1 Review of Drug Metabolism in Drug Discovery and Development

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1.1 SUMMARY

Drug metabolism is a physiological phenomenon in which xenobiotic compounds are chemically transformed into metabolites of the parent drug. Drug metabolism comprises a diverse set of chemical reactions within four general categories: oxidation, reduction, conjugation, and hydrolysis. These general categories of chemical reactions correspond to general categories of enzymes, which are responsible for catalyzing the reactions. The object of drug metabolism is to clear the xenobiotics from the body, so that the metabolites tend to be more polar and soluble than the parent drug, making them easier to excrete. Transporters are now recognized as a necessary component of drug metabolism, since they facilitate penetration of the parent drug into metabolizing organs and passage of ionic metabolites across cell membranes into the excreta. Drug metabolism is important in the clinical action of drugs because it is often the main means by which drugs are cleared from the body, so the rate of metabolism is one determinant of the elimination half-life of the drug. Drug metabolism is additionally important because the metabolites may have pharmacological or toxicological properties, which are superimposed on the clinical profile of the parent drug. For these reasons, the drug discovery process aims to design molecules with rates of metabolism appropriate for clinical use and pathways of metabolism which minimize side effects or toxicities attributable to metabolites. In clinical development, characterization of the metabolic

Encyclopedia of Drug Metabolism and Interactions, 6-Volume Set, First Edition.
Edited by Alexander V. Lyubimov.

pathways, the major circulating metabolites, and the enzymes that produce these metabolites is necessary for a full understanding of the clinical profile of a new drug. Accordingly, several types of clinical studies of metabolism are mandated for new drug registration, including identification and quantitation of major circulating metabolites, determination of the major pathways of clearance (CL) and their associated metabolic enzymes, characterization of drug–drug interactions based on metabolic phenomena, and assessment of the extent of excretion of drug-derived materials from the body.

1.2 INTRODUCTION

When an organic compound enters the human body, it is normally (i) utilized as a nutrient, (ii) directly excreted, or (iii) chemically modified and then excreted. In the case of a nutrient, the molecules enter specific biochemical pathways that either split them into small units followed by complete oxidation to generate energy (*catabolism*) or utilize them as precursors for constructing physiological molecules such as nucleic acids, polysaccharides, proteins, and triglycerides (*anabolism*). The overall process of utilization of nutrients is called *intermediary metabolism*. An example is the splitting of dietary fatty acids such as palmitic acid into two-carbon acetyl CoA units that can be oxidized in the mitochondria to produce energy (as ATP).

$$CH_3-(CH_2)_{14}-COOH \rightarrow 8CH_3CO-SCoA \rightarrow 16CO_2 + 12H_2O\ (+107ATP)$$

In cases of nonnutrient compounds (*xenobiotics*), however, pathways for significant energy production seldom exist, although in some cases the body is able to partially metabolize an organic compound for its energy content, as for instance with N-demethylation of drugs (Section 1.3).

$$R-NMe_2 + NADPH + O_2 \rightarrow R-NHMe + HCHO \rightarrow HCOOH + NADH \rightarrow CO_2 + NADH\ (\text{equivalent to } 3ATPs)$$

The methyl group is released as formaldehyde, which is further oxidized to formate and finally carbon dioxide, generating 2 mol of NADH. However, since 1 mol of NADPH must be invested for the metabolic demethylation, then the net energy production is 1 mol of NADH, equivalent to 3 mols of ATP. Most such reactions of xenobiotics are either energy-neutral (e.g., hydrolyses) or actually energy-consumptive (e.g., hydroxylations), since they produce no energy equivalents, but may use cofactors such as NADPH, PAPS, SAM (*S*-adenosine-L-methionine), or UDPGA, which require cellular ATP equivalents for their synthesis.

With the majority of xenobiotics, only a limited set of nonspecific chemical modifications is possible. This process is called *drug metabolism*, although it occurs with all absorbed foreign compounds, and not just drugs. To avoid confusion with intermediary metabolism, drug metabolism is sometimes called *biotransformation*. However, in fact, there is rarely any serious confusion between these two terms, and the term *biotransformation* is not really descriptive enough to convey a clear meaning in any event. So, most scientists working in this field simply call it *drug metabolism*. For the purposes of this chapter, we make no distinction between xenobiotic chemical

compounds that are unintentionally introduced into the body (e.g., natural plant alkaloids or environmental chemicals) and those that are intentionally dosed (e.g., medicinal drugs). The same CL mechanisms operate on all xenobiotics, and we use the term *drug metabolism* to describe the chemical modification of any nonphysiological compound. Drug metabolism occurs in all species, from bacteria to humans, but our primary focus in this chapter is the human phenomenon, only with reference to other species, as they are relevant to the process of discovery and development of new drugs. An increasing proportion of new drugs are proteins and nucleic acids (i.e., *biologics*), but the scope of this chapter is limited to the discussion of traditional small-molecule organic compounds.

The recognition that foreign substances may be metabolized in the body goes back almost two centuries, and an interesting history of the early discoveries is available in the form of a journal article [1] or website [2]. About 60 years ago, biochemists began to recognize drug metabolism as a distinct field of study. Soon, scientists in academia, pharmaceutical companies, and regulatory agencies realized that characterization of the metabolic fate of drugs was an important component in understanding their clinical profiles. Initially, it was sufficient to merely demonstrate that a dosed drug and/or its metabolites were eliminated from the body in a reasonable amount of time. Next, in the evolution of drug metabolism, there was a need to determine the chemical form of the major drug-related materials in the excreta. Today, the potential role of circulating metabolites in therapeutic action as well as toxicity has become apparent, and a sophisticated quantitative chemical, biochemical, pharmacological, and toxicological description of metabolism is required for the registration of new drugs.

Finally, we can ask "What is the object of drug metabolism?" As can be seen in subsequent sections, drug metabolism is more than just an attempt by the body to "eat" ingested foreign compounds. The existence of a complex, regulated, and interacting set of barriers and CL mechanisms suggests that the object is to chemically and physically limit the entry of these compounds to the body and facilitate their removal from the body. Those compounds that are not clearable by direct excretion in urine or feces are subject to sequential rounds of metabolism, which change their chemical and physical properties until they *can* be excreted. With these thoughts in mind, let us discuss in detail exactly what drug metabolism is and why it is important to the discovery and development of new drugs.

1.3 THE PHENOMENON OF DRUG METABOLISM

Drug metabolism comprises such a rich variety of chemical modifications of organic compounds that it is rare to find a drug that is not subject to some type of metabolic process. Of course, there is a kinetic component of the drug-metabolism process as well, so in some cases the metabolism occurs only slowly. For example, amiodarone is cleared from the body exclusively by metabolism [3], but because the metabolic process is very slow, this drug has a 55-day terminal half-life [4]. In other cases, the direct excretion process is much faster than metabolism and dominates CL. So we find that amoxicillin, for example, is mainly excreted as the intact parent drug in urine [5]. Nonetheless, most drugs are rapidly metabolized as the major route of CL from the body. Although the diverse manifold of possible reactions presents a complex array of

possibilities, we can recognize some patterns, so that it is possible to use relatively few terms to describe virtually all the transformations. In fact, there are only four major categories: oxidations, reductions, conjugations, and hydrolyses.

These four categories of chemical transformation arise from four broad classes of enzymes, which actually accomplish the transformation for a particular drug. It is common, if not universal, for a particular drug to be subject to more than one metabolic conversion. For example, dextromethorphan has two major metabolites, one resulting from N-demethylation and the other from O-demethylation (Fig. 1.1).

As one would expect, some metabolites are the product of sequential operation of two or more of these basic transformations, as shown for loratadine, which undergoes oxidative decarboethoxylation followed by aromatic hydroxylation and finally glucuronidation (Fig. 1.2).

Occasionally, a metabolic pathway apparently not conforming to this fourfold categorization is encountered, as shown in Fig. 1.3.

However, almost always, closer examination shows that the process was actually a diversion during operation of one of the four standard categories [8,9]. In the example in Fig. 1.3, pulegone is first hydroxylated on an allylic methyl group, followed by internal hemiketal formation and dehydrative aromatization to form menthofuran [10].

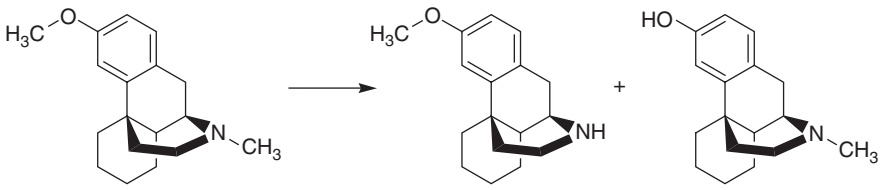

Figure 1.1 N- and O-demethylation of dextromethorphan [6].

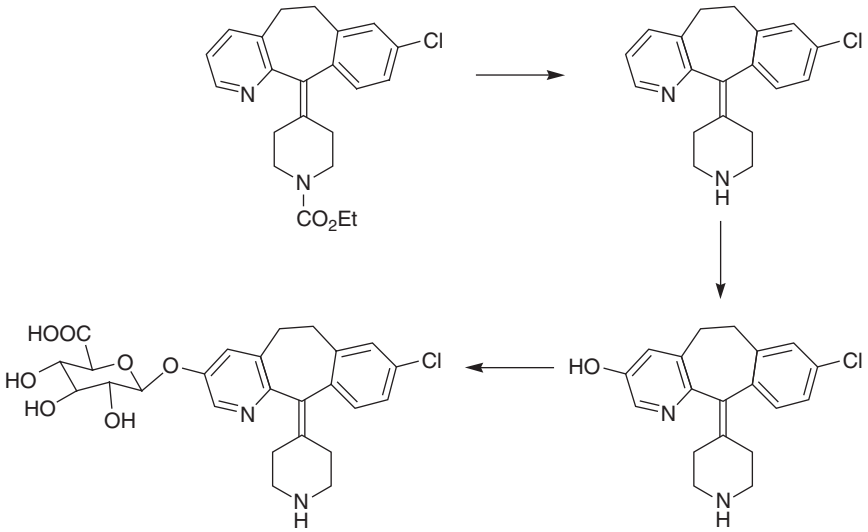

Figure 1.2 Metabolic pathway for loratadine [7].

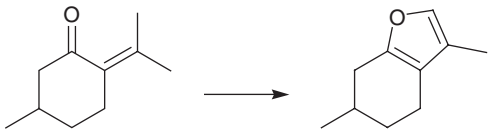

Figure 1.3 Ring closure of pulegone to menthofuran.

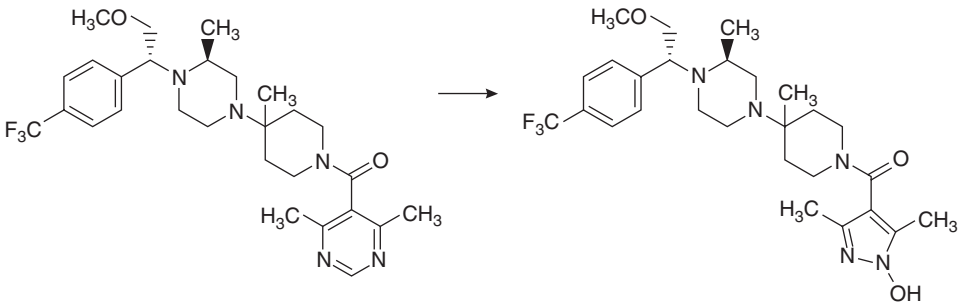

Figure 1.4 Ring contraction of vicriviroc [11].

A more vexing example is provided below by the contraction of a six-membered pyrimidine ring to a five-membered pyrazole ring in the anti-HIV drug vicriviroc (Fig. 1.4). Although no explanation for this reaction had been published, one can write a plausible, albeit complex, metabolic pathway linking the parent drug and the metabolite utilizing only known reactions from the four standard categories (details left as an exercise for the reader).

