1
Metabolism in Drug Development

Bernard Testa

1.1
What? An Introduction

Drug metabolism, and more generally xenobiotic metabolism, has become a major pharmacological and pharmaceutical science with particular relevance to biology, therapeutics, and toxicology, as abundantly explained and illustrated in a number of recent books [1–8] and reviews [9–18]. As such, drug metabolism is also of great importance in medicinal chemistry and clinical pharmacology because it influences the deactivation, activation, detoxification, and toxification of most drugs [19–22]. This broader pharmacological context will be considered in Section 1.2. There, I shall address the "Why?" question, namely "Why does drug metabolism deserve so much attention?"

Given the major impact of biotransformation reactions and resulting metabolites on the preclinical and clinical success or failure of drug candidates, it comes as no surprise that huge efforts are being deployed toward developing ever earlier and faster biological tools. Here, the objective is to assess as rapidly as possible the viability of such candidates. This brings us to the "How?" question (Section 1.3), namely "How to obtain useful data and predictions on the metabolism of candidates?" Although an overview of modern analytical technologies is provided in Chapter 19 of this book, a first focus here will be on the many factors affecting the fate of a drug. Having gathered many sound if narrow experimental results, drug researchers need to make sense of them. In other words, they seek the help of artificial intelligence to extract reliable information from experimental data and transform it into valuable knowledge permitting extrapolative predictions to new molecules. This, as the reader knows, is the focus of this multi-authored book, the present chapter serving as a bird's eye view of the field.

As much as we live in an artificial world of hardware and software, human beings, so we believe and hope, must remain masters of the game by defining objectives, being cognizant of limits, and interpreting as wisely as possible the predictions generated by machines. The point made in Section 1.4 will thus be a "Who?" question and conclusion, namely "Who among scientists are best able to assess the soundness and reliability of drug metabolism predictions?" Should

Drug Metabolism Prediction, First Edition. Edited by Johannes Kirchmair.

these be software specialists, chemists, biologists, or physicians? This section will end with a plea to pool competences and create teams whose total expertise will be greater than the sum of individual expertise.

1.2 Why? Metabolism in Drug Development

1.2.1 The Pharmacological Context

To put the present book in a global context, it appears useful to ponder the fate of medicines in the body and, more specifically, in the human body. The upper part of Figure 1.1 illustrates in schematic form the two aspects of the interactions between a xenobiotic and a biological system [15,23]. Note that a "biological system" is defined here very broadly and includes functional proteins (e.g., receptors), monocellular organisms and cells isolated from multicellular organisms, isolated tissues and organs, multicellular organisms, and even populations of individuals, be they uni- or multicellular. As for the interactions between a drug (or any xenobiotic) and a biological system, they may be simplified to

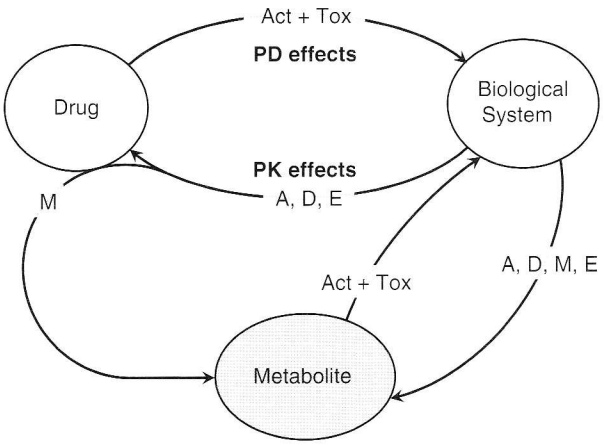

Figure 1.1 The upper part of this scheme illustrates the interaction between a drug (or any xenobiotic) and the organism (or any biological system). The salient point is the interdependence between pharmacodynamic processes ("what the drug does to the body," namely activity (Act) and toxicity (Tox)) and pharmacokinetic processes ("what the body does to the drug," namely absorption (A), distribution (D), metabolism (M = biotransformation), and excretion (E)). The lower part of the scheme is meant to make explicit the potential role of metabolites in the PD effects of a drug. It emphasizes that a metabolite, once formed, will also be involved in PK processes. More important, the figure highlights the fact that metabolite(s) may also play PD roles. Such roles are two, namely pharmacological activity and/or toxic effects (modified from Ref. [23]).

"what the compound does to the biological system" and "what the biological system does to the compound."

In pharmacology, one speaks of "pharmacodynamic (PD) effects" to indicate what a drug does to the body, and "pharmacokinetic (PK) effects" to indicate what the body does to the drug [24]. But one must appreciate that these two aspects of the behavior of xenobiotics are inextricably interdependent. Absorption, distribution, and excretion (abbreviated as ADE) will obviously have a decisive influence on the intensity and duration of pharmacodynamic effects, whereas metabolism (meaning biotransformation) will generate metabolites that may have distinct pharmacodynamic effects of their own. Conversely, by its own pharmacodynamic effects, a compound may affect the state of the organism (e.g., hemodynamic changes and enzyme activities) and hence its capacity to handle xenobiotics. Only a systemic approach as used in pharmacokinetic–pharmacodynamic (PKPD) modeling and in clinical pharmacology is able to grasp the global nature of this interdependence.

When looking in more detail at the behavior of a drug in the body, one finds a number of pharmacokinetic hurdles to be overcome before the sites of action can be reached. As schematized in Figure 1.2 for oral administration [25], a drug

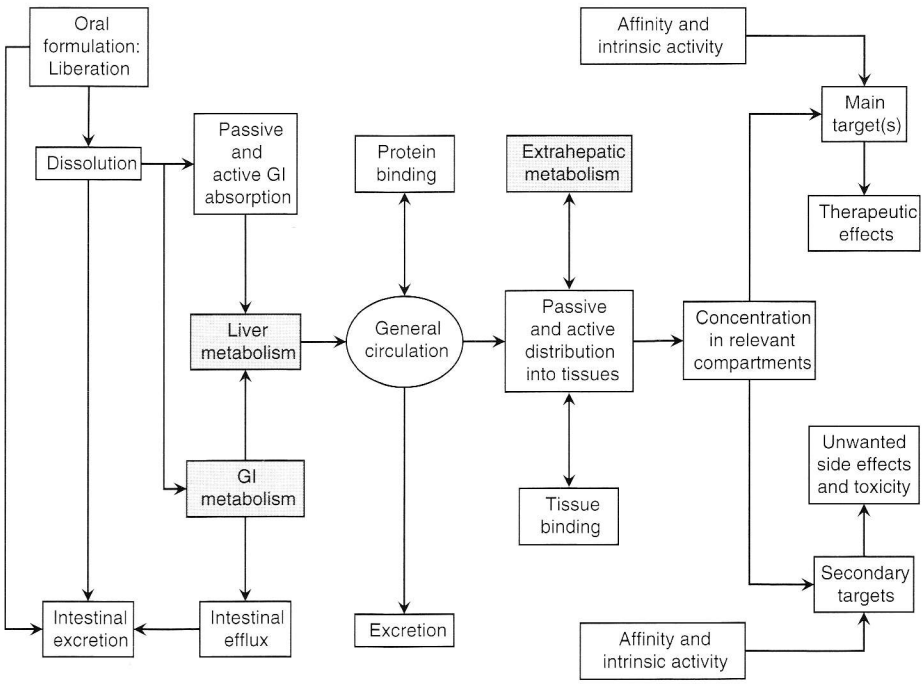

Figure 1.2 Schematic presentation of the fate of a drug in the body following oral administration. Metabolic processes are in darker gray boxes. Pharmacokinetic processes are in lighter gray boxes, and pharmaceutical and pharmacodynamic processes are in white ones (modified from Ref. [25]).

must (i) be liberated from its pharmaceutical form (often a tablet), (ii) dissolve in the gastrointestinal fluid, (iii) escape metabolism by the gut wall and flora, (iv) be absorbed through the intestinal wall passively (via permeation) and/or actively (via transporters), (v) escape excretion in the intestinal lumen by efflux transporters (mainly phosphoglycoprotein; see Chapter 15), (vi) escape metabolism in the blood while being transported to the liver via the portal vein, and finally (vii) escape metabolism in the liver before reaching the general circulation from which it will be cleared by equilibration in tissues, by extrahepatic metabolism, and by excretion (mainly urinary).

