REVIEW

The Biochemistry of Drug Metabolism – An Introduction
Part 1. Principles and Overview

by **Bernard Testa***[a]) and **Stefanie D. Krämer**[b])

[a]) Department of Pharmacy, University Hospital Centre (CHUV), Rue du Bugnon, CH-1011 Lausanne (e-mail: Bernard.Testa@chuv.ch)

[b]) Department of Chemistry and Applied Biosciences, ETH-Zurich, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich

Drug metabolism as a multidisciplinary science was born in the first half of the 19th century, when hippuric acid (the glycine conjugate of benzoic acid) was discovered in horse urine (hence its name). In 1841, it was discovered in the urine of a human after ingestion of 2 g of benzoic acid, an experiment that marked the beginning of human drug-metabolism studies [1][2]. Subsequent progress was impressive, but it remained restricted to a narrow circle of biochemists. It was only in the 1950s that drug metabolism really took off due to a convergence of factors including *a*) the progressive awareness among pharmaceutical scientists of the variety and significance of metabolic reactions, and the involvement of metabolites in unwanted drug effects; *b*) the groundbreaking studies of distinguished pioneers; *c*) the explosive development of analytic instrumentation; and *d*) the acknowledged scientific and didactic impact of a few books [3–6].

Since then, many books have appeared, most of them being edited ones offering expertly written reviews; some such books are listed in the *References* [7–20]. Other books were written by one or two authors, their import and tone being more unitarian and didactic (*e.g.*, [21–28]).

The present Work falls in the second category and summarizes the experience of its two authors as lecturers at the M.Sc. and Ph.D. levels. Modern computer technology now allows for lively and attractive teaching support, and we have attempted to transpose an entire course in *Powerpoint*™ format into a printed format. This was achieved by structuring it into seven *Parts* (see *Fig. 1.1*) consisting mainly of colored figures (*i.e.*, the original yet adapted slides), each with an extensive caption, plus a short introductory text, and an extensive bibliography. As a further original feature, the various *Parts* of the Work are first published as separate review papers before appearing in book form.

We hope readers will enjoy these features as much as we enjoyed delivering our lectures and preparing this Work.

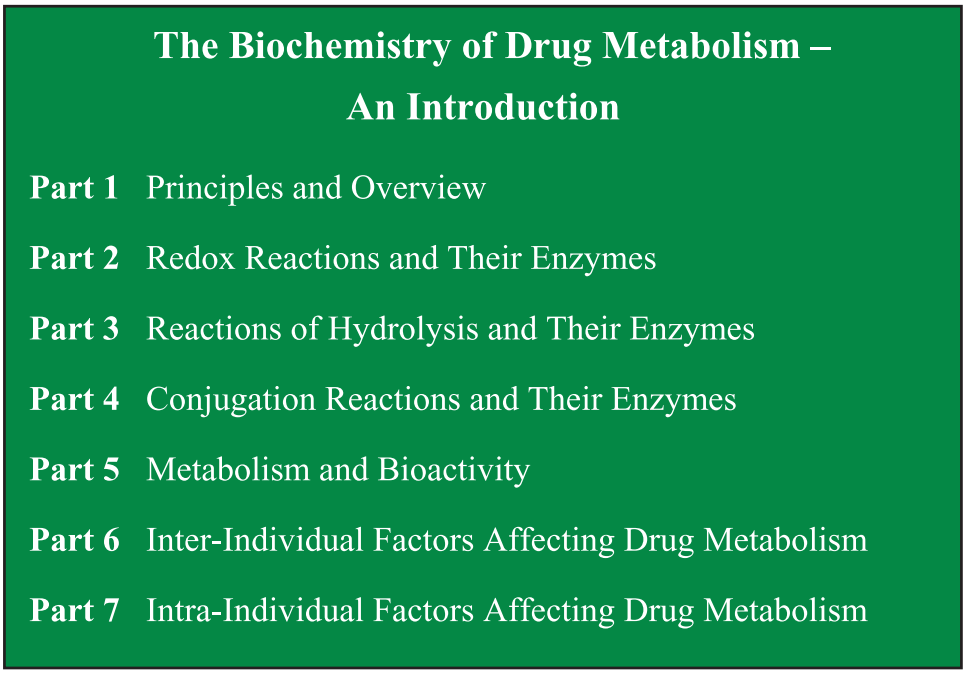


Fig. 1.1. The *Figure* presents the seven Parts of the book. These are being published first as seven separate reviews, and then together as a monograph. The construction of the entire work and the sequence of its Parts obeys a logic we found best adapted to our didactic mission and objectives. *Part 1* brings an overview and explains some basic principles. The core of drug metabolism, *i.e.*, its actors (the enzymes) and their actions (the metabolic reactions), are presented in *Parts 2*, *3*, and *4*. This is done by considering first oxidoreductases and their redox reactions (oxidations and reductions; *Part 2*), then hydrolases and reactions of hydrolysis (*Part 3*), and finally the vast diversity of conjugating enzymes (transferases) and their reactions of conjugation (*Part 4*). The pharmacological and toxicological consequences of drug and xenobiotic metabolism are explained in *Part 5*. The work ends with *Parts 6* and *7* which present in systematic form the many biological factors that influence (modulate) the metabolism of foreign compounds, namely inter-individual factors (which are 'written' in the genome of the organism; *Part 6*) and intra-individual factors (which change over time in a given organism; *Part 7*).

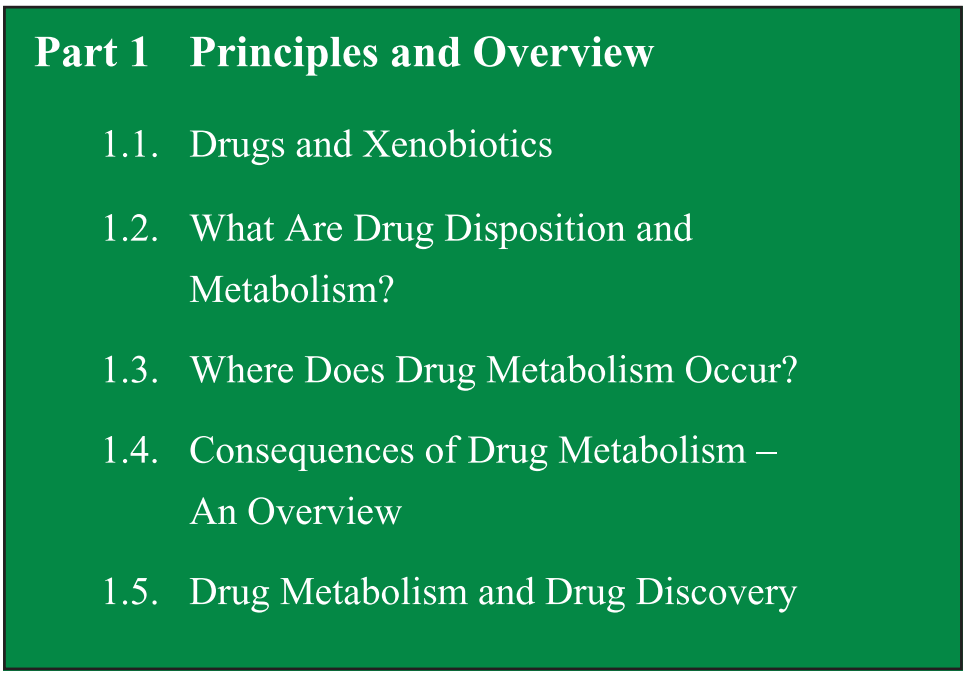


Fig. 1.2. The content of *Part 1* is summarized in this *Figure*. *Chapt. 1.1* defines xenobiotics and shows that drugs are but one class thereof. In other words, toxicological issues resulting from biotransformation (toxification) are a problem that goes well beyond medicinal compounds to encompass all foreign compounds our organism is exposed to. *Chapt. 1.2* examines the components of drug disposition, thus placing metabolism (= biotransformation) in the broader context of a drug's fate in the organism. We then take a brief look at where metabolism does occur in the body (*Chapt. 1.3*). This is followed (*Chapt. 1.4*) by a systematic overview of the consequences of biotransformation in terms of bioactivity (pharmacological and toxicological effects), pharmacokinetic-toxicokinetic behavior [29], and clinical effects. Finally, *Chapt. 1.5* takes a look at drug research, showing how and why drug metabolism has become so important in discovery and development. This Chapter also summarizes the *in vitro* biological methods and *in silico* tools used to assess or predict biotransformation.

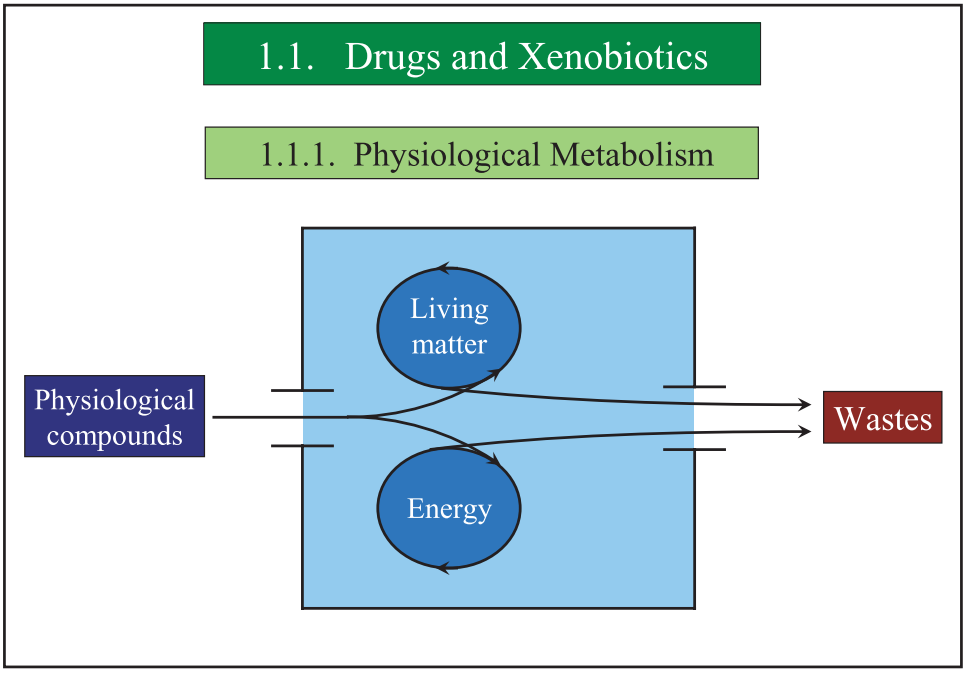


Fig. 1.3. This *Figure* opens *Chapt. 1.1*, whose aim is to define xenobiotics [21–23][25][26][30–32]. The definition is best approached by beginning with the *physiological metabolism*. Indeed, all organisms are open systems, *i.e.*, complex adaptive systems which maintain their low entropy content by extracting energy and 'building material' from a permanent flux of matter that enters them as physiological compounds, and exits as wastes and heat (plants obtain their energy directly from photons). After entering the organism, these physiological compounds (see *Fig. 1.4*) undergo catabolic and/or anabolic reactions. *Catabolic* (*degradation*) *reactions* liberate part of the energy content of these compounds and/or break them down to small building blocks (*e.g.*, amino acids). *Anabolic* (*synthetic*) *reactions* incorporate physiological compounds or some smaller components into living matter. The waste products resulting from physiological metabolism have a higher entropy content than the entering physiological compounds; they are, thus, of low or no value to the organism and are excreted mainly in the urine and feces.