Although many interesting chemical transformations are known in biochemistry, we limit our discussion here only to ones that have been demonstrated to occur with xenobiotics. Table 1.1 summarizes the main chemical reactions of human drug metabolism. Each metabolic reaction has been given a name descriptive of the overall chemical transformation that occurs, regardless of the internal mechanism by which the transformation was accomplished. However, in many cases, the metabolic reaction has a name commonly used in the published literature of drug metabolism. For example, "introduction of an oxygen atom at an aliphatic position" would typically be referred to as *Hydroxylation*, even though an oxygen atom, not a hydroxyl group was added to the molecule. The common name is given in parenthesis. Note that the drugs and metabolites are drawn in their unionized forms to better see the chemical transformation that has occurred. Although Table 1.1 is intended to be reasonably comprehensive for introductory purposes, it is not exhaustive. A number of unusual examples have been compiled elsewhere [8,9].

Finally, it is worth noting that some metabolic reactions can be reversed, leading to the phenomenon of *futile cycling*. A metabolite that is produced through one of the metabolic reactions in Table 1.1 may be reconverted to the parent drug by another metabolic reaction, with no net chemical transformation. An example is the conversion of a tertiary amine to an *N*-oxide (Reaction I.F). Since some *N*-oxides can be reduced to tertiary amines (Reaction II.B), the result is that the amine appears not to have been metabolized, when in fact two metabolic steps occurred [32]. The existence

TABLE 1.1 Chemical Transformations Comprising Drug Metabolism

I. Oxidations

A. Insertion of an oxygen atom into an aliphatic C–H bond (aliphatic hydroxylation) (Fig. 1.5)
$R_3C–H \rightarrow R_3C–OH$

B. Insertion of an oxygen atom into an aromatic C–H bond (aromatic hydroxylation) (Fig. 1.6)
Ar–H → Ar–OH Although this reaction is superficially equivalent to Reaction I.A, the underlying mechanism is quite different, justifying a separate designation.

C. Carbon–carbon bond cleavage (C-dealkylation) (Figs. 1.7 and 1.8)
$R–R' \rightarrow R–H$ or $R–CH_2–COOH \rightarrow R–COOH$

D. Carbon–heteroatom bond cleavage (N, O, or S-dealkylation) (Fig. 1.9)
$R_2CH–NR'_2 \rightarrow R_2C{=}O + R'_2N–H$
$R_2CH–OR' \rightarrow R_2C{=}O + R'O–H$
$R_2CH–SR' \rightarrow R_2C{=}O + R'S–H$
Although dealkylation is sometimes presented as a variant of Reaction I.A in which the alkyl group is merely hydroxylated followed by spontaneous dissociation to the observed amine, alcohol, or thiol and carbonyl products, the actual mechanism is distinct, justifying a separate designation.

E. Addition of an oxygen atom to an alkene or alkyne (epoxidation) (Fig. 1.10)

F. Addition of an oxygen atom to a heteroatom (N- or S-oxidation) (Fig. 1.11)
$R_3N \rightarrow R_3N \rightarrow O$
$R_2S \rightarrow R_2S \rightarrow O$
$R_2NH \rightarrow R_2N–OH$

G. Oxidative dehalogenation (Fig. 1.12)
para-$Y–C_6H_4–X \rightarrow$ *para*-$Y–C_6H_4–OH$
(X = F, Cl; Y = RNH, OH)

H. Dehydrogenations (Figs. 1.13 and 1.14)
$R_2CH–OH \rightarrow R_2C{=}O$
$R_2CH–CHR'_2 \rightarrow R_2C{=}CR'_2$

I. Aromatizations (Fig. 1.15)

II. Reductions

A. Hydrogenation of a carbonyl group (Fig. 1.16)
$R_2C{=}O \rightarrow R_2CH–OH$

B. Deoxygenation of *N*- or *S*-oxides (*N*- or *S*-oxide reduction) (Fig. 1.17)
$R_3N \rightarrow O \rightarrow R_3N$
$R_2S \rightarrow O \rightarrow R_2S$

C. Reductive dehalogenation (Fig. 1.18)
R–X → R–H (X = Br, Cl)

D. Reduction of N–N and N–O bonds
Nitro reduction: $Ar–NO_2 \rightarrow Ar–N{=}O \rightarrow Ar–NH–OH \rightarrow Ar–NH_2$
Azo reduction: $Ar–N{=}N–Ar \rightarrow Ar–NH–NH–Ar \rightarrow Ar–NH_2$

Isoxazole reduction (Fig. 1.19)

TABLE 1.1 (*continued*)

III. Adductions and Condensations (Conjugations)
A. Acetylation (Fig. 1.20)
$Ar–XH \rightarrow Ar–X–COCH_3$ (X = N or O)

B. Amide formation (Fig. 1.21)
$RCOOH \rightarrow RCONH–R'R'$ = glycine, taurine, and other amino acids

C. Glutathionation (Nucleophilic addition of the tripeptide glutathione (GSH (reduced glutathione), Glu-Gly-Cys-SH) to an electrophilic carbon atom in a xenobiotic molecule) (Fig. 1.22)
$R_3C–L \rightarrow R_3C–SG$ (L is a leaving group)

D. Glycosylation (Fig. 1.23)
$R_3C–XH \rightarrow R_3C–X–Glycosyl$
(X = O or N; Glycosyl = glucose, glucuronic acid; R_3C- may be aliphatic or aromatic)

E. Methylation (Fig. 1.24)
$R–XH \rightarrow R–X–Me$ (X = O or S)

F. Phosphorylation (Fig. 1.25)
Base-Ribose-OH + ATP → Base-Ribose-O + ADP

G. Sulfurylation (Sulfation) (Fig. 1.26)
$R_3C–OH \rightarrow R_3C–OSO_3H$ (R_3C may be aliphatic or aromatic)

IV. Hydrolyses
A. Esters, amides and phosphates (Fig. 1.27)
$RC(=O)–X + H_2O \rightarrow RC(=O)–OH(X = OR' \text{or } NR'_2)$
$RO–PO_2–OR' + H_2O \rightarrow RO–PO_2–OH$

B. Hydrolysis of glycosides
$R_3C–X–Glycosyl + H_2O \rightarrow R_3C–XH$
(X = O or N; Glycosyl = glucose, glucuronic acid; R_3C- may be aliphatic or aromatic)

C. Hydrolysis of epoxides (Fig. 1.28)

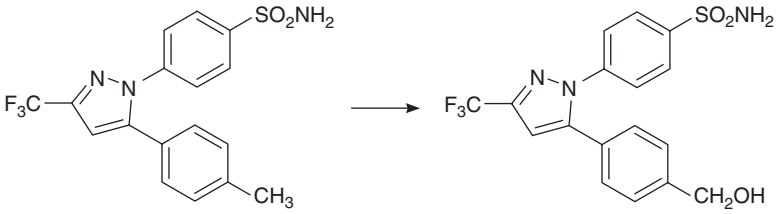

Figure 1.5 Benzylic hydroxylation of COX-2 inhibitor celecoxib [12].

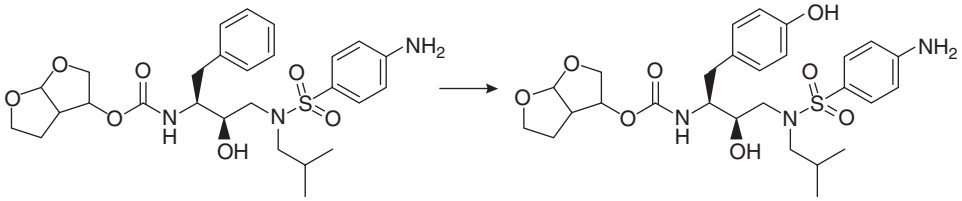

Figure 1.6 Phenyl hydroxylation of HIV protease inhibitor darunavir [13].

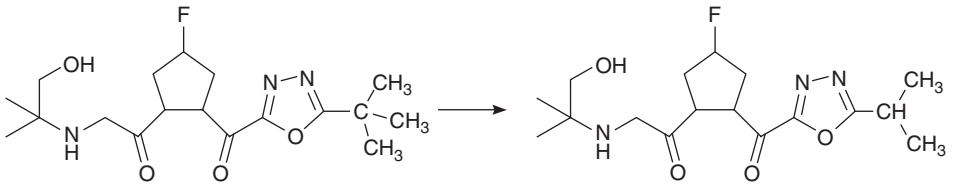

Figure 1.7 C-Demethylation of DPP4 inhibitor LC15-0133 [14].

Figure 1.8 β-Oxidation of thromboxane A_2 receptor antagonist (+)-*S*-145 [15].

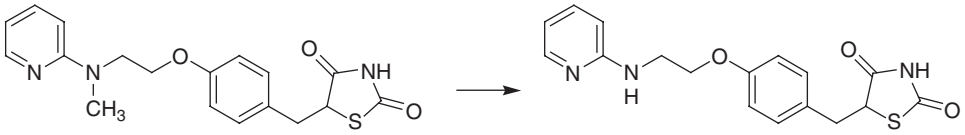

Figure 1.9 N-Demethylation of PPARγ agonist rosiglitazone [16].

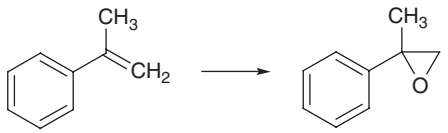

Figure 1.10 Epoxidation of plastics monomer α-methylstyrene [17].

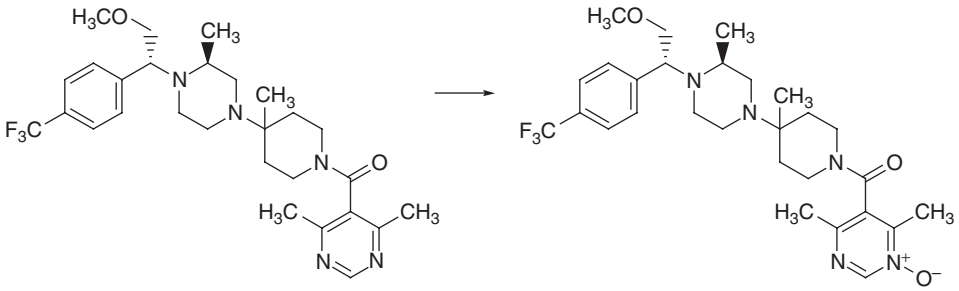

Figure 1.11 N-Oxidation of HIV entry inhibitor vicriviroc [11].

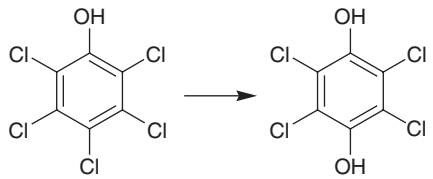

Figure 1.12 Dechlorination of pentachlorophenol [18].

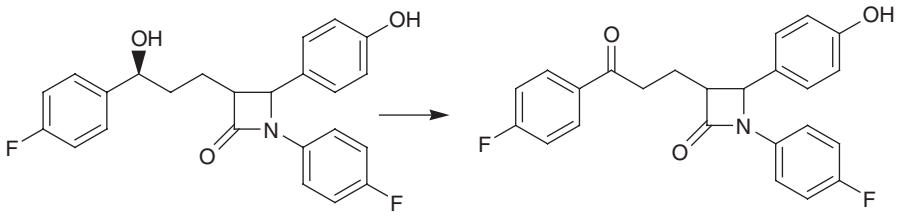

Figure 1.13 Dehydrogenation of anticholesterolemic drug ezetimibe to the corresponding ketone [19].

Figure 1.14 Dehydrogenation of anticonvulsant valproic acid [20].

