance

Monitoring of endogenous creatinine clearance has traditionally been used as a surrogate for measurement of glomerular filtration rate (GFR) and kidney injury, given the negligible protein binding of creatinine and its complete excretion unchanged into urine. In recent years, however, it has been recognized that changes in serum creatinine and creatinine clearance can occur without changes in other biomarkers of renal injury or without change in other markers of GFR, such as iothexol or cystatin C. Creatinine has been shown to be taken up by cationic renal transporters OCT2, MATE1, and MATE-2K, and there are multiple clinical examples in which the administration of drugs that inhibit these transporters result in an elevation of serum creatinine or decrease in creatinine clearance.¹²¹ Although it has been well documented that creatinine renal clearance is indeed higher than GFR, indicating that creatinine undergoes renal secretion, there is still controversy over which transporters are truly most important for this process in vivo. Recently creatinine has been determined a substrate of OAT2, with some reports indicating affinity for this transporter to be much greater than the cationic transporters; however, further clinical evidence is required to determine if inhibitors of OAT2 solely decrease creatinine renal secretion.^{37,122}

Blood-Brain Barrier and Blood-Cerebrospinal Fluid Barrier Transport

The blood-brain barrier (BBB) serves an important protective function for the CNS and presents both structural and metabolic barriers for xenobiotic penetration into the brain. The endothelial cells of the BBB form tight junctions with minimal fenestrations, limiting BBB penetration to drug molecules that are capable of transcellular diffusion. For entry of polar or charged essential nutrients such as amino acids and glucose, transport processes are necessary. Transporters present on the BBB for this function have been exploited to facilitate brain penetration of agents like levodopa, one of the most commonly used agents in Parkinson's disease, which is transported into the brain by the l-type amino acid transporter (LAT) 1.¹²³ MCT1 is also expressed at the BBB, and along with its role in the renal reabsorption of GHB, it is responsible for distribution of GHB into the brain, where it elicits its euphoric effects.¹²⁴ Other influx transporters expressed at the BBB include ENTs and OCTs,

which have known drug substrates and may therefore be involved in brain distribution of these drugs.³⁸ It should be noted that drug concentrations in the brain are not easily measured, and changes in brain concentrations do not generally result in any changes in plasma concentrations of the drug, making it difficult to assess the in vivo importance of transporters at the BBB. However, preclinical knockout models are particularly useful in this case, although extrapolation to humans is uncertain as knockout models represent an extreme scenario. These models have aided in assigning a clear limitation of brain penetration to transport by efflux transporters expressed at the BBB, including P-gp, BCRP, and MRPs. Mdr1 knockout mice display substantially higher brain/plasma concentration ratios for many P-gp substrates, with over 10-fold increases exhibited for digoxin, paclitaxel, and protease inhibitors compared to wild-type animals.^{125,126} Inhibition of P-gp has been used as a strategy to increase BBB penetration and has been demonstrated to be effective in animal models.^{126,127} The use of P-gp inhibitors in studies with human subjects shows similar, but much more modest, effects on brain penetration. This decreased effect is due in part to the lower inhibitor concentrations reached at doses used in human subjects.¹²⁸ Bcrp knockout animals have demonstrated the ability of this efflux transporter to limit brain penetration of drugs such as sorafenib.¹²⁹ Many anticancer drugs are co-substrates for P-gp and BCRP, and knockout of both transporters has demonstrated synergistic effects on brain accumulation of these drugs.^{26,129,130} The use of co-inhibitors represents a potential strategy for improving treatment of brain tumors with these agents.¹²⁷ There are various influx and efflux transporters present on the blood-cerebrospinal fluid barrier (BCSFB) as well, including P-gp and MCT1, as well as others not present at the BBB, such as PEPT2.³⁸ Given that the CSF is occasionally sampled in clinical studies as a surrogate for the rest of the CNS, it is important to recognize there

are differences in transporter expression at the BBB and the BCSFB, and even those transporters expressed on both barriers may be present to different extents and may serve different functions. For example, for P-gp substrates, it has been shown that P-gp serves to keep drug out of the brain at the BBB; however, it also pumps drug into the CSF, resulting in a disparity in the CSF-to-plasma and brain-to-plasma ratios for P-gp substrates.^{38,131,132}

Regulation of Transporters and Interindividual Variability

While inherent polymorphisms in transporter genes can be one factor in the interindividual variability of transporter substrate pharmacokinetics, other factors such as transcriptional regulation of transporters, effects mediated by nuclear receptors, can also contribute. While there is little evidence of in vivo induction with most transporters, there are nuclear receptors implicated to affect transporter expression in vitro or in human tissue samples (Table 4.5).^{133,134} One exception among these is P-gp, for which there are multiple examples of in vivo drug-drug interactions as a result of induction. Rifampin, a strong agonist of the nuclear receptor pregnane X (PXR), induces the expression of several enzymes and transporters including CYP3A4 and MDR1. During rifampin treatment, decreased drug exposure is observed with CYP3A4/MDR1 co-substrates such as cyclosporine, calcium channel blockers, and chemotherapeutic agents, leading to a complete loss of response for some of these substrates.^{135,136} Rifampin and other PXR agonists also result in interactions with P-gp substrates that are not metabolized by CYP3A4, such as digoxin, dabigatran, talinolol, and fexofenadine, demonstrating the in vivo relevance of P-gp induction.^{14,137-139} There also exists some in vitro and in vivo evidence for induction of OATPs by rifampin.¹⁴⁰⁻¹⁴² (Note that inhibitor vs. inductive effects of rifampin can be determined with single- and

multiple-dose rifampin administration, respectively.) Rifampin also increased the effect of metformin on blood glucose, suggesting induction of OCT1 in the liver as well.¹⁴³ While there is no in vitro evidence to support rifampin effects on renal transporters, renal secretion of metformin also increased in this study, suggesting induction of OCT2 or MATE1. While effects at nuclear receptors may be mediated by concomitant drugs, interesting effects on transporter and enzyme regulation also occur in the presence of disease states. As mentioned above, during cholestasis hepatic transporter expression changes facilitate bile acid secretion into the plasma by MRPs and Ost $\alpha\beta$ and decrease secretion into the bile by MRP2 and BSEP. This change in transporter function has been attributed to the effect of bile acids on the farnesoid X receptor (FXR), as well as PXR, causing upregulation of sinusoidal efflux transporters and downregulation of those at the canalicular membrane.¹⁴⁴ Changes in enzyme and transporter expression have also been demonstrated in other disease states, including cancer, chronic renal failure, inflammation, and epilepsy, which display considerable interindividual variability.¹⁴⁵⁻¹⁴⁷ Current literature supports the involvement of select nuclear receptors in the regulation of affected proteins in some of these conditions.¹⁴⁵ Along with changes in gene expression or structure, transporter function can also be affected by posttranslational modifications, such as glycosylation or phosphorylation, which can lead to rapid changes in transporter activity.⁷⁰ Understanding the role of these posttranscriptional modifications in interindividual variability is ongoing, along with regulatory effects with the aforementioned diseases, and the mechanisms by which transporter functions are altered will aid in the accurate dosing of drugs in patient populations.

Conclusions

Transporters have important roles in the pharmacokinetics of many therapeutic agents. Transport can result in drug-drug interactions, nonlinear pharmacokinetics, and interindividual variability. Changes in transporter function can translate into drug toxicity, although transporter interactions also have the potential to be beneficial for drug therapy by increasing bioavailability or decreasing clearance. Application of transport principles and understanding the effects of transport on drug substrate concentrations promote the safe and effective use of therapeutic agents. Further investigation of transporter effects in vivo and mechanisms in vitro is expected to reveal additional significant effects of drug transporters on drug disposition.

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CHAPTER 5

Principles of Biotechnology-Derived Drugs

Tanaji T. Talele and Vijaya L. Korlipara

Drugs covered in this chapter:

- Abciximab
- Ado-trastuzumab emtansine
- Alemtuzumab
- Ave9633
- Axicabtagene
- Bevacizumab
- Brentuximab vedotin
- Capromab pendetite
- Catumaxomab
- Cetuximab
- Clopidogrel
- Codeine
- Eculizumab
- 5-Fluorouracil
- Gemtuzumab ozogamicin
- Imgn901
- Imiciromab pentetate
- Infliximab
- Inotuzumab ozogamicin
- Irinotecan
- Isoniazid
- 6-Mercaptopurine

- Niraparib
- Nofetumomab
- Olaparib
- Pegloticase
- Rucaparib
- SAR3419
- Satumomab
- Tamoxifen
- Technetium-99m-arcitumomab
- Tisagenlecleucel
- Tolterodine
- Trastuzumab
- Trastuzumab-MCC-DM1
- Vemurafenib
- Voretigene neparvovec-rzyl
- Warfarin

Abbreviations

- AAV** adeno-associated virus
- ABC** ATP-binding cassette
- ADC** antibody-drug conjugate
- BRCA** breast cancer-associated gene
- Cas9** CRISPR-associated protein9
- CDR** complementarity-determining region
- CRISPR** clustered regularly interspaced short palindromic repeats
- DAR** drug-to-antibody ratio
- DPD** dihydropyrimidine dehydrogenase
- DSB** double strand break
- G6PD** glucose-6-phosphate dehydrogenase
- HAMA** human anti-mouse antibody

HDR homology-directed repair
HGP human genome project
MAb monoclonal antibody
NAT N-acetyl transferase
PARP poly (ADP-ribose) polymerase
PCR polymerase chain reaction
PEG polyethylene glycol
SNP single nucleotide polymorphism
TALEN transcription activator–like effector nuclease
TPMT thiopurine methyl transferase
UDP uridine diphosphate
UGT UDP glucuronyl transferase
URE upstream regulatory elements
ZFN zinc finger nuclease

Introduction

Pharmaceutical biotechnology generates basic scientific knowledge, useful therapeutic and diagnostic products, and promising methodologies for future research and clinical applications. The techniques of biotechnology lead to the development of novel therapeutics, improved methods of manufacturing pharmaceuticals, and significant contributions to our understanding of disease etiology, pathophysiology, and biochemistry. Genomics, transcriptomics, proteomics, pharmacogenomics, and metabolomics, the core approaches to pharmaceutical biotechnology, are making major contributions in three areas:

1. Identification of new genes
2. Identification of drug targets
3. Development of “personalized medicine”

Although many of the first biotechnology-derived therapeutics were initially used in acutely ill, hospitalized patients, the products of today’s pharmaceutical biotechnology have an impact on patient populations with chronic diseases such as rheumatoid arthritis, gout, and cancer.

Pharmacogenetics

The Human Genome Project (HGP), funded by the DOE (Department of Energy) and NIH (the National Institutes of Health), began in the early 1990s and was completed in the early 2000s.¹ The application of genetic information gained from the HGP to disease diagnosis and drug therapy is a major development that is bringing about a wealth of change including the potential to develop drugs for hundreds of rare, inherited Mendelian disorders. A major benefit of pharmacogenetic screening is a reduction in the health care costs resulting from more effective treatments with fewer side effects and higher cure rates achieved from the patients' treatment targeted to the molecular etiology of their disease. Mutations are the result of heritable, permanent changes in DNA base sequence. These usually come about by one of the following three mechanisms: substitution, addition, or deletion. The number of base pair changes involved in a single gene mutation can vary from a single base pair change or single nucleotide polymorphism (SNP) to a large number of gross changes in the gene structure involving insertion, deletion, or rearrangements of a very large number of base pairs.

Mutations result in a variety of changes to the gene product. If the base change occurs within the reading frame of the gene product, it may alter the amino acid sequence of the gene product (protein). SNPs have been defined as single base mutations that occur in 1% or more of the population.^{2,3} Many of these SNPs may be responsible for the production of a malfunctioning gene product that, in turn, is responsible for a serious disease. The well-used example is the A → T mutation in the β -globin gene, in which the GAG glutamic acid codon is changed to the GTG valine codon, resulting in abnormal aggregation of the hemoglobin molecules and causing sickle cell anemia. Another example is one form of β -thalassemia that results from a mutation of a CAG glutamine codon to the TAG stop codon early in the DNA sequence, resulting in premature termination of the translation process and total loss of the β -subunit of the adult hemoglobin molecule.

In principle, one is able to manipulate the therapeutic outcome of a patient population by separating patients who will not respond normally to a particular drug therapy from those who will, based on an analysis of established genetic biomarkers related to the drug's metabolic profile (Fig. 5.1). Patients who are homozygous for the genetic biomarkers indicating poor metabolism of the recommended therapeutic agent may, depending on the therapeutic window of the drug, exhibit a response resembling an overdose to the normal drug dose and require a reduced dose to achieve therapeutic levels, whereas patients carrying the biomarkers indicating extensive metabolism may exhibit a lack of efficacy at normal doses and require an increased dose of the drug to achieve therapeutic activity. The patients who are homozygous or heterozygous for the normal (wild-type) genetic biomarker may require no alteration of the "normal" dose of the drug for adequate therapeutic outcome.

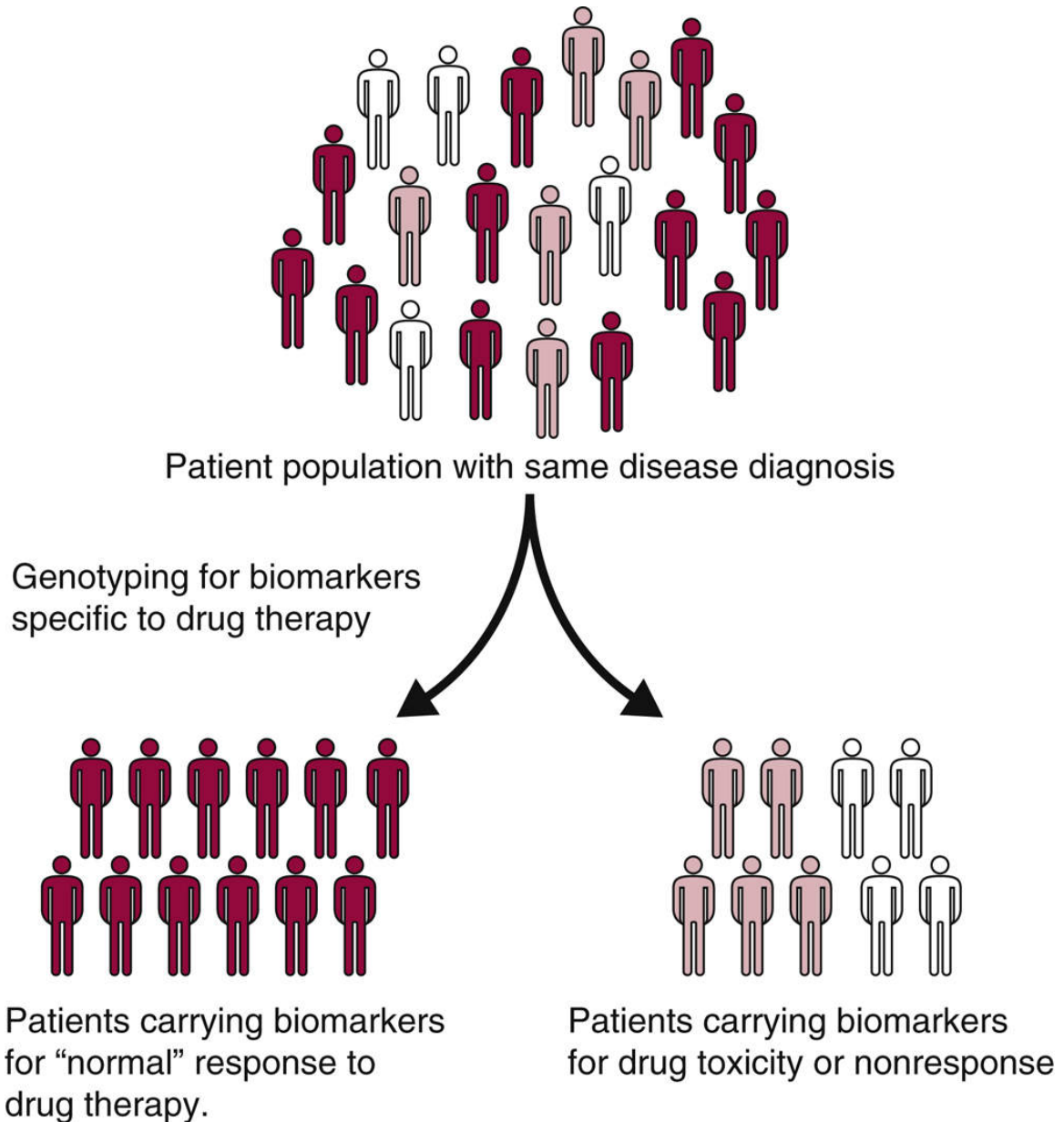


FIGURE 5.1 Manipulating therapeutic outcomes. Manipulating therapeutic outcomes through pharmacogenetic analysis of a population with the same disease diagnosis to eliminate patients with genetic biomarkers indicating an adverse response to a particular drug therapy.

The action of a drug on the body is a complex interaction involving the metabolism of drugs, the transport of drugs throughout the body, and the interaction of the drug with its target. The application of genetic variation to drug therapy centered on the variations seen in a number of drug metabolism enzymes, and much of the pharmacogenetic research has

developed with the phase I (oxidative) and phase II (conjugative) metabolic enzymes. Currently, considerable data relate SNPs in the coding and regulatory regions of these genes to poor metabolism of particular drugs that have the potential to result in drug overdoses in patients carrying the genetic variations and given the “normal” dose of drug. Transport proteins, such as the ATP-binding cassette (ABC) transporters, that move drugs through membrane barriers are critical in the absorption, distribution, and excretion of drugs and drug metabolites. Drug targets as well as the biochemical pathways following the drug-receptor interaction influence the drug response. Thus, the candidate genes for the therapeutic drug pathway include the genes for drug metabolism, drug transport, and drug response (Table 5.1).

TABLE 5.1
Specific Examples of the Application of Allelic Variation in Drug Therapy

Protein	Action	Drug	Therapy	Efficacy/toxicity
CYP450 2D6	Drug metabolism	Codeine	Pain	Efficacy
CYP450 2C9	Drug metabolism	Warfarin	Coagulation	Toxicity (hemorrhage)
Thiopurine S-methyltransferase	Drug metabolism	Mercaptopurine	Acute lymphoblastic leukemia	Toxicity (myelotoxic)
HER2/neu	Drug target	Herceptin	Breast cancer	Efficacy
BCR/ABL	Drug target	Gleevec	Chronic myelogenous leukemia	Efficacy
EGFR	Drug target	Erbitux	Colon cancer	Efficacy
Apolipoprotein E4	Marker	Tacrine	Alzheimer disease	Efficacy
Cholesteryl ester transferase	Marker	Statins	Atherosclerosis	Efficacy
ATP-binding cassette B1	Drug transport	Saquinavir	HIV	Efficacy
		Indinavir	Leukemia	
		Ritonavir		
		Daunorubicin		
		Etoposide		
UDP-glucuronosyltransferase 1A1	Drug metabolism	Irinotecan	Cancer	Toxicity (myelotoxic)

Protein	Action	Drug	Therapy	Efficacy/toxicity
N-Acetyltransferase 2	Drug metabolism	Isoniazid	Tuberculosis	Toxicity (hepatotoxic)
Pseudocholinesterase	Drug metabolism	Suxamethonium	Muscle relaxation during surgery	Toxicity (prolonged apnea)
CYP3A4/CYP2D6	Drug metabolism	Tamoxifen	Cancer	Efficacy
CYP2C19	Drug metabolism	Clopidogrel	Antithrombotic	Cardiovascular toxicity
CYP3A4/CYP2D6	Drug metabolism	Tolterodine	Antimuscarinic	Efficacy
Dihydropyrimidine dehydrogenase	Drug metabolism	5-Fluorouracil	Cancer	Toxicity
<i>BRAF</i> V600E	Drug target	Vemurafenib	Metastatic melanoma	Efficacy
<i>BRCA</i> -deficiency	Synthetic lethality	Olaparib, niraparib, rucaparib	<i>BRCA</i> -mutated cancers	Efficacy

ATP, adenosine triphosphate; *BRCA*, breast cancer–associated gene; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; HIV, human immunodeficiency virus.

Genomics

Genomics is the study of the full complement of genetic information, both coding and noncoding, in an organism's genome. The main genomics technique is the use of SNPs as biomarkers to inform clinicians about subtypes of disease that require differential treatments and provide pharmacists with information for selection of the best therapeutic methodology to effectively manage the disease as well as provide an indication of the patients at risk of experiencing adverse reactions or those who will not respond to a given drug dose. The general genotyping procedure for detecting known SNPs consists of polymerase chain reaction (PCR) amplification of the region of interest in the genome, discrimination of the alleles in that region, and detection of the discrimination products.

Transcriptomics

Transcriptomics is the technology behind the study of the full complement of messenger RNA (mRNA) transcripts in the cell's transcriptome and also

is known as expression profiling. Methods of expression profiling are either “open” or “closed.” Open systems do not require any advance knowledge of the sequence of the genome being examined; closed systems require some advance knowledge and usually involve the use of oligonucleotide or complementary DNA (cDNA) array hybridization technologies (the gene chip) and quantitative PCR ([Fig. 5.2](#)).