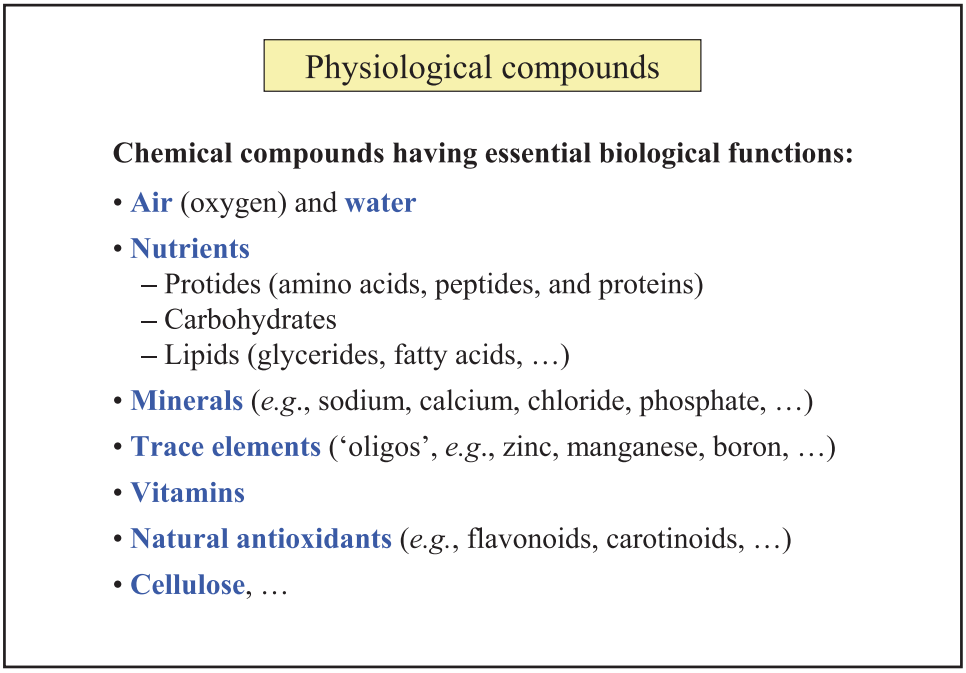


Fig. 1.4. The *Figure* defines *physiological compounds* as chemicals having essential biological functions, namely, which are indispensable to the survival of our body. Most of these compounds are listed here, beginning with the air we breathe and the water we take in. Note that differences exist between species, since oxygen, for example, is toxic to anaerobic microorganisms. Nutrients are conveniently classified into protides, carbohydrates, and lipids, but again some prokaryotes may not need them all. The list continues with the 'micronutrients', namely, inorganic compounds needed in modest (minerals) or trace amounts (oligo-elements). The list also contains compounds whose vital role was uncovered rather recently. These include those natural antioxidants which are not included among vitamins, *e.g.*, flavonoids and lycopene. It may well be that some of them are not indispensable individually, but representatives from different chemical classes are necessary, *i.e.*, acting by different mechanisms and differing in their hydro- and liposolubility. And finally, 'inert' compounds such as cellulose are now recognized to be vitally important in the long term.

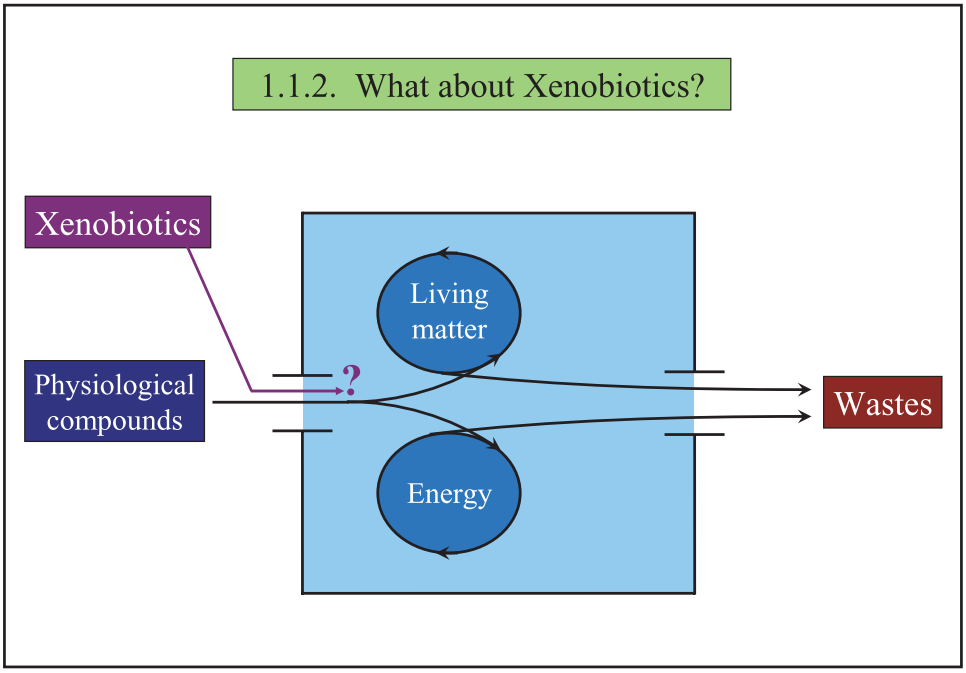


Fig. 1.5. This *Figure* completes *Fig. 1.3* by including xenobiotics and raising the question of their fate in the body. The word '*xenobiotic*' was coined in the early 1970s to indicate compounds that enter the body but have no physiological function and must, therefore, be eliminated [22]. This definition is correct but not complete, since there is also a tendency to view as xenobiotics endogenously produced compounds (endobiotics [32]) administered at relatively high doses, be it for medical or non-medical reasons. A proposed definition and a list of xenobiotics will be given in *Fig. 1.6*. To answer the question mark in the present *Figure* is the objective of the entire Work.

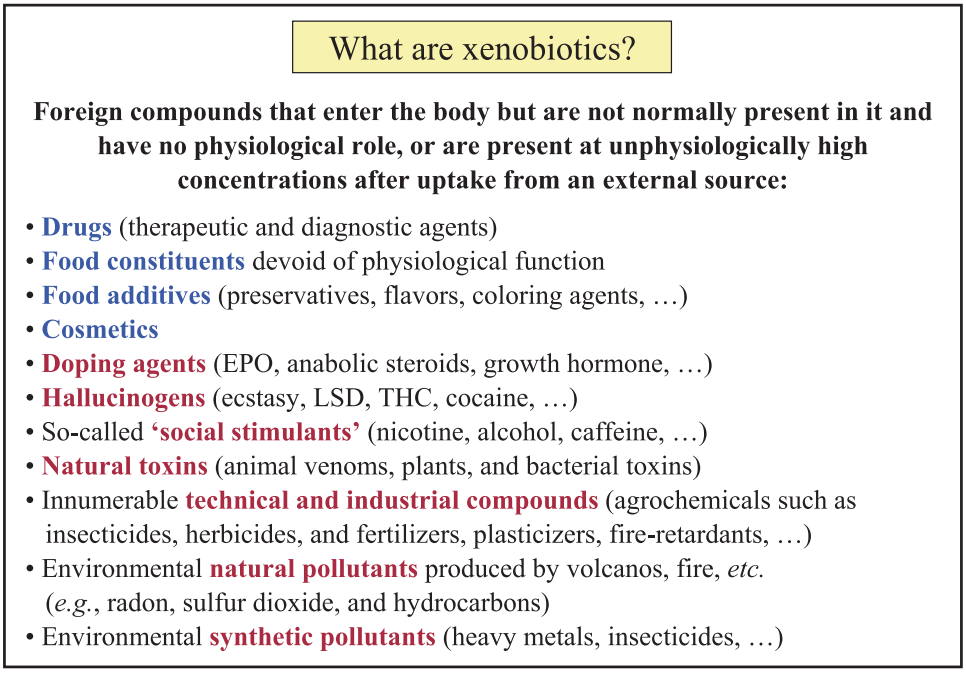


Fig. 1.6. Our proposed definition is shown here, although it may not satisfy everybody. As a result, the best way to grasp the meaning of the concept is to list all classes of chemicals viewed as xenobiotics [25]. The first, and for us central, class is obviously that of *drugs*, with the reminder that drugs are chemicals administered for preventive, therapeutic (treatment), or diagnostic purposes, and the further note that some endobiotics administered to patients (*e.g.*, L-Dopa, hormones such as insulin) are also drugs. Two further groups are the *innumerable chemicals present in our foods*, and *articles for personal hygiene*, be they natural compounds or synthetic additives. For example, it is recognized that a cup of coffee contains several hundreds of compounds many of which contribute to its characteristic flavor and odor. There are then the damaging compounds that are usually taken deliberately, *e.g.*, *doping agents* (including endobiotics such as testosterone), *hallucinogens*, and so-called '*social stimulants*' (nicotine and ethanol are certainly toxic, but caffeine in reasonable amounts should not be considered as damaging). The last groups are the more or less *toxic chemicals to which we are exposed involuntarily*, *e.g.*, natural toxins, industrial compounds, and pollutants of various origins. Most classes of xenobiotics listed here contain synthetic compounds, but natural compounds are almost everywhere in the list.

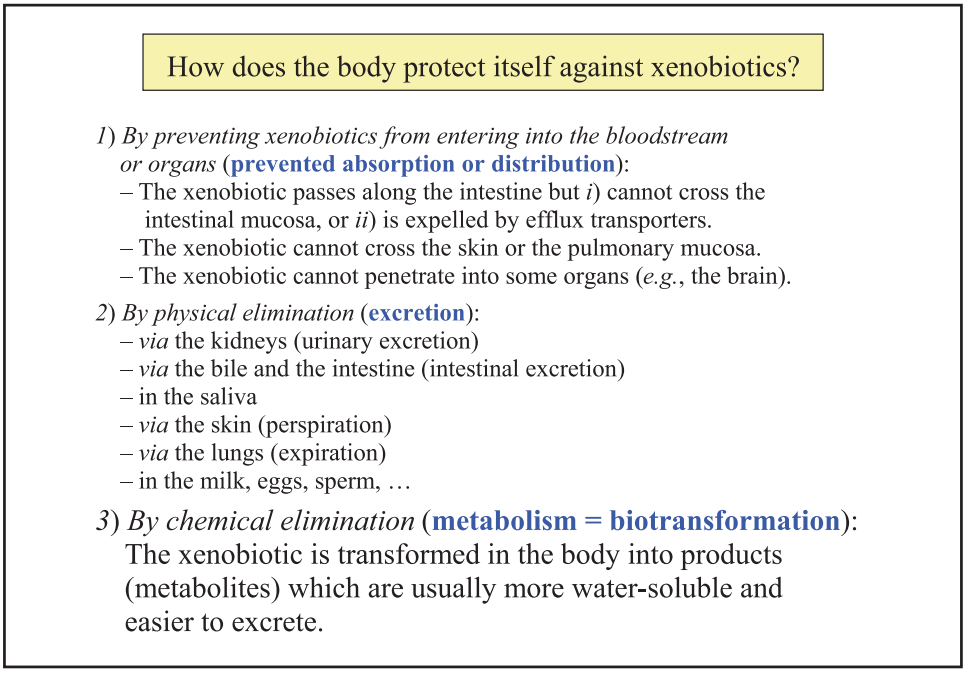


Fig. 1.7. Given that the endless accumulation of even nontoxic xenobiotics is incompatible with survival, natural selection led to the evolution of protective strategies of which metabolism (= biotransformation) is but one [33][34]. Indeed, we owe our current biological protection against xenobiotics to the innumerable natural xenobiotics in existence before the appearance of humankind [35–37]. Schematically, *three protective strategies* have emerged. Taken in a toxicokinetically relevant order, the first strategy is *inhibited entry* into the organism or a given organ. This prevention can be passive, relying on membranes acting as barriers, or active by transporter-mediated efflux. The second strategy is by *excretion* (*physical elimination*), which can be either passive (*e.g.*, urinary excretion) or active (transporter-mediated excretion, *e.g.*, into the bile). Note that a given compound can be barred entry or excreted by passive and active mechanisms acting simultaneously. The third strategy is the focus of this Work, namely *chemical elimination*, better known as '*metabolism*' as synonymous with '*biotransformation*'. Another meaning of the word 'metabolism' is that of 'disposition', namely the sum of the processes affecting the fate of a chemical in the body [29]; this meaning will not be used in this Work. The biotransformation strategy has evolved to increase the hydrophilicity of lipophilic xenobiotics, and hence facilitate their renal and biliary excretion. However, as will be illustrated repeatedly in this Work (mainly in *Part 5*), this strategy fails in a number of cases when biotransformation yields reactive or more lipophilic metabolites [38][39].