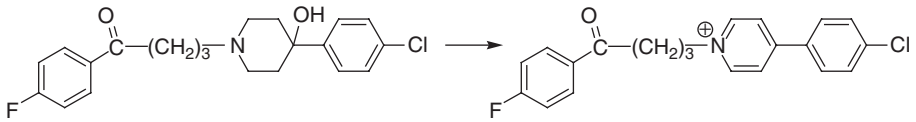

Figure 1.15 Conversion of neuroleptic agent haloperidol to pyridinium ion [21].

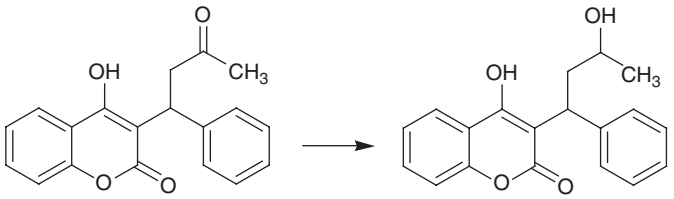

Figure 1.16 Reduction of the keto group of anticoagulant warfarin to a secondary alcohol [22].

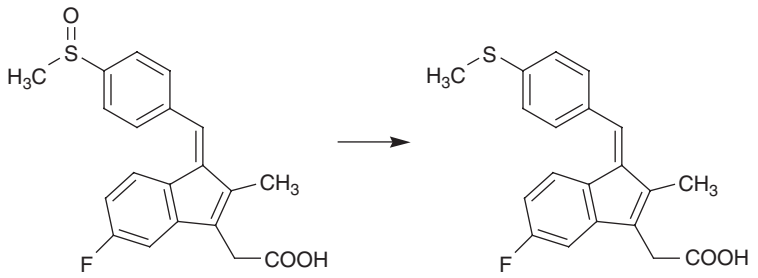

Figure 1.17 Reduction of sulfoxide group of NSAID sulindac to the sulfide [23].

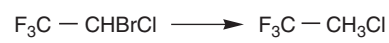

Figure 1.18 Reduction of general anesthetic halothane [24].

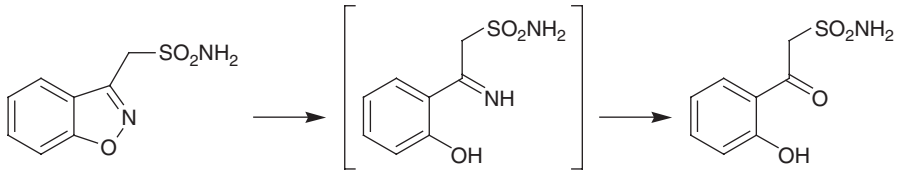

Figure 1.19 Reduction of the anticonvulsant zonisamide [25].

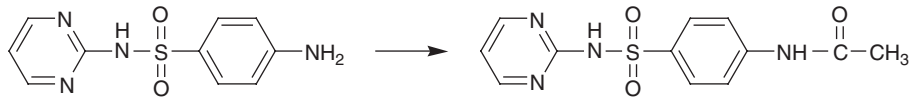

Figure 1.20 N-Acetylation of antiinfective sulfadiazine [26].

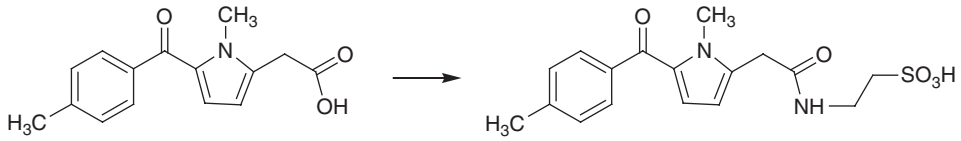

Figure 1.21 Taurine conjugation of NSAID tolmetin [27].

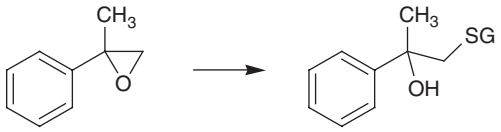

Figure 1.22 Addition of glutathione to α-methyl styrene oxide [17].

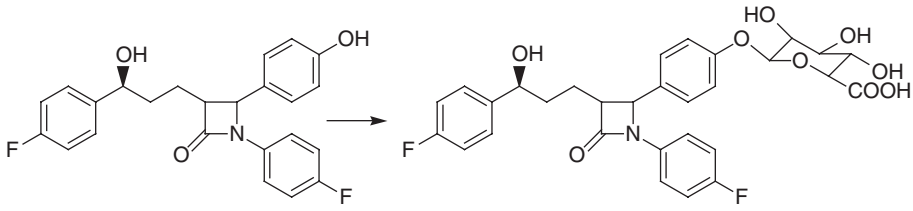

Figure 1.23 Phenolic glucuronidation of anticholesterolemic drug ezetimibe [19].

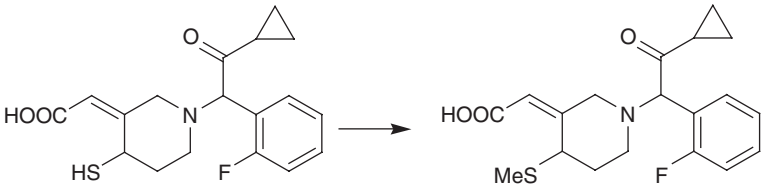

Figure 1.24 S-Methylation of active thiol metabolite of antithrombotic agent prasugrel [28].

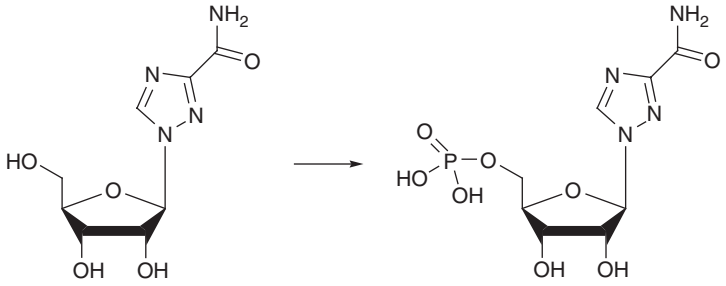

Figure 1.25 Activation of antiviral agent ribavirin by 5′-phosphorylation [29].

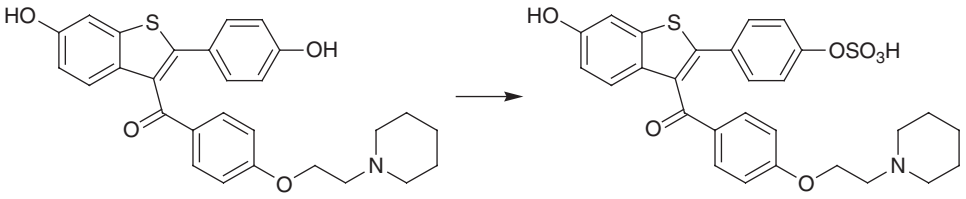

Figure 1.26 Phenolic sulfation of synthetic estrogen raloxifene [30].

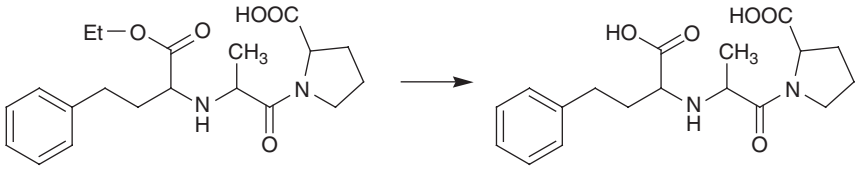

Figure 1.27 Conversion of the prodrug enalapril to active ACE inhibitor enalaprilat [31].

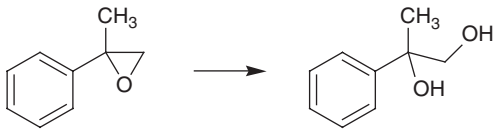

Figure 1.28 Hydrolysis of α-methylstyrene oxide [17].

of the separate oxidation and reduction steps can usually be demonstrated by *in vitro* experiments under controlled conditions. Futile cycling may also occur with the hydroxy-to-keto conversion, sulfation/desulfation, phosphorylation/dephosphorylation, and acetylation/deacetylation [33]. An important variation is seen with glucuronidation of a phenolic drug (Reaction III.D), followed by excretion of the *O*-glucuronide into the intestine through the bile. Intestinal bacteria often efficiently hydrolyze glucuronides back to the parent drug (Reaction IV.B), which may be reabsorbed through the intestinal wall [19]. This *entero-hepatic circulation* may be detected by an anomalous pharmacokinetic (PK) profile, and confirmed by collection of bile to confirm the presence of the glucuronide as well as by *in vitro* experiments showing that the parent drug is readily glucuronidated. Another special case of futile cycling is called *redox cycling*, in which hydroxylation of an aromatic ring on a drug produces a hydroquinone, which is then one-electron oxidized by molecular oxygen to a semiquinone radical and then a quinone [34,35]. Quinones are often easily metabolically reduced back to hydroquinones to start a new cycle, which may be repeated many times. This is an especially undesirable type of metabolism, because it amounts to the drug acting as a catalyst to convert oxygen to dangerous *reactive oxygen species* such as superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals. These several examples of futile cycling show that there can be "hidden" metabolism, and some genetically variant enzymes or drug–drug interactions may interfere with one of the counterbalancing reactions, causing unexpected metabolic effects. Thus, drug-metabolism scientists who are developing drug candidates with potentially reversible metabolic pathways normally investigate whether futile cycling is occurring *in vivo*.

1.4 THE DRUG DISCOVERY AND DEVELOPMENT PROCESS

The study of drug metabolism is a distinct academic science including basic research, specialized scientific societies, and dedicated journals. However, as the name suggests, the biggest application of the science of drug metabolism is in the understanding of the clinical actions of new therapeutic agents and in satisfying regulatory requirements for their registration. For this reason, the majority of metabolism data appearing in the literature for specific drugs results from industrial drug development. Accordingly, we briefly outline the new drug discovery and development process to provide the framework for discussion of this important application of drug metabolism.

1.4.1 Discovery

Basic research elucidates the complex biochemical events that comprise a physiological process such as regulation of blood glucose, and identifies key biochemical control points mediated by enzymes or receptors. Drug discovery begins with a new concept for therapeutic intervention of a disease based on the knowledge gleaned from such research. Most drugs exert their pharmacological action by modulating the activity of one of these enzymes or receptors within cells of the abnormally functioning organ. So the next step is *lead generation*, the design or discovery of a small molecule that will bind to the molecular target in such a way as to modulate its activity. Once a lead has been generated, medicinal chemists typically synthesize hundreds to thousands of analogs by systematically varying the structure to create a compound that has

been optimized with respect to potency and selectivity toward the molecular target, PK characteristics, and safety. Numerous *in vitro* and *in vivo* screening assays are established to determine which compounds have these properties in the acceptable range for the intended medical indication [36]. Since drug discovery normally takes place before the clinical phase, the human PK characteristics of a drug candidate must be inferred from *in vivo* animal models or from various human-derived *in vitro* systems [37]. The data output from these screens is used iteratively to design better molecules. Once a discovery compound has been identified with overall favorable "druglike" characteristics (good potency, selectivity, safety, and PK), it is designated as a *clinical drug candidate* and enters the development process [38].

1.4.2 Preclinical Development

Before a new chemical entity can be tested in humans, its safety in various *in vitro* and *in vivo* pharmacological and toxicity tests must be assessed [39]. This includes administration of high doses of the drug candidate to a rodent species (mice or rats) and a large animal species (dogs or monkeys), as well as *in vitro* tests of genotoxic potential (such as the Ames test). In parallel with the safety testing, a large-scale chemical synthetic process must be developed to produce the tens of kilograms of the drug candidate required to conduct clinical trials. Finally, a clinical formulation must be devised that provides adequate and reproducible exposure of subjects in the clinical trials, and animal PK studies are helpful in this design process. Data from the discovery and preclinical activities form the basis of a petition to government health authorities for permission to begin testing in humans. Once this dossier, called the *Investigational New Drug Application* (IND) in the United States, is approved, clinical trials may proceed [40].