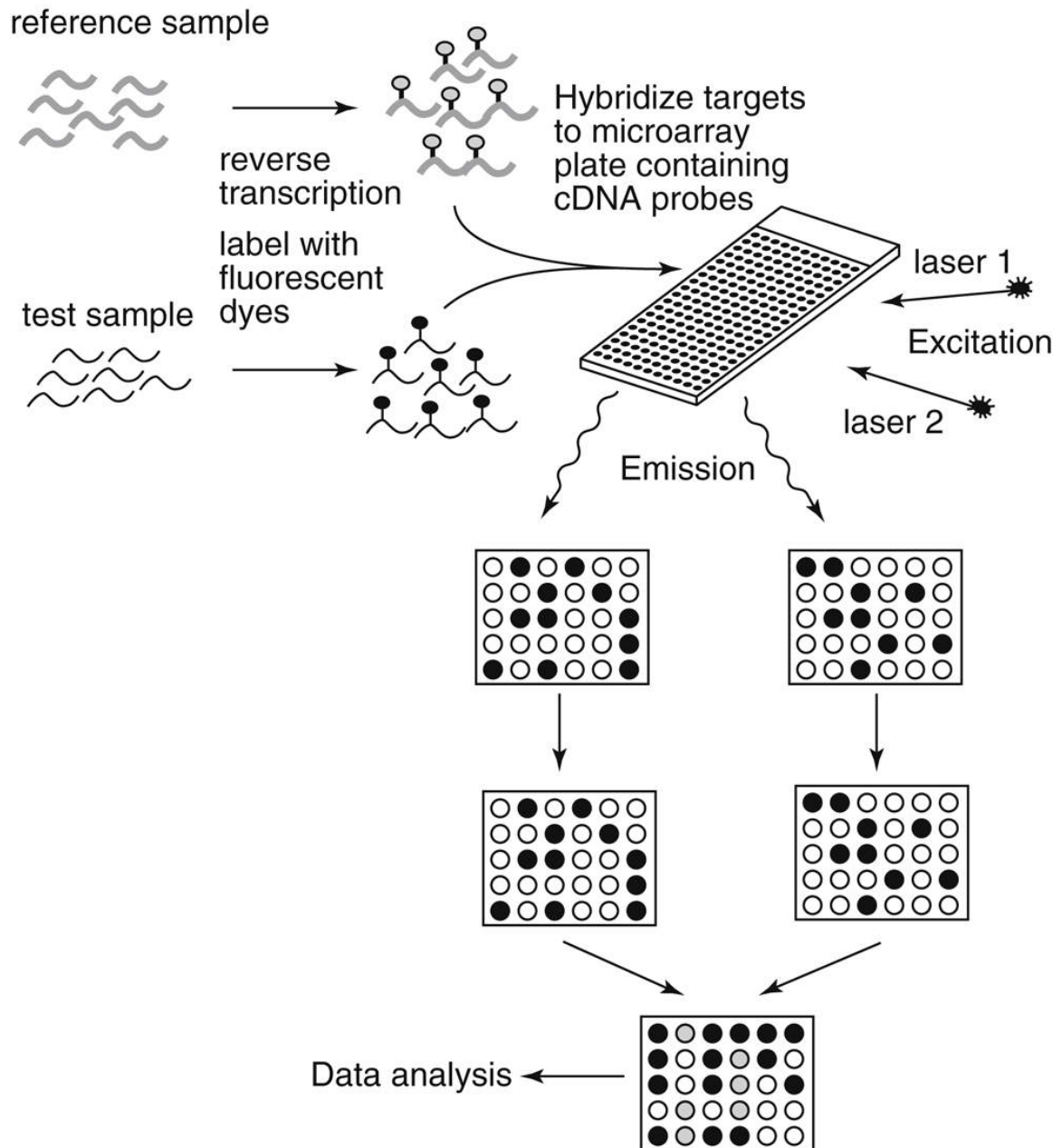


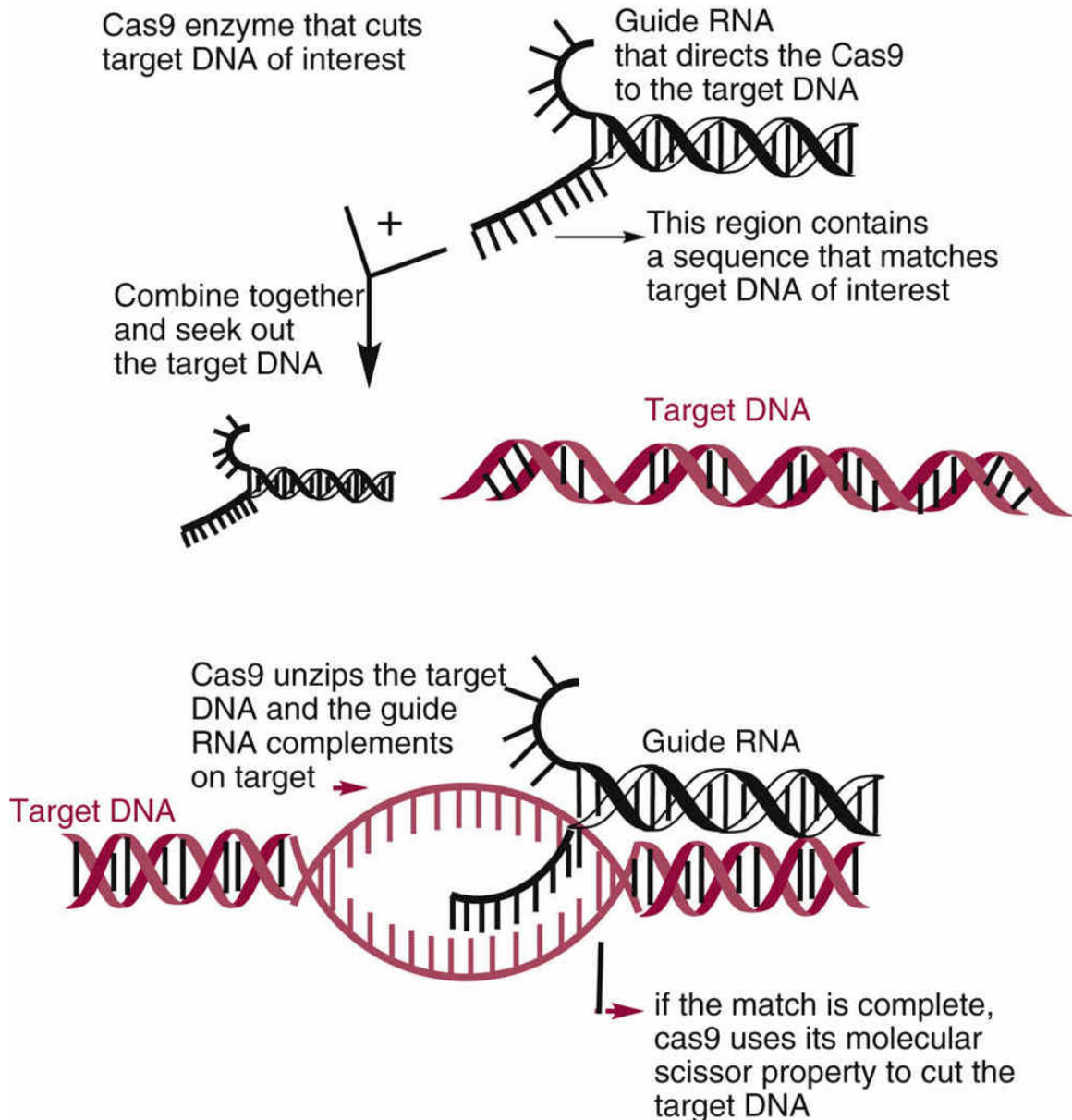
FIGURE 5.2 cDNA chip. Schematic representation of a cDNA microarray chip. Probes of interest are obtained from DNA clones and printed on coated glass microscope slides. Analysis of mRNA expression is carried out by taking total messenger RNA (mRNA) from both a test and control sample, fluorescently labeling the samples with either Cy3- or Cy5-dUTP during a single round of reverse transcription, pooling the fluorescent targets, and hybridizing to the immobilized probes on the array under very stringent conditions. Laser excitation of the incorporated targets yields an emission with a characteristic spectrum, which is measured using a scanning confocal laser microscope and analyzed using a computer program.

The cDNA arrays are prepared by spotting gene-specific PCR products, including full-length cDNAs, collections of partially sequenced cDNAs, or randomly chosen cDNAs from any library of interest onto a glass, silicon, gel, or bead matrix or a nylon or nitrocellulose membrane. The matrix (most often glass) is coated with polylysine, amino silanes, or amino-reactive silanes to assist in the attachment of the cDNA probes. The PCR products of the clones are purified and spotted onto the matrix by robots through contact printing or noncontact piezo or ink-jet devices. After cross-linking the probe to the matrix by ultraviolet irradiation, the probe is made single stranded by heat or alkali treatment. The mRNA target is prepared from both a test and reference sample by reverse transcription to produce cDNA, which is then labeled with fluorescent probes (one for the test and another for the reference). The fluorescent targets are pooled and hybridized to the probe array under very stringent conditions. Laser excitation of the hybridized samples on the matrix and comparison of the fluorescence intensity of the reference sample with the fluorescence intensity of the test sample using computer algorithms yield an emission characteristic of the increase or decrease of mRNA expression under test conditions.

CRISPR Technologies and Therapeutic Applications

Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein (Cas9) complex, a genome editing tool that holds promise for human gene therapy, was named as the “Breakthrough of the Year” by *Science* magazine in 2015.⁴ CRISPR technology is comprised of three components: (1) a guide RNA that targets a specific locus in the gene; (2) a Cas9 nuclease, which creates a DNA break at the locus; and (3) a DNA repair template to enable gene replacements and insertions.⁵ The use of this technology offers the ability to genetically manipulate the patient-derived stem cells to generate various disease models, including cystic fibrosis, Parkinson’s disease, cardiomyopathy, and ischemic heart disease. CRISPRs, together with Cas9 RNA-guided endonuclease proteins,

can be easily targeted to virtually any genomic location of choice by a short RNA guide. The guide sequence within these CRISPR RNAs typically corresponds to phage sequences, which constitute the natural mechanism by which CRISPR produces antiviral defense; however, it can be easily replaced by a sequence of interest to retarget the Cas9 nuclease. The principle behind CRISPR is illustrated in [Figure 5.3](#).⁶



Once DNA is cut, many molecular operations can be performed such as correct gene insertion for gene therapy as in sickle cell anemia, knockdown of gene to develop disease models for screening, transcriptional activation, and repression

FIGURE 5.3 CRISPR (clustered regularly interspaced short palindromic repeats) technology.

Some applications of CRISPR technologies include homology-directed repair (HDR), gene silencing, pooled genome-scale knockout screening, transient activation of endogenous genes (CRISPRa or CRISPRon), transient gene silencing or transcriptional repression (CRISPRi), DNA-free

CRISPR-cas9 gene editing, and embryonic stem cells and transgenic animals.⁶

CRISPR technology-based modified bacteriophages can be developed as a delivery vehicle for a CRISPR-cas9 gene editing system that targets and inactivates either virulence genes or the resistance genes themselves, leaving the rest of the microbiome intact. In another application, CRISPR can be used to target known bacterial resistance genes to deactivate them *in situ* and resensitize virulent bacteria, thus making existing antibiotics effective again.⁷

Although promising, CRISPR-based therapeutic development has a number of challenges that remain to be addressed. These include (1) a potential for the induction of off-target mutations at sites other than the intended target, (2) considerable safety concerns for CRISPR use in humans, (3) ethical concerns, (4) the fact that most diseases are not caused by single mutations, and (5) availability of safe carrier systems for CRISPR-cas9 delivery to human cells *in vivo*.

Proteomics

The array of proteins found within the cell, their interactions, and modifications hold the key to understanding biologic systems. The proteome is defined as a protein population of a cell characterized in terms of localization, posttranslational modification, interactions, and turnover, at any given time. The complexity of the proteome surpasses that of the genome. Proteomics is the technology behind the study of the total protein complement of a genome or the complete set of proteins expressed by a cell, tissue, or organism. The presence of an open reading frame in a DNA sequence indicates the presence of a gene, but it does not indicate gene transcription, RNA editing, translation, or posttranslational modification and the presence of isoforms. Analysis of the transcriptome does not indicate alteration of protein levels by proteolysis, recycling, and sequestration. Therefore, it is important to determine protein levels, protein expression, and protein-protein interactions directly.

Proteomics is divided into three main areas⁸:

1. Microcharacterization for large-scale identification of proteins and posttranslational modifications.⁸
2. Differential gel electrophoresis for comparison of protein levels.⁹
3. Protein-protein interaction studies using protein chips,¹⁰ mass spectrometry isotope-coded affinity tag technology,¹¹ and the yeast two-hybrid system.¹²

Metabolomics

Metabolomics is the technology behind the measurement of metabolite concentrations, fluxes, and secretions in cells and tissues (metabolome). Metabolomics is at the cross-roads of genotype/phenotype interactions, where the interrelationship of metabolic pathways is considered to be the fundamental component of an organism's phenotype. Metabolomics is distinguished by the fact that metabolites are not directly linked to the genetic code but actually are products of a concerted action of many enzyme networks and other regulatory proteins.^{13,14} Metabolites also are more complex than nucleic acids, which are composed of four nucleotides, or proteins, which in turn are composed of 20 different amino acids. As a result, metabolites cannot be sequenced like genes and proteins, and so their characterization requires that the structure of each metabolite be determined individually through description of elemental composition and stereochemical orientation of functional groups.

Metabolites are more than just products of enzyme-catalyzed reactions. They also are sensors and regulators of complex molecular interactions in the organism, and as a result, the composition of the metabolome can be altered by changes in the individual's environment. Therefore, a study of the metabolome is complicated not only by the uniqueness of the individual's genome but also by the uniqueness of the individual's environment. On the other side of the coin, however, one could consider the metabolome to be a very sensitive indicator of the individual's phenotype.^{15,16}

Metabolomics can be approached by using several different but related strategies:

1. Target analysis investigates the primary metabolic effect of a genetic variation, in which the analysis usually is limited to the substrate and/or product of the protein expressed by the altered gene.
2. Metabolic profiling limits the investigation to a number of predefined metabolites, usually in a specific metabolic pathway.
3. Metabolomics is a comprehensive analysis (both identification and quantification) of metabolites in a biologic system, which investigates the effect of multiple genetic variations on many different biochemical pathways.
4. Metabolic fingerprinting is a strategy to screen a large number of metabolites for biologic relevance in diagnostic or other analytical procedures in which it is important to rapidly distinguish between individuals in a population. Metabolic fingerprinting is the ultimate characterization of an individual's phenotype for disease diagnosis and drug therapy. Once the technologic problems of high-throughput metabolic analysis are developed, it will be a major competitor of SNP analysis in pharmacogenomics.

The analytical technologies used in metabolomic investigations are nuclear magnetic resonance and mass spectrometry alone or in combination with liquid or gas chromatographic separation of metabolites. Other techniques include thin-layer chromatography, Fourier transform infrared spectrometry, metabolite arrays, and Raman spectroscopy.

Pharmaceutical Biotechnology Methods

Pharmaceutical biotechnology is defined, at its most basic level, as the manipulation of nucleic acids in the production of therapeutic and diagnostic agents. In order to understand both DNA and RNA, the fundamental genetic material, it is important to understand the basic process that moves information from DNA to RNA. This process starts with transcription.

Transcription

The genetic information in DNA is transcribed to the intermediate RNA molecule that moves to the cytoplasm, where it directs the synthesis of the gene product it encodes using ribosomes. RNA differs chemically from DNA in that the deoxyribose sugar molecule in DNA is replaced by ribose and the thymine in DNA is replaced by uracil in RNA. Structurally, RNA contains both single-stranded runs and short, double helical regions.

There are several types of RNA molecules in the cell, three of which are highlighted here. Messenger RNA (mRNA) is transcribed from a particular DNA sequence. Transfer RNAs (tRNAs) are covalently linked to specific amino acids and carry the anticodon triplet that recognizes a complementary trinucleotide sequence of the mRNA that is specific for the amino acid that it carries. The ribosome contains both ribosomal RNA (rRNA) and proteins.

Several differences exist in the mechanism of transcription between prokaryotes and eukaryotes. These differences are important when evaluating if recombinant expression of a protein should be carried out in a eukaryotic or prokaryotic system. For our purposes, we describe transcription in a eukaryotic system.

Transcription is carried out by RNA polymerases, of which there are three types in eukaryotes. RNA pol I catalyzes the synthesis of rRNAs, RNA pol II is responsible for the synthesis of mRNA, and RNA pol III synthesizes tRNA. All three polymerases are large enzymes containing 12 or more subunits.

Each eukaryotic RNA polymerase copies DNA from the 3' end, thus catalyzing mRNA formation in the 5' → 3' direction and synthesizing RNA complementary to the antisense DNA template strand. The reaction requires the precursor nucleotides adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), and uridine triphosphate (UTP) and does not require a primer for initiation of transcription. The five stages of eukaryotic transcription include initiation, elongation and termination, capping, polyadenylation, and splicing.

Initiation

Eukaryotes have different RNA polymerase-binding promoter sequences than prokaryotes. The TATA consensus sequence of the eukaryotic promoter region is located 25-35 base pairs upstream from the transcription start site ([Fig. 5.4](#)). The low activity of basal promoters is greatly increased by the presence of upstream regulatory elements (UREs) located 40-200 base pairs upstream of the promoter sequence and includes the SP1 box, the CCAAT box, and the hormone response elements. Transcription from many eukaryotic promoters can be stimulated by control elements, called enhancers, located many thousands of base pairs away from the transcription start site.

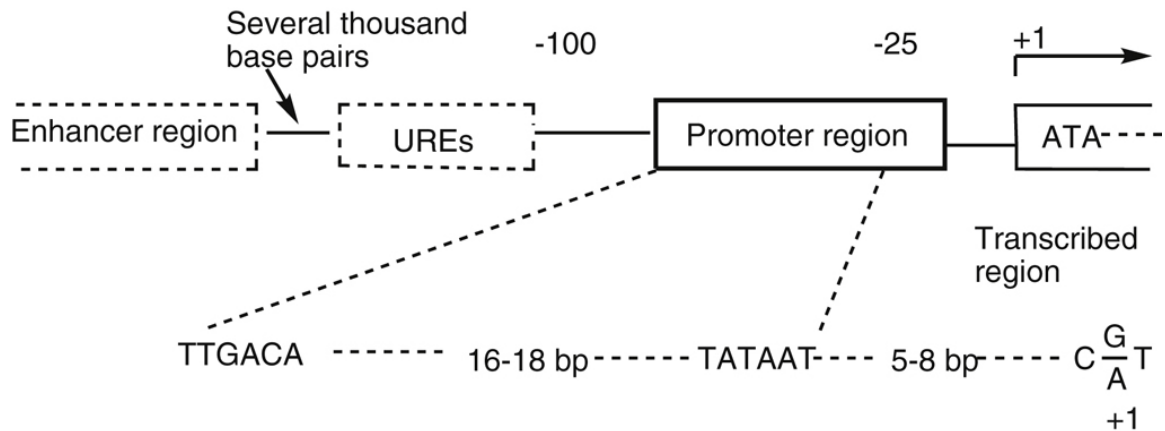


FIGURE 5.4 Schematic representation of the eukaryotic transcription unit showing the relationship between the promoter, upstream regulatory elements (UREs), and enhancer region. Note that transcription usually starts with a purine base in the 1 position.

Adapted from Turner PC, McLennan AG, Bates AD, White MRH. *Instant Notes in Molecular Biology*. New York: BIOS Scientific Publishers Ltd, Springer-Verlag; 1997; with permission.

Elongation and Termination

RNA polymerase moves along the DNA template until a terminator sequence is reached. The RNA molecule made from a protein-coding gene by RNA pol II in eukaryotes is called pre-mRNA. The pre-mRNA from a eukaryotic protein-coding gene is extensively processed within the nucleus before export to the cytoplasm.

Capping

At the end of polymerization, the 5' end of the pre-mRNA molecule is modified by addition of an N7-methyl guanine molecule (Fig. 5.5).

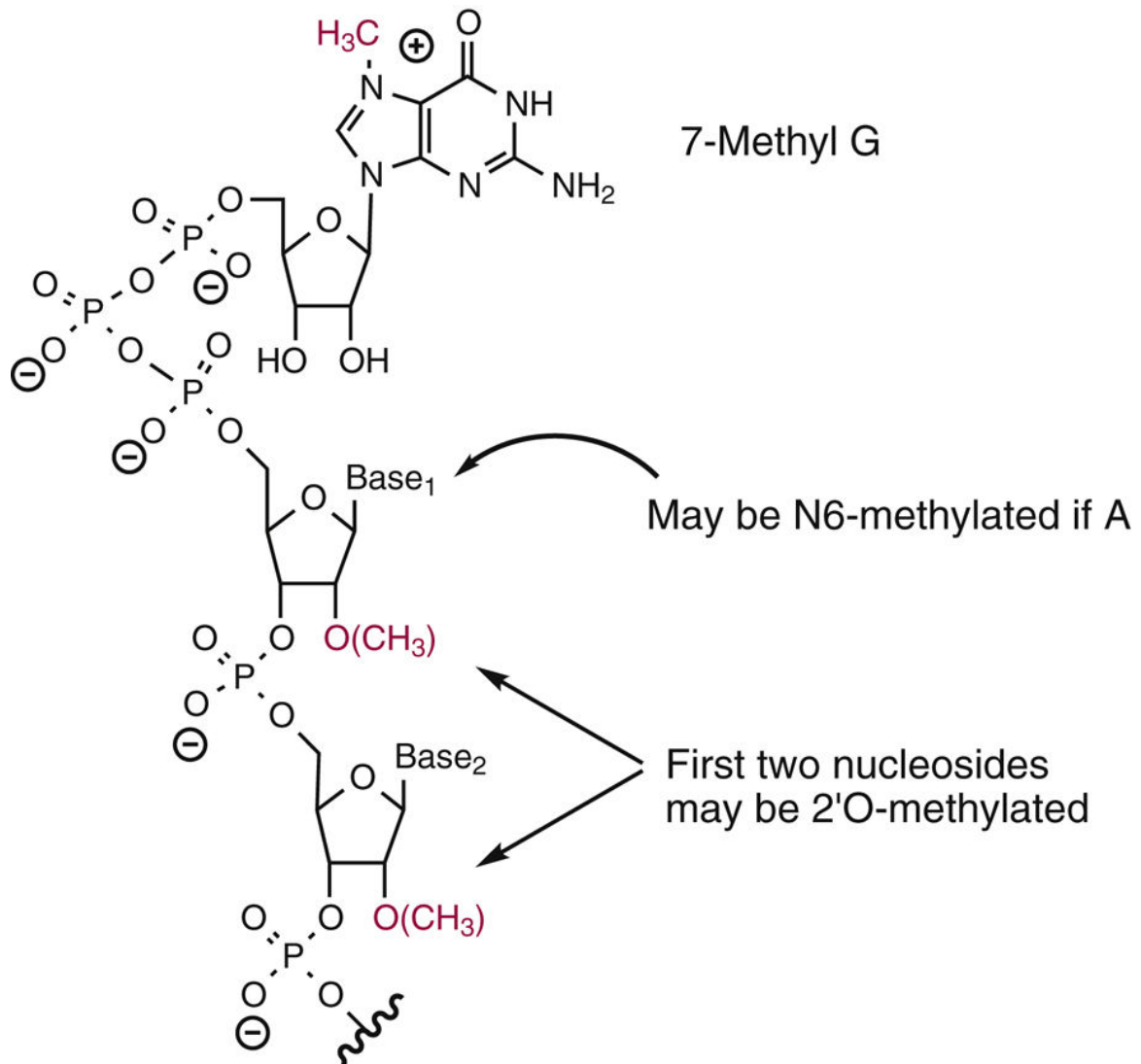


FIGURE 5.5 Chemical structure of the 5'-cap of mRNA (messenger RNA). Cap 0 consists of the 7-methylguanosine triphosphate attached to the 5' end of the mRNA. Cap 1 consists of the 7-methylguanosine and 2'O-methylation of the 5' base. Cap 2 consists of the 7-methylguanosine and the 2'O-methylation of the first two bases in the sequence.