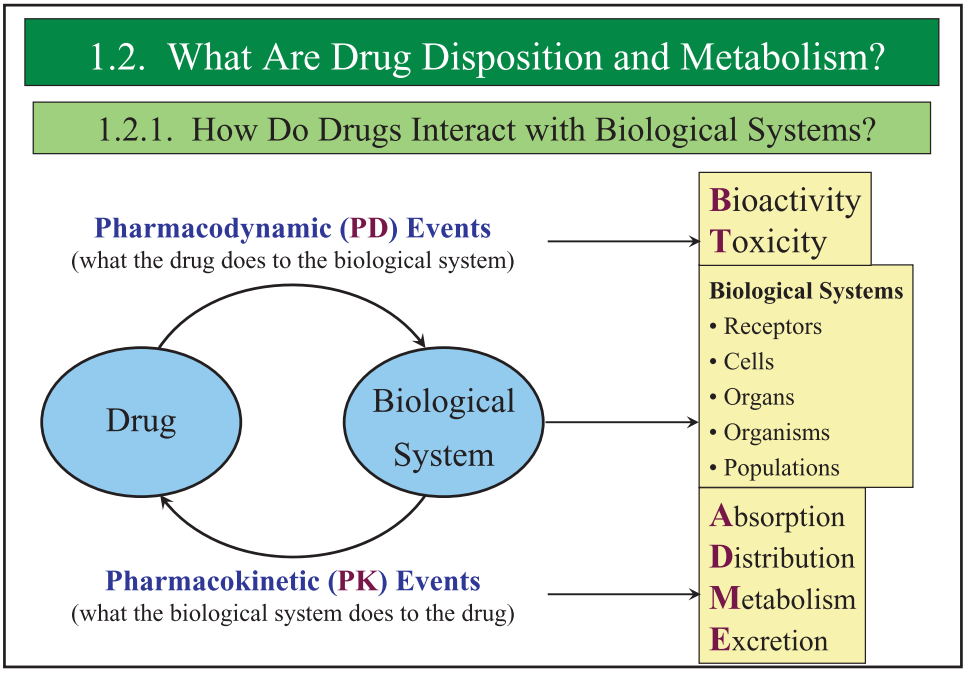


Fig. 1.8. *Chapt. 1.2* takes a closer look at drug metabolism and disposition by presenting definitions and placing these processes in a broader biological context. This *Figure* illustrates in schematic form the two aspects of the interactions between a xenobiotic and a biological system. Note that '*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 'what the compound does to the biosystem' and 'what the biosystem does to the compound'. In pharmacology, one speaks of '*pharmacodynamic effects*' to indicate what a drug does to the body, and '*pharmacokinetic effects*' to indicate what the body does to the drug. But one must appreciate that these two aspects of the behavior of xenobiotics are inextricably interdependent. Absorption, distribution, and elimination will obviously have a decisive influence on the intensity and duration of pharmacodynamic effects, whereas biotransformation will generate metabolites which 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, enzyme activities, *etc.*) and hence its capacity to handle xenobiotics [40]. Only a systemic approach as used in pharmacokinetic/pharmacodynamic (PKPD) modeling and in clinical pharmacology is capable of appreciating the global nature of this interdependence.

1.2.2. The Main Phases of Drug Disposition and Action

Phase	Processes	Research objectives
Pharmaceutical phase	• Disaggregation of pharmaceutical form • **L**iberation and dissolution of drug	Drug available for absorption (optimization of pharmaceutical availability)
Pharmacokinetic phase	• **A**bsorption, **D**istribution (incl. binding & storage) • **M**etabolism (biotransformation) • **E**xcretion	Drug available for action (optimization of biological availability)
Pharmacodynamic phase	Interaction of drug (and metabolites) with: • targets (sites) of therapeutic **A**ctivity (receptors, *etc.*) • sites of **T**oxic effects	Therapeutic effects (optimization of wanted effects, and minimization of unwanted effects)

Fig. 1.9. The pharmacokinetic (PK) processes of absorption, distribution, metabolism, and excretion, and the pharmacodynamic (PD) phenomena of bioactivity and toxicity, are now placed in a broader medicinal perspective by considering the three phases of a drug's action. The (chronologically) first phase, which was not mentioned up to this point, is the *pharmaceutical phase* during which the drug is liberated from the pharmaceutical form (and is dissolved in case of a solid form). In the schematic presentation of this *Figure*, the *pharmacokinetic* and *pharmacodynamic phases* are taken to be consecutive, which is misleading and in apparent contradiction with *Fig. 1.8*. Indeed, it is obvious that the PK and PD phases occur simultaneously, their separation in this *Figure* serving to draw a parallel with the main objectives of drug research. In other words, the three phases of drug disposition and action correspond quite logically to the three research objectives of pharmaceutical, biological, and therapeutic optimization.

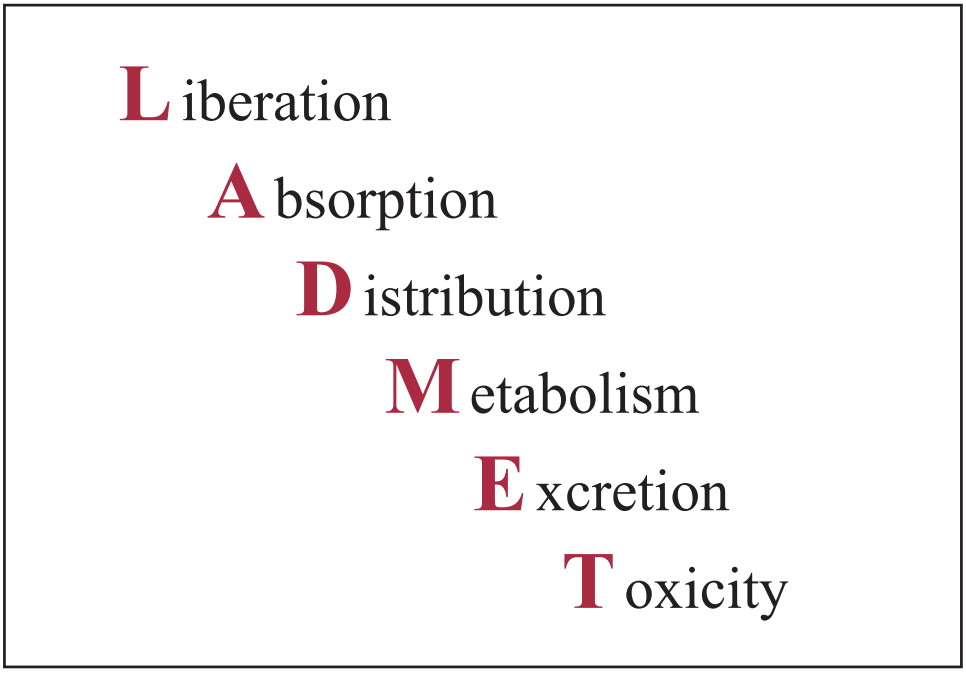


Fig. 1.10. This *Figure* offers an eye-catching reminder of the *LADMET* concept as the counterpart of '*Bioactivity*' in drug discovery and development (drug D&D). Since a number of decades, the pharmacy curriculum has extended the original pharmacokinetic core (ADME) to pharmaceutics and biopharmacy by including '*Liberation*'. More recently, drug researchers have come to realize that '*Toxicity*' could not be separated from the ADME core as a criterion of '*drugability*' and a major challenge in the optimization of *'drug-like' properties* [41].

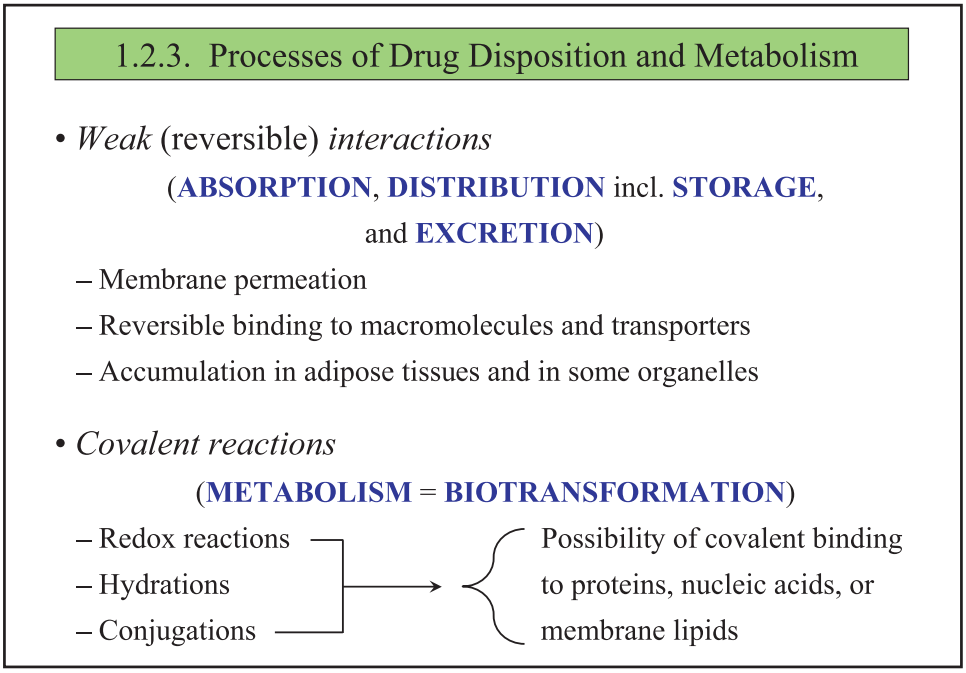


Fig. 1.11. The previous *Figures* have placed metabolism in the broader context of ADME, LADME, and ADMET. The message in the present *Figure* is to demonstrate that absorption, distribution (including storage), metabolism, and excretion can be examined in a common *physicochemical* context. Indeed, these pharmacokinetic phenomena show a bimodal distribution when arranged according to the energy levels involved. *Reversible interactions* such as membrane crossing, reversible binding to soluble proteins and transporters, and accumulation in adipose tissues and organelles involve weak energies in the approximate range of 10 to 60 kJ mol^{-1}. In contrast, metabolic reactions are irreversible in the sense that the formation of metabolites involves the cleavage and formation of (high-energy) *covalent bonds*, and occurs in the approximate range of 200 to 400 kJ mol^{-1}. Note also that redox and conjugation reactions sometimes generate *reactive metabolites* which will react spontaneously with proteins, nucleic acids, or membranes to form *adducts*. Such reactions caused by reactive metabolites are termed '*post-enzymatic*' and are of great toxicological significance [25], as discussed mainly in *Part 5*.