The continuously increasing significance of metabolism investigations in drug discovery and development cannot be fortuitous. This phenomenon owes much to the therapeutic and toxicological consequences of drug metabolism (Figure 1.1), which simultaneously drive, and are driven by, the huge methodological, factual, and conceptual advances in this discipline. The necessity of acquiring a thorough knowledge of the metabolism of developmental candidates is illustrated below by considering successively the contribution of metabolites to a drug's wanted activities, unwanted effects, and disposition in the body.

1.2.2 Consequences of Drug Metabolism on Activity

A drug is expected to have beneficial effects (it wouldn't be a drug otherwise) that can be caused by the parent compound (the drug itself) and/or by one or more metabolites. In a perspective of drug discovery, one can note that a number of metabolites of established drugs were found to have equivalent or improved therapeutic properties compared to their parent and have become useful drugs in their own right. Examples include desloratadine (from loratadine), cetirizine (from hydroxyzine), and oxazepam (from diazepam) [21,26]. Even more significant is the discovery of paracetamol, which has replaced phenacetin, its more toxic parent.

An important information in any drug's dossier is the activity (or lack thereof) of its metabolites [27]. What should be realized, however, is that "activity" is usually understood to imply the same pharmacological target as the parent molecule [21,26]. However, the activity of metabolites can also result from interaction with other pharmacodynamic targets sites not or poorly affected by the parent drug. Here, one finds a continuum of possibilities existing from one extreme (drugs having *no active metabolite*) to the other (intrinsically inactive *prodrugs*), with Table 1.1 listing a few examples.

To begin at the top of Table 1.1, *soft drugs* are defined as biologically active compounds (i.e., drugs) characterized by a fast metabolic inactivation to nontoxic metabolites [28,29]. As for sedative–hypnotic benzodiazepines, they fall into two categories [21]. Some have no active metabolite, for example, the 3-hydroxylated benzodiazepines such as lorazepam, oxazepam, and temazepam, which undergo *O*-glucuronidation and cleavage reactions. Other benzodiazepines such as diazepam have one or more active metabolite(s), sometimes

Table 1.1 Classification of drugs without or with active metabolites [21].

Parent drug	Active metabolite(s)
Examples of drugs without active metabolites	
Soft drugs	Designed to have none
Oxazepam and other 3-hydroxylated benzodiazepines	None known
Examples of drugs with one or more active metabolite(s)	
Diazepam	Nordazepam
Morphine	Morphine 6-*O*-glucuronide
Tramadol	*O*-Desmethyltramadol
Examples of drugs with one or more highly active metabolite(s)	
Cisplatin	Monoaqua and diaqua species
Encainide	*O*-Desmethyl encainide and 3-methoxy-*O*-desmethyl encainide
Tamoxifen	4-Hydroxytamoxifen and endoxifen
Examples of inactive medicinal compounds having one or more metabolite(s) accounting for total activity	
Prodrugs	Designed as such

long-acting ones. Morphine and tramadol are interesting examples of drugs having one or more active metabolite(s). The case of cisplatin is a special one because its monoaqua and diaqua metabolites are intrinsically much more reactive toward DNA but have poor cellular penetration because of their high polarity and reactivity [30].

Prodrugs represent by definition the extreme case of medicinal compounds whose complete, or practically complete, activity is ascribable to one or more metabolites [6,22,31–35]. Most prodrugs, in particular the carrier-linked ones, are activated by hydrolysis. Other types of prodrugs, also known as bioprecursors, are activated by redox reactions.

1.2.3
Adverse Consequences of Drug Metabolism

The toxicological consequences of the metabolism of drugs and other xenobiotics can be favorable (i.e., *detoxification*) or unwanted (i.e., *toxification*). The risks of toxification have now become a major issue in drug discovery and development, where minimizing metabolic toxification is given a high priority by screening for reactive intermediates and assessing toxicity, with metabonomics and toxicogenomics (see Chapter 16) being increasingly useful tools [21,36–48].

Table 1.2 introduces us to metabolic toxification (often but inadequately called bioactivation) by summarizing the main types and mechanisms of adverse drug reactions (ADRs). *On-target ADRs* result from an exaggerated response caused

Table 1.2 Types and mechanisms of adverse drug reactions [21].

Types	Mechanisms
1. On-target ADRs	Predictable in principle and generally dose dependent. Based on the pharmacology of the drug and its metabolite(s), often an exaggerated response or a response in a nontarget tissue
2. Off-target ADRs	Predictable in principle and generally dose dependent. Resulting from the interaction of the drug or a metabolite with a nonintended target
3. ADRs involving reactive metabolites	Predictable in principle and generally dose dependent. A major mechanism is covalent binding to macromolecules (adduct formation), resulting in cytotoxic responses, DNA damage, or hypersensitivity and immunological reactions. A distinct (and synergetic) mechanism is the formation of ROS) and oxidative stress
4. IDRs	Unpredictable, apparently dose independent, and rare (<1 case in 5000). They might result from a combination of genetic and external factors, but their nature is poorly understood. IDRs include anaphylaxis, blood dyscrasias, hepatotoxicity, and skin reactions

by drug overdosing or too high levels of an active metabolite; they are predictable in principle and generally dose dependent and are labeled as type A. *Off-target ADRs* result from the interaction of the drug or a metabolite with a nonintended target such as a receptor, an ion channel, or an enzyme. They also are predictable in principle and generally dose dependent. A highly relevant example is that of several lipophilic drugs belonging to various pharmacological classes that cause cardiotoxicity (QT prolongation) by blocking at therapeutic doses the human ERG potassium channel [49,50]. On-target and off-target ADRs are direct ones and pharmacological in nature.

ADRs caused by *reactive metabolites* are the ones of greatest concern in our context [36–48]. They involve covalent binding to macromolecules and/or oxidative stress after the formation of reactive oxygen species (ROSs). These ADRs are predictable (or rationalizable) in terms of the drug's or metabolite's structure, and they are generally dose dependent. They are often labeled as type C. *Idiosyncratic drug reactions* (IDRs) (also known as type B ADRs) are rare to very rare, unpredictable, and apparently dose independent. They are also poorly understood, yet they appear to be usually related to reactive metabolites [51].

A global vision of early molecular mechanisms of toxification and detoxification is offered in Figure 1.3 (modified from Ref. [23]). Many metabolites formed by redox or hydrolytic reactions are *nucleophiles*, for example, phenols and alcohols. Such metabolites are generally innocuous *per se* but may be further metabolized by various oxidoreductases to more reactive *electrophiles*. These cover a variety of chemical functionalities and form adducts with proteins and other biomacromolecules. Both nucleophiles and electrophiles can be trapped by typical

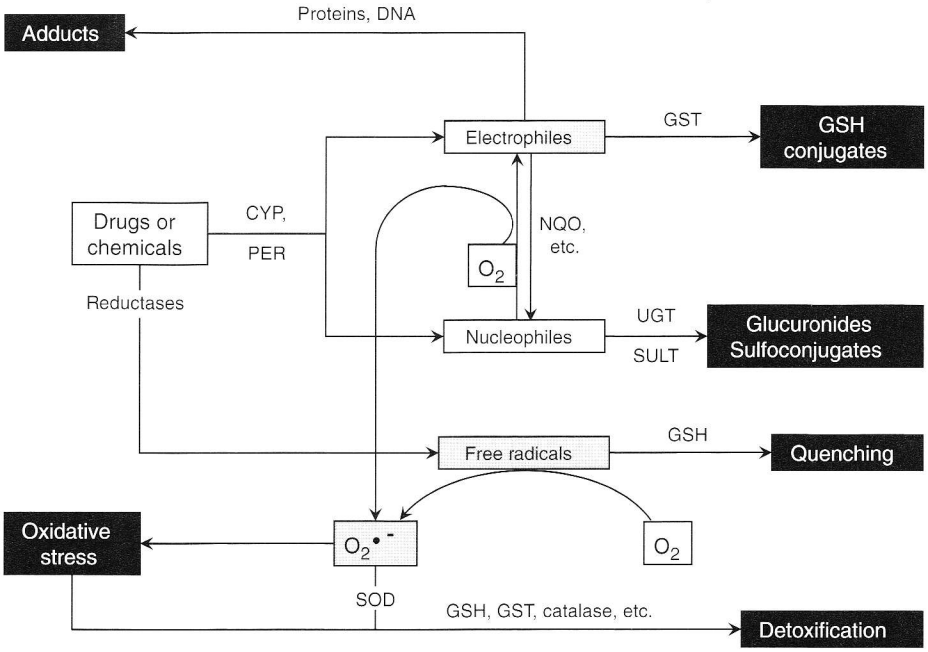

Figure 1.3 Early molecular mechanisms of toxification and detoxification. Oxidoreductases such as CYPs, peroxidases (PER), and reductases transform xenobiotics into nucleophiles, electrophiles, or free-radical species. The role of quinone reductases (NQOs) as a detoxifying enzyme is well documented [16]. Glutathione (GSH) is particularly effective in quenching free radicals. Nucleophiles and electrophiles are further conjugated by UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), or glutathione *S*-transferases (GSTs), although some conjugates have toxic potential. Free radicals reacting with molecular oxygen may reduce it to the superoxide anion radical, which in turn may be detoxified by superoxide dismutase (SOD) (modified from Ref. [23]).

reactions of detoxification, mainly glucuronidation and sulfonation for nucleophiles and glutathione conjugation for electrophiles. However, toxification is not restricted to adduct formation, and indeed Figure 1.3 mentions the formation of free radicals and ROSs as further mechanisms.