Polyadenylation

The 3' end of the pre-mRNA is generated by nuclease catalyzed cleavage followed by the addition of a run, or tail, of 100-200 adenosine nucleotides, resulting in what is called the poly (A) tail. Cleavage and polyadenylation require specific sequences in the DNA and its pre-mRNA transcript that are part of the transcription termination signal. The poly (A) tail helps

stabilize the mRNA molecule, reducing its sensitivity to 3'-nuclease activity.

RNA Splicing

Splicing is the precise excision of the intron sequences and joining of exons to produce a functional mRNA molecule.

Translation

Once the fully processed mRNA has been transported from the nucleus to the cytoplasm, protein synthesis occurs. The triplet genetic code carried on the mRNA is translated into a protein sequence by the ribosome. Amino acids are delivered to the ribosome by tRNAs that carry specific amino acids attached to their 3'-terminus based on the tRNA anticodon sequence. The anticodon complements the triplet codon sequence in mRNA, and 61 triplet sequences code for the 20 amino acids (Table 5.2). Three codons are nonsense or stop codons that terminate translation. The code is degenerate with 18 of the 20 common amino acids coded for by more than one codon. Two amino acids, Met (AUG) and Trp (UGG), have one unique codon each. From a fixed start point on the mRNA (start codon, AUG), which establishes the open reading frame, each group of three bases in the coding region of the mRNA represents a codon that is recognized by a complementary triplet on the tRNA molecule.

TABLE 5.2
The Genetic Code

First Position	Second Position				Third Position
	U	C	A	G	
U	Phe UUU	Ser UCU	Tyr UAU	Cys UGU	U
	Phe UUC	Ser UCC	Tyr UAC	Cys UGC	C
	Leu UUA	Ser UCA	Stop UAA	Stop UGA	A
	Leu UUG	Ser UCG	Stop UAG	Trp UGG	G
C	Leu CUU	Pro CCU	His CAU	Arg CGU	U

First Position	Second Position				Third Position
	U	C	A	G	
	Leu CUC	Pro CCC	His CAC	Arg CGC	C
	Leu CUA	Pro CCA	Gln CAA	Arg CGA	A
	Leu CUG	Pro CCG	Gln CAG	Arg CGG	G
A	Ile AUU	Thr ACU	Asn AAU	Ser AGU	U
	Ile AUC	Thr ACC	Asn AAC	Ser AGC	C
	Ile AUA	Thr ACA	Lys AAA	Arg AGA	A
	Met AUG	Thr ACG	Lys AAG	Arg AGG	G
G	Val GUU	Ala GCU	Asp GAU	Gly GGU	U
	Val GUC	Ala GCC	Asp GAC	Gly GGC	C
	Val GUA	Ala GCA	Glu GAA	Gly GGA	A
	Val GUG	Ala GCG	Glu GAG	Gly GGG	G

There are four stages in the protein synthesis in both prokaryotes and eukaryotes:

1. *Initiation*—assembly of the ribosome on an mRNA molecule.
2. *Elongation*—repeated cycles of amino acid addition.
3. *Termination*—recognition of the stop codon, release of the new protein chain, and breakdown of the synthetic complex.
4. *Posttranslational modification*—usually includes protein cleavage by carboxy or aminopeptidases and chemical modification such as acetylation, sulfonylation, phosphorylation, hydroxylation, lipidation, and/or addition of polysaccharides.

Genes

A gene is the segment of genomic DNA (gDNA) involved in producing a polypeptide chain. The mRNA assembled from the gene includes regions

preceding (the leader or 5'-untranslated region) and following (the trailer or 3'-untranslated region) the coding region as well as intervening sequences such as introns that are removed in the processing of the pre-mRNA. With the discovery of other processes that contribute to the penultimate sequence of the mature mRNA, the definition of a gene is evolving.¹⁷

Cloning and the Preparation of DNA Libraries

Two discoveries in the early 1970s provided breakthroughs in nucleic acid chemistry: the discovery of bacterial enzymes capable of cleaving nucleic acids at specific, palindromic (symmetrical) base sequences (Figs. 5.6 and 5.7) and the discovery of bacterial plasmids as vehicles (vectors) to amplify and carry the gene fragments produced by those restriction enzymes. Plasmids are small, circular, extrachromosomal nucleic acid molecules in bacteria that replicate independently within the bacterial cell. Restriction enzymes (and now PCR) are used to produce relatively small DNA fragments that are inserted into bacterial plasmid vectors, forming recombinant DNA (rDNA) molecules. The rDNA vectors are inserted into bacterial hosts, where the plasmid replicates with the bacteria, producing a large number of identical rDNA molecules known as clones, thus completing the process known as DNA cloning.

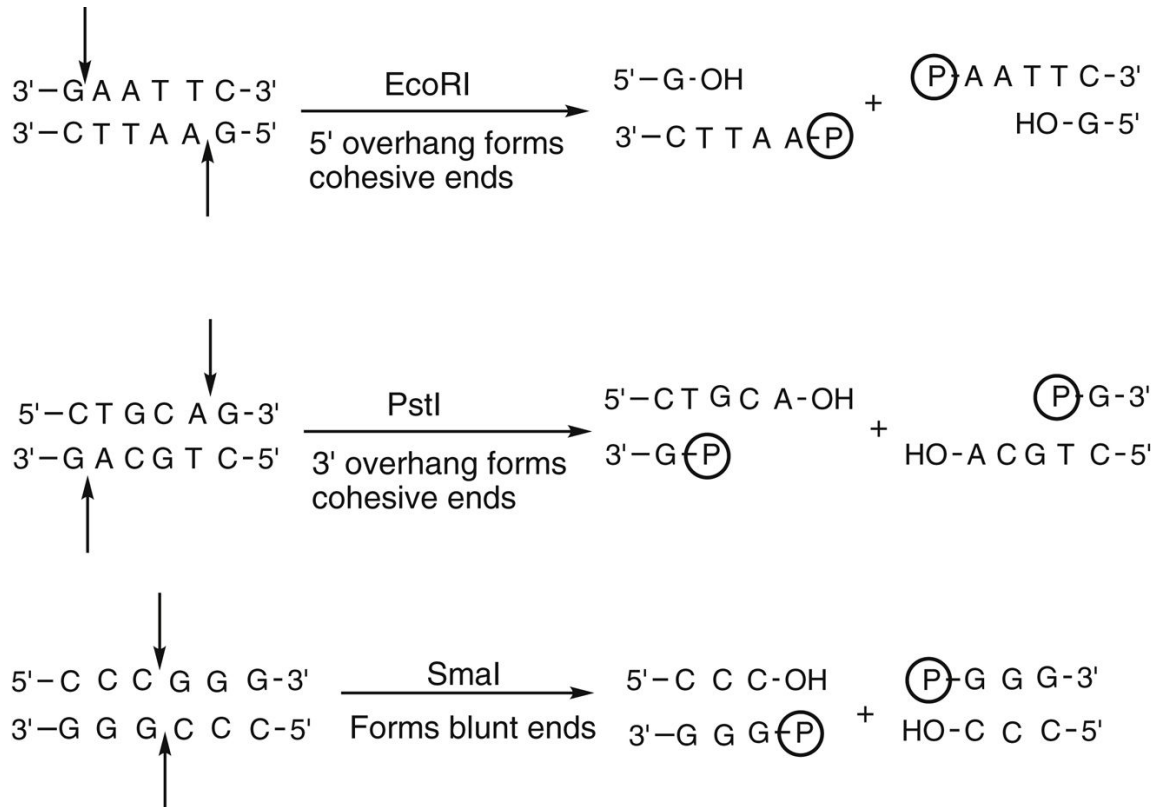


FIGURE 5.6 Actions of restriction endonucleases EcoRI, PstI, and SmaI at their recognition sequences. Note that EcoRI and PstI enzymes produce “sticky ends” with overlapping sequences, whereas SmaI produces “blunt ends” or nonoverlapping sequences.

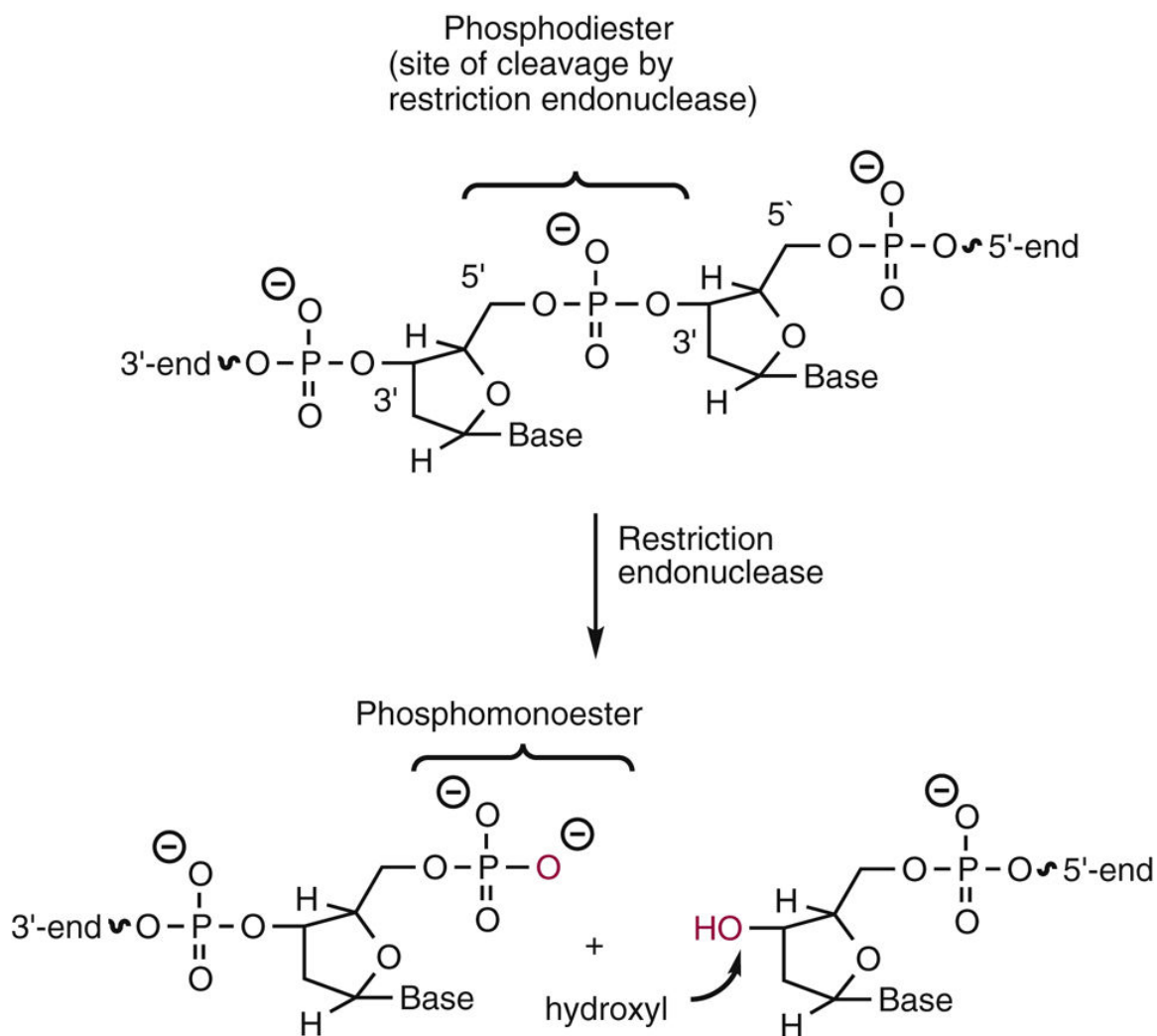


FIGURE 5.7 Cleavage by restriction endonuclease.

Sequencing DNA Fragments.

The two previous methods of nucleic acid sequencing, the Maxam and Gilbert method (chemical method)¹⁸ and Sanger's dideoxy method,¹⁹ have been superseded by the far faster massive parallel sequencing methods. In short, high-throughput sequencing involves breaking up gDNA into fragments and placing the individual fragments onto specially designed microbeads where the many copies of each fragment have been amplified. The amplified fragments are then loaded into the small wells of a substrate. As the wells are loaded with sample, reagents are pumped across the plate. The addition of the reagents results in an enzymatic reaction between complimentary bases in the DNA fragments, and a

fluorescent signal is released and read by a computational sequence analyzer.²⁰

Synthesizing Oligonucleotides.

The need for short oligonucleotides of known sequence has grown tremendously with the need for radiolabeled and fluorescently labeled probes to isolate and characterize nucleic acids. The phosphite triester and the phosphotriester methods are convenient solid-phase automated techniques for the synthesis of oligonucleotides ([Fig. 5.8](#)).²¹

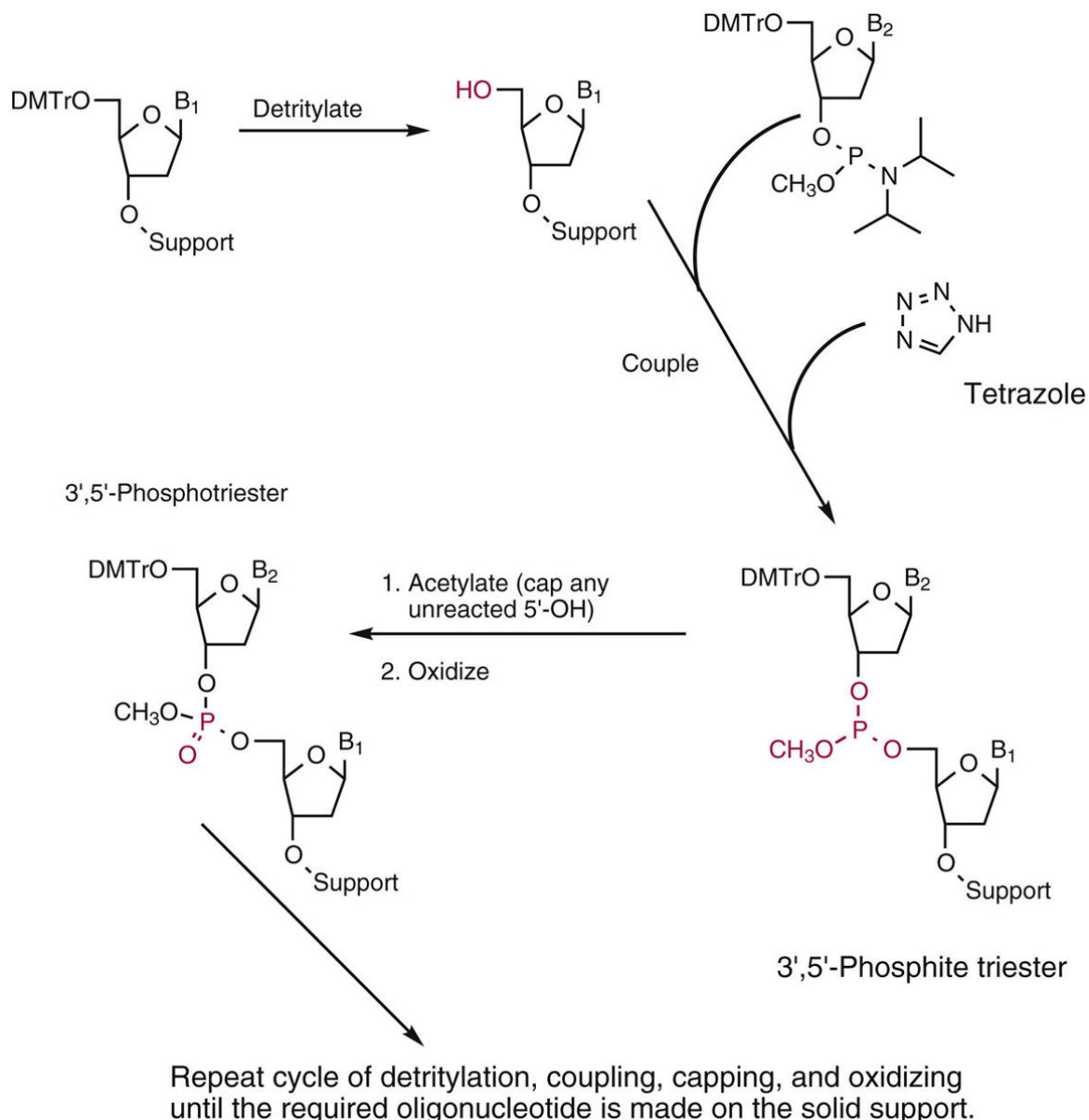


FIGURE 5.8 Solid-phase phosphate and phosphite triester method of oligodeoxyribonucleotide synthesis. DMTr, 4,4-dimethoxytrityl

Polymerase Chain Reaction (PCR).

Working with small quantities of nucleic acids isolated from cell and tissue sources is difficult, and there is often a need to amplify these sequences. PCR is used to amplify fragments of DNA without the need for cloning. The process can amplify samples that contain as little as a single nucleotide fragment used as template. There is a requirement for the

sequences flanking the boundaries of the fragment to be amplified so that oligonucleotide primers can be prepared.²²

Protein Synthesis Through Recombinant DNA.

Once the gene coding for the desired protein has been identified and isolated (Fig. 5.9), the genetic material is introduced into cells on a vector capable of DNA replication and initiation of transcription. A cloning vector is a carrier molecule, the vehicle that is used to insert foreign DNA into a host cell. Typically, vectors are genetic elements that can be replicated in a host cell separately from that cell's chromosomes. Bacterial plasmids are circular DNA of only a few thousand base pairs that replicate freely within the cells and are ideal for carrying the gene into the host organism. DNA fragments coding for the desired protein can be cloned from gDNA or cDNA and inserted into the vector that carries the code to synthesize the protein in the host.

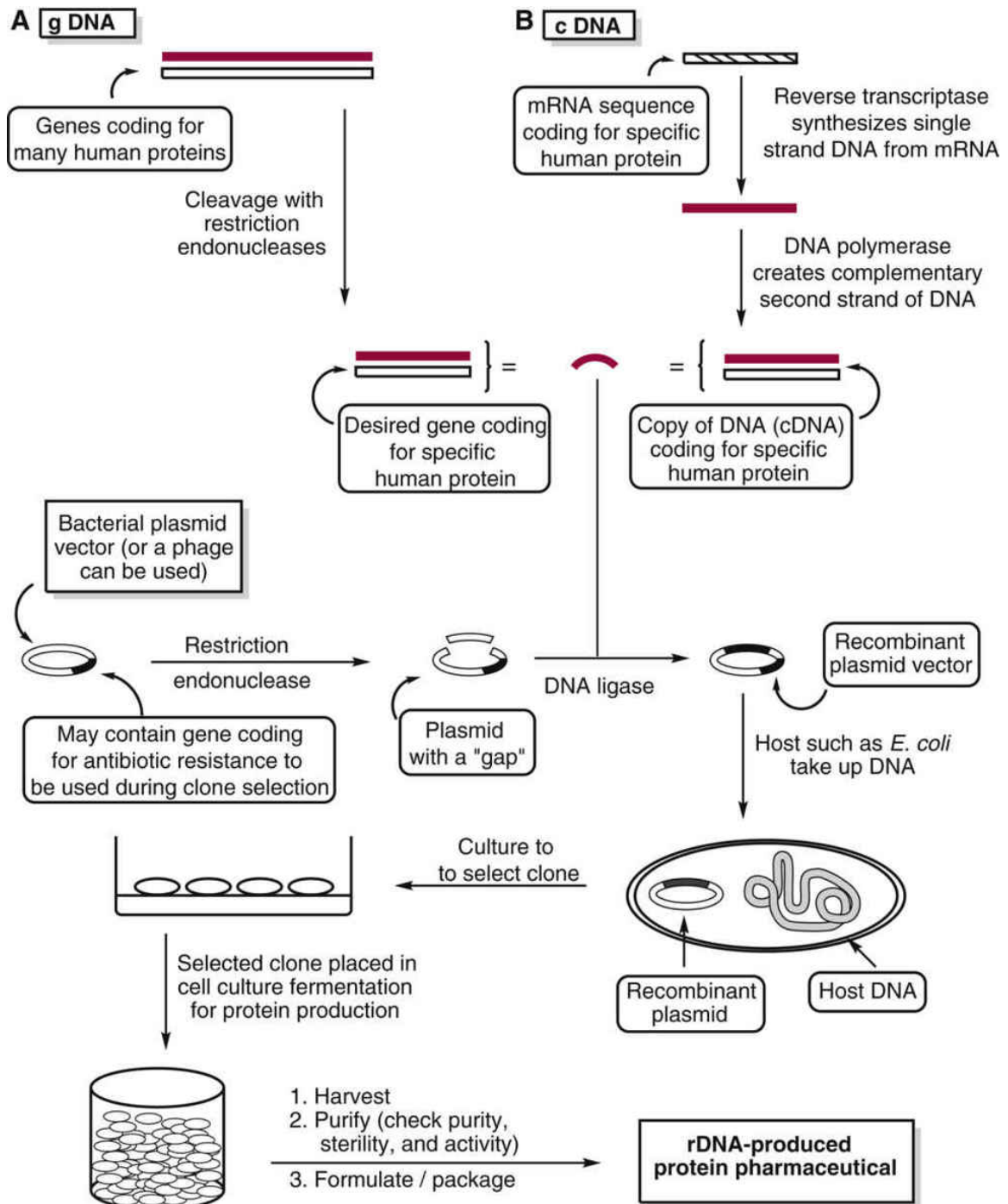


FIGURE 5.9 Summary of typical rDNA production of a protein from either (A) genomic DNA or (B) cDNA.