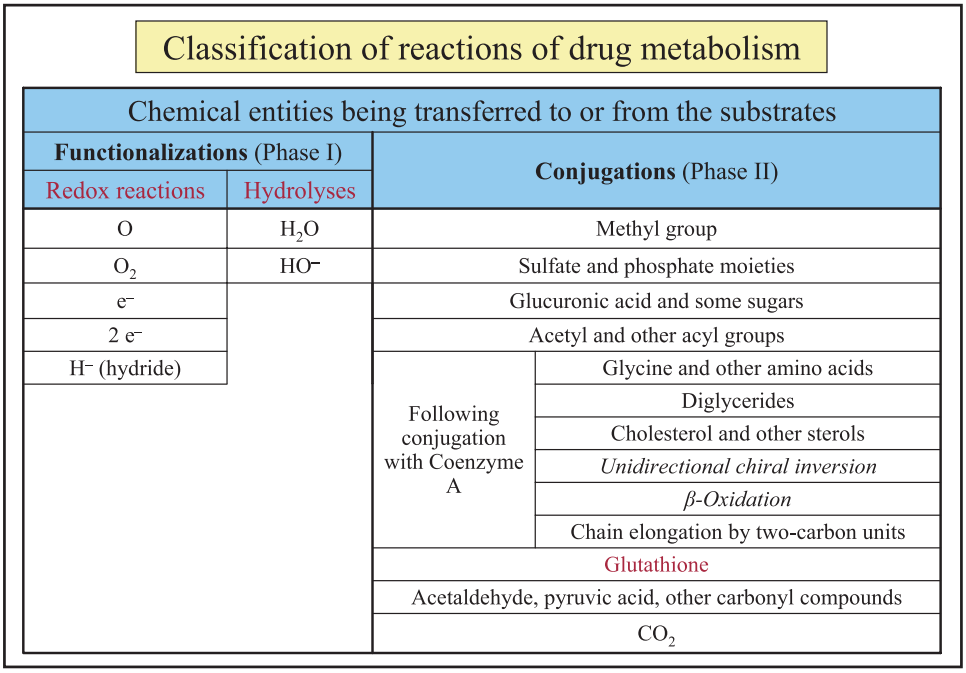
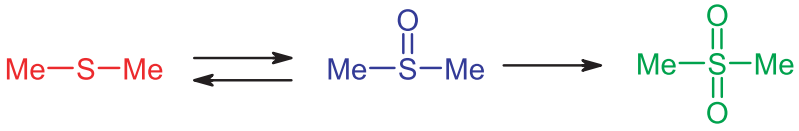


Fig. 1.12. Having moved one step closer to metabolism proper and to its chemical aspects, we can now enter the biochemistry of xenobiotic metabolism. A first classification is between 'phase I' and 'phase II'. While the classification is relevant and useful, the terminology is misleading and outdated, since 'phase II' reactions can occur without or before 'phase I'. We prefer to label 'phase I' as reactions of *functionalization*, and 'phase II' as reactions of *conjugation* [25][28][30][42][43]. The term 'functionalization' may be a source of confusion, since it means different things to different experts; in our mind, it implies the creation of a functional group or the modification of an existing one, and it includes the all important redox reactions (*Part 2*) and hydrolyses/hydrations (*Part 3*). The second major class is that of conjugations, which, as shown, involves a large variety of moieties which can be transferred to the substrate. The term 'conjugation' is used universally and without problem, and it will be defined in *Part 4* according to clear criteria. In the present *Figure*, reactions marked in red are those which, in a few well-defined cases, can occur *non-enzymatically* (*e.g.*, oxidation of polyphenols, hydrolyses of labile esters, glutathione conjugation of strong electrophiles) [44]. Unidirectional chiral inversion (*e.g.*, of profens) and *β*-oxidation (of fatty acid analogs) are written in italics since they are not conjugations *stricto sensu*, but deserve to be so classified, since a coenzyme A conjugate is the indispensable intermediate.

Example: The metabolism of dimethyl sulfoxide



1) **Dimethyl sulfoxide** (DMSO) is a solvent, an excipient and a former drug.

2) It enters the body through the skin.

3) At high percutaneous doses, it acts as an analgesic in muscles and joints.

4) One part of the dose is excreted in urine.

5) The other part is metabolized in the liver to **dimethyl sulfide** (by loss of one oxygen atom) and **dimethyl sulfone** (by gain of one oxygen atom).

6) **Dimethyl sulfone** is excreted in urine.

7) **Dimethyl sulfide** is volatile, has a garlic smell, and is excreted *via* the lungs in the expired air.

Fig. 1.13. A concrete and simple case is presented here to help readers get a feeling of some aspects of drug metabolism and its consequences. The example shown is that of dimethyl sulfoxide (DMSO). This highly polar liquid has exceptional solvent properties and is practically inert chemically and biologically, making it an (almost) ideal solvent in drug research. The compound was used in the 1960s as an analgesic in case of arthritis and arthrosis, being applied externally in undiluted form. This therapeutic use was soon discontinued due to the discovery of potential ocular toxicity in dogs, but it continues to be used at low percent concentrations as an excipient in gels and oitments due to its good pharmaceutical properties. The metabolism of DMSO is comparatively simple, consisting of irreversible oxidation to dimethyl sulfone, and reversible reduction to dimethyl sulfide. Whereas DMSO and dimethyl sulfone are excreted in urine, dimethyl sulfide is excreted partly *via* the lungs due to its volatility. This creates an esthetic problem given the pungent garlic smell of the sulfide (see [5]).

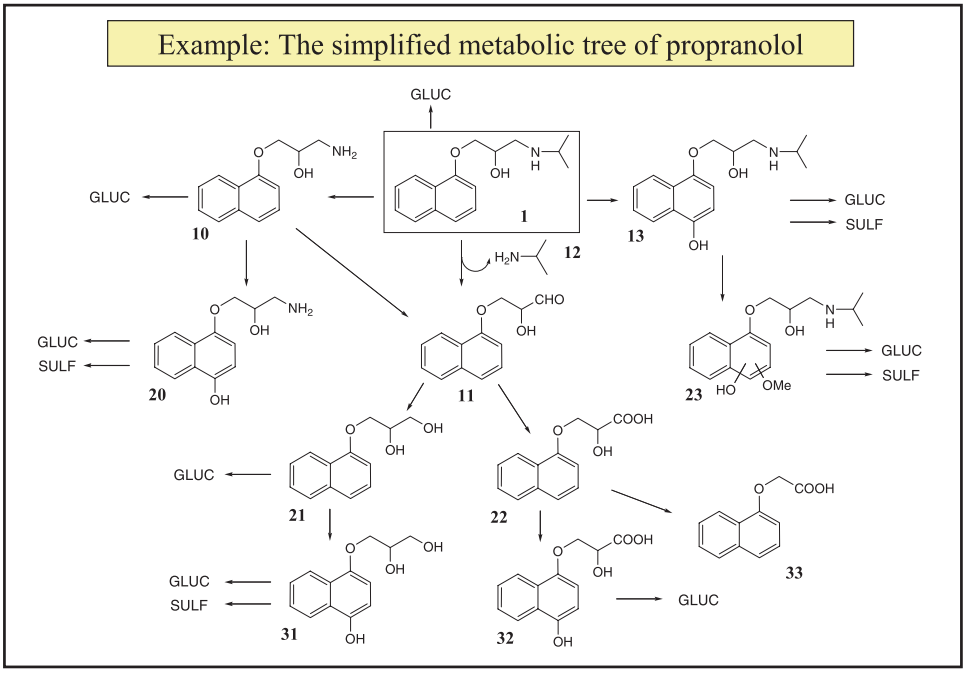


Fig. 1.14. Propranolol (**1**), the first β-blocking drug, is extensively metabolized in humans ($>90\%$ of an oral dose) [45]. Its major human metabolites are presented here to illustrate the concept and potential complexity of a *metabolic tree*. The primary metabolites of propranolol (**1**) are its *O*-glucuronide, the primary amine **10** resulting from oxidative *O*-dealkylation, the aldehyde **11** formed by a reaction of oxidative deamination which also liberates isopropylamine (**12**), and the phenol 4-hydroxypropranolol (**13**); minor positional isomers of **13** have also been characterized in humans. Note that the aldehyde **11** is also a secondary metabolite formed from the primary amine **10**. All the oxidative reactions so far are catalyzed by cytochromes P450 (CYPs, see *Part 2*). Secondary metabolites are the primary alcohol **21** and the carboxylic acid **22** formed from the aldehyde **11** by alcohol dehydrogenases and aldehyde dehydrogenases, respectively (see *Part 2*). Other secondary metabolites are the phenol **20** and isomeric phenols **23**. Tertiary metabolites include the two phenols **31** and **32**, and the minor α-naphthoxyacetic acid metabolite formed by oxidative chain-shortening. Many of these metabolites are also excreted as the *O*-glucuronide and the *O*-sulfate (see *Part 4*).

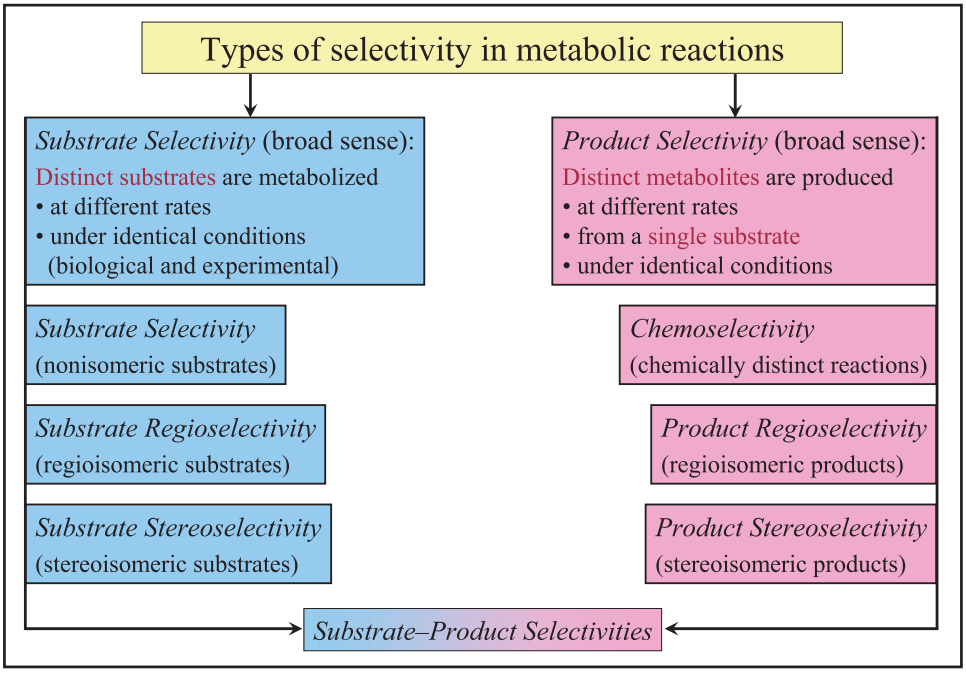


Fig. 1.15. In this work, the specificity of an enzyme will be taken to mean an ensemble of properties, the description of which makes it possible to specify the enzyme's behavior. In contrast, the present Work will apply the term selectivity to metabolic processes, indicating that a given metabolic reaction or pathway is able to select some substrates or metabolites from a larger set. In other words, the *selectivity of a metabolic reaction* is the expression of the *specificity of an enzyme*. Having clarified these definitions, we turn our attention to the various types of selectivities a metabolic reaction can show. When two or more substrates are metabolized at different rates under identical conditions, *substrate selectivity* is observed (left side of the *Figure*). Substrate selectivity is distinct from *product selectivity* (right side of the *Figure*), which is observed when two or more metabolites are formed at different rates from a single substrate under identical conditions. In other words, substrate-selective reactions discriminate between different compounds, while product-selective reactions discriminate between different groups or positions in a given compound. The *substrates being metabolized* at different rates may share various types of relationships. They may be chemically very or slightly different (*e.g.*, analogs, resulting in *substrate selectivity in a narrow sense*). Alternatively, the substrates may be isomers such as positional isomers (regioisomers, resulting in *substrate regioselectivity*), stereoisomers (diastereoisomers or enantiomers, resulting in *substrate stereoselectivity*, substrate diastereoselectivity (seldom used) or *substrate enantioselectivity*). Products formed at different rates in product-selective reactions may also share various types of relationships. Thus, they may be analogs (*product selectivity in a narrow sense*), regioisomers (*product regioselectivity*), or stereoisomers (*i.e.*, diastereoisomers or enantiomers, resulting in *product stereoselectivity*, product diastereoselectivity (seldom used) or *product*

enantioselectivity). And since Nature is never as simple as we would like it, the product selectivity displayed by two distinct substrates in a given metabolic reaction may be different, implying that product selectivity itself may be substrate-selective. The term *substrate–product selectivity* is used to describe such complex cases, which have been reported mainly for stereoselectivity. As presented here, these concepts are quite abstract and not straightforward to grasp. But their repeated application in *Parts 2* to *4* will reveal their usefulness [25][46].