A look at *toxicophoric groups* (also called toxicophores, toxophores, or toxophoric groups) is particularly illustrative of the unity that underlies their chemical diversity [39,46,47,52–54]. Indeed, the toxic potential of many toxicophores is explained by their metabolic toxification to electrophilic intermediates or to free radicals (Figure 1.3). In more detail, the major functionalization reactions that activate toxophoric groups include oxidation to electrophilic intermediates, reduction to free radicals, and autooxidation with oxygen reduction, which leads to superoxide, other ROSs, and reactive nitrogen species (RNSs). The electrophilic intermediates and the free radicals then react with bio(macro)molecules, producing critical or noncritical lesions. ROSs also react with unsaturated and mainly polyunsaturated fatty acids in membranes and elsewhere, leading to lipid

peroxidation. Of more recent awareness is the fact that some conjugation reactions may also lead to toxic metabolites, namely reactive acyl glucuronides or conjugates with deleterious physicochemical properties.

1.2.4 Impact of Metabolism on Absorption, Distribution, and Excretion

A common view is that metabolizing enzymes have evolved to transform xenobiotics into more hydrophilic metabolites and so facilitate their effective excretion mainly via the urinary and biliary routes. Physicochemical alterations due to metabolism are indeed documented, for example, with the marked decrease in basicity and lipophilicity upon cytochrome P450 (CYP)- or flavin-containing monooxygenase (FMO)-catalyzed *N*-oxygenation of tertiary amines [55,56] and the marked decrease in lipophilicity caused by the sulfoxidation of sulfides [57] and by the *O*-glucuronidation of alcohols and phenols [58].

The chemical and physicochemical differences between a drug and its metabolites cannot remain without pharmacokinetic consequences, in particular by affecting the distribution and excretion of the metabolites compared with the parent drug [59,60]. Intestinal absorption may also differ between drug and metabolites when biotransformation begins in the gut lumen and walls [61]. At the biological level, these pharmacokinetic differences may also be observed, for example, in the penetration and storage into target and nontarget tissues [62–65] and in the excretion by the urinary, biliary, or other routes. At the biochemical level, such pharmacokinetic differences are seen in passive membrane permeation [66,67], active influx and efflux transport [68], and binding to extracellular and intracellular macromolecules.

In a highly stimulating review article, Smith and Dalvie [69] have speculated about why metabolites circulate when one would expect their fast excretion from the circulation. With insight, the authors separated metabolites into highly lipid-permeable (i.e., highly lipophilic) and *poorly lipid-permeable* (i.e., hydrophilic) ones. Regarding the latter, they have stressed their transporter-mediated efflux from the liver, high plasma protein binding, and restricted distribution into tissues. And indeed, a coupling of conjugating enzymes and efflux transporters may well play a significant role in such a behavior (see Ref. [70] and Section 15.1). But there is more to the story because transferase–transporter coupling is believed to explain an efficient biliary excretion of *O*-glucuronides followed by intestinal hydrolysis and enterohepatic recycling. A particularly impressive example of this process was seen in postmenopausal women dosed with estradiol [71]. Both estradiol and its metabolite estrone were extensively *O*-glucuronidated and underwent enterohepatic cycling. An experimental proof of this phenomenon was seen in the time profile of the serum concentrations of both hormones. The time profile of estradiol showed a first phase of absorption–elimination with an approximate half-life of 2 h. This was rapidly followed by a second absorption phase that resulted in sustained levels of estradiol for 24 h and more. The serum concentration curve of the metabolically produced

estrone showed enterohepatic absorption phases after about 24 and 50 h, extending its half-life severalfold.

Highly lipophilic metabolites also impact on distribution and excretion, but in an unfavorable manner. Thus, a number of conjugates are esters formed by the acylation of endogenous hydroxy compounds with xenobiotic acyl-CoA cofactors [18,72–74]. Many of these conjugates are more lipophilic than the parent xenobiotic carboxylic acid, namely hybrid triglycerides, hybrid phospholipids, and sterol esters (cholesterol esters and bile acid esters). Their formation has been reported for a number of xenobiotic carboxylic acids using, for example, human or animal hepatocytes or adipocytes. *In vivo* evidence is also available, mostly in rats. A case in point is that of the widely used anti-inflammatory drug ibuprofen [75]. Note that such metabolites may also contribute to the residues of veterinary drugs found in animal tissues intended for human consumption [65].

1.3
How? From Experimental Results to Databases to Expert Software Packages

A number of criteria should be kept in mind when using expert computational tools. First and as the saying goes, the models on which these tools are based can be false, irrelevant, or at best incomplete (see Chapter 13). This may be due to intrinsic reasons such as an assumed linearity in cause-and-effect relations. In addition, there are extrinsic reasons as outlined below, in particular the reliability and validity range of the experimental data on which these models are themselves based. Thus, metabolic data are often obtained under controlled conditions, which means that a number of variables are set and kept constant and that large portions of the *space of possibilities* [76] are left unexplored (see Chapters 8, 17, and 18).

Furthermore, quantitative results are characterized by both their accuracy and their precision, two quality criteria regularly confused if not ignored by some experimentalists, as regular submission reviewers can testify. This issue will not be discussed further.

1.3.1
The Many Factors Influencing Drug Metabolism

A large variety of factors are known to influence drug metabolism in a quantitative and even qualitative manner. What is more, some of these factors also interfere with the effects of others, adding to their direct influence on drug metabolism an indirect and nonlinear component.

Table 1.3 presents a conventional classification dividing these factors into inter-individual and intra-individual ones [77–83]. Such a table, although self-explanatory and useful, is a static one that fails to inform on the dynamics of the many actions and interactions influencing the drug metabolism response. To this end, we turn to Figure 1.4, a highly simplified and schematic representation

Table 1.3 A classification of factors affecting drug metabolism [77,78].

Inter-individual factors	Intra-individual factors
Definition: Remain constant in a given organism	*Definition*: Vary over the lifetime of an organism
Cause: "Encoded" in the genome	*Cause*: Originate in physiological, pathological, and external influences
Consequences: Variations due to:	*Consequences*: Variations due to:
• species differences • gender differences • genetic polymorphisms	• age; biological rhythms; pregnancy; tissue characteristics, etc. • diseases; stress, etc. • nutrition; enzyme inhibition or induction by xenobiotics; drug–drug interactions, etc.

of such interactions. Our exploration of Figure 1.4 begins with the genome and the *inter-individual factors* it encodes. *Gene expression* is here the pivotal process, connecting genes as information carriers and gene products as actors. These gene products are of two types, namely (i) functional proteins as agents of

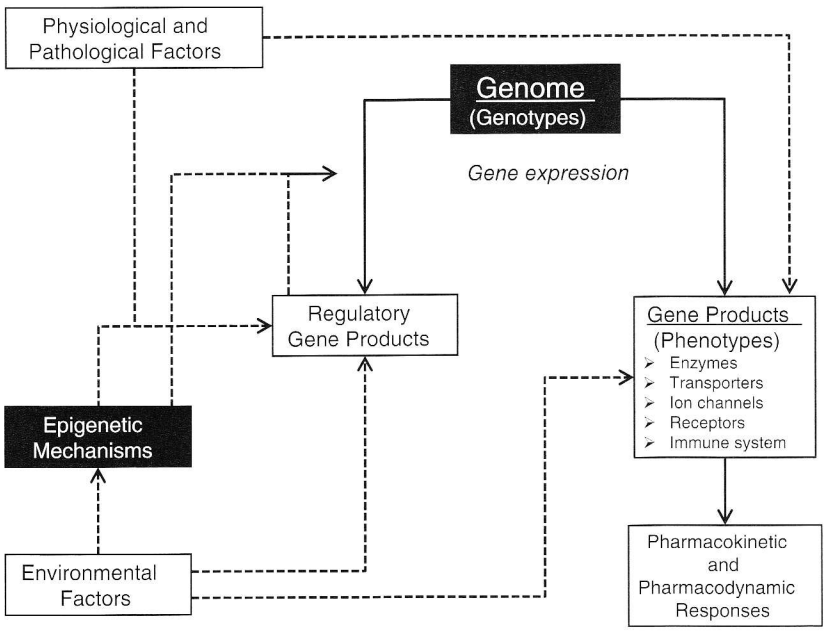

Figure 1.4 Schematic view of factors influencing pharmacodynamic and pharmacokinetic drug responses. Genes remain unaffected in their sequences, which account for differences seen between species, between genders, and in polymorphic populations (inter-individual differences). In contrast to this relative simplicity, the biochemical mechanisms underlying intra-individual differences are markedly more complex.