The vector containing the code for the target protein is then inserted into the host. Host cells are typically bacteria (e.g., *Escherichia coli*), eukaryotic yeast (e.g., *Saccharomyces cerevisiae* [baker's yeast]), or mammalian cell lines. The choice of host system is influenced primarily by the type of

protein to be expressed and by the key differences among the various host cells. Overall protein yields generally are much lower in mammalian cells, but in some cases, this may be the only system that produces the specific protein of interest. It should be noted that recombinant proteins produced in gram-negative bacteria may contain endotoxins.

The host cells containing the vector are grown under selection to identify a clone that contains the desired gene and is able to produce the best protein. The selected cloned cells are used as inoculum first for a small-scale cell culture/fermentation, which is then followed by larger fermentations in bioreactors. The medium is carefully controlled to enhance cell reproduction and protein synthesis. The host produces its natural proteins along with the desired protein, which may be secreted into the growth medium. The protein of interest can then be isolated from the fermentation, purified, and formulated to give a potential rDNA-produced pharmaceutical.

Protein Isolation and Purification.

The isolation and purification of the final protein product from the complex mixture of cells, cellular debris, medium nutrients, and other host metabolites is a challenging task. The structure, purity, potency, and stability of the recombinant protein must be considered. Often, sophisticated filtrations, phase separations, precipitation, and complex multiple-column chromatographic procedures are required to obtain the desired protein. Although isolation of the recombinant protein, produced in culture in relatively large amounts, generally is easier than isolating the native protein, ensuring the stability and retention of the bioactive three-dimensional structure (correct protein folding) of any biopharmaceutical is a more arduous task. In addition, recombinant proteins from bacterial hosts require removal of endotoxins, whereas viral particles may need to be removed from mammalian cell culture products. A discussion of these techniques is beyond the scope of this chapter; however, useful reviews on the analysis and chromatography²³ of biotechnology products are available as a resource for further information.

General Properties of Biotechnology-Produced Medicinal Agents

rDNA and hybridoma technologies have made it possible to produce large quantities of highly pure, therapeutically useful proteins. The rDNA-derived proteins and monoclonal antibodies (MAbs) are not dissimilar to the other protein pharmaceuticals or biopharmaceuticals that pharmacists have dispensed in the past. As polymers of amino acids joined by peptide bonds, the properties of these proteins differ generally from those of small organic molecule pharmaceuticals.

Stability of Biotech Pharmaceuticals

The instability of proteins, including protein pharmaceuticals, can be separated into three distinct classes. Chemical instability results from bond formation or cleavage yielding a modification of the protein and a new chemical entity. Photoinstability of protein drugs upon exposure to light results in chemical and physical instability. Physical instability involves a change to the secondary or higher order structure of the protein rather than a covalent bond-breaking modification.²⁴⁻²⁶

Chemical Instability

A variety of reactions give rise to the chemical instability of proteins, including hydrolysis, oxidation, racemization, β -elimination, and disulfide exchange (Fig. 5.10). Each of these changes may cause a loss of biologic activity. Proteolytic hydrolysis of peptide bonds results in fragmentation of the protein chain. It is well established that in dilute acids, aspartic acid residues in proteins are hydrolyzed at a rate at least 100-fold faster than that of other peptide bonds because of the mechanism of the reaction. An additional hydrolysis reaction is the deamidation of the neutral residue of asparagine and glutamine side chain linkages, forming the ionizable carboxylic acid residues aspartic acid and glutamic acid (Fig. 5.10A). This conversion may be considered primary sequence isomerization.²⁶

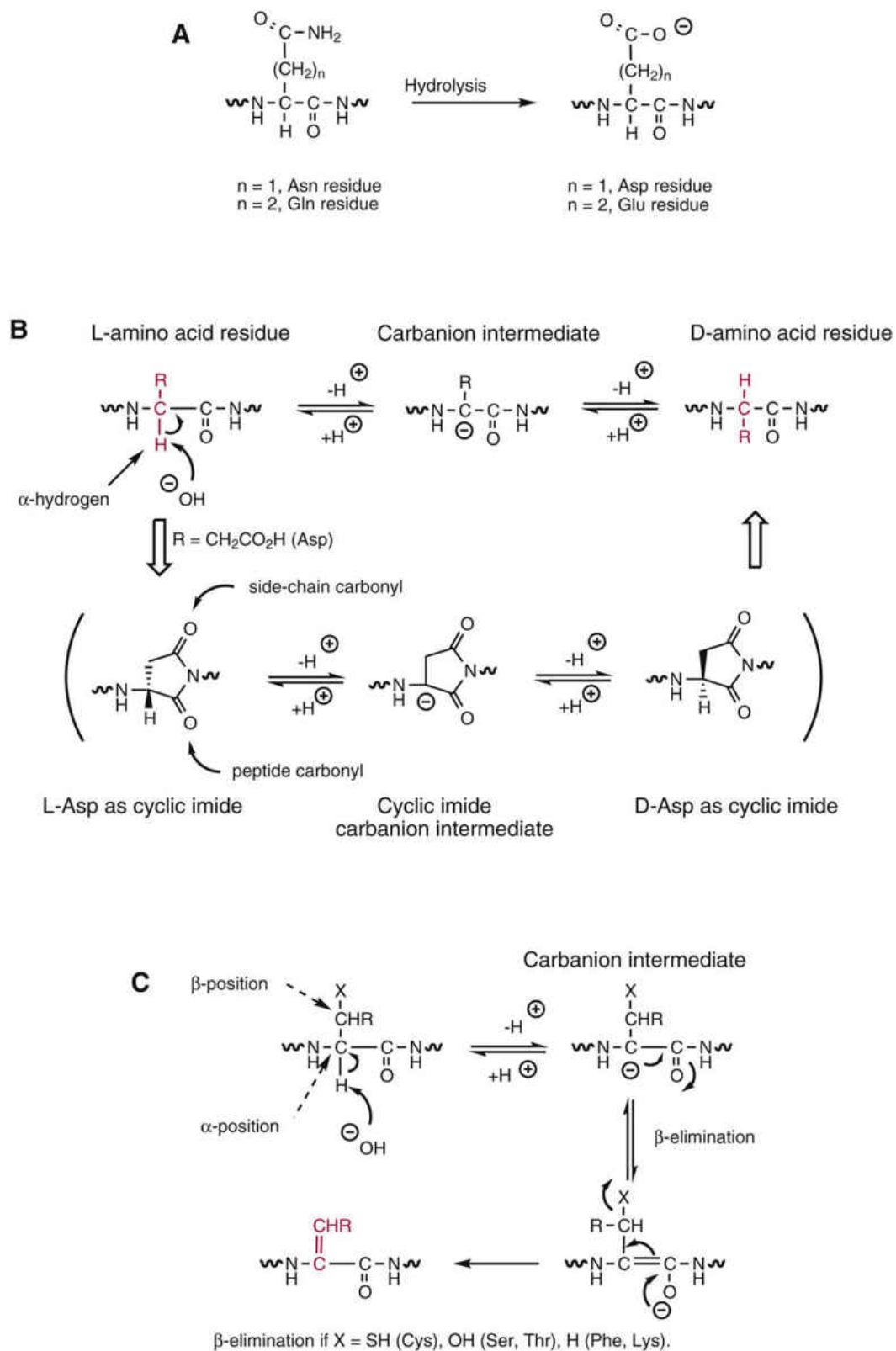


FIGURE 5.10 Chemical instability of protein biopharmaceuticals. (A) Hydrolysis, (B) base-catalyzed racemization, and (C) β-elimination.

Oxidative degradative reactions can occur to the side chains of sulfur-containing methionine and cysteine residues and the aromatic amino acid residues histidine, tryptophan, and tyrosine in proteins during their isolation and storage. The weakly nucleophilic thioether group of methionine (R-S-CH_3) can be oxidized at low pH by hydrogen peroxide as well as by oxygen in the air to the sulfoxide (R-SO-CH_3) and the sulfone ($\text{R-SO}_2\text{-CH}_3$). The thiol (sulfhydryl, R-SH) group of cysteine can be successively oxidized to the corresponding sulfenic acid (R-SOH), disulfide (R-SS-R), sulfinic acid ($\text{R-SO}_2\text{H}$), and, finally, sulfonic acid ($\text{R-SO}_3\text{H}$). A number of factors, including pH, influence the rate of this oxidation. Oxidation of histidine, tryptophan, and tyrosine residues is believed to occur with a variety of oxidizing agents, resulting in the cleavage of the aromatic rings.

Base-catalyzed racemization reactions may occur in any of the amino acids except achiral glycine to yield residues in proteins with mixtures of l- and d-configurations. The α -methine hydrogen is removed to form a carbanion intermediate (Fig. 5.10B). The degree of stabilization of this intermediate controls the rate of this reaction. Racemization generally alters the proteins' physicochemical properties and biologic activity. Also, racemization generates nonmetabolizable d-configuration forms of the amino acids. Aspartate residues in proteins racemize at a 10^5 -fold faster rate than when free, in contrast to the 2- to 4-fold increase for the other residues. The facilitated rate of racemization for aspartic acid residues is believed to result from the formation of a stabilized cyclic imide.

Proteins containing cysteine, serine, threonine, phenylalanine, and lysine are prone to β -elimination reactions under alkaline conditions (Fig. 5.10C). The reaction proceeds through the same carbanion intermediate as racemization. The reaction is influenced by a number of additional factors, including temperature and the presence of metal ions.

The interrelationships of disulfide bonds and free sulfhydryl groups in proteins are important factors influencing the chemical and biologic properties of protein pharmaceuticals. Disulfide exchange can result in incorrect pairings and major changes in the higher order structure

(secondary and above) of proteins. The exchange may occur in neutral, acidic, and alkaline media.

Photoinstability

The exposure of proteins to light and the ensuing chemical and physical degradation have been studied extensively for many years.²⁶ The peptide backbone, tryptophan, tyrosine, phenylalanine, and cysteine are the primary targets of photodegradation in proteins. Primary or type I photodegradation begins with absorption of light, resulting in excitation of an electron to higher energy singlet states. Absorption occurs through either the peptide backbone or by the amino acid side chains of tryptophan, tyrosine, phenylalanine, and cysteine. In aqueous solution, the absorption wavelengths are 180-230 nm for the peptide backbone, 280-305 nm for tryptophan, 260-290 nm for tyrosine, 240-270 nm for phenylalanine, and 250-300 nm for cysteine. Although tryptophan is present in relatively low abundance in proteins, it has the highest molar absorption coefficients and is therefore a major player in the photodegradation of protein drugs.

The major photolytic pathways of tryptophan in proteins are shown in [Figure 5.11](#). Following absorption of light, the excited state tryptophan will relax to the lowest energy singlet state followed by fluorescence, undergo intersystem crossing to the triplet state (³tryptophan), or eject an electron with formation of a tryptophan radical cation that will rapidly deprotonate to form the neutral tryptophan radical. The tryptophan radical may extract a hydrogen from a nearby tyrosine, repairing the tryptophan and forming a tyrosine phenoxy radical; react with oxygen, if present, forming a peroxy radical on the Trp; or react with nearby amino acids.

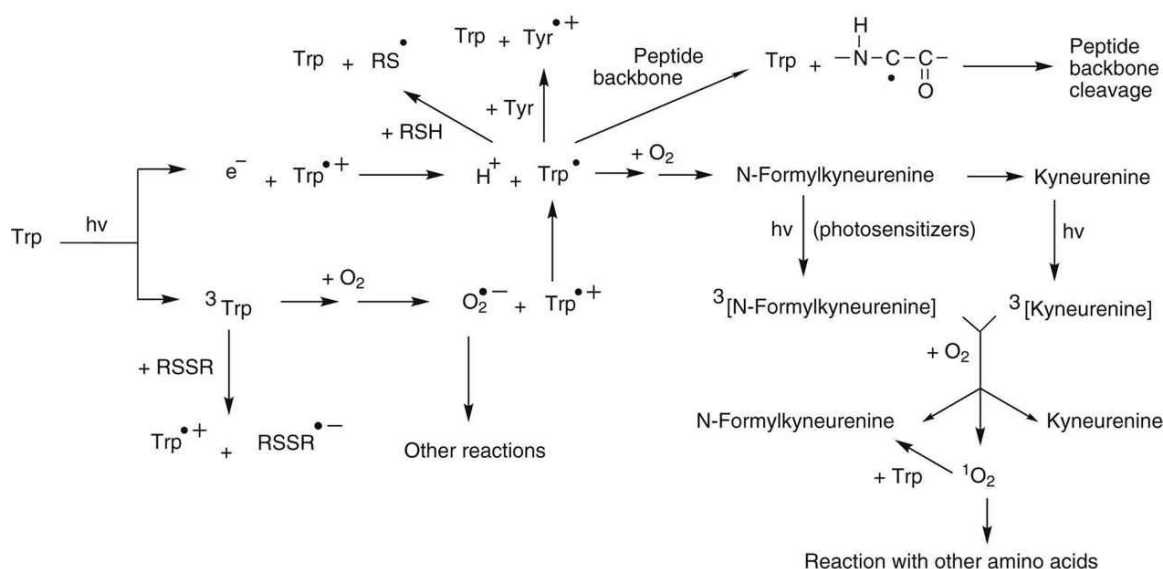
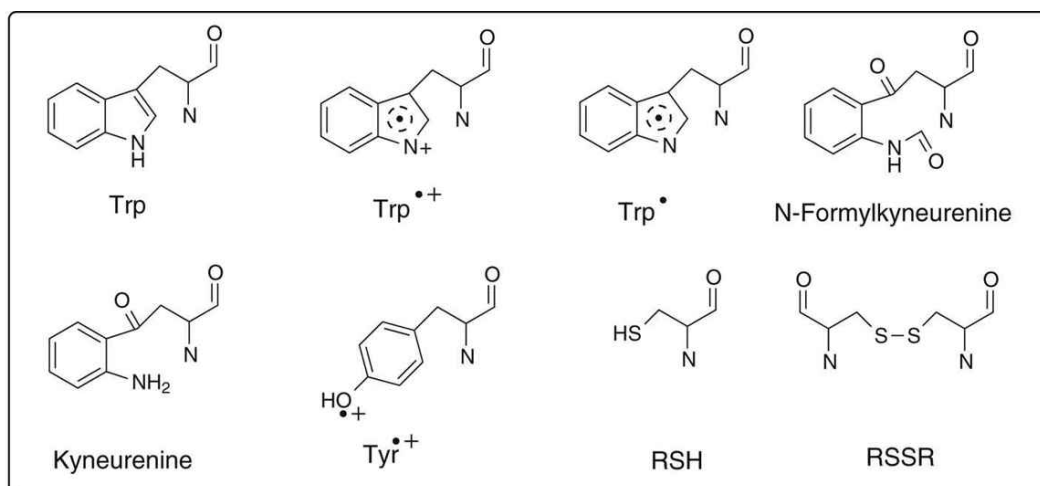


FIGURE 5.11 Structure of tryptophan (Trp), tyrosine (Tyr), and common photolytic pathways.

The ejected electron can become solvated forming an e^-_{aq} or migrate along the peptide backbone and react with cystine residues, forming a disulfide radical anion as discussed below. Under anaerobic conditions, the tryptophan triplet state generally either relaxes back to the ground state with formation of light at 420-500 nm or electron transfers to a nearby cystine with subsequent reactions as discussed earlier. In the presence of oxygen, formation of the tryptophan-based peroxy radical can undergo further reaction to produce N-formylkyneurenine and kyneurenine. Interestingly, the N-formylkyneurenine and kyneurenine absorb light at longer wavelengths than Trp, thereby acting as photosensitizers to visible

light, causing additional damage to the protein. Likewise, photodegradation of tyrosine, phenylalanine, cysteine, and histidine residues in proteins can occur.

Changes in the primary structure of the protein can result in changes in the secondary and tertiary structures, impacting long-term stability, bioactivity, and immunogenicity of the peptide and protein drugs. Complete protection of the proteins from light is the only method to prevent photodegradation. This is achieved with primary and secondary packaging containers such as cardboard boxes that block incoming light to the protein. Addition of excipients such as methionine is also used to further reduce the protein aggregation as in the case of darbepoetin alfa formulation. Methionine is known to react with peroxide to form methionine sulfoxide and likely reduces protein aggregation through its effect as a peroxide scavenger. Because photodegradation reactions occur through generation of singlet oxygen species, which are generated during the photolytic process by reaction of molecular dioxygen, some biopharmaceutical products, both liquid and lyophilized, are packaged in inert atmospheres.

Physical Instability

Generally not encountered in most small organic molecules, physical instability is a consequence of the polymeric nature of proteins. Proteins adopt secondary, tertiary, and quaternary structures, which influence their three-dimensional shape and, therefore, their biologic activity. Any change to the higher order structure of a protein may alter both. Physical instability includes denaturation, adsorption to surfaces, and noncovalent self-aggregation (soluble and precipitation). The most widely studied aspect of protein instability is denaturation. Noncovalent aggregation, however, is one of the primary mechanisms of protein degradation.²⁷

A protein, in principle, can be folded into a virtually infinite number of conformations. Denaturation occurs by disrupting the weaker noncovalent interactions that hold a protein together in its secondary and tertiary structures. Temperature, pH, and the addition of organic solvents and solutes may cause denaturation. The process can be reversible or irreversible. In general, denaturation affects the protein by decreasing

aqueous solubility, altering the three-dimensional molecular shape, increasing susceptibility to enzymatic hydrolysis, and causing the loss of the native protein's biologic activity.

Handling and Storage of Biotechnology-Produced Products

The preparation and administration of protein drugs of synthetic, recombinant, or hybridoma origin are dissimilar to the nonprotein pharmaceuticals. Proteins generally have more limited shelf stability. The average shelf life for a biotechnology product is 12-18 months, compared with more than 36 months for a low-molecular-weight drug. Although each individual biotechnology drug may be different, several generalizations can be made.

Proper storage of the lyophilized and the reconstituted drug is essential. Most of these drugs are expensive, so special care must be taken not to inactivate the therapeutic protein during storage and handling. The human proteins have limited chemical and physical stability, which is shortened on reconstitution. Expiration dating ranges from 2 hours to 30 days. The self-association of either native state or misfolded protein subunits may readily occur under certain conditions. This can lead to aggregation and precipitation and results in a loss of biologic activity. Self-association mechanisms depend on the conditions of formulation and may occur as a result of hydrophobic interactions.

Many of the biotechnology-produced drugs are stored refrigerated, but not frozen (2-8°C). In general, temperature extremes must be avoided. One example is the rDNA-produced, blood clot-dissolving drug alteplase. A recombinant version of a naturally occurring human tissue-type plasminogen activator, lyophilized alteplase is stable at room temperature for several years if protected from light. Freezing or exposure to excessive heat decreases the physical stability of the protein. Anything that causes denaturation or self-aggregation, even though labile peptide bonds are not broken, may inactivate the protein. Some pharmacy facilities may need to increase cold storage capacity to accommodate biotech storage needs. If the patient must travel any distance home after receiving the medication, the pharmacist should help package the biotechnology product according

to the manufacturer's directions. This may mean supplying a reusable cooler for the patient's use. Because the protein drug should not be frozen, the cooler should contain an ice pack rather than dry ice.

Some rDNA-derived pharmaceuticals, particularly the cytokines (e.g., the interferons, interleukin-2, and colony-stimulating factors), require human serum albumin in their formulation to prevent adhesion of the protein drug to the glass surface of the vial, which results in loss of protein. The amount of human serum albumin added varies with the biotech product. The vials should not be shaken to prevent foaming of the albumin, which causes protein loss or inactivation of the biotechnology-derived proteins. Care must be exercised in reconstituting protein pharmaceuticals. The diluent used for reconstitution of biotechnology drugs varies with the product and is specified by the manufacturer. Diluents can include normal saline, bacteriostatic water, and 5% dextrose. Several reviews of biotechnology drugs written for pharmacists contain additional information on the subjects of handling and storage.

Pharmacokinetic Considerations of Biotechnology-Produced Proteins

The processes of absorption into, distribution within, metabolism by, and excretion from the body (i.e., ADME) of biotechnology-produced pharmaceuticals are important factors affecting the time course of their pharmacologic effect. To deliver quality pharmaceutical care with biotech products, a pharmacist must be able to apply pharmacokinetic principles to establish and maintain a nontoxic, therapeutic effect. The pharmacokinetics of protein and peptide drugs differs in some pharmacokinetic aspects from those of the small molecule organic agents with which we are most familiar. Although a lengthy discussion of this topic is beyond the scope of this chapter, a brief overview of metabolism follows. Useful reviews are also available for further information.^{28,29}

The plasma half-life of most administered proteins and peptides is relatively short because they are susceptible to a wide variety of metabolic reactions. The enzymes involved in peptide bond hydrolysis and, thus, the degradation of peptides and proteins are known as peptidases and can be

found in the blood, in the vascular bed, in the interstitial fluid, on cell membranes, and within cells. These enzymes include carboxypeptidases (cleaves C-terminal residues), dipeptidyl carboxypeptidases (cleaves dipeptides from the C-terminus), aminopeptidases (cleaves N-terminal residues), and amidases (cleaves internal peptide bonds). For the most part, these enzymes are all specific for the natural amino acids of the L-configuration. Overall, the metabolic products of most proteins and peptides are not considered to be a safety issue. They generally are broken down into amino acids and reincorporated into new, endogenously biosynthesized proteins.

Metabolic oxidation reactions may occur to the side chains of sulfur-containing residues, similar to that observed for in vitro chemical instability. Methionine can be oxidized to the sulfoxide, whereas metabolic oxidation of cysteine residues forms a disulfide. Metabolic reductive cleavage of disulfide bridges in proteins may occur, yielding free sulfhydryl groups.