1.4.3 Clinical Development

Clinical trials are formally divided into four distinct, but temporally overlapping phases (I–IV), defined by the objectives of each phase [41].

Phase I tests the safety and tolerability of the drug candidate in normal healthy volunteer subjects. The primary goal is to determine the safety profile and the maximum dose that may be safely given to people. An additional goal is the determination of PK parameters, which are needed to plan the next phases. Other phase I PK studies include metabolism and elimination of the drug [42], detection of circulating metabolites of the drug [43], determination of the effect of food on orally administered drugs [44], formulation testing [45], and checking for PK interactions between coadministered drugs [46]. The most common form of PK drug–drug interaction is the competition of two drugs for the same CL mechanism, especially the CYP superfamily of drug-metabolizing enzymes [47]. These interactions, if present, place limitations on which drugs can ultimately be administered together clinically with the new agent [48]. All clinical PK studies rely on some method of quantitation of drug levels in blood or plasma, and the major bioanalytical technique in use today for this purpose is *liquid chromatography coupled to tandem mass spectrometry* (LC-MS/MS) [49,50]. Present technology permits determination of picogram-per-milliliter levels of drug in the presence of many interfering substances, with sample-to-sample cycle times <2 min.

Phase II investigates in limited numbers of patients whether the drug candidate modulates the pharmacological target (*proof of activity*) and whether this activity actually results in the desired therapeutic benefit (*proof of concept*). If proof of concept is established, several dose levels are next assessed for efficacy in patients with the disease to be treated. The optimum dose level is then chosen, based on a balance of benefit and safety. Although the main goal of phase II clinical trials is the determination of efficacy toward the disease indication, PK is a fundamental part of this goal, because to understand the clinical utility of the new drug candidate, it is necessary to define the temporal relationship between plasma levels and the pharmacodynamics of the beneficial effect (PK/PD). A secondary PK goal in phase II is to determine if the disease state affects the PK of the candidate drug compared to the PK seen in healthy subjects.

With an optimized dose identified, *phase III* tests the drug in much larger groups of patients to establish a comprehensive clinical profile of the new drug candidate. Phase III trials are intended to confirm a statistically significant benefit versus either placebo or, more usually, standard-of-care therapy. Additional phase III studies may investigate the variation of therapeutic response and safety in various severities of the disease and in special populations such as ethnic groups or comorbidities. Once phase III is complete, the sponsor of the drug candidate may submit an application (called the *New Drug Application* in the United States) for approval to government health authorities of all countries in which marketing is desired [51].

Phase IV usually occurs once the drug has been approved and is on the market. It involves testing in even larger populations and for longer periods of time, as well as testing against additional disease indications. However, monitoring patients for long-term safety issues (*pharmacovigilance*) may be the most important activity in phase IV, because the number of patients and the length of treatment allow detection of low frequency and/or long-term effects of the new drug that may not have been evident in phase III.

1.5 THE SIGNIFICANCE AND IMPORTANCE OF DRUG METABOLISM

Drug metabolism is centrally important to the clinical utility and effectiveness of most drugs. Metabolism is the main route of CL for nearly three-quarters of marketed drugs [53]. In fact, even the *absence* of metabolism is an important fact to be known about a drug's clinical profile. The importance of drug metabolism in drug action has four general aspects: CL of the drug from the body, pharmacological activity, toxicity, and drug interactions.

1.5.1 Clearance of Drugs from the Body

Persistence of the drug in the body is usually quantitated as the PK half-life ($t_{1/2}$) or the mean residence time (MRT), both of which are typically given in units of hours or days. Persistence is a function of the availability and capacity of the body's CL mechanisms, of which drug metabolism is often the most important.

In the frequent case that CL occurs by enzymatic metabolism of the drug, the rate of metabolism of the drug by the particular enzyme catalyzing the metabolic reaction

is determined by the Michaelis–Menten equation of enzyme kinetics (chapter titled ***Enzyme Kinetics of Drug-Metabolizing Reactions and Drug–Drug Interactions***).

$$\text{Rate} = \frac{V_{\max}[\text{Drug}]}{K_{\text{M}} + [\text{Drug}]} \tag{1.1}$$

In Equation 1.1, $V_{\max}$ is the rate that the enzyme can convert drug to metabolite when drug availability is not limiting (i.e., high drug concentrations), and K_{M} is a constant that determines the drug-concentration dependence of the enzyme rate. We can simplify Equation 1.1 by assuming that drug concentrations are always low compared to K_{M} (i.e., [Drug] $\ll K_{\text{M}}$), leading to Equation 1.2.

$$\text{Rate} = \frac{V_{\max}}{K_{\text{M}}}[\text{Drug}] \tag{1.2}$$

Thus, when the assumption of [Drug] $\ll K_{\text{M}}$ is obtained (a condition that is often true), the rate of metabolism is proportional to drug concentration (i.e., first-order kinetics), and in that case blood levels of the drug are proportional to dose. Since dose-linear PKs is usually a desirable property of a clinical drug, one of the selection criteria for new drug candidates during the discovery phase may be that K_{M}-values are high compared to the expected maximal clinical blood levels.

Because metabolizing enzymes are usually concentrated in a CL organ (e.g., liver), CL of a drug from the blood is governed by mass transport to the CL organ in addition to the enzymatic capacity of that organ to remove the drug from blood, as expressed in the following relationship.

$$\text{CL} = \frac{f_u \text{CL}_{\text{int}} Q}{f_u \text{CL}_{\text{int}} + Q} \tag{1.3}$$

In Equation 1.3, CL is the rate at which blood is cleared of the drug by the CL organ, with units of L/h. Intrinsic CL (CL_{int}) is the capacity of the organ to clear the drug in the absence of mass transport restrictions on presentation of the drug to the organ. Q is the rate of blood flow to the CL organ and f_u is the fraction of drug not bound to plasma proteins such as albumin [54].

CL can also be expressed as the concentration-normalized rate, so we may define the intrinsic ability of the enzyme to metabolize the drug as the intrinsic CL (CL_{int}) by dividing Equation (1.2) by [Drug].

$$\text{CL}_{\text{int}} = \frac{V_{\max}}{K_{\text{M}}} \tag{1.4}$$

Substituting Equation 1.4 into Equation 1.3 yields Equation 1.5, which shows that CL is a function of enzyme kinetics and organ blood flows.

$$\text{CL} = \frac{f_u \frac{V_{\max}}{K_{\text{M}}} Q}{f_u \frac{V_{\max}}{K_{\text{M}}} + Q} \tag{1.5}$$

Equation 1.5 can be further simplified by recognizing that many drugs are so-called "low CL" drugs, meaning that the intrinsic CL is low compared to the blood flow.

$$\mathrm{CL} = f_u \frac{V_{\max}}{K_{\mathrm{M}}} \tag{1.6}$$

Since half-life is a more intuitive concept for most scientists than CL, we note that for an ideal drug (i.e., one-compartment PKs), the half-life ($t_{1/2}$) of the drug in the body is related to its CL in a reciprocal fashion,

$$t_{1/2} = \frac{0.693 V_{\mathrm{d}}}{\mathrm{CL}} \tag{1.7}$$

where V_{d} is the volume of distribution. Substituting Equation 1.7 into Equation 1.6 yields the final expression.

$$t_{1/2} = \frac{0.693 V_{\mathrm{d}}}{f_u \frac{V_{\max}}{K_{\mathrm{M}}}} \tag{1.8}$$

Equation 1.8 shows that the values of the enzymatic constants $V_{\max}$ and K_{M} for a particular enzyme–drug pair will determine whether the drug is low or high CL and, therefore, whether the half-life is long or short. Note that Equation 1.8 was derived for the simple case in which [Drug] $\ll K_{\mathrm{M}}$ and $\mathrm{CL_{int}} \ll Q$ in order to arrive at an equation simple enough to be intuitively understood. Omitting those assumptions would make Equation 1.8 more mathematically complex, but would not alter the qualitative conclusion that the half-life of a drug is inversely related to the enzymatic rate of its metabolism. Equation 1.8 is also used in later sections to rationalize the phenomena of drug interactions, induction, and genetic variation in drug metabolism.

During the drug discovery process, Equation 1.8 provides a means for medicinal chemists to adjust the half-life of their drug candidates by changing the molecular structure to reduce $V_{\max}/K_{\mathrm{M}}$ (to increase half-life) or increase $V_{\max}/K_{\mathrm{M}}$ (to decrease half-life). For instance, if the half-life in animals is very short, it is likely to be short in humans as well. Thus, the chemists would try to design a molecule that is less metabolically labile by identifying the point on the molecule that is susceptible to rapid metabolism (the *metabolic hot spot*; e.g., a methoxy group subject to O-demethylation) and replacing it with a molecular configuration that is more metabolically stable (e.g., substituting OCF_3 for OCH_3). The $V_{\max}/K_{\mathrm{M}}$ ratio (i.e., $\mathrm{CL_{int}}$) of a series of modified molecules can be quickly checked with an appropriate *in vitro* system containing the metabolic enzyme responsible for the metabolism (e.g., cytochrome P450 in liver microsomes). Unfortunately, merely replacing the metabolized group with something that is less easily metabolized does not always lengthen the half-life, since metabolism sometimes shifts to another site on the molecule (*metabolic switching*). Thus, a series of molecules must usually be synthesized and tested to find one that is actually metabolized more slowly, and the ability to test many molecules quickly with metabolic enzymes rather than whole-animal studies greatly facilitates this aspect of drug design. Conversely, a candidate molecule may be metabolized so slowly that the expected human half-life would be unacceptably long. In this case, a common approach is for the chemists to introduce a *metabolic soft spot* to provide an easier point for metabolic enzymes to attack the molecule. Again, a series of modifications would usually be tested with enzymes to find one with the right $V_{\max}/K_{\mathrm{M}}$ ratio, as reflected in the observed half-life.

1.5.2 Pharmacological Activity of Metabolites

It is important to note that CL refers to the elimination from the body of a particular chemical compound, in this discussion the parent drug. Thus, chemical transformation to a metabolite is a form of CL, even though the same mass of drug-related material may remain in the body after the metabolism. Consequently, if the metabolite(s) are pharmacologically active, biological effects may continue even after the parent drug has been cleared. Since metabolites are often only subtly different in chemical structure from the parent drug (e.g., conversion of a tertiary amine to a secondary amine by N-demethylation), it is not surprising that some metabolites retain binding affinity for the biological target. These are called *active metabolites*, and they may contribute substantially to the overall pharmacological effect of the administered drug. For example, in the nonsedating antihistamine loratadine shown in Fig. 1.2, the descarboethoxy metabolite also displays prominent antihistaminic activity [7]. Accordingly, characterization and quantitation of metabolites and their residence times in the body are just as important as they are for the parent drug in developing a full understanding of the clinical profile of a new drug candidate.

Sometimes the parent compound itself has essentially no pharmacological activity and must be converted to an active form by metabolism in the body. Such compounds are called *prodrugs*, and they may be used to circumvent barriers of solubility or intestinal absorption. The inactive prodrug compound may have solubility favorable for preparation of a soluble intravenous formulation or membrane permeability that allows for good oral absorption. Once in the body, enzymes chemically change the prodrug to an active compound which is then available to the biological target. For example, as shown in Fig. 1.27 in Table 1.1, the pharmacologically active free acid enalaprilat is released from the inactive prodrug ester enalapril by intestinal, hepatic and plasma esterases. The difference between a compound producing an active metabolite and a prodrug, then, rests in whether the administered agent itself exhibits intrinsic pharmacological activity, but in both cases metabolism is involved in eliciting the full pharmacological action of the drug. An in-between case is that of the anticancer drug tamoxifen, which has an active four-hydroxy metabolite that is 30–100X more potent than tamoxifen itself. Thus, the extent to which an individual patient metabolizes tamoxifen affects the outcome of treatment [55].