Table 1.4 Classification of biological systems used in drug metabolism investigations [15].

Types of biosystems	Examples
Subcellular systems	Purified or expressed enzymes; organelles; homogenate fractions (e.g., S9, microsomes); blood serum and plasma
Cellular and tissue systems	Primary cell cultures (e.g., hepatocytes); tumor cell lines (e.g., Caco-2 cells); tissue slices; isolated perfused organs
In vivo systems	Multicellular organisms; batches of experimental animals; groups of individuals; collectives of patients; populations

pharmacodynamic and pharmacokinetic responses and (ii) regulatory gene products as activators or inhibitors of gene expression.

Intra-individual factors occupy the upper-right and lower-right corners of the figure. To some extent, they can act directly on functional proteins, specifically enzyme inhibition by drugs and other xenobiotics. But most effects of physiological, pathological, and external factors occur through an influence on gene expression via regulatory gene products. In this context, *epigenetic mechanisms* have become an exciting and seemingly unlimited field of research [84,85].

1.3.2 Acquiring and Interpreting Experimental Results

A schematic summary of biological systems used in drug metabolism studies is shown in Table 1.4 and goes from the simpler to more complex biosystems [15,86–89]. Although the latter are generally able to yield a large amount of biological information, they are also major consumers of resources and time. Large-scale studies using, for example, *clinical or ethnic populations* are useful mainly if genetic polymorphisms or intra-individual factors are the object of study. The results of such studies are seldom if ever used as a source of information in the development of predictive databases and algorithms. In contrast, investigations based on a modest number of human volunteers are (or should be) necessary to demonstrate the relevance of preclinical studies, namely (i) *in vitro* results obtained with subcellular and cellular systems and (ii) *in vivo* results obtained from batches of experimental animals, often highly inbred ones.

At the other end of the biocomplexity spectrum we find *subcellular systems* (Table 1.4), which are discussed in detail in Chapters 8, 17, and 18. Some such media are comparatively simple to obtain but will only be useful in biotransformation studies if a very limited range of metabolic reactions are under examination (e.g., hydrolyses in the case of human blood plasma). As with purified or expressed enzymes, they require rather sophisticated techniques for their production but will yield highly valuable knowledge and can be used in high-throughput assays. The same is true for the most popular biosystems in *in vitro* drug metabolism studies, namely tissue homogenates, fractions thereof (e.g., S9), and isolated organelles. However, there is a poorly investigated problem here,

namely the potential biophysical perturbations experienced by enzymes transferred from a highly crowded intracellular environment [90] to a dilute medium.

The above issue brings us to *cellular biosystems* [91,92] (discussed in more detail in Chapters 8, 17, and 18). In our scheme, their complexity is intermediate between that of subcellular and *in vivo* systems (Table 1.4), although in another context cells have been aptly compared to a "bottomless pit of complexity" [93]. Cellular biosystems generally possess a broad variety of drug-metabolizing activities and are rich if obviously incomplete models of *in vivo* systems. Similar to cell homogenates, they can be secured from eukaryotic organisms differing in some inter-individual or intra-individual factor, yielding what is best defined as *ex vivo* systems. From data gathered in an extensive meta-analysis of the literature [9], it was found that about 10% of investigations were performed using cellular or tissue systems. This appears as a modest percentage given the efficiency and versatility of these systems.

The point I want to make in this subsection is the necessity for developers of predictive expert tools to check the reliability and validity range of the experimental data fed into their database. To this end, distinct quality criteria need to be defined for qualitative and quantitative data. Also, the influence of inter- and intra-individual factors should be taken into account and when possible used to segregate metabolic data. This analysis and annotation of data is clearly a major task but a necessary one to improve the predictive capacity of expert software.

1.3.3 Expert Software Tools and Their Domains of Applicability

In which domains of drug metabolism is a given predictive software package designed to be useful? This certainly is the initial question to be asked when planning to use such software packages. Table 1.5 presents a classification of predictive tools and a personal view of their capabilities.

In a simplified manner, one can distinguish between two types of methods to predict drug and xenobiotic metabolism, namely specific ("local") tools and comprehensive ("global") ones [15,94–101]. *Specific tools* apply to simple biological systems (e.g., single enzymes) and/or to single metabolic reactions, and they may or may not be restricted to rather narrow chemical series of analogs. Such methods include quantitative structure–metabolism relationships (QSMRs) based on structural and physicochemical properties [59,102]. Quantum mechanical calculations (see Chapters 7 and 11) may also shed light on structure–metabolism relationships (SMRs) and generate parameters to be used as independent variables in QSMRs [103], revealing, for example, correlations between rates of metabolic oxidation and energy barrier in H-atom abstraction [104]. Three-dimensional QSMR (3D-QSMR) methods yield a partial view of the binding/catalytic site of a given enzyme as derived from the 3D molecular fields of a series of substrates or inhibitors (the training set; see Chapters 9 and 13). In other words, they yield a "photographic negative" of such sites and will allow a quantitative prediction for novel compounds structurally related to the training set.

Table 1.5 Specific ("local") and comprehensive ("global") *in silico* predictive tools [15].

Methods	Examples of applications
Specific ("local") *in silico* tools, applicable to (i) series of analogs or structurally heterogeneous compounds and (ii) a single metabolic reaction	
QSMRs (e.g., linear, multilinear, multivariate); 3D-QSMRs (e.g., CoMFA, Catalyst, GRID/GOLPE)	• Prediction of affinities, rates of metabolism • Inhibitory potency
Quantum mechanical (MO) methods (*ab initio*, semi-empirical)	• Regioselectivity of the reaction • Mechanism of the reaction • Relative rates of metabolism
Molecular modeling and docking	• Ligand behavior • Regioselectivity of the reaction
Expert packages combining docking, MO, and 3D-QSMRs (e.g., MetaSite)	• Regioselectivity of the reaction
Comprehensive ("global") *in silico* tools, applicable to (i) series of structurally heterogeneous compounds and (ii) versatile biological systems	
Meta-packages combining (i) docking, 3D-QSMRs, MO and (ii) a number of reactions (e.g., MetaDrug)	• Nature of first-generation metabolites, with an index of probability or likelihood • Flagging of reactive or adduct-forming metabolites • Enzyme induction and inhibition
Databases (Metabolite, Biotransformations)	• Nature of first-generation metabolites, with an index of probability or likelihood • Flagging of reactive or adduct-forming metabolites
Expert software and their databases (METEOR, META, MetabolExpert)	• Nature of first-generation metabolites, with an index of probability or likelihood • Flagging of reactive or adduct-forming metabolites

Molecular modeling of xenobiotic-metabolizing enzymes, combined with *in silico* docking (see Chapter 10), affords another approach to rationalize and predict drug–enzyme interactions [105]. Its application to drug metabolism was made possible by the crystallization and X-ray structural determination of CYPs, first bacterial and now human ones. Although such pharmacophoric models cannot yet give highly accurate quantitative affinity predictions, they nevertheless afford fairly reliable answers as to the relative accessibility of target sites in the substrate molecules. The 3D models of a large number of mammalian and mostly human CYPs are now available, as well as some other xenobiotic-metabolizing enzymes (see Chapter 5).

The last specific tools mentioned in Table 1.5 are expert tools combining several methods, for example, pharmacophore models (obtained by three-dimensional quantitative structure–activity relationship (3D-QSAR) modeling), protein models (obtained by molecular modeling), and docking [106–108]. Other powerful combinations are (i) 3D models obtained by molecular modeling and (ii) sophisticated QSMR approaches based on multivariate analyses of parameters obtained from molecular interaction fields (MIFs), as found in the MetaSite

algorithm [94,109,110]. MetaSite is a specific package in the sense that it is currently restricted to the major human cytochromes P450. At the end of the procedure, the atoms of the substrate are ranked according to their accessibility and reactivity. In other words, MetaSite takes the 3D stereoelectronic structure of both the enzyme and the ligand into account to prioritize the potential target sites in the molecule (see Chapter 9).