Biotechnology Drug Delivery

Protein-based pharmaceuticals, whether produced by biotechnology or isolated from traditional sources, present challenges to drug delivery because of the unique demands imposed by their physicochemical and biologic properties. Although a detailed discussion of this topic is beyond the scope of this chapter, a brief overview follows. Useful reviews also are available for further information.³⁰⁻³²

Delivery of high-molecular-weight, biotechnology-produced drugs into the body is difficult because of the poor absorption of these drugs, the acid lability of peptide bonds, and the rapid enzymatic degradation of these drugs in the body. In addition, protein pharmaceuticals are susceptible to physical instability, complex feedback control mechanisms, and peculiar dose-response relationships.

Given the limitations of today's technology, the strongly acidic environment of the stomach, the peptidases in the gastrointestinal tract, and the barrier to absorption presented by gastrointestinal mucosal cells preclude successful oral administration of most protein and peptide drugs.

Therefore, administration of all the biotechnology-produced protein drugs and some natural or synthetic peptides currently is parenteral (by intravenous, subcutaneous, or intramuscular injections) to provide a better therapeutic profile. Manufacturers supply most of these drugs as sterile solutions without a preservative. In such cases, it is recommended that only one dose be prepared from each vial to prevent bacterial contamination.

Novel solutions to overcome delivery problems associated with biotechnology protein products and natural or synthetic peptide drugs are being explored. Oral drug delivery approaches in development for proteins and peptide drugs include conjugated systems (e.g., PEGylation with polyethylene glycol), amino acid substitution, liposomes, microspheres, erythrocytes as carriers, and viruses as drug carriers.

PEGylation is frequently used to improve the pharmacokinetic properties of biotechnology-produced drugs.³³ This method involves the attachment of a flexible strand or strands of polyethylene glycol (PEG) to a protein. PEG is a simple, water-soluble, nontoxic polymer that is nonimmunogenic, is readily cleared from the body, and has been approved for human administration by mouth, injection, and topical application. The most common process used for PEGylation of protein is to activate the PEG with functional groups suitable for reaction with lysine and N-terminal amino groups. PEGylated proteins are less rapidly broken down by the body's enzymes than are the unmodified proteins. PEG can extend the duration of action of proteins and peptides in the body from minutes, to hours, to days, depending on the PEG molecule used. By increasing the biologic half-life and improving the efficacy of proteins in the body, these modifications can reduce the frequency of injections a patient requires. They also reduce the rapidity and intensity of the body's immune reaction against molecules such as interferon. Several PEGylated protein products (e.g., pegvisomant, certolizumab pegol, peginterferon $\alpha 2a$) are currently marketed, and many are under development.

When metabolism studies indicate a predominant cleavage site, attempts can be made to replace that residue with another that retains the receptor-binding activity of the protein/peptide drug while yielding enhanced

resistance to peptidase activity. Often, this can be accomplished by replacing the offending L-residue with its enantiomer, the D-amino acid, or another D-residue. Many peptidases are unable to cleave peptide bonds consisting of a “fraudulent” D-amino acid, and peptides containing such changes are designed as a means to increase their half-life. Also, the replacement of an L-amino acid with L-proline or N-methylation of the amide nitrogen offers the possibility of generating a peptide that is more resistant to enzymatic hydrolysis. The introduction of pseudopeptide bonds³⁴ and the design of retro-inverso peptides³⁵ are two examples of strategies that can afford peptidase resistance to peptide drugs.

Adverse Effects

An important consideration in the pharmaceutical care of a patient being administered a biotechnology-produced medicinal agent is the potential for adverse reactions. Many of the protein drugs are biotechnology-derived versions of endogenous human proteins normally present, on stimulus, in minute quantities near their specific site of action. Therefore, the same protein administered in much larger quantities may cause adverse effects not commonly observed at normal physiologic concentrations. Careful monitoring of patients administered biotechnology-produced drugs is critical for the health care team.

Immunogenicity

The immune system may respond to an antigen, such as a protein pharmaceutical, by triggering the production of antibodies. Biotechnology-derived proteins may possess a different set of antigenic determinants (i.e., regions of a protein recognized by an antibody) because of structural differences between the recombinant protein and the natural human protein.³⁶ Factors that can contribute to this immunogenicity include lack of or incorrect glycosylation, amino acid modifications, and amino acid additions and deletions. A number of recombinant proteins produced with bacterial vectors contain an N-terminal methionine in addition to the natural human amino acid sequence. Bacterial vector-derived recombinant protein preparations also may contain small amounts of immunoreactive bacterial polypeptides as possible contaminants. Additionally,

immunogenicity may result from proteins that are misfolded, denatured, or aggregated.

Production of Peptide and Protein Drugs

The production of many of important peptides and proteins, via synthetic procedures as well as through biotechnology, involves the pharmaceutical industry. Of necessity, peptides of low to moderate molecular weight, chemically modified peptides, and those containing pseudopeptide bonds or fraudulent amino acids will continue to be made by synthetic procedures rather than through biotechnology. Many of these peptides and proteins are available as chemical analogs, and these also are discussed, particularly the chemical changes made and the implications of these changes to the resulting biologic action (see [Chapter 34](#)).

Monoclonal Antibodies

Introduction to Antibodies

The cell-mediated branch of the immune system includes the antibody-secreting B cells or plasma cells.³⁷ Antibodies or immunoglobulins are soluble proteins that are produced in response to an antigenic stimulus. As part of the normal immune system, each B cell produces as many as 100 million antibody proteins (polyclonal antibodies) directed against bacteria, viruses, and other foreign invaders. Antibodies act by binding to a particular antigen, thereby “tagging” it for removal or destruction by other immune system components.

The production of antigen-“neutralizing” antibodies or immunoglobulins and the detection of a sufficient antibody titer are important concepts for an understanding of vaccinations and exposure to antigens. The humoral response to an antigen involves the creation of memory B cells and the transformation of B lymphocyte into plasma cells that serve as factories for the production of secreted antibodies. Approximately 4 days after initial contact with an antigen (immunization), immunoglobulin M (IgM) antibodies (one of five types of immunoglobulin structure) appear and then peak approximately 4 hours later. Approximately 7 days after exposure, IgG, the major class of circulating immunoglobulin, appears. The antibodies bind to the antigen and affect additional immune system-mediated events, “neutralizing” the antigen and leading to its elimination. The concentration of an immunoglobulin specific for a given antigen at a given time is referred to as the antibody titer and may be a measure of the effectiveness of the initial antigen exposure/vaccination to elicit immunologic memory.

MAbs are monovalent antibodies that bind to the same epitope and are produced from a single B-lymphocyte clone. Some of the improvements that can be done on Abs include minimizing immunogenicity and enhancing antigen-binding affinity, effector function, and pharmacokinetic profile.³⁸ One can reduce immunogenicity by minimizing nonhuman sequences by creating chimeric, humanized, or human versions of Abs.

Antigen-binding affinity can be enhanced by using phage display libraries to isolate MAbs with strong affinities to the antigen.

Antibody Structure

Antibodies are glycoproteins. The simplest structure of an immunoglobulin molecule consists of two identical long peptide chains (the heavy chains) and two identical short polypeptide chains (the light chains) interconnected by several disulfide bonds. The selectivity of any immunoglobulin for a particular antigen is determined by its structure and, specifically, by the variable or antigen-binding regions (Fig. 5.12). Enzymatic digestion of the antibody with papain yields the functional human antibody (Fab) fragment, which contains the antigen-binding sites, and the Fc fragment, which specifies the other biologic activities of the molecule.

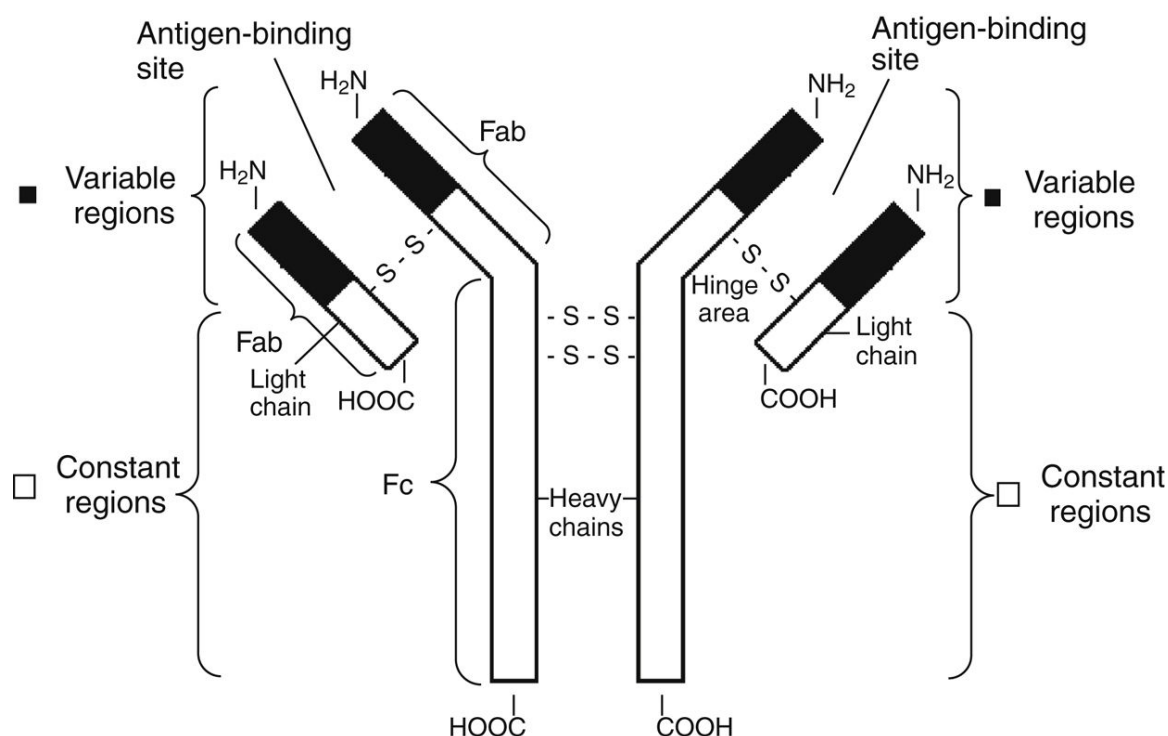


FIGURE 5.12 Schematic model of an antibody molecule.

Hybridoma Technology

MAbs are ultrasensitive, hybrid immune system–derived proteins designed to recognize specific antigens. Nobel Laureates Kohler and Milstein first reported MAbs in 1975.³⁹ MAbs have been used in laboratory diagnostics, site-directed drugs, and home test kits. The B lymphocyte produces a wide range of structurally diverse antibody proteins with varying degrees of specificity in response to a single antigen stimulus. Because of their structural diversity, these antibodies would be called polyclonal antibodies. MAbs are homogeneous hybrid proteins produced by a selected, single clone of an engineered B lymphocyte. They are designed to recognize specific sites or epitopes on antigens.

Hybridoma technology (the technology used to produce MAbs) consists of combining or fusing two different cell lines: a myeloma cell (generally from a mouse) and a plasma spleen cell (B lymphocyte) capable of producing an antibody that recognizes a specific antigen (Fig. 5.13). The resulting fused cell, or hybridoma, possesses some of the characteristics of both original cells: the myeloma cell's ability to survive and reproduce in culture (immortality) and the plasma spleen cell's ability to produce antibodies to a specific antigen.

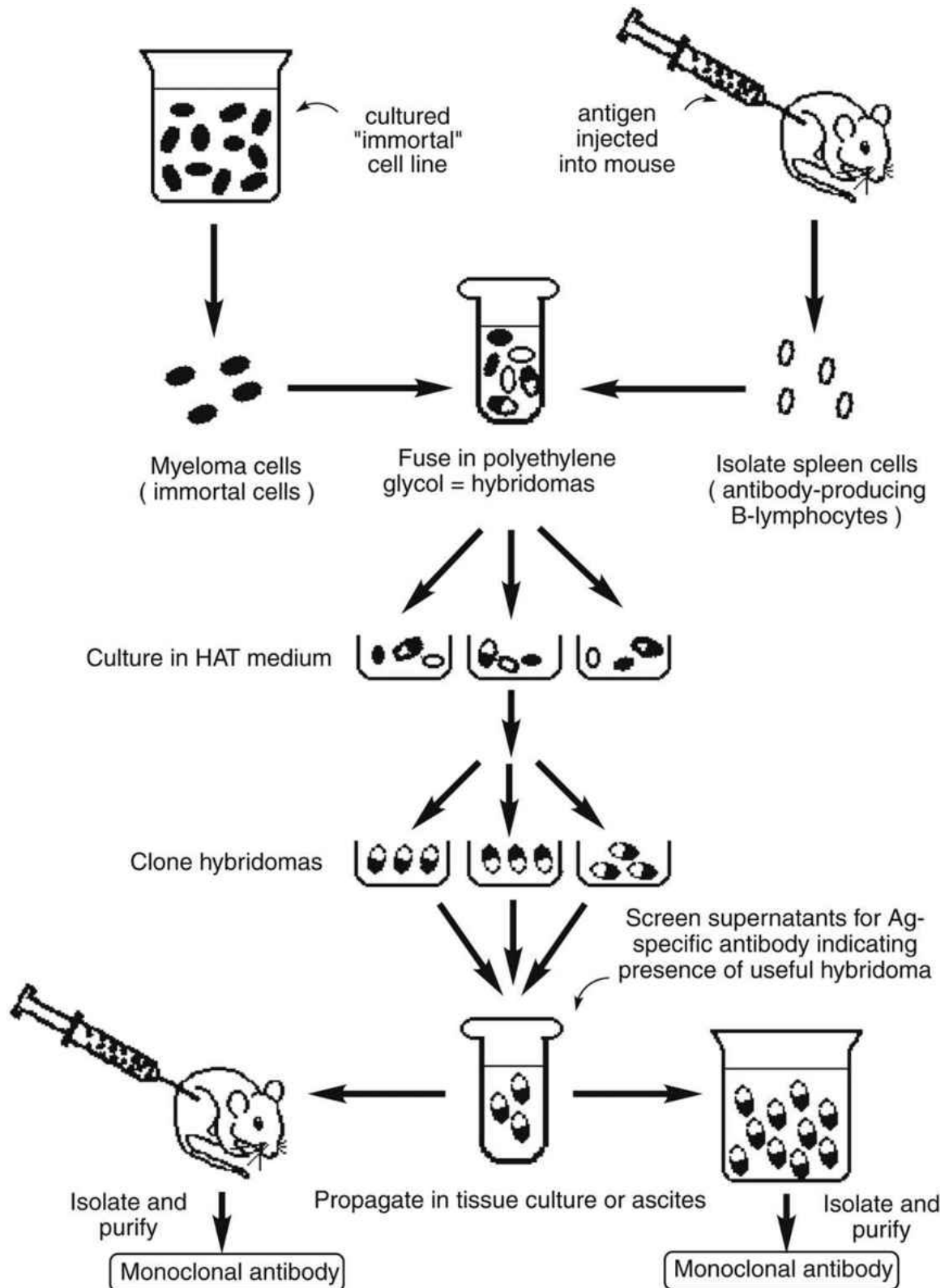


FIGURE 5.13 Outline of hybridoma creation and monoclonal antibody (MAb) production.

Monoclonal antibodies are more attractive than polyclonal antibodies for diagnostic and therapeutic applications because of their increased specificity of antigen recognition. Thus, they can serve as target-directed “homing devices” to find and attach to the targeted antigen. Developments in hybridoma technology have led to highly specific diagnostic agents for home use in pregnancy testing and ovulation prediction kits; laboratory use in detection of colorectal cancer, ovarian cancer, and others; and design of site-directed therapeutic agents, such as trastuzumab to combat metastatic breast cancer and abciximab as an adjunct for the prevention of cardiac ischemic complications.

Monoclonal Antibody Immunogenicity

Nearly 25 years after the pioneering work of Kohler and Milstein, MAbs began to realize their therapeutic potential.⁴⁰ Until recently, most MAbs were murine proteins based on their production. Initial clinical trials of murine MAbs showed that these mouse proteins were highly immunogenic in patients after just a single dose. Human patients formed antibodies to combat the foreign MAb that was administered. The human anti-mouse antibody response is known as HAMA. So, far from being the “magic bullets” that were proposed, immunogenic murine MAbs were useless in chronic therapy. Thus, a different approach was needed to eliminate the unwanted immune response (HAMA) in patients.

The variable regions of an immunoglobulin must be of a specific chemical structure with the ability to bind to the antigen they “recognize.” The part within the variable region that forms the intermolecular interactions with the antigen is the complementarity-determining region (CDR). The variable domains of antibody light and heavy chains contain three CDRs each.

It was determined that immune responses against the mouse-produced MAbs were directed against both the variable and the constant regions of the antibody. Human and murine antibodies are very homologous in chemical structure. Thus, a MAb with decreased immunogenicity can be engineered by replacing the mouse constant regions of an IgG with human constant regions, making the antibody less mouselike. In practice, what

generally occurs is that the variable heavy chain and variable light chain domains (CDRs) of human immunoglobulins are replaced with those of a murine antibody, which possesses the requisite antigen specificity. This “chimeric” MAb will retain its ability to recognize the antigen (a property of the murine MAb) and retain the many effector functions of an immunoglobulin (both murine and human) but will be much less immunogenic (a property of a human immunoglobulin). A chimeric MAb, containing approximately 70% human sequence, will have a longer half-life than its murine counterpart in a human patient. Therapeutic MAbs that are chimeric include catumaxomab (for malignant ascites), cetuximab (for colorectal cancer), and infliximab (for Crohn’s disease).

The discovery of the conserved structure of antibodies, particularly IgGs, across many species suggested the possibility of chimeric antibodies, and the realization that the homology extended to the antigen-binding site facilitated the engineering of humanized immunoglobulins. Advances in phage display technology and the production of transgenic animals has led to the production of humanized or fully human MAbs.³⁹ Functional human antibody fragments (e.g., Fab fragments) can be displayed on the surface of bacteriophages. A bacteriophage, also called a phage, is a virus that infects bacteria. The expression of these human antibody fragments on the phage surface has facilitated efficient screening of large numbers of phage clones (phage display) for antigen-binding specificity.⁴¹ Once a fragment with the requisite antigen specificity is selected, it can be isolated and engineered into a humanized MAb (replacing up to 95% of the murine protein sequence) or a fully human MAb (100% human sequence). Transgenic strains of mice have been genetically engineered to possess most or all of the essential human antibody genes. Thus, on immunization with a foreign antigen, the transgenic mice will develop humanized or fully human antibodies in response. Both of these techniques, although very complex and expensive, have yielded FDA-approved, humanized antibody pharmaceuticals, such as alemtuzumab (for lymphocytic leukemia), bevacizumab (for metastatic colorectal cancer), eculizumab (for paroxysmal nocturnal hemoglobinemia), and trastuzumab (for refractory breast cancer). The half-life of humanized antibodies is dramatically

enhanced (from hours to weeks), and immunogenicity is drastically reduced.

Monoclonal Antibody Diagnostic Agents

Several ultrasensitive diagnostic MAb-based products have enjoyed great success; these include a variety of imaging agents for the detection of blood clots and cancer cells. A monoclonal Fab fragment, technetium-99m-arcitumomab (CEA-Scan), can detect the presence and indicate the location of recurrent and metastatic colorectal cancer. Colorectal cancer and ovarian cancer can be detected with satumomab pendetide (OncoScint CR/OV). Capromab pendetide (ProstaScint) is used for detection, staging, and follow-up of patients with prostate adenocarcinoma. Small-cell lung cancer can be detected with nofetumomab (Verluma). The first imaging MAb for myocardial infarction is imiciromab pentetate (MyoScint).

Monoclonal Antibody–Based, In-Home Diagnostic Kits

The strong trend toward self-care, coupled with a heightened awareness by the public of available technology and an emphasis on preventive medicine, has increased the use of in-home diagnostics. MAb specifically minimizes the possibilities of interference from other substances that might yield false-positive test results. The antigen being selectively detected by MAb-based pregnancy test kits is human chorionic gonadotropin, the hormone produced if fertilization occurs and that continues to increase in concentration during the pregnancy. [Table 5.3](#) lists some examples of MAb-containing in-home pregnancy test kits as well as some examples of MAb-based, in-home ovulation prediction kits.

TABLE 5.3
Some MAb-Based In-Home Test Kits

Manufacturer	Product Distributor	Positive End Point
Pregnancy		
Answer Plus	Carter Products	Plus in test window = +
Answer Quick & Simple	Carter Products	Plus in test window = +

Manufacturer	Product Distributor	Positive End Point
Clear Blue Easy	Unipath	Blue line in large window = +
Clear Blue Easy One Min.	Unipath	Blue line in large window = +
Conceive	Quidel	Pink to purple test line = +
1 Step E.P.T.	Warner Lambert	Pink color in test and control = +
Ovulation		
Answer Quick & Simple	Carter Products	Purple stick line darker than reference = +
Conceive 1 Step	Quidel	Pink to purple test line darker than reference = +
First Response 1 Step	Carter Products	Purple test line darker than reference = +
Clear Plan Easy	Unipath	Blue test line in large window similar or darker than line in small window = +
Ovukit Self Test	Quidel	White to shades of blue compared to LH surge guide = +
OvuQuick	Quidel	Test spot appears darker than reference = +
Q Test	Quidel	Purple test line darker than reference = +

Data from Pray W. *Nonprescription Product Therapeutics*. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2006:778-780 ; Quattrocchi E, Hove I. Ovulation & pregnancy home testing products. *U.S. Pharmacist*. 1998;23:54-63 ; and Rosenthal WM, Briggs GC. Home testing and monitoring devices. In: Allen LV Jr, Berardi RR, DeSimone EM II, et al., eds. *Handbook of Nonprescription Drugs*. Washington, D.C.: American Pharmaceutical Association; 2000:917-942 .

LH, luteinizing hormone; MAb, monoclonal antibody.