1.5.3 Toxicity of Metabolites

A variation on the concept of active metabolites is the case in which the metabolite has different pharmacological activities from those of the parent drug. These different activities arise from binding of the metabolite to receptors other than those that the parent binds, including being less selective for binding to the particular receptor subtype for which the parent drug was optimized. These additional undesired activities would manifest as *adverse effects* of the drug and could range from mild to severe. Some metabolites can even display frank toxicity, such as cyanide metabolically released from the industrial chemicals acetonitrile and acrylonitrile [56,57] or the nephrotoxin thioformamide formed from the antihelminthic thiabendazole [58].

An important but currently poorly understood type of toxicity due to metabolism results from the production of *reactive metabolites* [59]. These are metabolites that have chemical reactivity that causes them to react with normal physiological compounds present in cells. The normal cellular components with which these metabolites

react are typically nucleophiles (amines, thiols, alcohols, phenols, or nucleobases) and range from small molecules such as glutathione to macromolecules such as proteins or nucleic acids. In each case, the normal component is chemically altered in such a way that its usual function in cellular biology is altered or disrupted. We can, somewhat arbitrarily, further distinguish between reactive metabolites and reactive intermediates. A reactive *intermediate* is so unstable that it immediately reacts with chemical moieties inside the active site of the enzyme that formed it. Conversely, a reactive *metabolite* is stable enough to be released from the active site of the enzyme that formed it and, is thus, potentially able to react with any target molecule in the cell. Numerous examples of reactive intermediates are known, especially with the cytochrome P450 class of enzymes, which are often victims of irreversible metabolism-based inhibition because of the formation of extremely reactive electrophilic intermediates resulting from oxidation of certain drugs. An example is the reactive oxirene resulting from oxidation of the acetylene functional group of ethynylestradiol by CYP3A5 [52]. Likewise, many examples of reactive metabolites have been reported, of which the classic example is *N*-acetyl phenyliminoquinone (NAPIQ), formed by sequential oxidations and conjugations of the commonly used painkiller acetaminophen (paracetamol) [59]. NAPIQ has been shown to covalently bind to an array of intracellular nucleophiles.

With each type of metabolite-derived toxicity, characterization of metabolites would be necessary to fully understand the clinical profile of a drug. International regulatory authorities have recognized this fact by issuing guidances to developers of new drugs, requiring characterization of the toxicological properties of major metabolites circulating in blood in humans. The central dictum is that these major human metabolites must also be present in the circulation of preclinical species used for assessment of safety of new drugs, at exposure levels at least equal to those in humans. Metabolites that are unique to humans may require synthesis and direct dosing for assessment of safety in animals [60,61].

1.5.4 Drug Interactions Related to Metabolic Processes

The final area of importance of human drug metabolism is the occurrence of *PK drug–drug interactions* arising from modulation by one drug (the *perpetrator* drug) of the catalytic activity of a drug-metabolizing enzyme that is involved in the CL of a second drug (the *victim* drug). These modulations take two forms: *inhibition* and *induction* (chapters titled ***Enzyme Kinetics of Drug-Metabolizing Reactions and Drug–Drug Interactions***).

Inhibition arises from the fact that drug-metabolizing enzymes are generally nonspecific and typically recognize multiple drugs as substrates. Thus, when two or more drugs are in the body simultaneously, they may act as competitive substrates for one of these enzymes (chapter titled ***Enzyme Kinetics of Drug-Metabolizing Reactions and Drug–Drug Interactions***). Modifying Equation 1.8 by the form of the Michaelis–Menten equation that describes competitive inhibition, we can derive Equation 1.9, which shows the effect on the half-life of a drug by a competitive drug, I.

$$t_{1/2} = \frac{0.693 V_{\mathrm{d}}}{f_u \dfrac{V_{\max}}{K_{\mathrm{M}}\left(1+\dfrac{[\mathrm{I}]}{K_{\mathrm{i}}}\right)}} \tag{1.9}$$

Equation 1.9 shows that the clinical effect of the presence of a second drug I with an inhibition constant K_i is to increase the elimination half-life of a drug, depending on the magnitude of the ratio $[I]/K_i$. Often there is also a concomitant increase in C_{max}. The increased exposure of the patient to the victim drug may result in adverse events or toxicity, so the drug–drug interactions due to inhibition of metabolism are an important safety concern for regulatory authorities. Accordingly, another metabolism-related selection criterion for new drugs is that $[I]/K_i$ should be $\ll 1$, and K_i-values are routinely measured for the most important drug-metabolizing enzymes when a sponsor proposes to bring a new drug candidate to clinical trials. Equation 1.9 assumes *reversibility* of the mutual inhibition between two drugs, so an important adjunct to measurement of K_i-values is the determination that the new drug candidate does not exhibit *metabolism-based inhibition*, which is *irreversible* in nature (also called *time-dependent inhibition*). Assessment of drug–drug interactions due to inhibition of CL by a metabolism-based inhibitor involves a different, more complex mathematical approach [62].

Drugs may also pharmacokinetically interact because of upregulation of the steady-state level of a metabolic enzyme, a phenomenon known as *enzyme induction* (chapter titled ***Transcriptional Regulation of Cytochrome* P450 *Genes***). The upregulation can occur through a variety of mechanisms, including (i) activation of xenobiotic-sensing receptors resulting in increased expression of the enzyme [63] and (ii) decrease of the cellular degradation rate of the enzyme. Induction usually requires several days of exposure to the inducing drug before the cell fully responds to the stimulus and a new steady-state level of the enzyme is attained. In Equation 1.8, V_{max} may be factored into $k_{cat} \cdot E_{tot}$, where k_{cat} is the catalytic rate constant of the enzyme and E_{tot} is the total amount of enzyme present in the system. If E_{tot} in the CL organ is increased by induction, then the total activity, as measured by V_{max} will increase. An increase in V_{max} translates to an increase in CL of the victim drug, a decrease in half-life and a possible decrease in C_{max} is obtained. This reduced exposure may result in loss of efficacy of the victim drug, so induction of drug-metabolizing enzymes is also an important concern for regulatory authorities. Since the induced enzyme may also be responsible for the metabolism of the inducing drug itself, this phenomenon is sometimes manifest as an adaptive response called *autoinduction*. Sequential doses of an autoinducing drug lead to lower C_{max}- and AUC-values of that drug. It is possible for autoinduction to be so severe that the drug becomes ineffective after a few doses. Thus, it is important in drug discovery to detect potent enzyme inducers and avoid advancement of such compounds into clinical development. Potent inducers of cytochrome P450 enzymes can be eliminated by higher-throughput *in vitro* assays with gene-reporter assays linked to receptors such as PXR (pregnane X receptor) (Section 1.6.5).

1.6 THE BIOCHEMICAL PROCESS OF DRUG METABOLISM

1.6.1 Anatomical Sites of Drug Metabolism

Where does drug metabolism occur in the body? Some level of drug-metabolism activity can be found in essentially every tissue, but the highest specific activities are associated with organs that interface with the environment: gut, liver, kidneys, skin, lungs, nasal mucosa, and so on. This makes sense if we accept that the object of drug

metabolism is to minimize systemic exposure of the body to xenobiotics. Thus, the first line of defense is to prevent or at least limit entry into the body. This is the function of the various efflux transporters that pump chemicals that have permeated the first layer of cells in these organs. We may consider drug metabolism to be the second line of defense, since generally the enzymes of drug metabolism are intracellular, so that only compounds not efficiently excluded by efflux transporters are subject to drug metabolism. This distinction of first and second lines of defense is somewhat arbitrary, since xenobiotics are not all subject to efflux transport or metabolism during initial organ penetration, but it provides a useful conceptual framework for thinking about the process and is reasonably accurate for many drugs.

Since the greatest absolute amounts of xenobiotics usually arrive by oral ingestion, the intestine and liver are of special importance for the barrier function, and the majority of drug-metabolizing capacity may be found there. An orally ingested xenobiotic must survive stomach acid and intestinal digestive enzymes such as proteases, lipases, and other hydrolases. Then it faces the intestinal wall that is designed to be impermeable to foreign substances, especially highly charged, hydrophilic, or high molecular weight compounds. And those molecules that pass through these two barriers still must elude the efflux transporters. Nonetheless, the majority of xenobiotics actually do survive this gauntlet of exclusion processes and are absorbed into the body, explaining why another type of high capacity barrier (drug metabolism) is necessary in intestine and liver. The process of interception and elimination of molecules that manage to penetrate the intestinal epithelium in order to prevent their ultimate entry into the systemic circulation is called the *first-pass effect*. The physiological purpose of intestine and liver drug metabolism is to *maximize* the first-pass effect. Conversely, the problem faced by the medicinal chemists and pharmaceutical scientists is to *minimize* the first-pass effect, so that a high percentage of the dosed medicine can actually reach the systemic blood stream. Thus, in order to achieve good oral bioavailability, drug discovery teams usually seek to design molecules that are metabolized at only moderate rates.

Once a drug enters the bloodstream, the body begins clearing the compound either by direct excretion or by metabolism followed by excretion. Both of these processes occur in the liver and kidney, but with organ differences. The liver can utilize uptake transporters to bring compounds from the blood into liver cells, where they can be directly excreted through the action of efflux transporters or metabolized by the versatile hepatic drug-metabolizing enzymes. The kidney, on the other hand, can directly excrete a drug into urine by the process of glomerular filtration and by secretion into urine from renal cells by efflux transporters. Kidney also contains a number of drug-metabolizing enzymes, but not the same set or the same densities as liver. Again, for many drugs the direct excretion processes in liver and kidney are ineffective for CL, and molecules must first be metabolized. Metabolism frequently makes a drug more polar and water soluble, facilitating transporter-mediated excretion. In addition, polar metabolites are usually less bound to plasma proteins compared to the parent drug, facilitating their filtration in the kidney. In fact, in this context, metabolism is historically described as occurring in two steps, *phase I* and *phase II*, although more recently this distinction has come into question [64]. Phase I enzymes introduce a polar site into the molecule, for instance, by hydroxylation at a hydrophobic region of the molecule or by dealkylation of an amine or ether functionality to uncover a secondary amine or hydroxyl group. Sometimes phase I metabolism sufficiently increases water solubility that the polar metabolite can either diffuse out of the cell membrane directly into bile or blood

or move to a transporter and be secreted. At other times, however, the metabolite is still too insoluble to be mobilized and continues to mainly associate with cellular membranes. These hydrophobic metabolites may then be recognized as substrates by the phase II enzymes, which add a very polar or even ionic group to make a secondary, conjugated metabolite that is usually excretable in bile or urine. Examples of these more soluble conjugates are glucuronides and sulfates, both of which are negatively charged at pH 7. Thus, we see that metabolism by phase I and/or phase II enzymes followed by excretion of the solubilized metabolites is the most important means of CL of the majority of xenobiotics. A more complete statement of the problem faced by a drug discovery team, then, is to find the right balance between metabolism slow enough that the first-pass effect is low but fast enough that compounds not directly excretable can be metabolized and excreted in a reasonable amount of time.