Comprehensive expert software packages are in principle applicable to versatile biological systems (i.e., to any enzyme and reaction) and to any chemical compound [15,98]. As shown in the second part of Table 1.5, this is the ultimate goal of meta-packages combining docking, 3D-QSMR, and molecular orbital (MO) methods, not for a single enzyme but for the largest possible majority of them. The inclusion of other functional proteins such as transporters can also be envisaged. Combining several specific models to form a meta-model is a most appealing (if ambitious) strategy, and much work remains to be done before such approaches can be seen as genuinely comprehensive. MetaDrug appears as a promising step in this direction [111,112]. As reviewed by Hawkins [113], one approach to global prediction of metabolism is to use databases in the form of either knowledge-based software or predictive, rule-based one. These databases can be searched to retrieve information on the known metabolism of compounds with similar structures or containing specific moieties. Predictive, knowledge-based packages attempt to portray the metabolites of a compound based on knowledge rules, defining the most likely products [113]. Existing software packages of this type are, for example, MetabolExpert, META, and METEOR, which are discussed in Chapter 12.

1.3.4 Roads to Progress

Given the wide range of methods available today (Table 1.5), which significant advances in predictive drug metabolism can medicinal chemists hope for in a reasonable future? A number of items are proposed here:

1) Numerous enzyme superfamilies and families play a role in drug metabolism [2], but the relative involvement of these enzymes, in both quantitative and qualitative terms, remains a matter of debate [23]. When listening to some medicinal chemists, one may get the feeling that drug metabolism begins and ends with CYPs and that the word "metabolism" implicitly implies "by cytochrome P450." A recent meta-analysis does indeed confirm the primary role of CYP-catalyzed reactions, but it also demonstrates the marked role of non-CYP enzymes (i.e., other oxidoreductases, hydrolases, transferases). Thus, almost 60% of first-generation metabolites are indeed produced by CYPs, but the contribution of this superfamily strongly decreases in the second (ca. 30%) and mainly third and higher generations (ca. 20%). This relative decrease is compensated by an increased involvement of *transferases and some non-CYP oxidoreductases* [9]. More

attention should be given in predictive packages, and especially in specific (local) tools, to some major enzyme (super)families such as dehydrogenases, flavin-containing monooxygenases, peroxidases, hydrolases, UDP-glucuronosyltransferases, sulfotransferases, and glutathione *S*-transferases.

2) The recognition of *toxophoric groups* appears as an indispensable asset of expert predictive tools [21]. Much experimental evidence has been gathered [53], and useful computational models are available [114–116]. However, there is much room for improvement in flagging reactive metabolites or metabolic intermediates.
3) Existing tools are quite competent in suggesting first-generation metabolites. These, of course, will remain hypothetical ("unconfirmed positives") until proven present by experiment ("confirmed positives"). Strictly speaking, "false positives" do not exist because a possibility, however minute, always remains for improved analytical tools to detect them. The real problem lies with "false negatives," namely unpredicted yet later confirmed metabolites. These result from gaps in our knowledge or in a package's database; in the latter case, such gaps, after being identified, can help developers improve their product. In my view, a quality index based on false negatives should be a main criterion to assess expert predictive tools.
4) Most predictive packages classify their predicted metabolites according to an *index of probability or likelihood* [117]. It seems that most misclassifications result from missing information or inadequate weighing of probabilities among metabolites.
5) Following improvements in items 3 and 4 above, there is a serious need to develop automatic prediction of *second- and later generation metabolites*, being aware that the probability of misleading predictions would grow exponentially and prohibitively with the number of generations considered.
6) Assuming item 5 above to be reasonably accounted for, a versatile predictive package should be able to organize the most probable metabolites into a *realistic metabolic tree*. As an aside, I am worried to note how many good experimental papers in drug metabolism summarize their findings with an aberrant metabolic tree!
7) In metabolic predictions, molecular factors are usually taken into account in a satisfactory, if incomplete, manner [99] (Figure 1.5). However, and to the best of my knowledge, no current tool is able to take species and other biological factors credibly into account [101]. One can only hope that in several years or a few decades, enough experimental evidence will have been published to allow polymorphisms and a few intra-individual factors to be taken into account.

1.4
Who? Human Intelligence as a Conclusion

Having taken a bird's eye view of experimental biosystems and predictive software packages, let us now conclude by dedicating some words to the

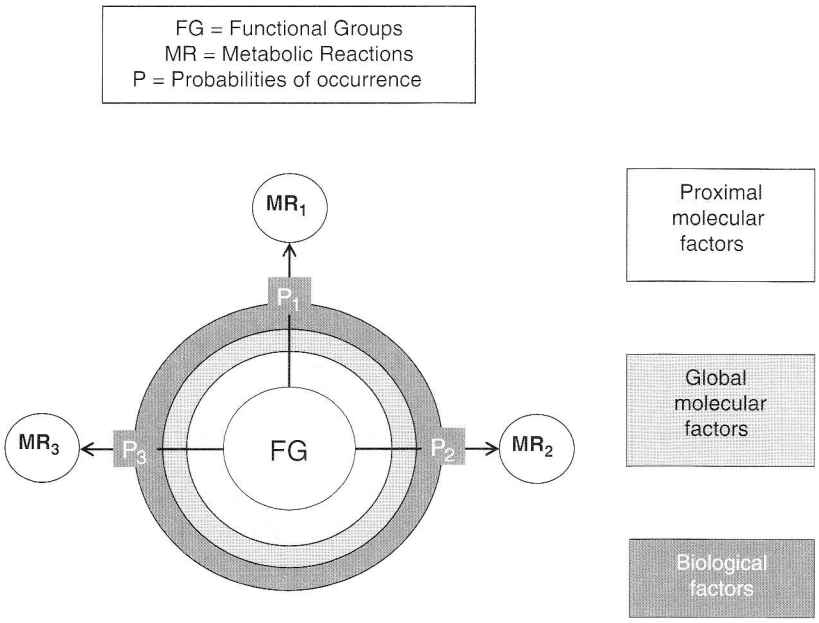

Figure 1.5 Summary of factors influencing the metabolism of drugs and other xenobiotics. Both proximal (i.e., functional groups) and global (i.e., molecular) properties are partly taken into account in predictive software. In contrast, the many biological factors (see Table 1.3) are poorly considered, if at all, mainly because of a paucity of usable experimental data (modified from Ref. [101]).

central actor in drug metabolism studies and predictions, namely the human expert [118].

Experimental drug metabolism is obviously a multidisciplinary science because it draws on *chemistry* (physical, organic–synthetic, analytical, medicinal, etc.), *biology* (biochemistry, enzymology, genetic, epigenetics, etc.), and *pharmacology* (molecular, clinical, pharmacokinetic, toxicology, therapeutics, etc.). Add to this list the *computational components* (software development, MO computations, QSMRs, 3D-QSMRs, structure–property relationships, drug design, homology modeling, molecular modeling and docking, etc.), and you end up with drug metabolism prediction as a research front drawing on an impressively broad range of disciplines.

Companies engaged in creating and developing predictive software packages rely on published results to feed their databases and extract SMRs. This, however, is no trivial task because it necessitates competence in biochemistry, pharmacology, and analytical chemistry. As for medicinal chemists, their major role is in unveiling SMRs to extract additional information from metabolic data and improve the quality of predictions. Programmers create, develop, and upgrade predictive software packages, while experts in drug metabolism must define and apply quality criteria to monitor progress. Such upgrading must be a continuous

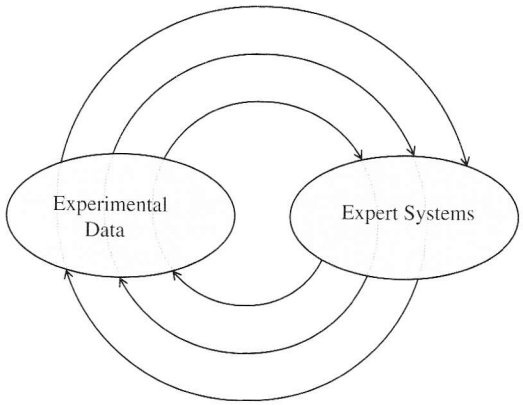

Figure 1.6 The spiral of progress in the creation, development, and updating of software packages for drug metabolism prediction (modified from Ref. [118]).

one in terms of both algorithms and databases content, being based in particular on a constant influx of new data and so generating a spiral of progress as sketched in Figure 1.6.