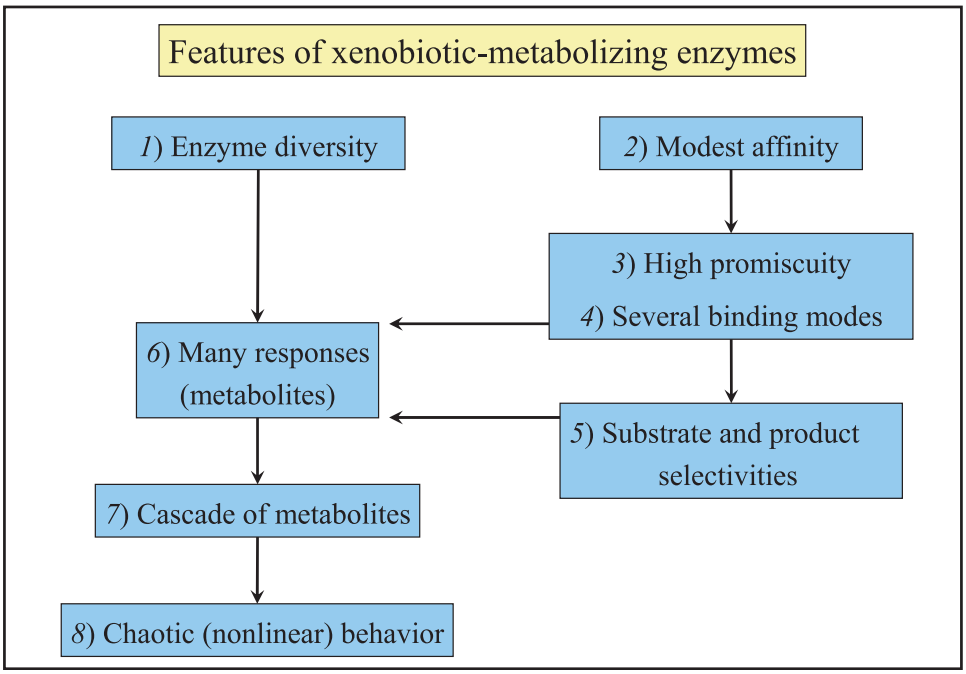


Fig. 1.16. Having introduced metabolic reactions in previous *Figures*, we now take our first look at the agents of these reactions, namely the *enzymes*. Six main classes of enzymes are recognized based on the reactions being catalyzed [47], namely *Oxidoreductases* (EC 1) which catalyze oxidoreduction reactions; *Transferases* (EC 2) which transfer a group from a donor to an acceptor; *Hydrolases* (EC 3), which catalyze the hydrolytic cleavage of C–O, C–N, C–C, and some other bonds; *Lyases* (EC 4) which cleave C–C, C–O, C–N, C–S, and other bonds by elimination, leaving double bonds or rings, or conversely add groups to double bonds; *Isomerases* (EC 5) which catalyze geometric or structural changes within one molecule; *Ligases* (EC 6) which catalyze the joining together of two molecules coupled with the hydrolysis of a pyrophosphate bond in ATP. The vast majority of enzymes known to act on xenobiotics belong to oxidoreductases (*Part 2*), hydrolases (*Part 3*), and transferases (*Part 4*). As exemplified in *Fig. 1.14* with propranolol (**1**), a single substrate

usually yields several (often many) metabolites which are produced '*parallel-wise and series-wise*'. Such a cascade of metabolites allows for *nonlinear responses* (chaotic behavior) in the sense that small causes can have large effects, and large cause can have small effects (see Caption to *Fig. 1.42*). As for the production of many metabolites from a given substrate, this is caused by two factors, namely *a*) the variety and diversity of enzymes that act on a given substrate, and *b*) the product selectivity of a given reaction. In turn, these two factors can be explained as a consequence of the low affinity and the promiscuity (*i.e.*, the capacity to recognize and metabolize a large structural variety of substrates) of xenobiotic-metabolizing enzymes toward their substrates. However, the core factor is the property of promiscuity shown by xenobiotic-metabolizing enzymes. Indeed, this property has been favored by Evolution, since it broadens the chemical space of potential substrates; but promiscuity comes at a cost, the trade-off being reduced turnover.

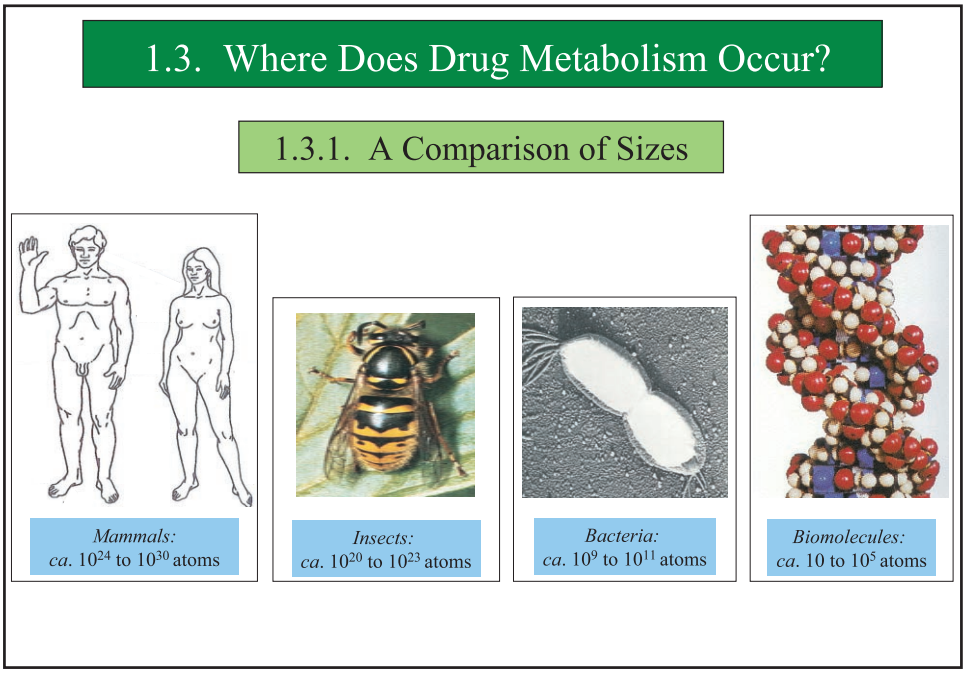


Fig. 1.17. This *Figure* opens *Chapt. 1.3* in which the biological sites of drug metabolism will be summarized. Here, and in the spirit of *Adrian Albert* [21], our readers are offered a comparison of sizes as a reminder of the huge differences in scale and complexity between human subjects and biomolecules. Our voyage from the macro-world to the microworld begins with the moving image of a human couple as carried into interstellar space by the NASA spacecraft Pioneer 10 launched March 3, 1972. While the linear dimensions of an insect are *ca.* 2–3 order of magnitude smaller than human ones, their volume/weight/number of atoms is smaller by *ca.* 8 orders of

magnitude. About 10–15 orders of magnitude are lost when comparing the volume of insects and bacteria. And biomolecules (micro- as well as macromolecules) are 5–19 orders of magnitude lighter than bacteria. The point we want to make here is that biological phenomena at the macroscopic-medical level are often explainable (if only in part) by underlying biochemical processes at the microlevel, but cannot be deduced from them with acceptable certainty. Given the biochemical focus of the Work, caution is urged when trying to infer macroscopic consequences.

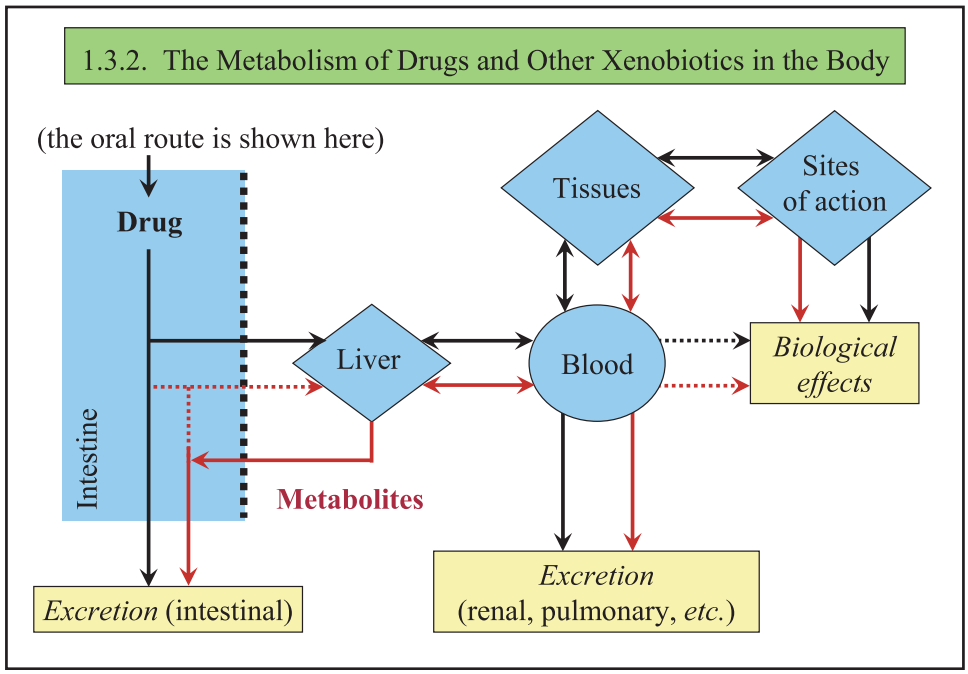


Fig. 1.18. Where does drug metabolism occur? This *Figure* does not answer the question, but it brings us one step closer to it by schematizing the fate of a drug in the body. The *black arrows* represent the drug itself (*i.e.*, the parent compound), whereas the *red arrows* represent its metabolites. Assuming oral administration, part or all of the dose is *absorbed* intestinally, arrives in the liver from where it will be *distributed* by the blood into the tissues and its sites of action. *Reversible binding* to blood and tissue constituents is an important component of a drug's fate [48]. As shown, *metabolite formation* can occur in the intestine and in the liver. However, metabolism in the blood and in peripheral tissues is also a possibility (see *Fig. 1.19*). In most cases, *excretion* of a large fraction of a dose is from the blood *via* the kidneys, minor fractions possibly being excreted *via* the lungs (volatile compounds), skin, saliva, milk [49], *etc.* Excretion of the unabsorbed fraction is intestinal; biliary excretion of some metabolites (mainly large anions) can also result in fecal excretion of part of the dose [50]. The

phenomenon of *enterohepatic cycling* is worth a mention. Glucuronides of sufficient molecular weight (in humans, $>ca.$ 500) undergo biliary excretion. When hydrolysis by the intestinal bacteria is possible (this is the case for *O*-glucuronides; see *Part 4*), the phenol or alcohol so liberated can be re-absorbed, reach the liver and circulate again.

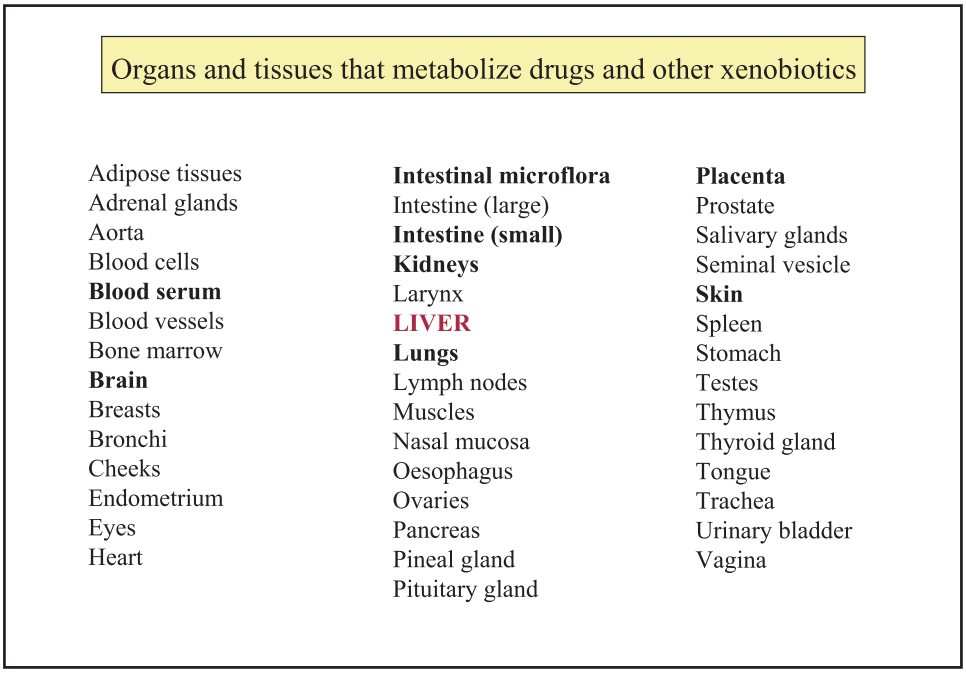


Fig. 1.19. The *liver* has been called the 'chemical factory of the body', and indeed it is an organ whose function is to breakdown and synthetize compounds, xenobiotics included. Most drug-metabolizing enzymes are expressed in the liver, and at comparatively high levels. When introducing drug-metabolizing organs of secondary or tertiary importance, it becomes important to consider these two criteria namely *a*) the variety of enzymes expressed, and *b*) the levels of expression. To visualize the two criteria, one can just think of a histogram with each enzyme being a bin. In the liver, most bins are occupied, and at relatively high levels. The *organs and tissues of secondary importance* (bold in the *Figure*) either express most xenobiotic-metabolizing enzymes at comparatively lower levels (*e.g.*, the brain), or express a limited number of enzymes at relatively high levels (*e.g.*, blood and the kidneys). *Tissues and organs of tertiary importance* express low or very low levels of xenobiotic-metabolizing enzymes. However, their significance should not be underestimated, since they may be involved in the bioactivation or toxification of a few specific substrates. Taken globally, the list in this *Figure* includes almost all organs, a notable exception being the ossified organs (bones and teeth) whose xenobiotic-metabolizing activity appears all but impossible to investigate.