1.6.2 Subcellular Localization of Drug Metabolism

Taking hepatocytes as the archetypical drug-metabolizing cells, the greatest variety of reactions and capacity for CL are in the smooth endoplasmic reticulum (SER) and the cytosol. Certain special reactions, such as β-oxidation, occur mainly in the mitochondria and peroxisomes. Accordingly, when we wish to study metabolism in an *in vitro* system so that we can control most variables, we use hepatocytes to simultaneously evaluate all possible metabolic routes. Often, however, we want to limit the experimental question to only certain metabolic pathways. In that case, we need to use a subcellular fraction. Hepatocytes can be lysed by homogenization of liver tissue, followed by differential centrifugation to segregate the desired subcellular fraction [65]. By this means, we can prepare the so-called *S-9 fraction* (supernatant from $9,000 \times g$ sedimentation), which contains *cytosol* and liposomal fragments of the smooth endoplasmic reticulum called *microsome*s. *S*-9 provides convenient access to almost all important drug-metabolism pathways. Microsomes can be separated from cytosol by more forceful sedimentation of the *S*-9 fraction at $105,000 \times g$, allowing us to separately interrogate both the soluble and membrane-bound drug-metabolism enzymes.

1.6.3 The Enzymes of Drug Metabolism

As mentioned above, almost all drug metabolism is mediated by enzymes that fall into a few general classes. Table 1.2 summarizes these classes and a few important features of each enzyme type in the class. In most cases, it is easy to match up an enzyme type with each of the reactions shown in Table 1.1, and the two tables have been arranged in parallel to facilitate this matching. In drug development, it is necessary to identify the enzymes mediating the metabolism of drug candidates, because this information promotes an understanding of several aspects of the clinical behavior of drugs, including genetic variation, drug–drug interactions, time-dependent inhibition, autoinduction, and the effect of disease on a patient's ability to clear the drug.

Most of the enzymes in Table 1.2 exist in *superfamilies* of closely related proteins, often called *isozymes* or *isoforms,* with different but sometimes overlapping substrate preferences. The enzymes in a superfamily are typically given a designation of the form "*n*L*m*", where "*n*" and "*m*" are numbers (1, 2, 3, etc.) and "L" is a letter (A, B, C, etc.). The "*n*" designates the family, "L" the subfamily, and "*m*" the specific

TABLE 1.2 Summary of Major Enzymes Mediating Human Drug Metabolism

Oxygenases, Oxidases, and Dehydrogenases

Cytochrome P450 *(CYP)*[a]
Typical reaction: $R–H + O_2 + NADPH + H^+ \rightarrow R–OH + H_2O + NADP^+$
Prosthetic group: Heme
Required cofactors: O_2, NADPH, CPR, and cytochrome b_5 (with some substrates)
Tissue expression: Liver and most other tissues
Subcellular localization: Endoplasmic reticulum

Flavin-Containing Monooxygenase (FMO)[b]
Typical reaction: $R_3N + O_2 + NADPH + H^+ \rightarrow R_3N^+–O^- + H_2O + NADP^+$
Prosthetic group: FAD
Required cofactors: O_2 and NADPH
Tissue expression: Liver
Subcellular localization: Endoplasmic reticulum

Aldehyde Oxidase (AOX)[c]
Typical reaction: $RCH{=}O + H_2O + O_2 \rightarrow RCOOH + H_2O_2$
Prosthetic group: Molybdenum, [2Fe–2 S] centers and flavin
Required cofactors: O_2
Tissue expression: Liver and other tissues
Subcellular localization: Cytosol

Monoamine Oxidase (MAO)[d]
Typical reaction: $RCH_2NH_2 + H_2O + O_2 \rightarrow RCH{=}O + NH_3 + H_2O_2$
Prosthetic group: Flavin
Required cofactors: O_2
Tissue expression: Many tissues
Subcellular localization: Mitochondria

Xanthine Oxidase (XOR)[e]
Typical reaction: xanthine $+ H_2O + O_2 \rightarrow$ uric acid $+ H_2O_2$
Prosthetic group: Molybdenum, [2Fe–2 S] centers and flavin
Required cofactors: O_2
Tissue expression: Liver
Subcellular localization: Cytosol

Alcohol Dehydrogenase (ADH)[f]
Typical reaction: $R–CH_2–OH + NAD^+ \rightarrow R–CH{=}O + NADH + H^+$
Prosthetic group: None
Required cofactors: NAD^+
Tissue expression: Liver
Subcellular localization: Cytosol

Aldehyde Dehydrogenase (ALDH)[g]
Typical reaction: $R–CH{=}O + NAD^+ + H_2O \rightarrow R–COOH + NADH + H^+$
Prosthetic group: None
Required cofactors: NAD^+
Tissue expression: Liver
Subcellular localization: Cytosol

TABLE 1.2 (*continued*)

Reductases

Aldo-Keto Reductase (AKR)[h]
Typical reaction: RCH=O or $R_2C=O + NADPH + H^+ \rightarrow RCH_2OH$ or $R_2CHOH + NADP^+$
Prosthetic group: None
Required cofactors: NADPH
Tissue expression: Liver and other tissues
Subcellular localization: Cytosol

Cytochrome P450 *(CYP)*[i]
Typical reaction: $ArN \rightarrow O + NADPH + H^+ \rightarrow ArN + H_2O + NADP^+$
Prosthetic group: Heme
Required cofactors: NADPH and CPR (hypoxic conditions)
Tissue expression: Liver and most other tissues
Subcellular localization: Endoplasmic reticulum

Cytochrome P450 *Reductase (CPR)*[j]
Typical reaction: $ArN \rightarrow O + NADPH + H^+ \rightarrow ArN + H_2O + NADP^+$
Prosthetic group: FMN and FAD
Required cofactors: NADPH
Tissue expression: Liver and most other tissues
Subcellular localization: Endoplasmic reticulum

Quinone Reductase (NQO1)[k]
Typical reaction: quinone + NADPH → hydroquinone + $NADP^+$
Prosthetic group: Flavin Required Cofactor: NADPH
Tissue expression: Liver and other tissues
Subcellular localization: Cytosol

Transferases

N-Acetyl Transferase (NAT)[l]
Typical reaction: $Ar–NH_2 + AcSCoA \rightarrow Ar–NHCOCH_3 + CoASH$
Prosthetic group: None
Required cofactors: AcSCoA
Tissue expression: Liver and other tissues
Subcellular localization: Cytosol

Adenosine Kinase (AK)[m]
Typical reaction: Base-Ribose-OH + ATP → Base-Ribose-OPO_2H_2 + ADP
Prosthetic group: None
Required cofactors: ATP
Tissue expression: Liver and other tissues
Subcellular localization: Cytosol

Catecholamine O-Methyl Transferase (COMT)[n]
Typical reaction: A catechol + SAM → a guaiacol + *S*-adenosyl-L-homocysteine
Prosthetic group: None
Required cofactors: SAM
Tissue expression: Liver and other tissues
Subcellular localization: Both cytosol and endoplasmic reticulum

Glutathione S-Transferase (GST)[o]
Typical reaction: R–X + GSH → R–SG + HX (X is a leaving group such as halogen)

(*continued overleaf*)

TABLE 1.2 (*continued*)

Prosthetic group: None
Required cofactors: GSH
Tissue expression: Liver, kidney, and other tissues
Subcellular localization: Cytosol

Sulfotransferase (SULT)[p]
Typical reaction: Ar–OH + PAPS → Ar–OSO_3H + adenosine-3′, 5′-diphosphate
Prosthetic group: None
Required cofactors: PAPS
Tissue expression: Liver and intestine
Subcellular localization: Cytosol

Thiopurine S-Methyl Transferase (TPMT)[q]
Typical reaction: A thiopurine-SH (or other R–SH) + SAM → a thiopurine-SMe + *S*-adenosyl-L-homocysteine
Prosthetic group: None
Required cofactors: SAM
Tissue expression: Liver, kidney, and erythrocytes
Subcellular localization: Cytosol

UDP-Glucuronosyl Transferase (UGT)[r]
Typical reaction: R–OH or Ar–OH + UDPGA → alkyl or aryl β-D-glucuronide + UDP
Prosthetic group: None
Required cofactors: UDPGA
Tissue expression: Liver and intestine
Subcellular localization: Endoplasmic reticulum

Condensation

Amino Acid Conjugation[s]
Typical reaction:
Ar–COOH + H_2N–CHR–COOH + ATP → Ar–CONH–CHR–COOH + AMP + $H_4P_2O_7$
Prosthetic group: None
Required cofactors: glycine or taurine, ATP, and CoASH
Tissue expression: Liver
Subcellular localization: Mitochondria

Hydrolases

Alkaline phosphatase (AP)[t]
Typical reaction: RO–PO_3H_2 + H_2O → ROH + $HOPO_3H_2$
Prosthetic group: None
Required cofactors: None
Tissue expression: Intestinal lumen
Subcellular localization: Epithelial brush border

Amidase (AADAC)[u]
Typical reaction: Ar–$NHCOCH_2R$ + H_2O → Ar–NH_2 + RCH_2COOH
Prosthetic group: None
Required cofactors: None
Tissue expression: Liver
Subcellular localization: Endoplasmic reticulum

Arylsulfatase (ARS)[v]
Typical reaction: Ar–OSO_3H + H_2O → Ar–OH + $HOSO_3H$

TABLE 1.2 (*continued*)

Prosthetic group: None
Required cofactors: None
Tissue expression: Liver
Subcellular localization: Endoplasmic reticulum and lysosomes

Carboxylesterase (CES)[w]
Typical reaction: $RCOOR' + H_2O \rightarrow RCOOH + R'OH$
Prosthetic group: None
Required cofactors: None
Tissue expression: Liver and many other tissues
Subcellular localization: Cytosol and endoplasmic reticulum

Epoxide hydrolase (EH)[x]
Typical reaction: Epoxide + $H_2O \rightarrow$ *trans*-1,2-diol
Prosthetic group: None
Required cofactors: None
Tissue expression: Liver and other tissues
Subcellular localization: Endoplasmic reticulum

β-*Glucuronidase* (β-*Gluc)*[y]
Typical reaction: Alkyl or aryl β-D-glucuronide + $H_2O \rightarrow$ D-glucuronic acid + alcohol or phenol
Prosthetic group: None
Required cofactors: None
Tissue expression: Liver, kidney, and intestinal lumen
Subcellular localization: Endoplasmic reticulum and lysosomes in liver and kidney; secreted by *Escherichia coli* in intestine

[a]Reference: Volume I, chapter titled ***Structure and Function of Cytochrome* P450 *Enzymes***; also Ref. 66.
Web Links: http://www.bing.com/reference/semhtml?title=Cytochrome_P450&qpvt=cytochrome+P450&src=abop&q=cytochrome+P450&fwd=1.
Accessed 2010 June 1.
http://www.brenda-enzymes.info/php/result_flat.php4?ecno=1.14.14.1.
Accessed 2010 June 1.
[b]Reference: Volume efI, chapter titled ***Flavin-Containing Monooxygenase: The Role of Flavin-Containing Monooxgenase in Lead Design and Selection***; also Ref. 67.
Web Links: http://en.wikipedia.org/wiki/Flavin-containing_monooxygenase.
Accessed 2010 June 1.
http://www.brenda-enzymes.info/php/result_flat.php4?ecno=1.14.13.8.
Accessed 2010 June 1.
[c]Reference: Volume I, chapter titled ***Molybdenum-Containing Hydroxylases***; also Ref. 68.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=1.2.3.1.
Accessed 2010 June 1.
[d]Reference: Volume I, chapter titled ***Amine Oxidases and Reductases***; also Ref. 69.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=1.4.3.4.
Accessed 2010 June 1.
[e]Reference: Volume I, chapter titled ***Molybdenum-Containing Hydroxylases***; also Ref. 70.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=1.17.3.2.
Accessed 2010 June 1.
[f]Ref. 71.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=1.1.1.1.
Accessed 2010 June 1.
[g]Ref. 72.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=1.2.1.3.
Accessed 2010 June 1.