Similar thoughts apply to pharmaceutical companies large and small engaged in drug discovery and development. Some drug discovery groups will use predictive software like they were black boxes, while others will carefully assess and critically interpret their output. Here also a broad range of competence is called for, primarily in organic chemistry, biochemistry, enzymology, and pharmacology.

What the above says is simply that a comparable pool of competence is needed to create predictive software or to use them in drug discovery and development. It may even be that the more ambitious the project, the broader and more varied the necessary team of specialists. Not to mention the challenge of team leaders to understand and coordinate the work of their colleagues. Human intelligence is indeed served by artificial intelligence, but it must remain in charge. This is what the present book is about.

References

1 Anzenbacher, P. and Zanger, U.M. (eds) (2012) *Metabolism of Drugs and Other Xenobiotics*, Wiley-VCH Verlag GmbH, Weinheim, Germany.

2 Testa, B. and Krämer, S.D. (2008/2010) *The Biochemistry of Drug Metabolism*, vol. **1**, *Principles, Redox Reactions, Hydrolyses*, vol. **2**, *Conjugations, Consequences of Metabolism, Influencing Factors*, Wiley-VCH Verlag GmbH, Weinheim, Germany.

3 Uetrecht, J.P. and Trager, W.F. (2007) *Drug Metabolism – Chemical and Enzymatic Aspects*, Informa Healthcare, New York.

4 Lash, L.H. (ed.) (2005) *Drug Metabolism and Transport*, Humana Press, Totowa, NJ.

5 Silverman, R.B. (2004) *The Organic Chemistry of Drug Design and Drug Action*, 2nd edn, Academic Press, San Diego, CA.

6 Testa, B. and Mayer, J.M. (2003) *Hydrolysis in Drug and Prodrug Metabolism – Chemistry, Biochemistry and Enzymology*, Wiley-VCH Verlag GmbH, Weinheim, Germany.

7 Ioannides, C. (ed.) (2002) *Enzyme Systems that Metabolise Drugs and Other Xenobiotics*, John Wiley & Sons, Ltd, Chichester.

8 Testa, B. (1995) *The Metabolism of Drugs and Other Xenobiotics – Biochemistry of Redox Reactions*, Academic Press, London.

9 Testa, B., Pedretti, A., and Vistoli, G. (2012) Reactions and enzymes in the metabolism of drugs and other xenobiotics – a meta-analysis of the primary literature. *Drug Discovery Today*, **17**, 549–560.

10 Kadlubar, S. and Kadlubar, F.F. (2010) Enzymatic basis of phase I and phase II drug metabolism, in *Enzyme- and Transporter-Based Drug–Drug Interactions* (eds K.S. Pang, A.D. Rodrigues, and R.M. Peter), Springer, New York, pp. 3–25.

11 Testa, B. (2010) Principles of drug metabolism, in *Burger's Medicinal Chemistry, Drug Discovery and Development*, 7th edn, vol. **1** (eds D.J. Abraham and D.P. Rotella), John Wiley & Sons, Inc., Hoboken, NJ, pp. 127–166.

12 Trager, W.F. (2007) Principles of drug metabolism 1: redox reactions, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 87–132.

13 Testa, B. (2007) Principles of drug metabolism 2: hydrolysis and conjugation reactions, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 133–166.

14 Totah, R.A. and Rettie, A.E. (2007) Principles of drug metabolism 3: enzymes and tissues, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 167–191.

15 Testa, B. and Krämer, S.D. (2006) The biochemistry of drug metabolism – an introduction. Part 1: principles and overview. *Chemistry & Biodiversity*, **3**, 1053–1101.

16 Testa, B. and Krämer, S.D. (2007) The biochemistry of drug metabolism – an introduction. Part 2: redox reactions and their enzymes. *Chemistry & Biodiversity*, **4**, 257–405.

17 Testa, B. and Krämer, S.D. (2007) The biochemistry of drug metabolism – an introduction. Part 3: reactions of hydrolysis and their enzymes. *Chemistry & Biodiversity*, **4**, 2031–2122.

18 Testa, B. and Krämer, S.D. (2008) The biochemistry of drug metabolism – an introduction. Part 4: reactions of conjugation and their enzymes. *Chemistry & Biodiversity*, **5**, 2171–2336.

19 Kalgutkar, A.S., Dalvie, D., Obach, R.S., and Smith, D.A. (2012) *Reactive Drug Metabolites*, Wiley-VCH Verlag GmbH, Weinheim, Germany.

20 Rendic, S. and Guengerich, F.P. (2012) Contributions of human enzymes in carcinogen metabolism. *Chemical Research in Toxicology*, **25**, 1316–1383.

21 Testa, B. and Krämer, S.D. (2009) The biochemistry of drug metabolism – an introduction. Part 5: metabolism and bioactivity. *Chemistry & Biodiversity*, **6**, 591–684.

22 Ettmayer, P., Amidon, G.L., Clement, B., and Testa, B. (2004) Lessons learned from marketed and investigational prodrugs. *Journal of Medicinal Chemistry*, **47**, 2393–2404.

23 Testa, B. (2009) Drug metabolism for the perplexed medicinal chemist. *Chemistry & Biodiversity*, **6**, 2055–2070.

24 Testa, B. (1987) Pharmacokinetic and pharmacodynamic events: can they always be distinguished? *Trends in Pharmacological Sciences*, **8**, 381–383.

25 van de Waterbeemd, H. and Testa, B. (2009) Introduction: the how and why of

bioavailability research, in *Drug Bioavailability – Estimation of Solubility, Permeability, Absorption and Bioavailability*, 2nd edn (eds H. van de Waterbeemd and B. Testa), Wiley-VCH Verlag GmbH, Weinheim, Germany, pp. 1–6.

26 Fura, A., Shu, Y.Z., Zhu, M., Hanson, R. L., Roongta, V., and Humphreys, W.G. (2004) Discovering drugs though biological transformation: role of pharmacologically active metabolites in drug discovery. *Journal of Medicinal Chemistry*, **47**, 4339–4351.

27 Yengi, L.G., Leung, L., and Kao, J. (2007) The evolving role of drug metabolism in drug discovery and development. *Pharmaceutical Research*, **24**, 842–858.

28 Bodor, N. and Buchwald, P. (2000) Soft drug design: general principles and recent applications. *Medicinal Research Reviews*, **20**, 58–101.

29 Bodor, N. (1984) Novel approaches to the design of safer drugs: soft drugs and site-specific chemical delivery systems, in *Advances in Drug Research*, vol. **13** (ed. B. Testa), Academic Press, London, pp. 255–331.

30 Lippert, B. (ed.) (1999) *Cisplatin – Chemistry and Biochemistry of a Leading Anticancer Drug*, Verlag Helvetica Chimica Acta, Zurich.

31 Albert, A. (1958) Chemical aspects of selective toxicity. *Nature*, **182**, 421–422.

32 Rautio, J. (ed.) (2010) *Prodrugs and Targeted Delivery – Towards Better ADME Properties*, Wiley-VCH Verlag GmbH, Weinheim, Germany.

33 Testa, B. (2009) Prodrugs: bridging pharmacodynamic/pharmacokinetic gaps. *Current Opinion in Chemical Biology*, **13**, 338–344.

34 Raito, J., Kumpulainen, H., Heimbach, T., Oliyai, R., Oh, D., Järvinen, T., and Savolainen, J. (2008) Prodrugs: design and clinical applications. *Nature Reviews Drug Discovery*, **7**, 255–270.

35 Smith, D.A. (2007) Do prodrugs deliver? *Current Opinion in Drug Discovery and Development*, **10**, 550–559.

36 Oesch-Bartlomowicz, B. and Oesch, F. (2007) Mechanisms of toxification and detoxification that challenge drug candidates and drugs, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 193–214.

37 Stevens, J.L. (2006) Future of toxicology – mechanisms of toxicity and drug safety: where do we go from here? *Chemical Research in Toxicology*, **19**, 1393–1401.

38 Hop, C.E.C.A., Kalgutkar, A.S., and Soglia, J.R. (2006) Importance of early assessment of bioactivation in drug discovery. *Annual Reports in Medicinal Chemistry*, **41**, 369–381.