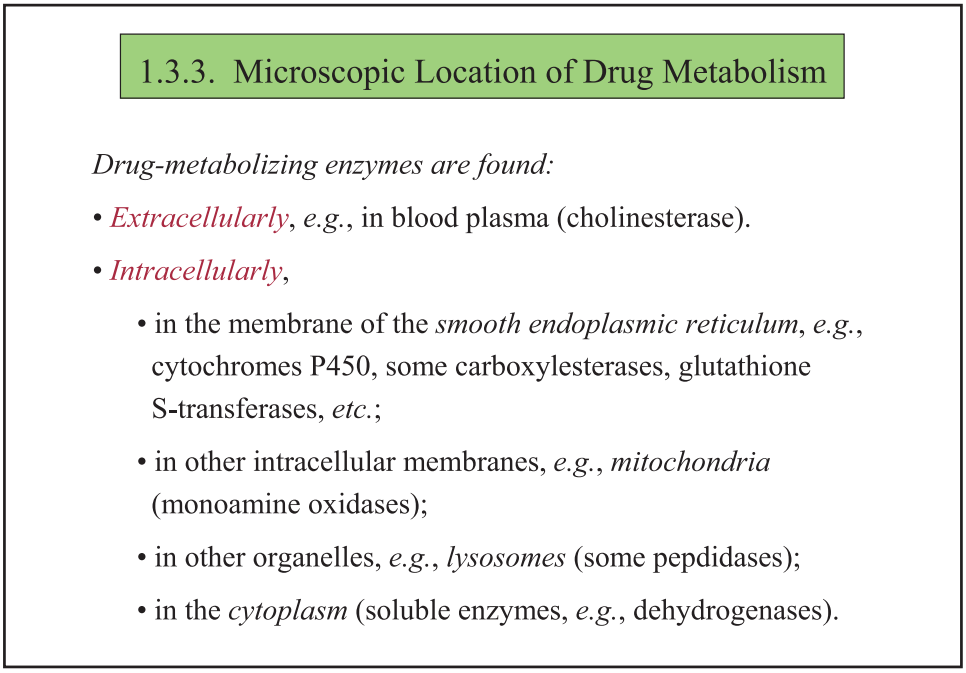


Fig. 1.20. This *Figure* is the continuation of the former one by considering the cellular location of xenobiotic-metabolizing enzymes. Indeed, blood plasma is an important and easily accessible *extracellular fluid* which contains high levels of hydrolases such as cholinesterase (EC 3.1.1.8) and paraoxonase (EC 3.1.8.1), and, for example, a copper-containing amine oxidase (EC 1.4.3.6). However, the vast majority of xenobiotic-metabolizing enzymes are found *intracellularly* (see the previous *Figure* for a list of organs). The *endoplasmic reticulum* (ER) is the location of the most important xenobiotic-metabolizing enzymes such as cytochromes P450 (CYPs, EC 1.14.14.1), glutathione transferases (EC 2.5.1.18), and glucuronyltransferases (EC 2.4.1.17) [51]. How the ER is transformed into microsomes is explained in *Fig. 1.22*. Some enzymes are located in *other organelles* (*e.g.*, mitochrondria and lysosomes). Soluble enzymes (*e.g.*, alcohol dehydrogenase, EC 1.1.1.1) are found in the *cytoplasm*.

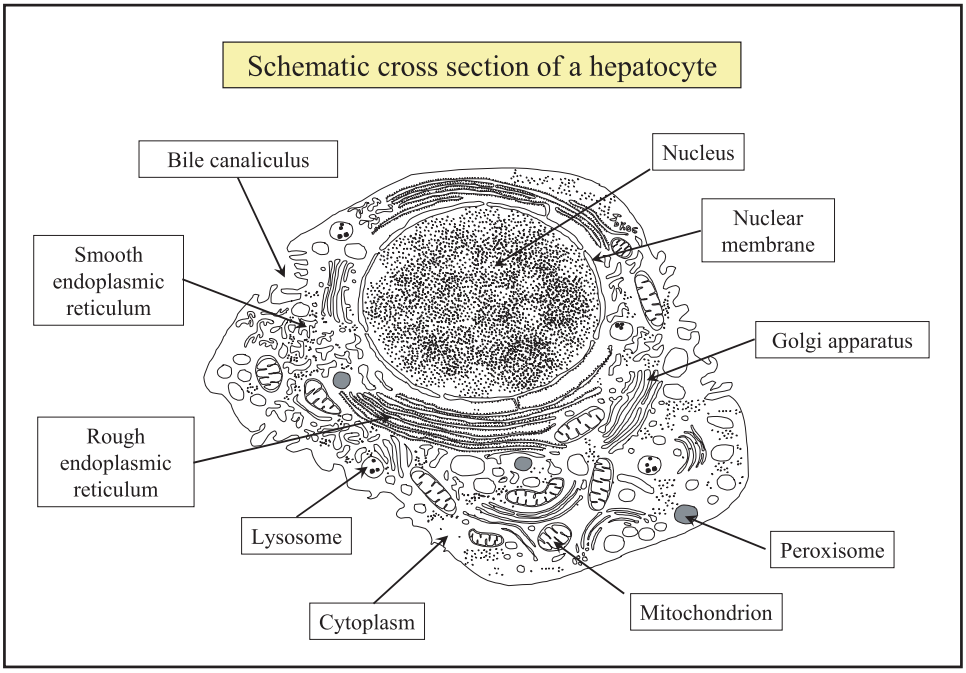


Fig. 1.21. The cellular components mentioned in the previous *Figure* are shown in this schematic representation of a hepatocyte. In addition to the *ER*, *mitochondria*, *lysosomes*, and *cytoplasm* mentioned above, one notes that some weak CYP activities have also been found in the *nuclear membrane*. Taken as a whole, this *Figure* makes it clear that a cell is an entity densely packed with strongly interacting components.

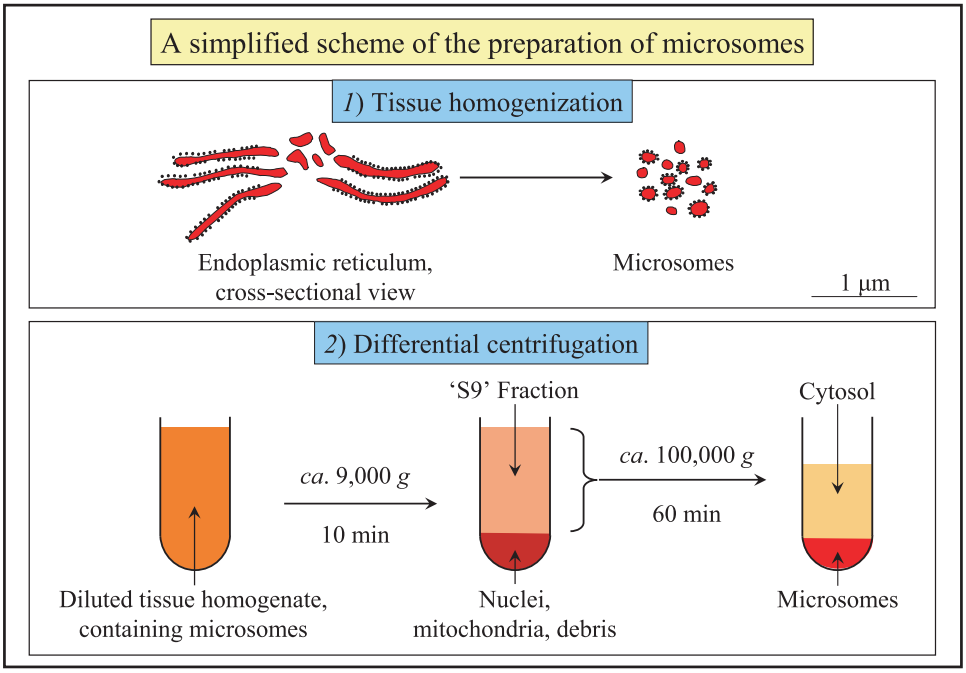


Fig. 1.22. *In vitro investigations* are an essential aspect of drug metabolism studies [52]. The models used are, in decreasing order of biological complexity, isolated organs, liver slices, cell cultures (mainly hepatocytes), subcellular preparations, and isolated enzymes. Among these, subcellular preparations offer an excellent compromise between information yield on the one hand, and, on the other hand, ease of use, low material consumption and throughput. This *Figure* offers a schematic presentation of the preparation of metabolically relevant subcellular fractions [20]. The tissue to be used (fragments of liver or other organs, hepatocytes, *etc.*) is first *homogenized*. This breaks up the endoplasmic reticulum into small spheres visible under the microscope and called *microsomes*. A first centrifugation removes debris, nuclei, and mitochondria, which can be further separated and isolated. The supernatant is called '*S9*' (an abbreviation of *9,000*-g *supernatant*) and is of particular interest, since it is made of microsomes and *cytoplasm*, and hence contains most of the xenobiotic-metabolizing enzymes present in the tissue. Ultracentrifugation then separates the cytoplasm (now called *cytosol*) and the microsomes. The entire procedure is carried out at low temperature (*ca.* 4°C). The microsomal pellet can now be resuspended in a buffer, supplemented with the necessary cofactors, and incubated with the substrate.

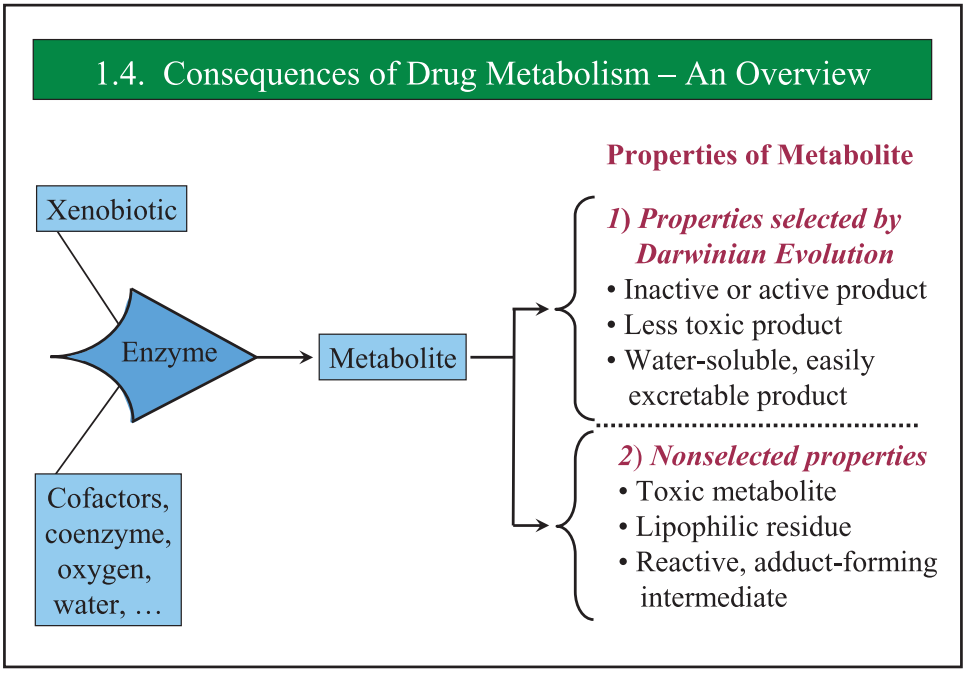


Fig. 1.23. *Chapt. 1.4* is dedicated to a short overview of the consequences of drug and xenobiotic metabolism. As discussed in *Fig. 1.7*, xenobiotic-metabolizing enzymes are believed to have arisen by *co-evolutionary arm race* between *herbivores* and *plants*, with plants evolving chemical defences (alkaloids, terpenoids, *etc.*), which decreased (however slightly) their probability of being consumed, and herbivores evolving counter-strategies to detoxify and excrete these chemicals [35]. Protection against *natural environmental toxins* (*e.g.*, heavy metals, sulfur dioxide, aliphatic and aromatic hydrocarbons, see *Fig. 1.6*) must also have provided a selective advantage not only to herbivores, but to any monocellular or multicellular organism. In other words, Evolution has favored the appearance and fine-tuning by random mutations of enzymes able to recognize and detoxify potentially detrimental xenobiotics of huge chemical diversity. As shown here, *beneficial effects* to the organism included the inactivation–detoxification of toxins and the facilitated excretion of useless compounds. But as we shall see, *exceptions* do exist in the sense that some metabolites can be reactive, more toxic, or more lipophilic than the parent xenobiotic. As a result, innumerable examples now exist of the beneficial or detrimental consequences of drug metabolism in pharmacology, toxicology, and pharmacokinetics.