Antibody-Drug Conjugates

Paul Ehrlich, in late 1800s and early 1900s, pioneered the studies in immunology as well as chemotherapy and introduced the concept of “magic bullets.” It is now known that cancer cells differ from normal cells as a consequence of genomic mutations in oncogenes and/or tumor suppressor genes.⁴² Consequently, certain antigens are specifically expressed on the surface of human tumor cells, which can be targeted by MAbs. Binding of MAbs to such tumor cells surface antigens is highly specific and triggers cell death by many mechanisms, including abrogation of tumor cell signaling and apoptosis, complement-dependent cytotoxicity, and inhibition of tumor vasculature. Despite many cell-killing mechanisms, MAbs often have limited antitumor activity as single agents, particularly against solid tumors.⁴³ Conventional chemotherapeutic agents on the other hand suffer from systemic toxicity and a lack of tumor specificity. This has paved the way for the development of antibody-drug conjugates (ADCs).⁴⁴ ADCs are tripartite drugs containing a tumor-specific MAb conjugated to a potent cytotoxic agent via a stable linker. Advantage of an ADC is that it selectively delivers a cytotoxic agent to the cancer cells while sparing the normal cells offering a better balance between safety and efficacy.

There has been an explosion in ADC research over the last 10 years.⁴⁵ Early attempts to develop ADCs were plagued with limited success due to low drug potency, high antigen expression on normal cells, and unstable nature of the linker used for attaching the drug to the MAb.⁴⁶ Currently two ADCs are approved by the US FDA for the treatment of cancer: ado-trastuzumab emtansine (Kadcyla) and brentuximab vedotin (Adcetris), and more than 30 are in clinical development.

Various Aspects of Designing ADCs

The selected MAb should target a well-defined antigen that is highly expressed at the tumor site but minimally expressed in the normal tissue to ensure efficient killing of tumor cells by ADCs while limiting general toxicity. Bifunctional linkers carrying attachment sites for both the MAb

and cytotoxic drug are used to join the two components. Existing linker attachment strategies typically depend on the modification of solvent-accessible cysteine or lysine residues on the MAb, which leads to heterogeneous ADC populations with variable drug:MAb ratios. While low drug loading reduces potency, high drug loading can negatively impact pharmacokinetics (PK). The drug:MAb ratios can also significantly influence the efficacy of ADCs. Moreover, the linker must remain stable in systemic circulation to minimize adverse effects yet rapidly cleave once ADC binds to its intended target antigen. Once antigen is recognized and ADC binds to it, ensuing ADC-antigen complex is internalized via a receptor-mediated endocytosis.⁴⁷ Once inside the cell, the drug is released by one of several mechanisms, including nonenzymatic hydrolysis or enzymatic cleavage of the linker or via degradation of the antibody.

Linker technology and stability:

The two major classes of linkers being widely used in ADC drug development include cleavable and noncleavable linkers that utilize different mechanisms for release of the drug payload from the MAb (Fig. 5.14). The cleavable linkers are of three types: (1) lysosomal protease-sensitive linkers, (2) acid-sensitive linkers, and (3) glutathione-sensitive linkers.

1. Lysosomal protease-sensitive linkers. Lysosomal proteases such as cathepsin B particularly recognize and cleave a dipeptide bond as in a valine-citrulline linker (Fig. 5.15) to release the free drug from the ADC.⁴⁸ Several ADCs evaluated in the clinic including FDA-approved brentuximab vedotin (for Hodgkin lymphoma) contain such a linker because it provided optimal balance between plasma stability and subsequent cleavage by intracellular protease.⁴⁹
2. Acid-sensitive linkers (Fig. 5.15). These types of linkers exploit the low pH (~5) in the lysosomal compartment to initiate hydrolysis of an acid-labile hydrazone group of the linker and subsequent release of the drug payload from the ADC. Hydrazone linkers have been used in the discovery and development of Mylotarg (anti-CD33

calicheamicin conjugate) and in inotuzumab ozogamicin (anti-CD22 calicheamicin conjugate)^{50,51}; however, both of these ADCs were withdrawn from the market due to toxicities related to the unstable nature of the hydrazone linker.⁵²

3. Glutathione-sensitive linkers (Fig. 5.15). These linkers take advantage of the higher concentration of glutathione inside the cell relative to that in the plasma. Therefore, a disulfide bond in these linkers is relatively stable in the bloodstream; however, it is capable of undergoing reduction by intracellular glutathione, which ultimately leads to release of the free drug from ADCs. The increased plasma stability of a disulfide bond in the circulation is a result of insertion of a sterically crowded *gem*-dimethyl group adjacent to the disulfide bond to prevent premature cleavage in the plasma.^{53,54} Similar to other linkers described above, glutathione-sensitive linkers are also used in several clinical ADC candidates, including SAR3419 (anti-CD19 maytansine conjugate), IMGN901 (anti-CD56 maytansine conjugate), and AVE9633 (anti-CD33 maytansine conjugate).⁵¹

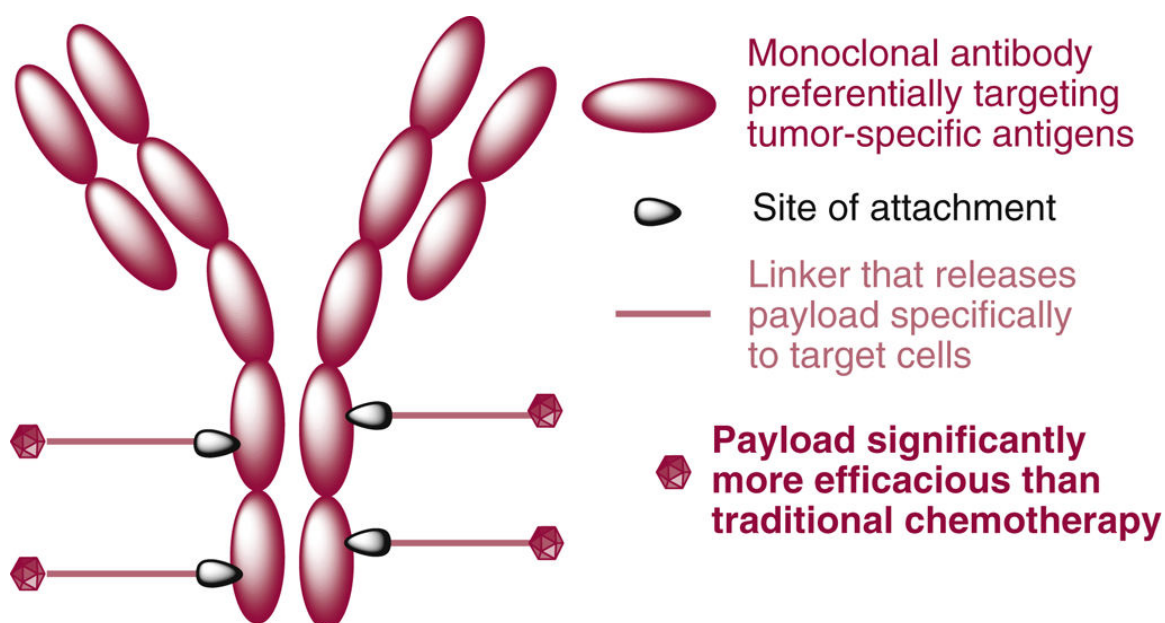


FIGURE 5.14 Schematic representation of key components of an antibody-drug conjugate (ADC).

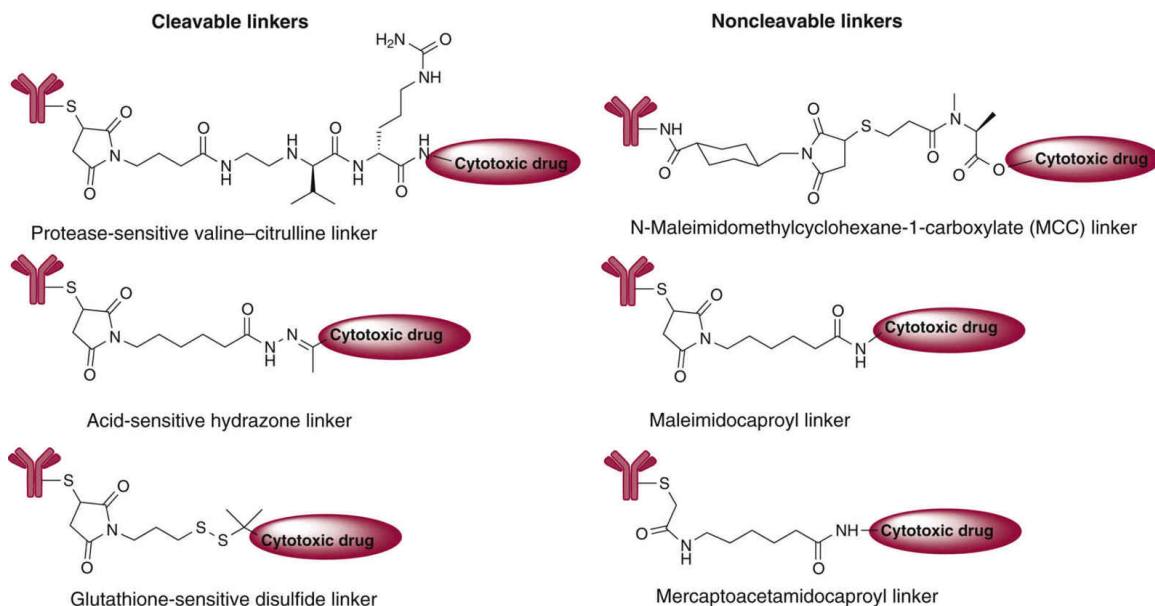


FIGURE 5.15 A snapshot of cleavable and noncleavable linkers used in antibody-drug conjugate (ADC) development.

The noncleavable linker strategy depends on complete degradation of the MAb upon internalization of the ADC, which results in release of free drug molecules that still carry a linker moiety and amino acid residue from the MAb (Fig. 5.15). Noncleavable linker strategies are useful to those payloads that exert their antitumor effect despite being chemically modified. Noncleavable linker strategy has been effectively used in the development of Kadcyla (trastuzumab-MCC-DM1, for metastatic breast cancer), wherein the released modified payload (lysine-MCC-DM1) demonstrated comparable potency to DM1 alone.⁵⁵ Noncleavable linkers have greater stability in the plasma as compared to cleavable linkers.

Conjugation Strategies: Chemical Conjugation

This strategy involves covalent bonding of a reactive moiety pendant to the drug-linker to the target antibody via an amino acid residue side chain, usually the ϵ -amine of lysine. This strategy has been successfully used for the development of gemtuzumab ozogamicin (Mylotarg; for acute myeloid leukemia).⁵⁶ Another approach involves a two-step process wherein surface lysine residues on the target antibody are first modified to insert a reactive functional group such as a maleimide, and subsequent conjugation to the drug-linker moiety containing a suitable reactive handle

such as thiol group.⁵⁷ This strategy was used for the development of trastuzumab-MCC-DM1 (Kadcyla; for metastatic breast cancer). Yet another approach involves controlled reduction of existing disulfide bonds in the target antibody to liberate free cysteine residues, which are further reacted with a maleimide attached to the drug-linker. This approach was successfully used in development of brentuximab vedotin (Adcetris; for Hodgkin lymphoma).⁵⁸ The abovementioned approaches are random and as a result produce heterogeneous mixtures of conjugated antibodies with highly variable drug-to-antibody ratios (DARs). Therefore, techniques that allow production of site-specific conjugation with robust DARs are highly desirable.

Site-specific Conjugation

This strategy allows production of homogeneous ADCs. Three major strategies for site-specific conjugation are (1) insertion of cysteine residues in the target antibody sequence by site-directed mutagenesis, (2) insertion of an unnatural amino acid residue with a bio-orthogonal reactive handle, and (3) enzymatic conjugation. As compared to conventional conjugation-derived ADCs, ADCs generated through drug-linker conjugation with the surface cysteine residues showed minimal heterogeneity, defined DAR, and improved pharmacokinetic and pharmacodynamic parameters.

Bystander Killing by ADCs

A bystander killing mechanism operates when cytotoxic species comes out of the tumor cell and diffuse and kill nearby cancer cells.⁵⁹

Vaccines

There are two types of immunization: active immunization and passive immunization. Active immunization is the induction of an immune response either through exposure to an infectious agent or by deliberate immunization with a vaccine (vaccination) made from the microorganism or its products to develop protective immunity. Passive immunization involves the transfer of products produced by an immune animal or human (preformed antibody or sensitized lymphoid cells) to a previously

nonimmune recipient host, usually by injection. Sufficient active immunity may take days, several weeks, or even months to induce (possibly including booster vaccinations), but it generally is long-lasting (even lifelong) through the clonal selection of genetically specific immunologic memory B and T lymphocytes. Passive immunity, although often providing effective protection against some infection, is relatively brief, lasting only until the injected immunoglobulin or lymphoid cells have disappeared (a few weeks or months). Thus, vaccines enable the body to resist diseases caused by infectious agents. In response to an injection of vaccine, the immune system makes antibodies, which recognize surface antigens found in the vaccine. If the subject is later exposed to a virulent form of the virus, the immune system is primed and ready to eliminate it. Many viral vaccines are produced from the antigens isolated from pooled human plasma of virus carriers. Vaccinations are among the most cost-effective and widely used public health interventions. Although generally safe, the minimal risk of vaccine-produced infections can be eliminated by administration of highly purified vaccine antigens of recombinant origin. The different types of vaccines are described below, and the vaccines currently available in the United States are listed in [Table 5.4](#).

TABLE 5.4
Some FDA-Approved Vaccines

Generic Name	Trade Name	Vaccine Type	Protection Against
Anthrax	BioThrax	Inactivated	Anthrax
Chicken pox	Varivax	Live attenuated	Chicken pox
Diphtheria	Pediarix	Toxoid	Diphtheria
<i>Haemophilus influenzae</i> type b (Hib)	ActHib, PedvaxHib	Conjugate	<i>H. influenzae</i>
Hepatitis A	Havrix, Vaqta	Inactivated	Hepatitis A
Hepatitis B	Engerix-B, Recombivax	Recombinant subunit	Hepatitis B
HepA-HepB	Twinrix	Inactivated recombinant	Hepatitis A and B
Herpes zoster	Zostavax	Live attenuated	Herpes zoster
Human papilloma virus	Gardasil, Cervarix	Recombinant	Cervical cancer

Generic Name	Trade Name	Vaccine Type	Protection Against
Influenza	Fluarix, Fluvirin, Fluzone, Flulaval, Afluria, Agriflu, FluMist	Inactivated live attenuated	Influenza
Japanese encephalitis	Ixiaro, JE-Vax	Inactivated	Japanese encephalitis
Measles	Attenuvax	Live attenuated	Measles
Meningococcal conjugate	Menactra, Menomune, Menveo	Inactivated	Meningococcal
Mumps	Mumpsavax	Live attenuated	Mumps
Pertussis	Boostrix	Subunit	Pertussis
Pneumococcal	Prevnar, PCV13, Pneumovax23	Conjugate	Pneumococcal
Polio	Ipol	Inactivated	Polio
Sipuleucel-T	Provenge	Autologous	Prostate cancer
Rabies	Imovax, Rabavert	Inactivated	Rabies
Rotavirus	Rotarix, RotaTeg	Live attenuated	Rotavirus
Rubella	Meruvax	Live attenuated	Measles
Tetanus toxoid (TT)		Toxoid	Tetanus
Typhoid	Typherix, Vivotif Berna, Typhim Vi	Inactivated	Typhoid
Varicella	Varivax	Live attenuated	Varicella
Vaccinia (smallpox)	ACAM2000	Live attenuated	Smallpox
Yellow fever	YF-Vax	Live attenuated	Yellow fever

Live, Attenuated Vaccines

To make a live, attenuated vaccine, the disease-causing organism is grown under special laboratory conditions that cause it to lose its virulence or disease-causing properties.

Inactivated Vaccines

Inactivated vaccines are produced by killing the disease-causing microorganism with chemicals or heat.

Subunit Vaccines

Sometimes vaccines developed from antigenic fragments are able to evoke an immune response, often with fewer side effects than might be caused by a vaccine made from the whole organism.

Toxoid or Inactivated Toxins

A toxoid is an inactivated toxin, the harmful substance produced by a microbe. Many of the microbes that infect people are not themselves harmful. It is the powerful toxins they produce that can cause illness. To

inactivate such powerful toxins, vaccine manufacturers treat them by chemical means (formalin solution) and irradiation to completely cripple any disease-causing ability.

Conjugate Vaccines

The bacteria that cause some diseases, such as pneumococcal pneumonia and certain types of meningitis, have special outer coats. These coats disguise antigens so that the immature immune systems of infants and younger children are unable to recognize these harmful bacteria. In a conjugate vaccine, proteins or toxins from a second type of organism, one that an immature immune system can recognize, are linked to the outer coats of the disease-causing bacteria. This enables a young immune system to respond and defend against the disease agent.

DNA Vaccines or Naked Vaccine

Genes encoding antigens of an infectious organism are expressed by the host's own cells. Genes are inserted into a bacterial plasmid under the control of a mammalian promoter. The chimeric plasmid is either directly injected into muscle or the DNA is conjugated to a solid matrix such as gold particles.

Recombinant Vector Vaccines

A vaccine vector, or carrier, is a weakened virus or bacterium into which harmless genetic material from another disease-causing organism can be inserted.

Pharmacogenomics and Personalized Medicine

Personalized medicine is defined as the utilization of molecular biomarkers from an individual's genome, transcriptome, proteome, and metabolome under the influence of the individual's envirome in the assessment of predisposition to disease, screening and early diagnosis of disease, assessment of prognosis, pharmacogenomic prediction of therapeutic drug efficacy and risk of toxicity, and monitoring of illness until the final therapeutic outcome is determined. Biomarkers serve as critical tools for disease detection and subsequent monitoring. For example, gene mutations, alterations in gene transcription and translation, and alterations in their protein products can all serve as specific biomarkers for disease.⁶⁰ Furthermore, mass spectrometry-driven proteomic analysis plays an important role in rapid detection of disease-specific biomarkers and proteomic patterns of various tissues and body fluids.⁶¹

The concept of personalized medicine was anticipated by Sir William Osler (1849-1919), a well-known Canadian physician during his time. He recognized that "variability is the law of life, and as no two faces are the same, so no two bodies are alike, and no two individuals react alike and behave alike under the abnormal conditions we know as disease." Personalized medicine has rapidly advanced the prediction of disease incidence as well as the prevention of prescribing the incorrect drug based on a person's clinical, genetic, and environmental information. The goal of personalized medicine is optimizing the medical care and outcomes for each patient.⁶²

Pharmacogenomics uses genomic tools to understand the genotype effects of relevant genes on the behavior of a drug, as well as the effects of a drug on gene expression. The best examples of successful pharmacogenomic applications are presented below.

Drug efficacy is not solely influenced by variations in drug-metabolizing genes but also by polymorphisms in genes that encode drug receptors and transporters. Polymorphisms and alleles in major phase I drug-metabolizing enzymes (CYP450s) and phase II drug metabolizing-

enzymes (UDP-glucuronyl transferases, thiopurine S-methyltransferases [TPMT]) can be genotyped to aid physicians in individualizing treatment doses for patients on therapeutics metabolized through the products of these genes.

The oral anticoagulant warfarin is prescribed for the long-term treatment and prevention of thromboembolic events. An investigation of the pharmacokinetic and pharmacodynamic drug properties of warfarin indicated the additive involvement of two genes when determining the dosage. One of these genes encodes for CYP2C9, which is responsible for the metabolic clearance (~80%) of the pharmacologically potent S-enantiomer of warfarin (Fig. 5.16). There are three allele types, wild-type CYP2C9*1 and nonsynonymous polymorphisms *2 and *3, and both CYP2C9*2 and *3 code for CYP2C9 enzymes with reduced enzymatic activity leading to increased warfarin half-life and subsequent significant clinical influence on warfarin sensitivity and severe bleeding events.⁶³ A 10-fold difference in warfarin clearance was observed between groups of individuals having the genotype of the highest metabolizer (CYP2C9*1 homozygote) and lowest metabolizer (CYP2C9*3 homozygote).⁶⁴

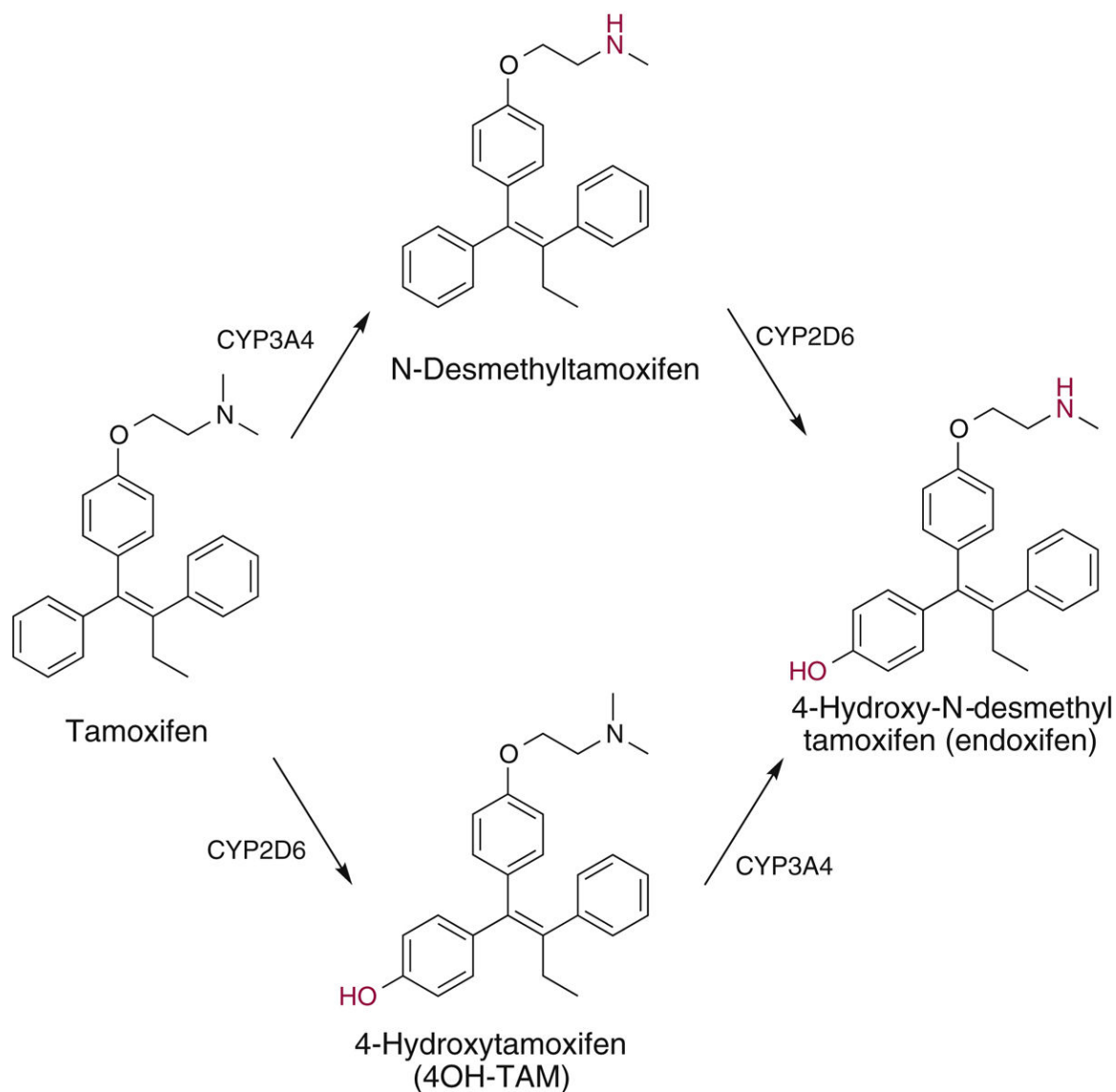


FIGURE 5.17 Tamoxifen metabolic pathway.