39 Guengerich, F.P. and MacDonald, J.S. (2007) Applying mechanisms of chemical toxicity to predict drug safety. *Chemical Research in Toxicology*, **20**, 344–369.

40 Shu, Y.-Z., Johnson, B.M., and Yang, T.J. (2008) Role of biotransformation studies in minimizing metabolism-related liabilities in drug discovery. *The AAPS Journal*, **10**, 178–192.

41 Tang, W. and Lu, A.Y.H. (2010) Metabolic bioactivation and drug-related adverse effects: current status and future directions from a pharmaceutical research perspective. *Drug Metabolism Reviews*, **42**, 225–249.

42 Evans, D.C., Watts, A.P., Nicoll-Griffith, D.A., and Baillie, T.A. (2004) Drug–protein adducts: an industrial perspective on minimizing the potential for drug bioactivation in drug discovery and development. *Chemical Research in Toxicology*, **17**, 3–16.

43 Zhou, S., Chan, E., Duan, W., Huang, M., and Chen, Y.-Z. (2005) Drug bioactivation, covalent binding to target proteins and toxicity relevance. *Drug Metabolism Reviews*, **37**, 41–213.

44 Park, B.K., Kitteringham, N.R., Maggs, J.L., Pirmohamed, M., and Williams, D.P. (2005) The role of metabolic activation in drug-induced hepatotoxicity. *Annual Reviews in Pharmacology and Toxicology*, **45**, 177–202.

45 Uetrecht, J. (2009) Immune-mediated adverse drug reactions. *Chemical Research in Toxicology*, **22**, 24–34.

46 Bolton, J.L., Trush, M.A., Penning, T.M., Dryhurst, G., and Monks, T.J. (2000) Role

of quinones in toxicology. *Chemical Research in Toxicology*, **13**, 135–160.
47 Sallustio, B.C., DeGraaf, Y.C., Weekley, J. S., and Burcham, P.C. (2006) Bioactivation of carboxylic acid compounds by UDP-glucuronosyltransferases to DNA-damaging intermediates: role of glycoxidation and oxidative stress in genotoxicity. *Chemical Research in Toxicology*, **19**, 683–691.
48 Stone, M.P., Huang, H., Brown, K.L., and Shanmugan, G. (2011) Chemistry and structural biology of DNA damage and biological consequences. *Chemistry & Biodiversity*, **8**, 1571–1615.
49 Recanatini, M., Poluzzi, E., Masetti, M., Cavalli, A., and De Ponti, F. (2005) QT prolongation through hERG K^+ channel blockade: current knowledge and strategies for the early prediction during drug development. *Medicinal Research Reviews*, **25**, 133–166.
50 Aronov, A.M. (2007) In silico models to predict QT prolongation, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 933–955.
51 Esser, C. (2007) Immunotoxicology, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 215–229.
52 Kazius, J., McGuire, R., and Bursi, R. (2005) Derivation and validation of toxicophores for mutagenicity prediction. *Journal of Medicinal Chemistry*, **48**, 312–320.
53 Kalgutkar, A.S., Gardner, I., Obach, R.S., Shaffer, C.L., Callegari, E., Henne, K.R., Mutlib, A.E., Dalvie, D.K., Lee, J.S., Nakai, Y., O'Donnell, J.P., Boer, J., and Harriman, S.P. (2005) A comprehensive listing of bioactivation pathways of organic functional groups. *Current Drug Metabolism*, **6**, 161–225.
54 Blagg, J. (2006) Structure–activity relationships for *in vitro* and *in vivo* toxicity. *Annual Reports in Medicinal Chemistry*, **41**, 353–367.
55 Caron, G., Carrupt, P.A., Testa, B., Ermondi, G., and Gasco, A. (1996) Insight into the lipophilicity of the aromatic *N*-oxide moiety. *Pharmaceutical Research*, **13**, 1186–1190.
56 Caron, G., Ermondi, G., Boschi, D., Carrupt, P.A., Fruttero, R., Testa, B., and Gasco, A. (1999) Structure–property relations in the basicity and lipophilicity of arylalkylamine oxides. *Helvetica Chimica Acta*, **82**, 1630–1639.
57 Caron, G., Gaillard, P., Carrupt, P.A., and Testa, B. (1997) Lipophilicity behavior of model and medicinal compounds containing a sulfide, sulfoxide or sulfone moiety. *Helvetica Chimica Acta*, **80**, 449–462.
58 Giroud, Y., Carrupt, P.A., Pagliara, A., Testa, B., and Dickinson, R.G. (1998) Intrinsic and intramolecular lipophilicity effects in *O*-glucuronides. *Helvetica Chimica Acta*, **81**, 330–341.
59 Testa, B., Crivori, P., Reist, M., and Carrupt, P.A. (2000) The influence of lipophilicity on the pharmacokinetic behavior of drugs: concepts and examples. *Perspectives in Drug Discovery and Design*, **19**, 179–211.
60 Lavé, T. and Funk, C. (2007) In vivo absorption, distribution, metabolism, and excretion studies in discovery and development, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 31–50.
61 Artursson, P., Neuhoff, S., Matsson, P., and Tavelin, S. (2007) Passive permeability and active transport models for the prediction of oral absorption, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 259–278.
62 Barton, P., Austin, R.P., and Fessey, R.E. (2007) In vitro models for plasma binding and tissue storage, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 321–340.

63 Liu, X., Chen, C., and Hop, C.E. (2011) Do we need to optimize plasma protein and tissue binding in drug discovery? *Current Topics in Medicinal Chemistry*, **11**, 450–466.

64 Abbott, N.J. (2007) In vitro models for examining and predicting brain uptake of drugs, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 301–320.

65 Reeves, P.T. (2010) Drug residues. *Handbook of Experimental Pharmacology*, **199**, 265–290.

66 Sugano, K. (2007) Artificial membrane technologies to assess transfer and permeation of drugs in drug discovery, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 453–487.

67 Liu, X., Testa, B., and Fahr, A. (2011) Lipophilicity and its relationship with drug passive permeation. *Pharmaceutical Research*, **28**, 962–977.

68 Scherrmann, J.M. (2007) The biology and function of transporters, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 51–85.

69 Smith, D.A. and Dalvie, D. (2012) Why do metabolites circulate? *Xenobiotica*, **42**, 107–126.

70 Jeong, E.J., Liu, X., Jia, X., Chen, J., and Hu, M. (2005) Coupling of conjugating enzymes and efflux transporters: impact on bioavailability and drug interactions. *Current Drug Metabolism*, **6**, 455–468.

71 Vree, T.B. and Timmer, C.J. (1998) Enterohepatic cycling and pharmacokinetics of oestradiol in postmenopausal women. *Journal of Pharmacy and Pharmacology*, **50**, 857–864.

72 Vickery, S. and Dodds, P.F. (2004) Incorporation of xenobiotic carboxylic acids into lipids by cultured 3T3-L1 adipocytes. *Xenobiotica*, **34**, 1025–1042.

73 Dodds, P.F. (1995) Xenobiotic lipids: the inclusion of xenobiotic compounds in pathways of lipid biosynthesis. *Progress in Lipid Research*, **34**, 219–247.

74 Fears, R. (1986) Lipophilic xenobiotic conjugates: the pharmacological and toxicological consequences of the participation of drugs and other foreign compounds as substrates in lipid biosynthesis. *Progress in Lipid Research*, **24**, 177–195.

75 Mayer, J.M. and Testa, B. (1997) Pharmacodynamics, pharmacokinetics and toxicity of ibuprofen enantiomers. *Drugs of the Future*, **22**, 1347–1366.

76 Mlodinow, L. (2009) Chapter 3, in *The Drunkard's Walk – How Randomness Rules our Lives*, Penguin Books, London.

77 Krämer, S.D. and Testa, B. (2008) The biochemistry of drug metabolism – an introduction. Part 6: inter-individual factors affecting drug metabolism. *Chemistry & Biodiversity*, **5**, 2465–2578.

78 Krämer, S.D. and Testa, B. (2009) The biochemistry of drug metabolism – an introduction. Part 7: intra-individual factors affecting drug metabolism. *Chemistry & Biodiversity*, **6**, 1477–1660.

79 Mugford, C.A. and Kedderis, G.L. (1998) Sex-dependent metabolism of xenobiotics. *Drug Metabolism Reviews*, **30**, 441–498.

80 Loi, C.M. and Vestal, R.E. (1998) Drug metabolism in the elderly. *Pharmacology and Therapeutics*, **36**, 131–149.

81 Labrecque, G., Beauchamp, D., Vanier, M.C., and Smolensky, M.H. (1997) Chronopharmacokinetics. *Pharmaceutical News*, **4**, 17–26.