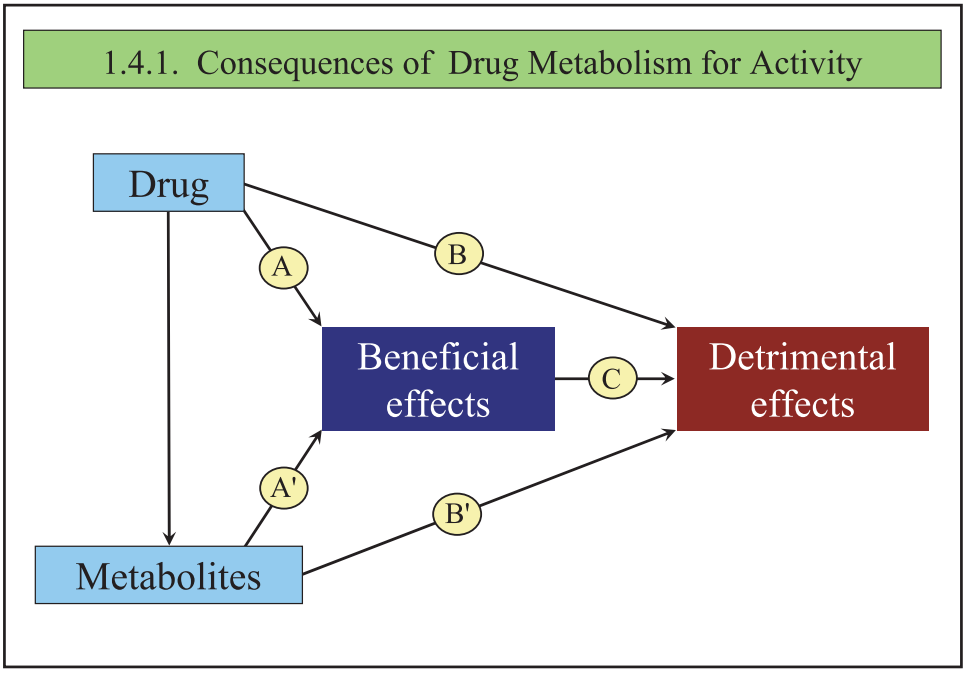


Fig. 1.24. We begin with the consequences of drug metabolism on global activity. As schematized here, a drug is expected to have *beneficial effects* (it would not be a drug otherwise) which can be caused by the parent compound (the drug itself) and/or one or more metabolites (arrows *A* and *A′*). But drug and/or metabolite(s) can also have *detrimental effects*. Interestingly, it is not always realized that such detrimental effects can be of two origins. One possibility (arrows *B* and *B′*) is for the drug and/or metabolite(s) to have side-effects resulting from interaction with biological targets different from the site of wanted action. A highly relevant example is that of several lipophilic drugs belonging to various pharmacological classes which cause cardiotoxicity (QT prolongation) by blocking at therapeutic doses the human ERG potassium channel [53]. Another and more subtle example is provided by antifungal azoles, which work by inhibiting a fungal cytochrome P450 (CYP51), thereby interfering with ergosterol biosynthesis. However, some of these drugs also inhibit human cytochromes P450 (CYP2C9, also 2D6 and 3A4) involved in drug metabolism, thereby causing potentially severe drug–drug interactions [54][55] (as discussed in *Part 7*). The other cause of detrimental effects is shown by arrow *C*, which, in plain language, means 'too much of a good thing is a bad thing'. For example, administering a β-blocker can be very useful to reduce hypertension, but overdosing will 'overshoot' and result in orthostatic hypotension.

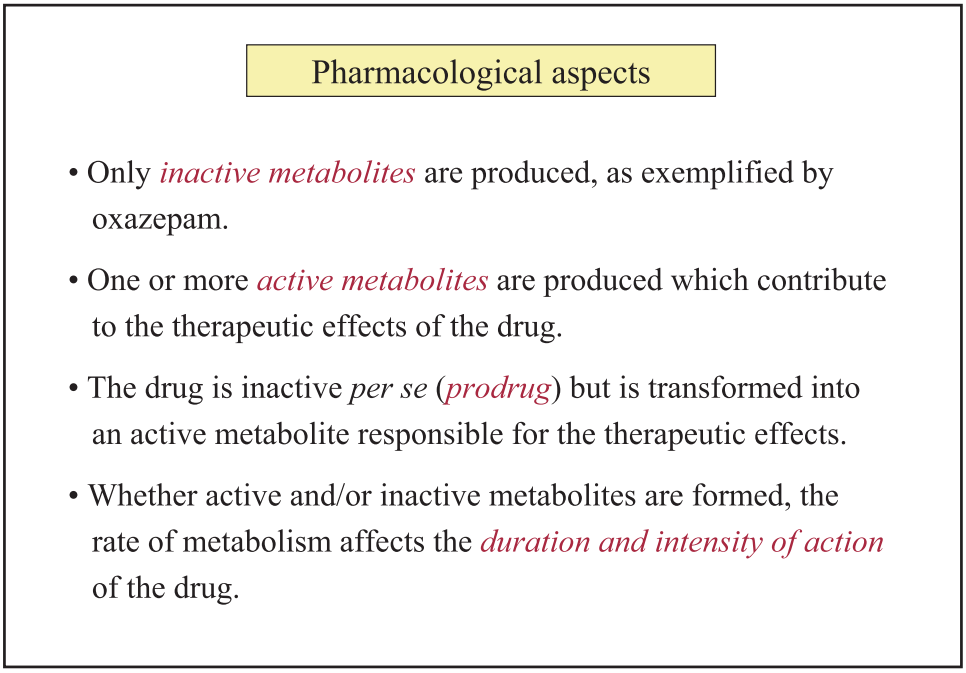


Fig. 1.25. The pharmacological aspects of drug metabolism can be quite varied and have a major impact in therapy. Numerous drugs have *active metabolites* [56][57] whose activity needs to be evaluated by two criteria. These criteria are *a*) does the metabolite have the same mechanism of action as the parent drug, and *b*) if yes, how does it compare quantitatively? Another case is that of drugs having *no pharmacologically active metabolite*. Interestingly, this situation is far rarer than assumed, a clear example being that of oxazepam whose metabolites are all inactive. At the other extreme, we find the 'drugs' that are inactive *per se* but are rapidly transformed into a bioactive metabolite; the name 'produgs' is used to label such therapeutic agents [28][58][59] (see *Part 5*).

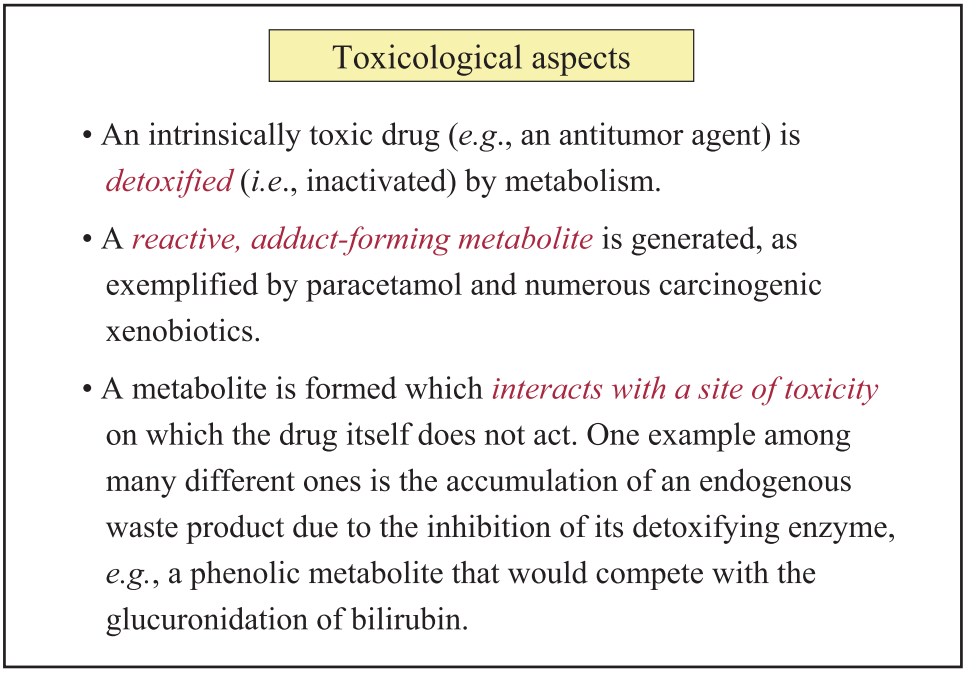


Fig.1. 26. The toxicological aspects of drug metabolism are even more important than pharmacological ones. Note, in particular, that the previous *Figure* is specifically addressed to drugs, whereas toxicology concerns all xenobiotics and not drugs exclusively. As we shall see in detail in *Part 5* and as summarized in this *Figure*, a number of toxicological consequences of xenobiotic metabolism are known. First, a xenobiotic (or a drug such as some antitumor agents) may be highly reactive and undergo *detoxification* by metabolism. The opposite is also true and unfortunately quite frequent, with some drugs and numerous chemicals undergoing *toxification* (arrow *B′* in *Fig. 1.24*). A first case is when the metabolite is chemically reactive and able to *bind covalently to biotargets* such as membrane lipids, proteins, or nucleic acids [60][61]. Many other cases of toxification do not involve adduct-forming metabolites, but simply metabolites whose structure allows them to *interact with a site of toxicity* on which the drug itself does not act. A number of such cases are known, but we believe that many more remain to be understood at the biomolecular level.

1.4.2. Pharmacokinetic Aspects of Drug Metabolism

Pharmacokinetic scenarios		
Drugs and other xenobiotics	Biotransformation	Excretion unchanged
Hydrophilic	minor	major
Lipophilic	major	minor
Very lipophilic	very minor	very minor

Fig. 1.27. A number of scenarios and consequences emerge when examining the pharmacokinetic aspects of drug metabolism. *Metabolic scenarios* can be simplified as shown here, with hydrophilic xenobiotics undergoing limited biotransformation but direct excretion, and lipophilic ones being extensively metabolized but poorly excreted as such. These four schematic scenarios are entirely in line with a *Darwinian Evolution* toward enzymes acting preferentially on poorly excretable, lipophilic xenobiotics to produce more hydrophilic, easily excretable metabolites (see *Fig. 1.23*). But it is humankind's misfortune that Evolution could not prepare us for our own creations, namely, synthetic xenobiotics of such high lipophilicity that our body is not equipped to excrete or metabolize them in any significant amount (see the next *Figure*).