[h] Refs 73 and 74.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=1.1.1.2.
Accessed 2010 June 1.
[i] Ref. 75.
Web Links: http://www.bing.com/reference/semhtml?title=Cytochrome_P450&qpvt=cytochrome+P450&src=abop&q=cytochrome+P450&fwd=1.
Accessed 2010 June 1.
http://www.brenda-enzymes.info/php/result_flat.php4?ecno=1.14.14.1.
Accessed 2010 June 1.
[j] Ref. 76.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=1.6.2.4.
Accessed 2010 June 1.
[k] Ref 35.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=1.3.1.74.
Accessed 2010 June 1.
[l] Ref. 77.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=2.3.1.5.
Accessed 2010 June 1.
[m] Ref. [78]; other kinases such as deoxycytodine kinase (DCK) can also carry out phosphorylations of nucleosides 79
[n] Ref. 80.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=2.1.1.6.
Accessed 2010 June 1.
[o] Reference: Volume I, chapter titled ***Functional Genomics of the Human Glutathione Transferases***; also Ref. 81.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=2.5.1.18.
Accessed 2010 June 1.
[p] Reference: Volume I, chapter titled ***Sulfotransferases***; also Ref. 82.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=2.8.2.1.
Accessed 2010 June 1.
[q] Ref. 83.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=2.1.1.67.
Accessed 2010 June 1.
[r] Reference: Volume I, chapter titled ***UDP-Glucuronosyltransferases: Pharmacogenetics, Functional Characterization, and Clinical Relevance***; also Ref. 84.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=2.4.1.17.
Accessed 2010 June 1.
[s] Reference: Volume I, chapter titled ***Amino Acid Conjugation: A Novel Route of Xenobiotic Carboxylic Acid Metabolism in Man***; also Ref. 85.
[t] Ref. 86.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=3.1.3.1.
Accessed 2010 June 1.
[u] Reference: Volume I, chapter titled ***Carboxylesterases***; also Ref. 87.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=3.5.1.4.
Accessed 2010 June 1.
[v] Ref. 88.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=3.1.6.1.
Accessed 2010 June 1.
[w] Reference: Volume I, chapter ***Carboxylesterases***; also Ref. 89.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=3.1.1.1.
Accessed 2010 June 1.
[x] Reference: Volume I, chapter titled ***Epoxide Hydrolases***; also Ref. 90.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=3.3.2.9.
Accessed 2010 June 1.
[y] Refs 91–93.
Web Link: http://www.brenda-enzymes.info/php/result_flat.php4?ecno=3.2.1.31.
Accessed 2010 June 1.

enzyme. The most widely recognized example is the cytochrome P450 (CYP) superfamily (chapter titled ***Structure and Function of Cytochrome* P450 *Enzymes***). The human genome is known to contain 57 functional CYP genes [66], of which approximately a dozen encode enzymes mainly involved in drug metabolism [94]. Arguably, the most important of the cytochrome P450 enzymes is CYP3A4, often truncated to just 3A4 when the context is understood to be a CYP enzyme. Among the "drug-metabolizing" CYPs, it is common for more than one to be capable of metabolizing a particular drug, although one particular CYP enzyme may make the biggest contribution to the metabolism [11]. Thus, for drug-development purposes, a complete description of the enzymes mediating metabolism must include not only which enzyme type is involved (in this case, CYP) but also which particular CYP enzymes are most responsible for the metabolism of the candidate drug (e.g., enzymes 2 C9, 3A4, and 3A5). This *reaction phenotyping* is a rigorous process of testing the ability of various CYP enzymes to metabolize the drug candidate, with multiple reagents, techniques and sources of enzymes [94] (see Volume III). Reaction phenotyping is required for most of the important drug-metabolizing enzymes, to the extent that the state of knowledge of each particular enzyme type allows. For instance, as with the CYPs, drugs metabolized by UDP-glucuronosyl transferase (UGT) also require reaction phenotyping [95]. Many drug-metabolizing enzymes have been cloned and heterologously expressed and are commercially available as single enzymes, greatly facilitating reaction phenotyping and other preclinical drug-metabolism purposes [96]. It is also possible to directly isolate the requisite enzymes from human liver [97], but this is seldom done since it is slow, labor intensive, and requires a source of fresh human tissue.

In addition to the existence of multiple enzymes in a given family, some of the enzymes exhibit allelic variants in their genes across populations. Some of these variants translate to proteins that have reduced or no catalytic activity, but in other cases the variant allele is the result of gene duplication leading to overexpression of the active enzyme. This phenomenon is called *genetic polymorphism*, and it can result in individuals who are *poor metabolizers* (homozygous for the defective gene), *intermediate metabolizers* (heterozygous), *extensive metabolizers* (homozygous for the wild-type gene), and *ultrarapid metabolizers* (carriers of the gene-duplication allele). Since the CYP enzymes are so important in human drug metabolism, polymorphism in the CYPs is especially significant [98] (chapter titled ***Cytochrome* P450 *Polymorphisms***). As mentioned in Section 1.5.4, V_{max} in Equation 1.8 may be factored into $k_{cat} \cdot E_{tot}$, allowing us to rationalize the effect of genetic polymorphisms on the half-lives of drugs that mainly depend on the polymorphic enzyme for their metabolism. Some genetic polymorphisms result in expression of inactive enzyme (i.e., $k_{cat} \sim 0$), while in other cases there is reduced expression (i.e., $E_{tot} \sim 0$). In either case, V_{max} is reduced, and individuals carrying such an allele exhibit increased half-life compared to the general population. The opposite is true for the ultrarapid metabolizers, where the active enzyme is overexpressed (i.e., $E_{tot} \gg$ normal population). These very real effects on the clinical PKs of some drugs in certain individual patients are important reasons to characterize the enzymology of metabolism of new drug candidates. Consequently, during the discovery process, we try to design compounds that do *not* mainly depend on a single, polymorphic enzyme for their CL.

1.6.4 The Role of Transporters in Drug Metabolism

Transporters are ubiquitous proteins in the body, which move molecules from one side of a barrier membrane to the other (see Volume II). Again taking hepatocytes as the typical drug-metabolizing cell, *uptake* transporters move drug molecules from the blood to the interior of the hepatocytes, while *efflux* transporters export drug or metabolite molecules from the cell interior to the blood or bile [99]. Some drug molecules readily penetrate cell membranes by passive diffusion, while others, with poor membrane permeability characteristics, may require the assistance of a transporter. Uptake transporters, then, could be called *phase 0* of drug metabolism, since they move drugs into cells, where the main drug-metabolizing activities are located.

Analogously, efflux transporters could be called *phase III* of drug metabolism. Once metabolism has occurred, removal of drug-derived material is not complete until the metabolites are moved from the interior of the cells to the excreta (bile, feces, and urine). The chemical modifications that make metabolites more water soluble also make them less permeable through cell membranes. Thus, the efflux transporters complete the process for metabolites that cannot independently diffuse through membranes and out of cells. A particularly important efflux transporter is *P-glycoprotein* (P-gp or MDR1), located on the intestinal epithelium, liver canaliculae, kidney proximal tubules, and brain endothelium. This transporter can pump many types of xenobiotic molecules and metabolites out of cells as a means of exclusion or elimination [100].

As with drug-metabolizing enzymes, transporters are subject to the phenomena of competitive inhibition and induction, creating a second type of metabolism-based drug–drug interaction [101]. Also, most, if not all, transporters have allelic variants resulting in polymorphic functionality [102]. This complicated *metabolism-transporter interplay* [103] has drawn the attention of the scientific community, regulatory authorities, and drug developers [100]. Thus, *transporter phenotyping* is now also recognized as an important component of a complete understanding of the behavior of drugs and metabolites in the body.

1.6.5 Biological Aspects of Drug Metabolism

The activities of human drug-metabolizing enzymes are modulated by a number of factors and conditions (see Volume IV). As mentioned in Section 1.5.4, many drug-metabolizing enzymes are upregulated by chronic exposure of liver and other organs to xenobiotics, thereby promoting the metabolic CL of the foreign chemical. This upregulation occurs at the transcriptional level under the control of cytoplasmic or nuclear receptors which sense the presence of the xenobiotics inside cells and activate gene expression for a complement of enzymes and proteins related to drug metabolism [63] (chapter titled ***Transcriptional Regulation of Cytochrome* P450 *Genes***). Three important receptors for upregulation of drug-metabolizing enzymes are *AhR* (aryl hydrocarbon receptor), *CAR* (constitutive androstane receptor), and *PXR* (pregnane X receptor). The names of these receptors are not informative as to their activators or functions. AhR is a cytoplasmic receptor that is activated by some polycyclic aromatic compounds, such as benzo[*a*]pyrene, resulting in upregulation of CYPs 1A and 1B, GST (glutathione *S*-transferase), NQO1, and UGT1A enzymes (see Table 1.2 for enzyme abbreviations). This set of induced enzymes would be important for metabolic CL of benzo[*a*]pyrene and other aromatic compounds, so that continuous exposure of

a person to inducers such as benzo[*a*]pyrene would result in increased capacity of the person to clear such compounds. CAR is a nuclear receptor activated by phenobarbital and other drugs, resulting in upregulation of CYPs 2B and 3A, GST, and UGT1A enzymes. PXR is particularly important in clinical drug metabolism, since it is activated by many compounds (e.g., dexamethasone and rifampicin) and is the principal mechanism of upregulation of the important enzyme CYP3A4. Other enzymes/transporters upregulated by PXR include SULT1A, UGT1A, and MDR1. Since activation of these receptors can have profound effects on the clinical utility of a drug, *in vitro* assessment of the human induction profile is usually a screening requirement during the discovery process and is normally expected by regulatory authorities during registration of a new drug. If *in vitro* or animal data indicated the likelihood of enzyme induction, phase I clinical studies would be mounted to rigorously define the induction potential.

Physiological conditions such as menstruation and pregnancy affect drug metabolism, possibly necessitating dosage adjustments of medications taken during these times [104,105]. Similarly, drug-metabolizing enzymes and transporters are variably expressed during the stages of fetal development [106] (chapter titled ***Sex Differences in Drug Metabolism***). Enzyme levels continue to change through childhood, adulthood, and old age [107]. The enzymes of drug metabolism are even different in men and women, leading to gender differences in PKs [108]. Section 1.6.3 introduced the idea of genetic polymorphisms in many of the enzymes of drug metabolism, and it follows as a natural consequence that the corresponding alleles occur with higher frequency in some human populations (i.e., *pharmacogenetics*), making the nature and extent of drug metabolism variable across ethnic and genetic groups [109].

Disease states can effect an individual's ability to metabolize drugs, especially in inflammation and infection, where released cytokines can modulate levels of CYP enzymes [110]. Diet can increase or decrease the activities of drug-metabolizing enzymes and transporters, probably due to the effects of phytochemicals operating through several molecular mechanisms [111]. Even the time of day can make a difference in the ability of an individual to clear drugs, since enzyme expression and physiological processes such as blood flows are subject to *circadian rhythms* [112].

Finally, in light of the discussion above, it should be no surprise that the enzymes of animals are different from those of humans, both in amino acid sequences of the proteins and expression levels of the active enzymes (chapter titled ***Species Differences of Drug-Metabolizing Enzymes***). This has implications for human drug development because preclinical drug metabolism information is often used to help design and select drug candidates, but preclinical studies are performed by definition in animals, not humans. Thus, because the animal drug-metabolizing enzymes may have different activities and specificities from those of human, conclusions from animal studies are compromised. *In vitro* drug-metabolism studies with human hepatocytes avoid the problem of interspecies differences, but introduce the problem of *in vitro* to *in vivo* extrapolation. In response to this need for reliable *in vivo* human-relevant drug metabolism information to be available preclinically, various humanized transgenic mouse strains have been developed [113,114], but the reliable use of such models is still being explored.