82 Boullata, J. and Armenti, V.T. (eds) (2004) *Handbook of Drug–Nutrient Interactions*, Humana Press, Totowa, NJ.

83 Rodrigues, A.D. (ed.) (2002) *Drug–Drug Interactions*, Dekker, New York.

84 Carey, N. (2012) *The Epigenetics Revolution – How Modern Biology Is Rewriting Our Understanding of Genetics, Disease and Inheritance*, Icon Books, London.

85 Leader, J.E., Wang, C., Fu, M., and Pestell, R.G. (2006) Epigenetic regulation of nuclear steroid receptors. *Biochemical Pharmacology*, **72**, 1589–1596.

86 Ballard, P., Brassil, P., Bui, K.H., Dolgos, H., Petersson, C., Tunek, A., and Webborn, P.J.H. (2012) The right compound in the right assay at the right time: an integrated discovery DMPK strategy. *Drug Metabolism Reviews*, **44**, 224–252.

87 van de Waterbeemd, H. (2002) High-throughput and *in silico* techniques in drug metabolism and pharmacokinetics. *Current Opinion in Drug Discovery and Development*, **5**, 33–43.

88 Bertrand, M., Jackson, P., and Walther, B. (2000) Rapid assessment of drug metabolism in the drug discovery process. *European Journal of Pharmaceutical Sciences*, **11**, S61–S72.

89 Parmentier, Y., Bossant, M.J., Bertrand, M., and Walther, B. (2007) In vitro studies of drug metabolism, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 231–257.

90 Minton, A.P. (2005) Influence of macromolecular crowding upon the stability and state of association of proteins: predictions and observations. *Journal of Pharmaceutical Sciences*, **94**, 1668–1675.

91 Riley, R.J. and Kenna, J.G. (2004) Cellular models for ADMET predictions and evaluation of drug–drug interactions. *Current Opinion in Drug Discovery and Development*, **7**, 86–99.

92 Nurse, P. (2003) Understanding cells. *Nature*, **424**, 883.

93 Davies, P. (2013) What's really going on in those cancer cells? *New Scientist*, **217** (2898), 24–25.

94 Caron, G., Ermondi, G., and Testa, B. (2007) Predicting the oxidative metabolism of statins. An application of the MetaSite® algorithm. *Pharmaceutical Research*, **24**, 480–501.

95 Kirchmair, J., Williamson, M.J., Tyzack, J. D., Tan, L., Bond, P.J., Bender, A., and Glen, R.C. (2012) Computational prediction of metabolism: sites, products, SAR, P450 enzyme dynamics, and mechanisms. *Journal of Chemical Information and Modeling*, **52**, 617–648.

96 Afzelius, L., Hasselgren Arnby, C., Broo, A., Carlsson, L., Isaksson, C., Jurva, U., Kjellander, B., Kolmodin, K., Nilsson, K., Raubacher, F., and Weidolf, L. (2007) State-of-the-art tools for computational site of metabolism predictions: comparative analysis, mechanistical insights, and future applications. *Drug Metabolism Reviews*, **39**, 61–86.

97 Egan, W.J. (2007) Computational models for ADME. *Annual Reports in Medicinal Chemistry*, **42**, 449–467.

98 Jolivette, L.J. and Ekins, S. (2007) Methods for predicting human drug metabolism. *Advances in Clinical Chemistry*, **43**, 131–176.

99 Testa, B., Vistoli, G., and Pedretti, A. (2005) Musings on ADME predictions and structure–activity relations. *Chemistry & Biodiversity*, **2**, 1411–1427.

100 van de Waterbeemd, H. and Gifford, E. (2003) ADMET *in silico* modelling: towards prediction paradise? *Nature Reviews Drug Discovery*, **2**, 192–204.

101 Testa, B. and Cruciani, G. (2001) Structure–metabolism relations, and the challenge of predicting biotransformation, in *Pharmacokinetic Optimization in Drug Research – Biological, Physicochemical and Computational Strategies* (eds B. Testa, H. van de Waterbeemd, G. Folkers, and R. Guy), Wiley-VCH Verlag GmbH, Weinheim, Germany, pp. 65–84.

102 Winiwarter, S., Ridderström, M., Ungell, A.L., Andersson, T.B., and Zamora, I. (2007) Use of molecular descriptors for absorption, distribution, metabolism, and excretion predictions, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 531–554.

103 Athersuch, T.J., Wilson, I.D., Keun, H.C., and Lindon, J.C. (2013) Development of quantitative structure–metabolism (QSMR) relationships for substituted anilines based on computational chemistry. *Xenobiotica*, **43**, 792–802.

104 Jones, J.P., Mysinger, M., and Korzekwa, K.R. (2002) Computational models for cytochrome P450: a predictive electronic

model for aromatic oxidation and hydrogen atom abstraction. *Drug Metabolism and Disposition*, **30**, 7–12.

105 Dong, D. and Wu, B. (2012) Substrate selectivity of drug-metabolizing cytochrome P450s predicted from crystal structures and *in silico* modeling. *Drug Metabolism Reviews*, **44**, 1–17.

106 de Graaf, C., Vermeulen, N.P.E., and Feenstra, K.A. (2005) Cytochrome P450 *in silico*: an integrative modeling approach. *Journal of Medicinal Chemistry*, **48**, 2725–2755.

107 De Groot, M.J., Lewis, D.F.V., and Modi, S. (2007) Molecular modeling and quantitative structure–activity relationship of substrate and inhibitors of drug metabolism enzymes, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 809–825.

108 Brown, C.M., Reisfeld, B., and Mayeno, A. N. (2008) Cytochromes P450: a structure-based summary of biotransformations using representative substrates. *Drug Metabolism Reviews*, **40**, 1–100.

109 Cruciani, G., Carosati, E., De Boeck, B., Ethirajulu, K., Mackie, C., Howe, T., and Vianello, R. (2005) MetaSite: understanding metabolism in human cytochromes from the perspective of the chemist. *Journal of Medicinal Chemistry*, **48**, 6970–6979.

110 Zhou, D., Afzelius, L., Grimm, S.W., Andresson, T.B., Zauhar, R.J., and Zamora, I. (2006) Comparison of methods for the prediction of the metabolic sites for CYP3A4-mediated metabolic reactions. *Drug Metabolism and Disposition*, **34**, 976–983.

111 Ekins, S., Andreyev, S., Ryabov, A., Kirillov, E., Rakhmatulin, E.A., Bugrim, A., and Nikolskaya, T. (2005) Computational prediction of human drug metabolism. *Expert Opinion in Drug Metabolism and Toxicology*, **1**, 303–324.

112 Stranz, D.D., Miao, S., Campbell, S., Maydwell, G., and Ekins, S. (2008) Combined computational metabolite prediction and automated structure-based analysis of mass spectrometric data. *Toxicology Mechanisms and Methods*, **18**, 243–250.

113 Hawkins, D.R. (2007) Comprehensive expert systems to predict drug metabolism, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 795–807.

114 Greene, N. (2007) Computational models to predict toxicity, in *ADME-Tox Approaches*, vol. **5** (eds B. Testa and H. van de Waterbeemd), *Comprehensive Medicinal Chemistry*, 2nd edn (eds J.B. Taylor and D.J. Triggle), Elsevier, Oxford, pp. 909–932.

115 Valerio, L.G. and Long, A. (2010) The *in silico* prediction of human-specific metabolites from hepatotoxic drugs. *Current Drug Discovery Technologies*, **7**, 170–187.

116 Marchant, C.A., Briggs, K.A., and Long, A. (2008) *In silico* tools for sharing data and knowledge on toxicity and metabolism: Derek for Windows, Meteor, and Vitic. *Toxicology Mechanisms and Methods*, **18**, 177–197.

117 T'jollyn, H., Boussery, K., Mortishire-Smith, R.J., Coe, K., De Boeck, B., Van Bocxlaer, J.F., and Mannens, G. (2011) Evaluation of three state-of-the-art metabolite prediction software packages (Meteor, MetaSite, and StarDrop) through independent and synergistic use. *Drug Metabolism and Disposition*, **39**, 2066–2075.

118 Testa, B. (2001) Pharmacokinetic lead optimization: fine art vs. blind technology, in *Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical and Computational Strategies* (eds B. Testa, H. van de Waterbeemd, G. Folkers, and R. Guy), Wiley-VCH Verlag GmbH, Weinheim, Germany, pp. 615–625.