Tolterodine [(*R*)-*N,N*-diisopropyl-3-(2-hydroxy-5-methylphenyl)-phenylpropanamine] is an antimuscarinic drug for the treatment of urinary urge incontinence and other symptoms associated with an overactive bladder. Two different oxidative metabolic pathways, hydroxylation and *N*-dealkylation, have been identified in humans (Fig. 5.18). Hydroxylation to the pharmacologically active 5-hydroxymethyl metabolite (5-HM) is catalyzed by CYP2D6, whereas the *N*-dealkylation pathway is catalyzed by CYP3A4. Further oxidation of 5-HM catalyzed by alcohol and aldehyde dehydrogenases yields the carboxylic acid of tolterodine and its *N*-dealkylated form, along with *N*-dealkylated 5-HM.

As described previously, CYP2D6 is subject to genetic polymorphism, with important implications for drugs that are metabolized by this enzyme such as tolterodine. Clinical studies have demonstrated that individuals with reduced CYP2D6-mediated metabolism represent a high-risk group in the population, with a propensity to develop adverse drug effects. In fast metabolizers, the mean systemic clearance of tolterodine was found to be 44 L/h, yielding a half-life of 2-3 hours. In contrast, poor metabolizers have a fivefold lower clearance and a mean half-life of 9 hours, which results in a sevenfold higher maximum serum concentration of tolterodine at steady state.⁶⁶

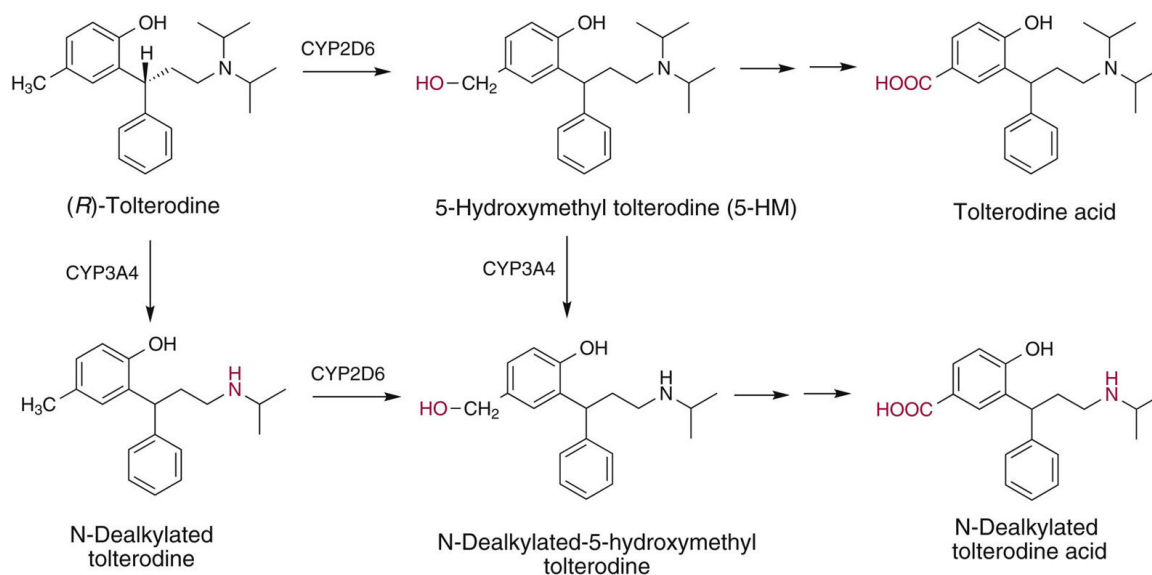


FIGURE 5.18 Tolterodine metabolic pathway.

Codeine is a prodrug whose analgesic property is primarily due to its metabolic conversion to the central analgesic morphine (Fig. 5.19). This metabolic conversion is catalyzed by CYP2D6 enzyme. Loss-of-function variations in CYP2D6 can lead to a poor analgesic response, and patients carrying such a variation are considered poor metabolizers and receive little therapeutic benefit from codeine. About 5%-10% of Caucasians are CYP2D6 poor metabolizers; the percentage is approximately 2%-3% in other racial and ethnic groups.⁶⁷ On the other hand, variations that result in increased metabolic activity of CYP2D6 will lead to enhanced conversion of codeine to morphine with concomitant increased analgesic

response. Patients who carry such variations are at risk for opioid toxicity, which includes moderate to severe central nervous system depression. Therefore, drugs containing codeine such as CYP2D6 ultrarapid metabolizers carry a product label that include warnings such as “may experience overdose symptoms such as extreme sleepiness, confusion or shallow breathing, even at labeled dosage regimens,” and encourages physicians to “choose the lowest effective dose for the shortest period of time and inform their patients about the risks and the signs of morphine overdose”.

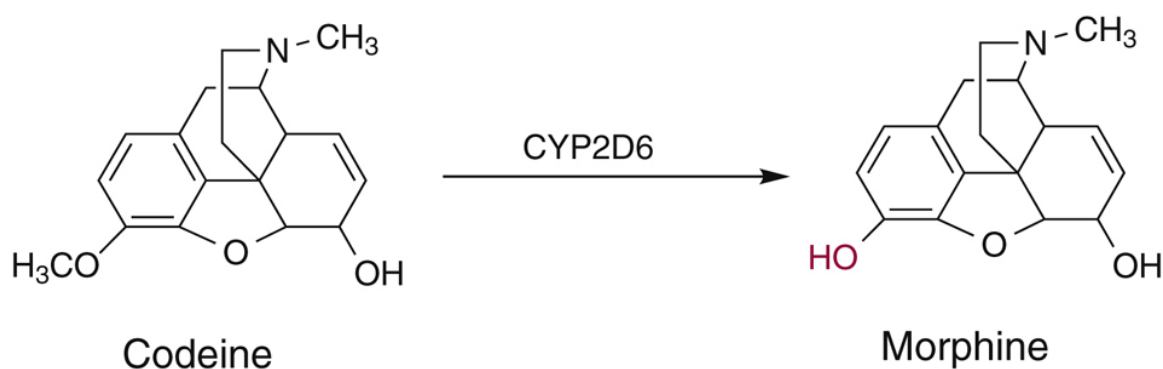


FIGURE 5.19 Codeine metabolism to morphine.

Clopidogrel, a thienopyridine derivative, is used for the prevention of recurrent thrombosis in patients with myocardial infarction and percutaneous coronary intervention with stent implantation. Clopidogrel response has been shown to vary widely, both interindividually and interethnically. CYP2C19 metabolizes clopidogrel to its pharmacologically active metabolite (Fig. 5.20). Therefore, patients who carry the loss-of-function *CYP2C19**2 alleles are particularly at higher risk for producing major cardiovascular events such as bleeding as compared to noncarriers.⁶⁸

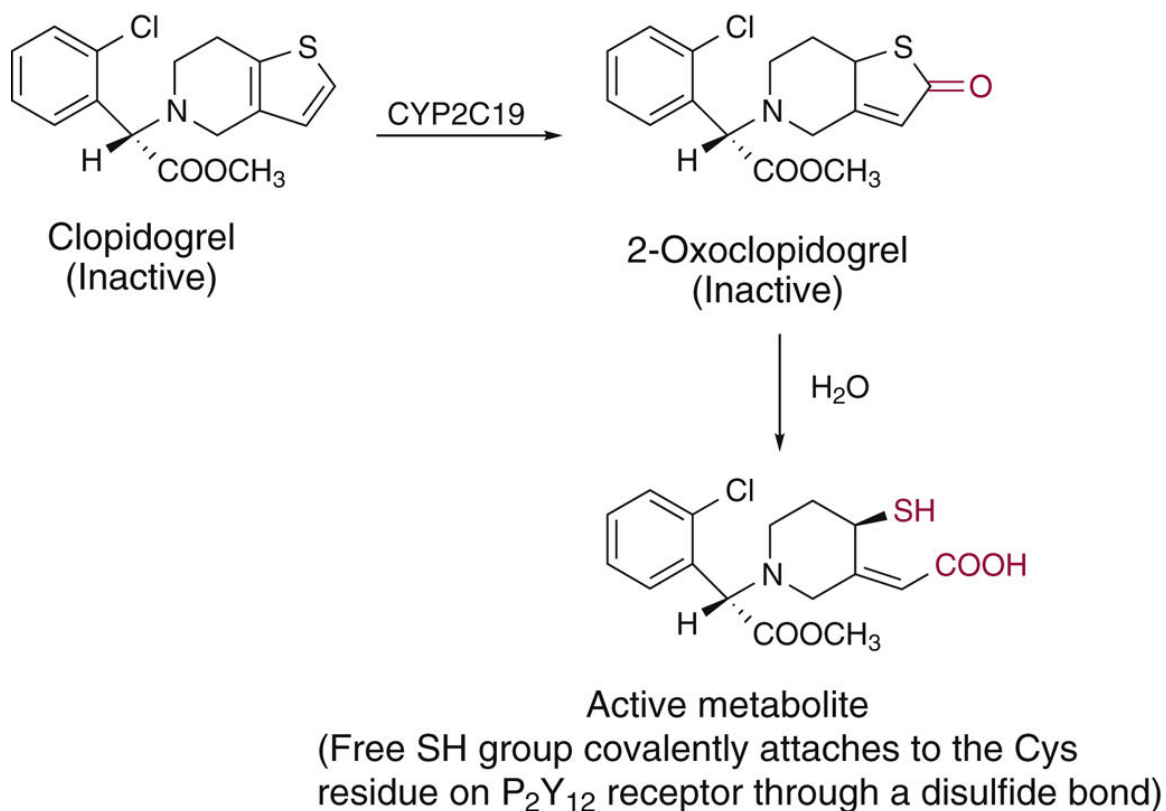


FIGURE 5.20 Clopidogrel metabolism to active metabolite.

Irinotecan (also known as CPT-11 or Camptosar) is an approved topoisomerase I inhibitor used to treat patients with metastatic colon cancer. Acute and delayed diarrhea and neutropenia often occur after treatment with irinotecan. Diarrhea following treatment with irinotecan occurs due to the excretion of an active metabolite (SN-38:10-hydroxy-7-ethyl-camptothecin) initially into the bile and subsequently the colon (Fig. 5.21; for more details, see Chapter 33: Drugs Used to Treat Neoplastic Diseases). Irinotecan treatment is associated with an increased frequency of severe and potentially life-threatening toxicity among patients with genetic polymorphisms that markedly reduce glucuronidation of SN-38. Patients with decreased capacity to glucuronidate SN-38 (e.g., patients homozygous for specific UDP-glucuronosyl transferase 1A1 [UGT1A1] genotypes such as *UGT1A1**28 [TA7]) are at increased risk for severe neutropenia after treatment with irinotecan or SN-38 than patients with the wild-type sequence.⁶⁹

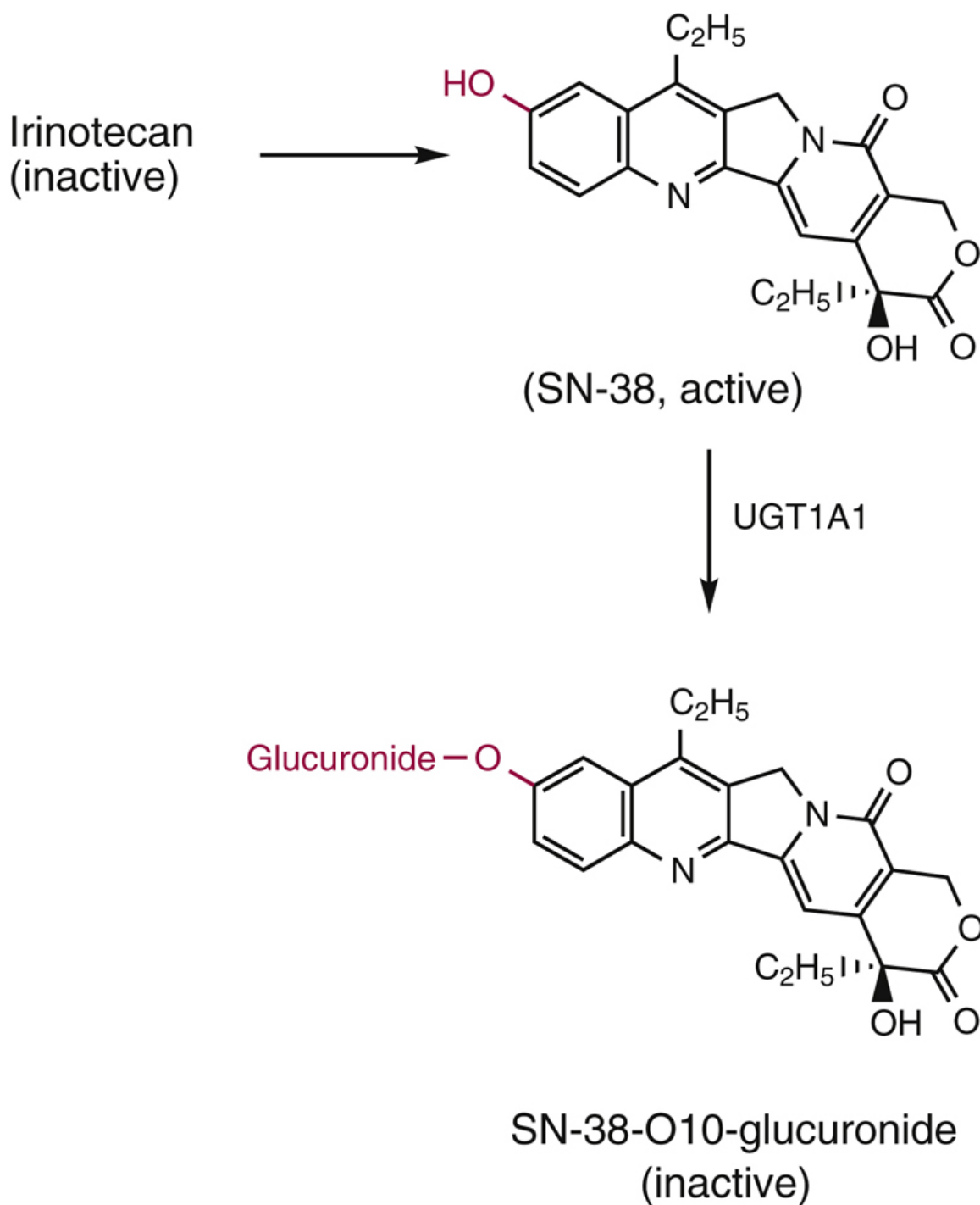


FIGURE 5.21 Metabolic activation of irinotecan.

TPMT is a key enzyme involved in the metabolism of azathioprine (AZA) and 6-mercaptopurine (6-MP), two widely used drugs to treat leukemia, rheumatic diseases, inflammatory bowel disease, and solid organ transplantation.⁷⁰ AZA is metabolized to 6-MP, the active metabolite of

AZA. 6-MP can be inactivated by either xanthine oxidase (XO) or TPMT to nontoxic metabolites (Fig. 5.22). TPMT-deficient patients carrying the nonfunctional TPMT*2, TPMT*3A, and TPMT*3C alleles are at higher risk for producing severe hematologic toxicity, and consequently TPMT-deficient patients require substantial reduction in dose.⁷¹

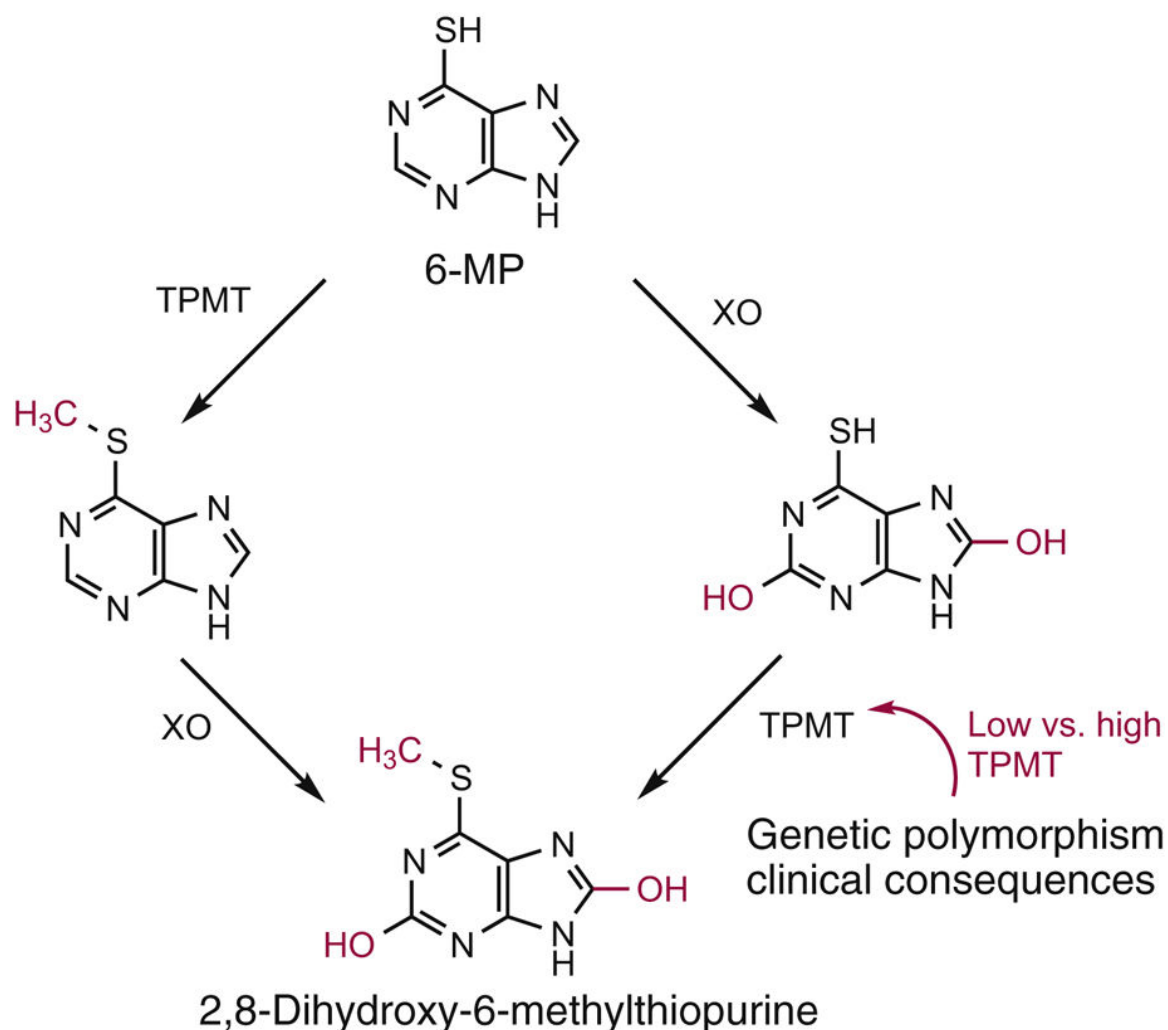


FIGURE 5.22 Metabolism of 6-mercaptopurine (6-MP) by thiopurine methyltransferase (TPMT) and xanthine oxidase (XO).

Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme that metabolizes 5-fluorouracil (5-FU), a widely used anticancer drug (Fig. 5.23). A genetic deficiency of the DPD enzyme can lead to toxicity to 5-FU due to reduced clearance, and thus, this drug should not be used in patients with DPD deficiency.^{72,73}

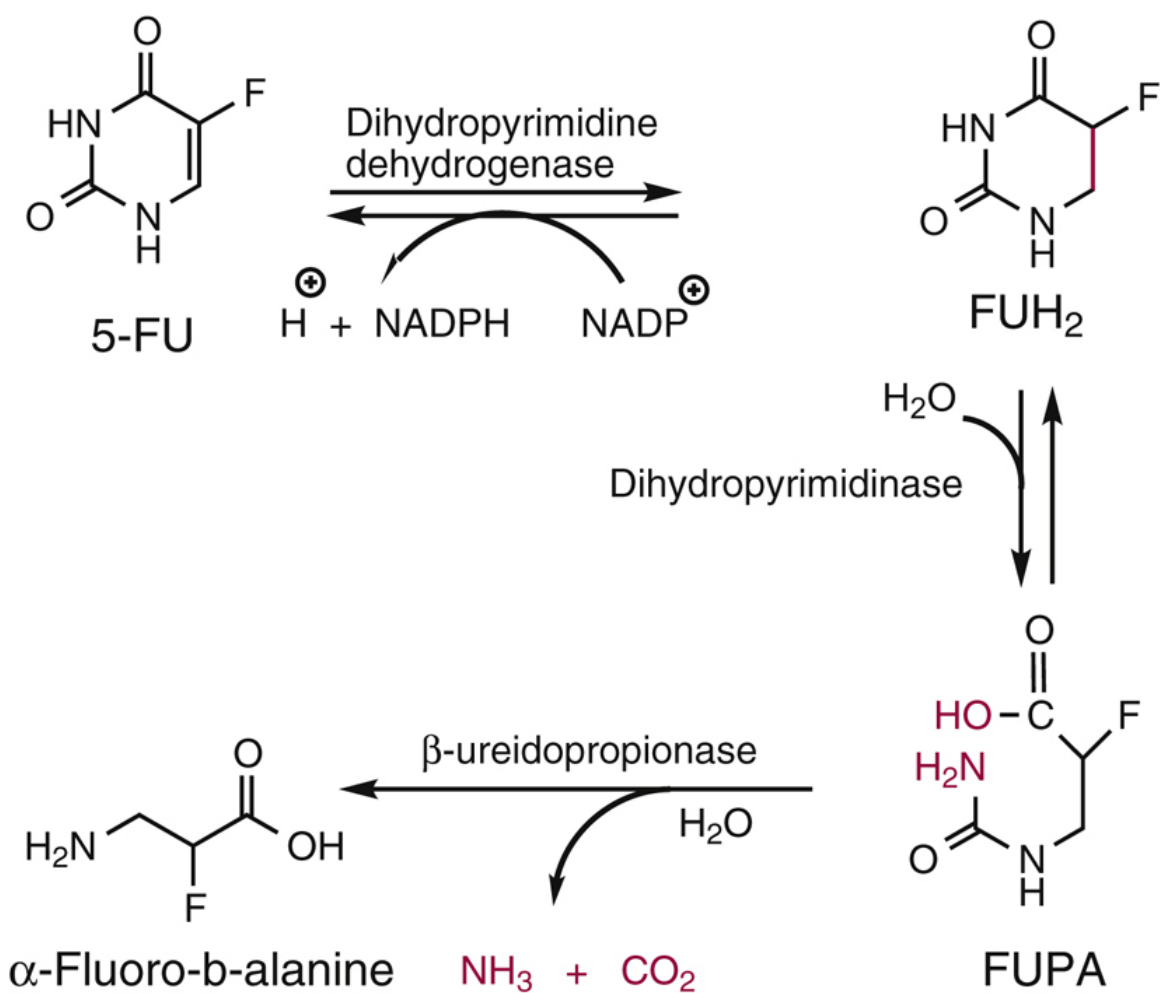


FIGURE 5.23 5-Fluorouracil (5-FU) inactivation by dihydropyrimidine dehydrogenase (DPD).

The human N-acetyl transferases (NATs) are responsible for the catalytic transfer of an acetyl group from acetyl coenzyme A to arylhydrazines as in isoniazid (Fig. 5.24).⁷⁴ Functional polymorphisms have been observed in two human NAT genes, *NAT1* and *NAT2*, leading to altered enzyme activity. Based on NAT activity, patients can be classified into two phenotypes: fast acetylators (wild-type NAT acetylation activity) and slow acetylators (reduced NAT enzyme activity). Polymorphisms in the *NAT1* (i.e., *NAT1**14, *15, *17, *19, and *22) and *NAT2* (e.g., *NAT2**5, *6, *7, *10, *14, and *17) result in slow acetylation phenotype.⁷⁵ Since *NAT2* plays an important role in deactivation of isoniazid, its response is dependent on the presence/absence of a particular *NAT2* genotype. Slow acetylators are presented with increased risk of isoniazid-induced hepatitis.⁷⁶

lethality: cancer cells that have lost key *BRCA* gene functions depend on backup DNA repair pathways such as PARP, and by inhibiting PARP, such dependence is exploited to preferentially kill the cancer cells while sparing normal cells.)⁷⁹

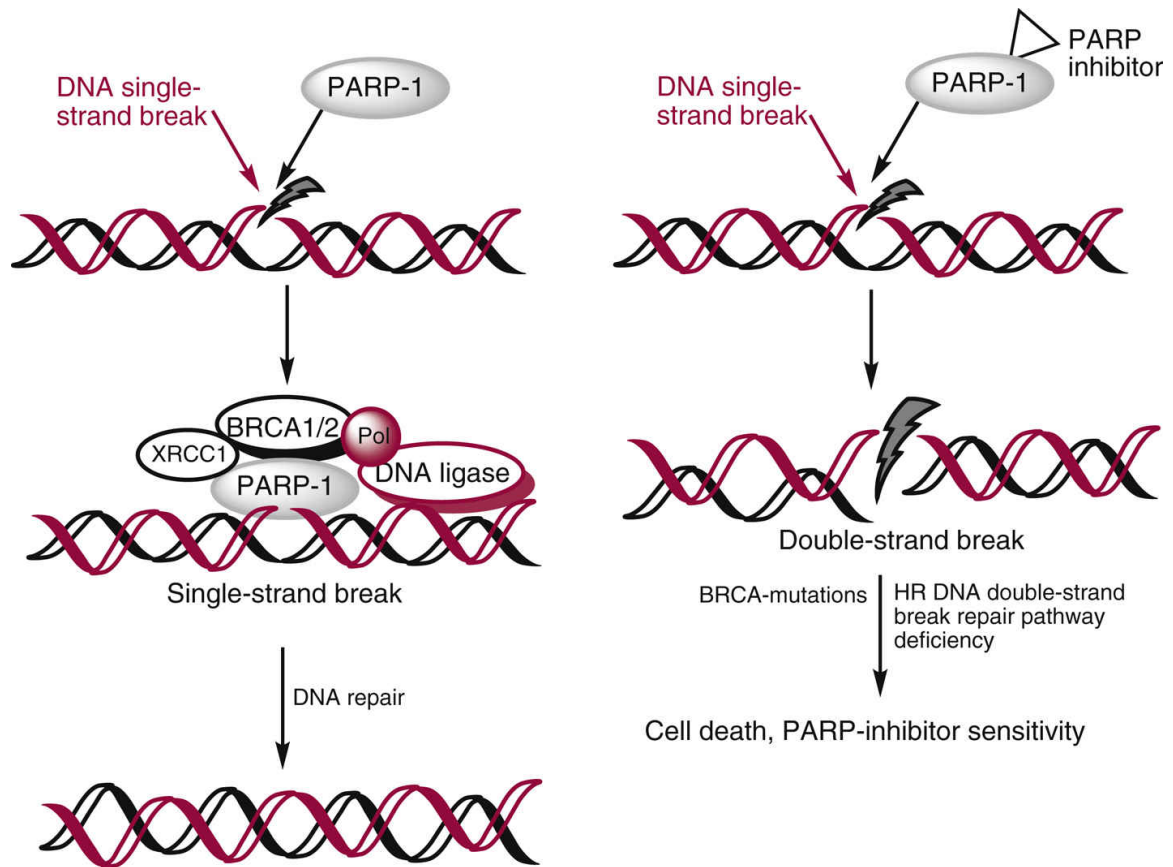


FIGURE 5.25 Role of poly (ADP-ribose) polymerase-1 (PARP-1) in DNA single-strand break repair and PARP-inhibitor sensitivity in patients with breast cancer-associated gene (*BRCA*) mutations (homologous recombination [HR] deficiency).

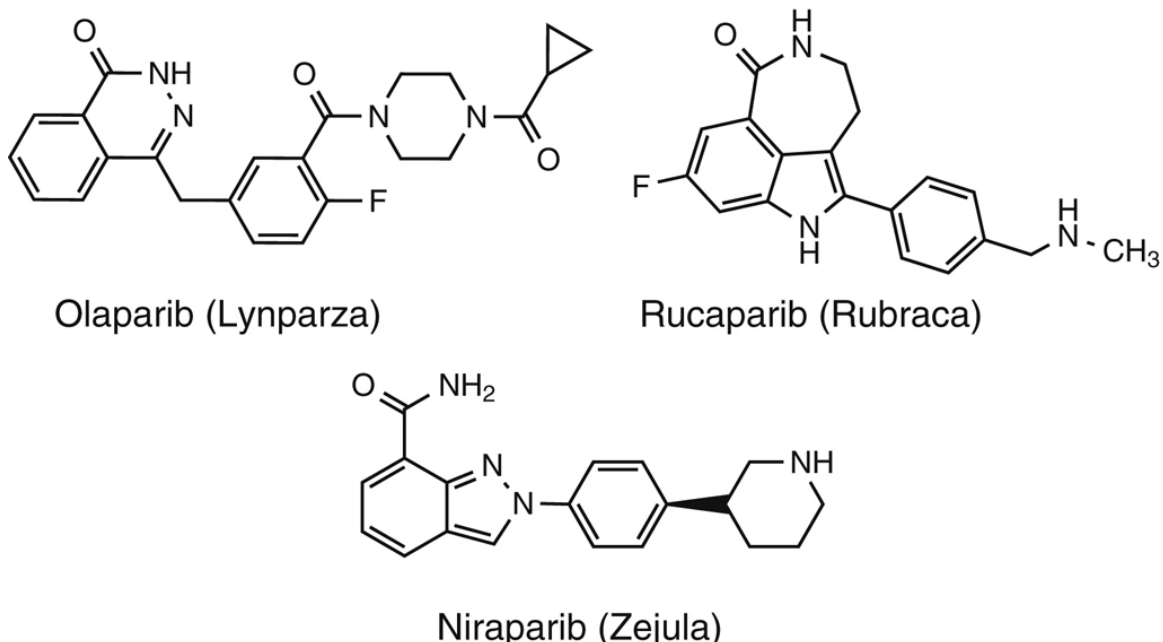


FIGURE 5.26 Structures of FDA-approved PARP-1 (poly [ADP-ribose] polymerase-1) inhibitors.

Pegloticase (Krystexxa), a pegylated uricase enzyme approved for the treatment of chronic gout, metabolizes uric acid to allantoin, a highly water soluble product that is easily excreted by the kidney (Fig. 5.27). With each molecule of uric acid degradation there is a simultaneous production of one molecule of hydrogen peroxide, which leads to overwhelming oxidative stress and subsequent hemolytic anemia and methemoglobinemia. Normally, oxidative stress is counteracted by the presence of glucose-6-phosphate dehydrogenase (G6PD) through production of cellular reducing equivalents such as NADH and glutathione. Pegloticase treatment of patients with G6PD deficiency is thus precluded.⁸⁰

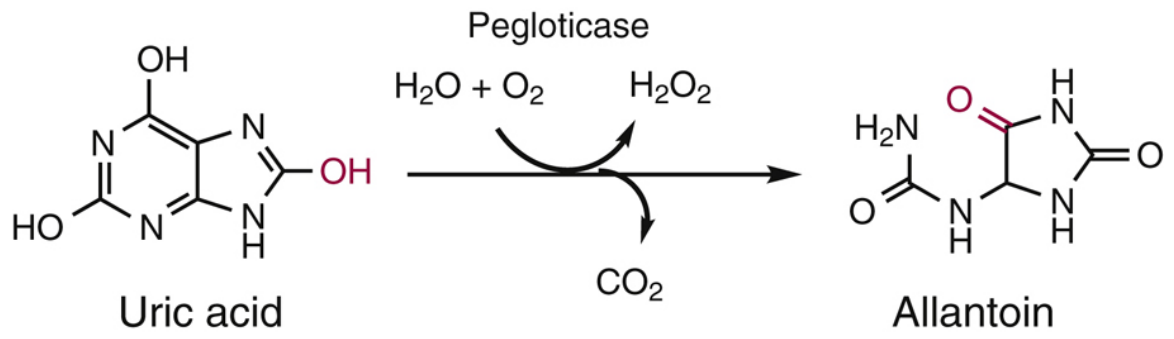


FIGURE 5.27 Degradation of uric acid by pegloticase.

Gene Therapy

Translation of gene therapy concepts to patient care began nearly three decades ago.⁸¹ In principle, gene therapy can produce a lasting and potentially curative clinical benefit. After numerous setbacks due to therapy-related toxicities, the studies are bearing fruit with approvals of gene therapy products by the US FDA beginning in 2017.⁸² A major problem encountered in gene therapy was similar to the problem encountered in all forms of drug therapy—the assurance of drug efficacy through efficient delivery of the therapeutic agent to its biologic target in a fully functional form. Gene therapy is unique in the sense that it is the product of gene expression, the protein, and not the gene itself that is the therapeutic agent. Hence, we must not only deliver the gene to its proper target but also assure that when the gene reaches its target, it will arrive in a form that will produce the therapeutic agent in such a form that it, too, will be assured of reaching its specified target.⁸³⁻⁸⁵

Gene therapy studies have traditionally focused on direct *in vivo* administration of viral vectors such as replication-defective retroviruses and adeno-associated virus (AAV). The steps (administration, delivery, and expression) involved in the delivery of the therapeutic gene are shown in Figure 5.28.⁸⁶ The field is further fueled by the availability of robust gene editing technologies such as CRISPR, zinc finger nuclease (ZFN), and transcription activator–like effector nuclease (TALEN) and *ex vivo* approaches using genetically engineered T cells⁸⁷ and hematopoietic stem cells. Three gene therapy products have been approved by the US FDA since August 2017, ushering in a new approach to the treatment of serious and life-threatening diseases. Tisagenlecleucel (Kymriah) and axicabtagene ciloleucel (Yescarta) are chimeric antigen receptor T-cell immunotherapies, in which patient's immune cells are removed,

injected with DNA outside the body, and returned to the bloodstream for the treatment of B-cell malignancies. Voretigene neparvovec-rzyl (Luxturna), the latest gene therapy product to be approved, is an AAV vector for *in vivo* treatment of congenital blindness. In addition, a number of gene therapy products are under various phases of clinical investigation for different disease conditions including severe combined immunodeficiency disease due to adenosine deaminase deficiency (CD34⁺ cells transduced with adenosine deaminase gene), glioblastoma (Toca511, a retroviral replicating vector), age-related macular degeneration (AAV2-sFLT01), Parkinson's disease (CERE-120: AAV delivery of neurturin), advanced osteocarcinoma (Her2 chimeric antigen receptor expressing T cells), chronic lymphocytic leukemia (CLL) (autologous T lymphocytes engrafted with a chimeric antigen receptor targeting the kappa light chain), Alzheimer disease (CERE-110: AAV delivery of nerve growth factor), acute lymphoblastic leukemia (ALL) (LV CD19 4-1BB CAR-T), multiple myeloma (gamma-RV BCMA CD28 CAR-T), HIV (ZFN CCR5 electroporation), beta-thalassemia (LV antisickling beta-hemoglobin), and hemophilia B (AAV8-Factor IX).^{82,88}

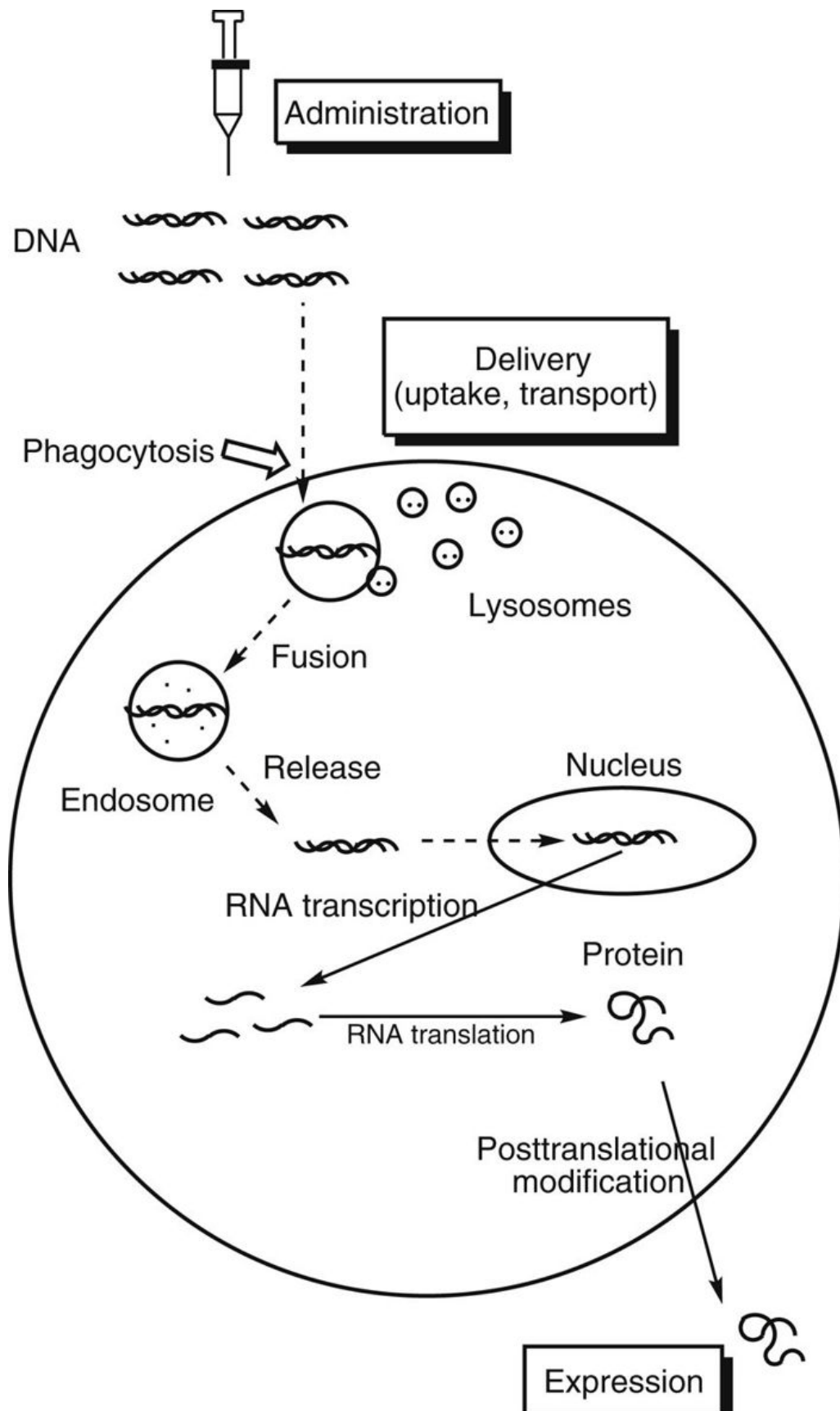


FIGURE 5.28 Steps in gene therapy. Gene therapy involves the steps of administration, delivery, and expression. (Note: dashed arrows represent uptake and transport.)

Summary

Completion of the HGP in 2003 resulted in the elucidation of the entire sequence of the 3 billion base pairs in the human genome, estimated to contain some 25,000 genes. The full impact that this scientific advance will have on our lives has yet to be determined, but the social, legal, ethical, and economic issues are certain to be extensive and complex. The manipulation and analysis of the genomic information obtained from the HGP is paving the path for transformative biomedical developments. DNA-based tests are among the first commercial medical applications. Gene tests can be used to diagnose and confirm disease, provide prognostic information about the course of disease, and predict the risk of future disease in healthy individuals.

Knowledge of genes involved in diseases, disease pathways, and drug response sites will result in the discovery of novel therapeutic targets beyond the 500 or so that were known prior to the completion of the HGP, providing a tremendous opportunity for the discovery of drugs that work through previously unexplored mechanisms.

Discovery of genomic biomarkers can help determine the type of drug therapy. For example, one of the most commonly used predictive biomarkers in cancer is immunohistochemical staining for the presence of the estrogen receptor in breast cancer. Only estrogen receptor-positive breast tumors are likely to respond to antihormonal therapy. Identification of biomarkers for various diseases can help in the selection of patients most likely to benefit from a potential drug, which in turn will speed the design of clinical trials that are more efficient and have the potential to reduce drug approval time and associated costs. Predicting toxicity is another important outcome of the availability of the genomic information.

For example, patients who have mutations in the TPMT gene will metabolize the chemotherapeutic mercaptopurine drugs at a reduced rate and can be overdosed on their treatment. Similarly, an SNP in the coding region of genes expressing drug-metabolizing CYP450 enzymes results in either poor (overdose) or fast (subtherapeutic dose) metabolism of drugs.

The future will see an altered form of health care using a genetic information infrastructure to contain costs and predict outcomes, create advanced personalized therapies, and develop a predict-and-manage paradigm of health care. Recent years have witnessed the introduction of a steady stream of biotechnology-derived drugs. The trend is expected to continue for the foreseeable future. The expiration of patents on biotechnology-derived drugs will introduce an era of more affordable biosimilars or follow-on biologics.⁸⁹ A biosimilar is defined by the World Health Organization as a biotherapeutic agent, which is similar in terms of quality, safety, and efficacy to an already licensed biotherapeutic (innovator) product. Unlike small molecules, biologics are much more complex and produced in living cells through rDNA technologies rendering their exact replication impossible. Consequently, the approval process of a biosimilar is highly regulated and requires a comparability exercise between biosimilar and innovator product in terms of physicochemical and biological comparability (quality studies), preclinical comparability (*in vitro* and *in vivo* studies), and clinical comparability.^{90,91} The first biosimilar product, Zarxio (filgrastim), was FDA approved in 2015. Eight additional biosimilars were approved by the end of 2017 as shown in [Table 5.5](#).

TABLE 5.5
FDA-Approved Biosimilars

Drug Name	Approval Date	Indication

Drug Name	Approval Date	Indication
Zarxio (filgrastim)	March 2015	Increases white blood cells to reduce the risk of infection
Inflectra (infliximab)	April 2016	As a tumor necrosis factor blocker to treat inflammatory diseases
Erelzi (etanercept)	August 2016	As a tumor necrosis factor blocker to treat inflammatory diseases
Amjevita (adalimumab)	September 2016	As a tumor necrosis factor blocker to treat inflammatory diseases
Renflexis (infliximab)	May 2017	As a tumor necrosis factor blocker to treat inflammatory diseases
Cyltezo (adalimumab)	August 2017	As a tumor necrosis factor blocker to treat inflammatory diseases
Mvasi (bevacizumab)	September 2017	Vascular endothelial growth factor-specific angiogenesis inhibitor to treat wide range cancers
Ogivri (trastuzumab)	December 2017	HER2/neu receptor antagonist for treating breast and gastric cancers
Ixifi (infliximab)	December 2017	As a tumor necrosis factor blocker to treat inflammatory diseases

Data from

<https://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/Biosimilars/ucm580432.htm>.

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