1.7 THE CHEMICAL CHARACTERIZATION OF DRUG METABOLISM

1.7.1 ADME Studies

The acronym ADME stands for absorption, distribution, metabolism, and excretion. A human ADME study gives a complete description of how much of a drug candidate enters the body by a particular route of administration such as oral dosing, where the compound distributes within the body, what metabolites are formed and in what quantities, and what length of time is required for complete excretion of drug-related materials in feces and urine (see Volume II). A typical human ADME study is based on administration of a low dose of radiolabeled drug candidate (almost always carbon-14), followed by monitoring and quantitating radioactivity in various biofluids, tissues, and excreta to track drug and metabolites as they are absorbed, distributed, metabolized, and excreted.

In reality, of course, for the usual oral route of administration, we do not get complete information in any of these four areas. First, let us consider the "A" part of ADME. Radioactivity, consisting of parent drug and metabolites, recovered in the urine could only have come from intestinally absorbed parent drug and represents a minimum value for absorption. However, radioactivity recovered in the feces cannot be interpreted, since it contains biliary-secreted parent drug, metabolites, and unabsorbed parent drug. The summed radioactivity in urine and feces, then, does not provide an unambiguous estimate of the extent of absorption. Thus, an oral ADME study does not, by itself, necessarily give a reliable estimate of how much of an orally administered drug was intestinally absorbed. A parallel intravascular dose can provide a way to estimate the oral absorption, since the entire dose is known to have reached the systemic circulation in that case. By making the usually reasonable assumption of non-route-dependent elimination processes, we can proportionally scale the observed amounts of drug-related materials appearing in the urine to estimate the fraction of the oral dose that was actually absorbed (provided that the total urinary excretion of parent drug and metabolites was not trivial). Usually, however, an intravascular dose is not included in a human ADME study.

The situation is equally problematic for the "D" in ADME: distribution. In animal studies, it is possible to dissect a dosed animal and measure the radioactivity content of each organ as an indication of the extent and location of distribution of the parent drug in the body. However, this is impossible for a clinical study, so technically a human ADME study is really just an AME study. In most cases, we assume that the distribution of drug to human organs is similar to distribution to animal organs.

The "M" part of the ADME study is probably the most complete and reliable, but even here there may be serious deficiencies with some drugs. Radioactivity allows us to find and quantitate metabolites in blood, urine, feces, and other samples, and modern spectroscopic techniques make exact structure determination virtually assured. However, with some drugs, a fraction of the radioactivity may be retained in the body for a prolonged period, perhaps as covalently attached drug residues from reactive metabolites. In these cases, radioactivity only appears in the excreta after the biological target of the covalent attachment has been broken down by degradation processes. Thus, information about the chemical identity of the reactive metabolite is lost. Furthermore, metabolites recovered in feces may have been produced from unabsorbed parent drug

or further transformed by intestinal microflora, obscuring the origin of these metabolites. Nonetheless, despite these deficiencies, human ADME studies, especially when supplemented by other studies such as animal ADME, human PKs, and enzyme phenotyping, provide the best overall picture of the handling of the drug by the human body. This is the reason that radiolabeled ADME studies are routinely required by regulatory authorities for registrational and product labeling purposes. For example, a recently published human ADME study of [^{14}C]-prasugrel identified over three dozen metabolites, which were rationalized by a scheme of metabolic pathways that showed the process for formation of the metabolite mainly responsible for the pharmacological activity. Exhaustive collection of urine and feces showed that $\sim$ 95% of the drug and metabolites was excreted within 10 days. Since about 70% was recovered in the urine, intestinal absorption of the oral dose of prasugrel must have been at least 70%. [28].

1.7.2 Metabolite Structure Elucidation

Characterization of the exact chemical structure of the major metabolites of a drug candidate is the foundation for understanding human drug disposition. The chemical structure may provide clues to the possible pharmacological or toxicological properties of the metabolite, and these can be investigated by independent synthesis because the structure is known. In most cases, the structure of each metabolite tells which type of enzyme accomplished the metabolic transformation, as can be demonstrated by comparison of the reactions in Table 1.1 to the enzymes in Table 1.2. It is relatively straightforward from that point to determine which particular enzyme of the family for that type of enzyme was the actual catalyst for the chemical transformation. Once the particular enzyme in the family is identified, then a set of characteristics for that drug immediately become apparent, such as which drugs will likely show interactions, whether polymorphic kinetics are likely to be observed, and whether induction is likely to be a clinical issue. Therefore, rigorous determination of metabolite chemical structure is absolutely essential when characterizing a new clinical candidate.

Metabolite structure elucidation requires the most advanced spectroscopic techniques (Volume V). This is because most such structure determination is accomplished with nano- or microgram quantities of metabolite in a chromatographic mobile phase matrix or with a somewhat impure sample when actual isolation must be accomplished. Chemical reactions are seldom used as a means of determining metabolite structures except in special cases of derivatization to facilitate chromatography or spectroscopy. The workhorse analytical method for metabolite separation and detection is LC combined with *radiometry* [115]. Structural identification of metabolites is mainly accomplished with *mass spectrometry* (MS) coupled with LC, so that metabolites are separated and introduced one-by-one into the inlet of the mass spectrometer (LC-MS). Several types are available depending on the exact information sought [116]. When necessary, *nuclear magnetic resonance spectroscopy* (NMR) is a powerful adjunct to MS that can typically provide structural information which is difficult or impossible to obtain from MS alone, such as the exact position of a hydroxyl group introduced by a CYP enzyme [117].

Since enzymes are involved in almost all metabolic transformations, we typically expect to see stereoselectivity of the metabolism when the parent drug or the metabolite contains one or more stereogenic centers [118]. The significance of *stereochemistry* in metabolism comprises four distinct phenomena. First, a drug which has a stereogenic

center (e.g., *R*,*S*-warfarin) may show differential rates, metabolic pathways and enzyme preference between the R- and S-stereoisomers [119]. Second, a drug lacking a stereogenic center may be metabolized in such a manner that a chiral center is introduced, and the enantiomeric metabolites are unlikely to be produced in equal proportions. For example, the achiral drug risperidone is hydroxylated by CYP enzymes to produce both *R*- and *S*-9-hydroxyrisperidone metabolites [119]. Third, a drug containing a stereogenic center may undergo metabolism that eliminates chirality at that carbon atom. For example, the stereogenic secondary alcohol functional group in ezetimibe is oxidized to an achiral ketone (see Fig. 1.13 in Table 1.1). Finally, a drug containing a stereogenic center may undergo *stereochemical inversion*. For example, chiral "profen" drugs such as ibuprofen are metabolically inverted such that the pharmacologically inactive R-isomers are converted to the active S-isomers [120,121]. In this last example, the subtle change of structure, although important for fully understanding the activity of the drug, would go undetected by ordinary achiral means of chromatography and structural analysis. Therefore, inversion of configuration represents a type of metabolism that must be specifically investigated by chiral methods on the "parent"-drug chromatographic peak.

Since the exact nature of the metabolic reactions observed to occur with a particular drug determines which enzymes are involved and whether active, toxic, or reactive metabolites are generated, a natural exercise is to try to predict preclinically which reactions will be most important for a given drug candidate in humans. Much effort has been devoted to this endeavor and considerable progress has been made, but these computational predictions have limitations and are not yet completely reliable [122]. Nonetheless, predictive software has been documented to be of value in avoidance of certain metabolic liabilities during the design of new drug candidates [123].

1.7.3 Quantitation of Extent of Metabolism

The fraction of the absorbed dose that is metabolized before excretion is called the *extent of metabolism*. Although the fraction of the total drug-related material represented by metabolites in blood is frequently equated with the extent of metabolism, this is clearly not correct, since the concentrations of metabolites in blood compared to the concentration of parent drug are determined not only by the extent of metabolism but also by the the PK CLs and volumes of distribution of the metabolites relative to the parent drug. Correct assessment of the extent of metabolism involves summing up the amounts of all metabolites excreted in urine and feces and expressing as a percentage of the administered dose. Since most marketed drugs are orally administered, then when absorption from the intestinal tract is incomplete, unabsorbed parent drug is subject to bacterial metabolism because of the microorganisms in the large intestine. It is often difficult to distinguish whether certain metabolic reactions such as hydrolysis and reduction occurred systemically or in the intestinal lumen. A related ambiguity occurs when a reversible type of metabolism such as glucuronidation or N-oxidation occurs in the liver followed by secretion into the intestine through the bile. Bacterial enzymes often can hydrolyze glucuronides and reduce *N*-oxides back to parent drug, which would be recovered in the feces. In that case, we would underestimate the extent of metabolism by assuming that the parent drug recovered in feces had not been metabolized. Sampling of bile in animal studies can alert us to the probability

of reversible metabolites in bile. However, while it is possible to obtain human bile samples as well, this is not routinely done in the absence of a reason more compelling than just detection of reversible metabolism. For these reasons, it may be difficult to reliably assess the true extent of human metabolism in certain cases.

1.7.4 Elimination of Drug-Related Materials from the Body

The "E" of the ADME study is measured as the sum of radioactivity recovered in both urine and feces after exhaustive collection, typically two weeks for an average drug. Obviously, drugs exhibiting a long PK half-life might require more than two weeks to be completely excreted. In other cases, incomplete recovery of radioactivity may indicate that metabolites are retained for a prolonged period, though it might also have just been the result of losses during collection of urine and feces. A reasonable expectation for total recovery of excreta over two weeks from human subjects is about 80–90%, so that recoveries which are significantly lower than that may indicate prolonged retention of metabolites, possibly as drug residues covalently bound to tissues [124]. In animal ADME studies, the residual radioactivity can be measured in carcass to resolve the ambiguity of long-retained metabolites versus simple losses, and this information can be helpful in understanding a human mass balance study with low recovery. A drug that clearly accumulates in the body, as either parent drug or metabolites, is a cause for concern and a possible reason for discontinuation of further clinical development.

1.8 CONCLUSION

Drug metabolism is nominally a chemical event, and the purely chemical aspects of the study of the process, such as separation and rigorous structural identification of the metabolites, require the most advanced chromatographic and spectroscopic chemical techniques. The chemical outcome of drug metabolism is important because the metabolites produced may have pharmacological or toxicological properties of their own, independent of those of the parent drug. However, drug metabolism arises from a complex set of biological phenomena that is only manifest at the end as a chemical transformation of a drug molecule. The biological aspects are equally important as the chemical aspects in contributing to the overall clinical profile of a drug. These biological aspects include the rate of enzymatic conversion of drug to metabolite, which largely determines the biological half-life, the particular enzymes that are responsible for the conversion, because some enzymes are subject to genetic polymorphism, and the regulation of those enzymes and their associated cofactors. Metabolism-based drug–drug interactions, which can seriously compromise the clinical usefulness of a drug, represent a higher-order feature of the biological side of drug metabolism. These clinical consequences of drug metabolism are the reasons that regulatory authorities require a complete description of the metabolic fate of a new drug candidate. Furthermore, the desire to design "druglike" molecules to minimize metabolism-related difficulties in clinical development explains why drug discovery organizations expend considerable effort to characterize the metabolism of research molecules before selecting the best one to enter clinical development.

ABBREVIATIONS

AcSCoA	Acetyl Coenzyme A
AMP	Adenosine Monophosphate
ADP	Adenosine Diphosphate
ATP	Adenosine Triphosphate
CoASH	Coenzyme A
CPR	Cytochrome P450 Reductase
FAD	Flavin Adenine Dinucleotide
FMN	Flavin Mononucleotide
GSH	Reduced Glutathione
NAD^+	Nicotinamide Adenine Dinucleotide
NADH	Reduced Nicotinamide Adenine Dinucleotide
$NADP^+$	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Reduced Nicotinamide Adenine Dinucleotide Phosphate
PAPS	Phosphoadenosine Phosphosulfate
SAM	*S*-Adenosine-L-Methionine
UDP	Uridine Diphosphate
UDPGA	Uridine Diphosphate Glucuronic Acid

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