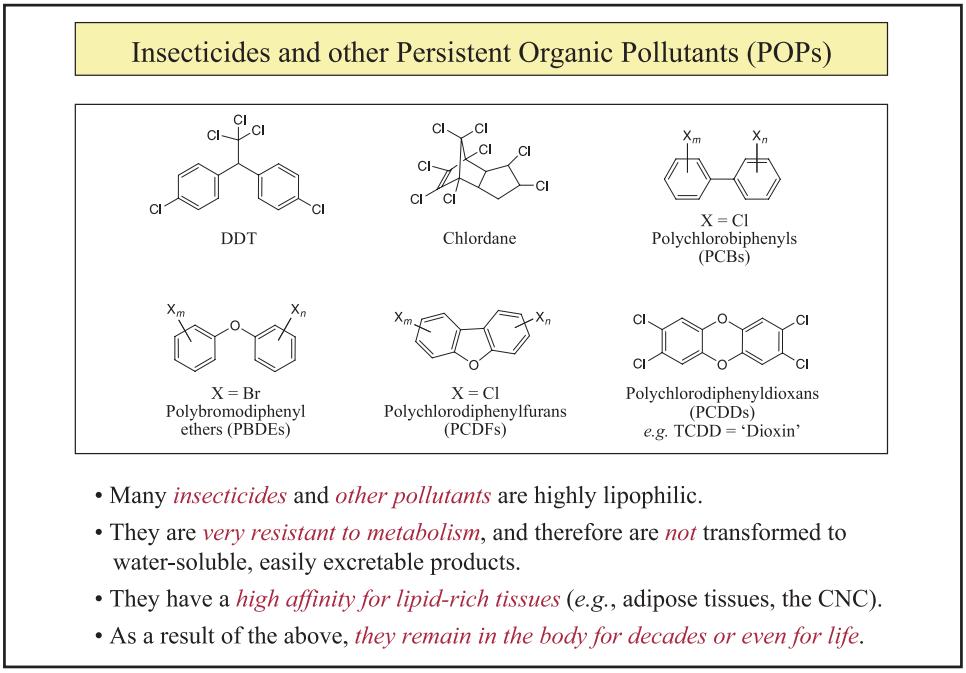


Fig. 1.28. The highly lipophilic compounds alluded to in the previous *Figure* are eliminated very slowly from the body [62]. A small fraction may be metabolized over years and excreted *via* the bile. Mammalian females may also excrete a fraction of their body load with their milk, thus putting their progeny at risk. But a majority of the load will tend to remain as residue in the adipose tissues and nervous system, often for life [37]. The vast majority of such compounds are environmental xenobiotics known as *POP*s (persistent organic pollutants) [63]. They include a number of insecticides such as DDT ('dichloro-diphenyl-trichloroethane') and many industrial pollutants such as polyhalogenated biphenyls, diphenyl ethers, diphenylfurans, and diphenyldioxanes. The use of a number of such POPs is now prohibited or severely restricted, at least in environmentally mindful countries, but the ecosphere is already badly polluted and will remain so for centuries.

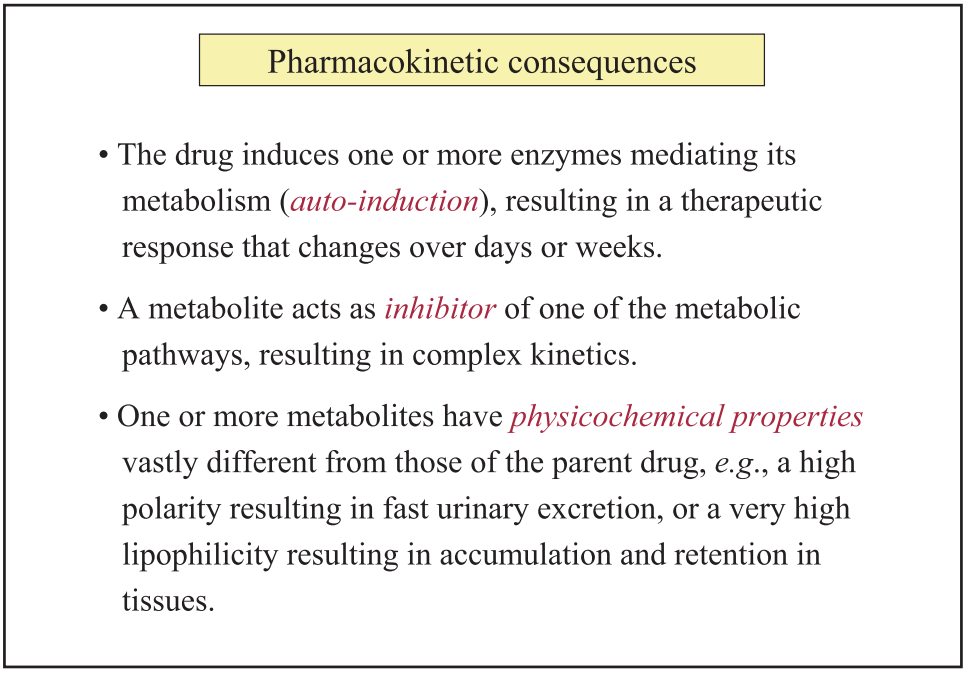


Fig. 1.29. An important consequence of drug metabolism is its pharmacokinetic impact. By this, we mean two aspects. First, a drug may affect its own disposition by *inducing an enzyme* involved in its metabolism. This is well illustrated with the antiepileptic carbamazepine, which induces its own CYP3A4-catalyzed oxidation such that its half-life in humans is reduced about two- to threefold or even more after repeated administration [64]. A different case is seen when a metabolite *inhibits* one of the metabolic pathways of the drug. This will result in a complex kinetics and render dose adjustment more difficult. Some phenolic metabolites, for example, may inhibit cytochromes P450, but this type of situation does not appear to be well documented. The last scenario summarized in the *Figure* concerns metabolites whose *physicochemical properties* differ greatly from those of the parent compound, resulting in a vastly different disposition, be it distribution, storage and/or excretion. The case of highly lipophilic residues is particular interest and will be exemplified in *Part 4*.

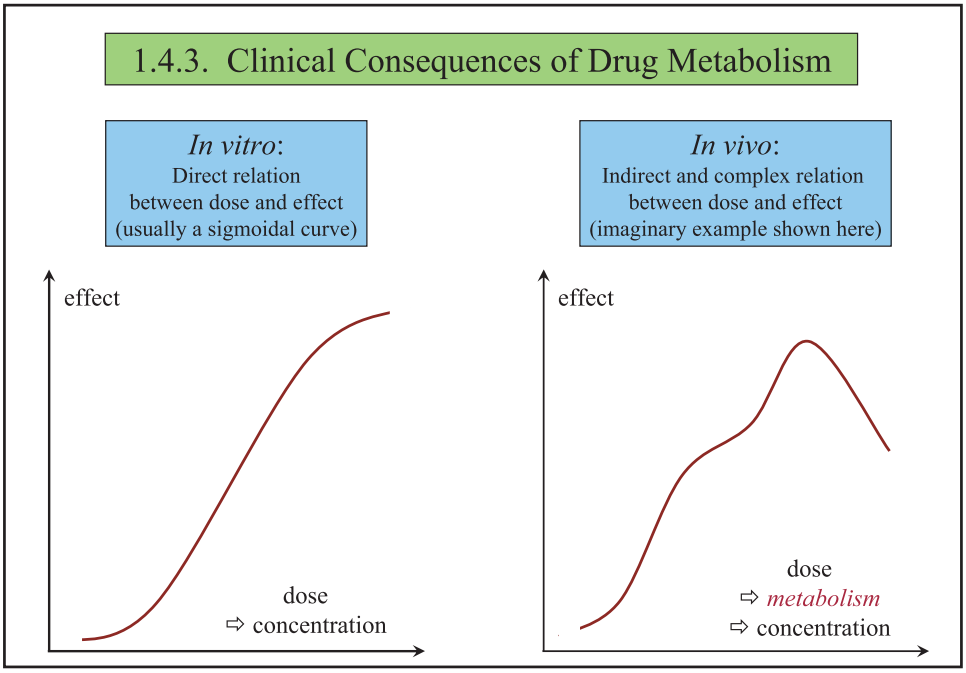


Fig. 1.30. The message in this *Figure* is a simple one, that dose–effect relations may differ greatly between *in vitro* and *in vivo* investigations due to metabolism being an interfering factor. In *in vitro* assays, there is a linear relation between the dose and the concentration, resulting in a direct relation (usually sigmoidal as shown) between concentration and effect. In *in vivo* situations, metabolism may blur the picture, rendering the dose–effect relation an indirect and complex one (an imaginary example is shown here). This is particularly true in clinical settings involving highly heterogeneous populations of patients, hence, the need for therapeutic drug monitoring and medical experience.

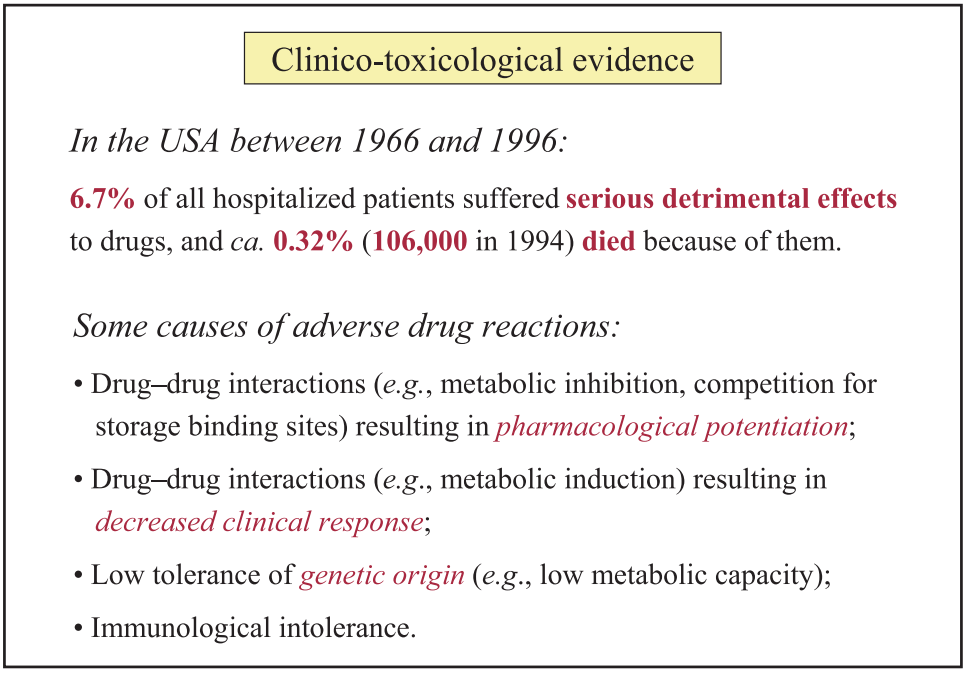


Fig. 1.31. The upper part of this *Figure* shows some sobering data on drug toxicity [65]. The incidence of detrimental effects due to inadequate pharmacotherapy is appaling, as is the number of deaths. Major questions are the predictability and avoidability of such damaging effects, and whether they are iatrogenic (caused by the medical persons). In the lower part of the *Figure*, we list the major causes of *adverse drug reactions* (ADRs) related to metabolism. *Drug–drug interactions* come first, resulting either in *pharmacological potentiation* (apparent overdosing) or *decreased clinical response* (apparent underdosing). Both situations can obviously be life-threatening, especially with narrow-margin drugs. Drug–drug interactions should be avoidable, at least when well documented and in patients receiving a very limited number of medicines. The same is no longer true in most clinical situations with patients receiving five, ten, or more different drugs each day. Low tolerance due to *genetic causes* is a main justification for pharmacogenomic studies [66]. Here, again, some level of predictability is possible by phenotyping or genotyping patients, and by the systematic use of therapeutic drug monitoring. The truly unpredictable metabolism-related toxicity is immunological intolerance, *e.g.*, an allergic reaction to an antigenic hapten–protein conjugate (a hapten being an adduct-forming reactive metabolite). The mechanisms of *immunotoxicity* begin to be understood [67]. An example of trans-acylation potentially leading to allergic reactions will be discussed in *Part 4*.

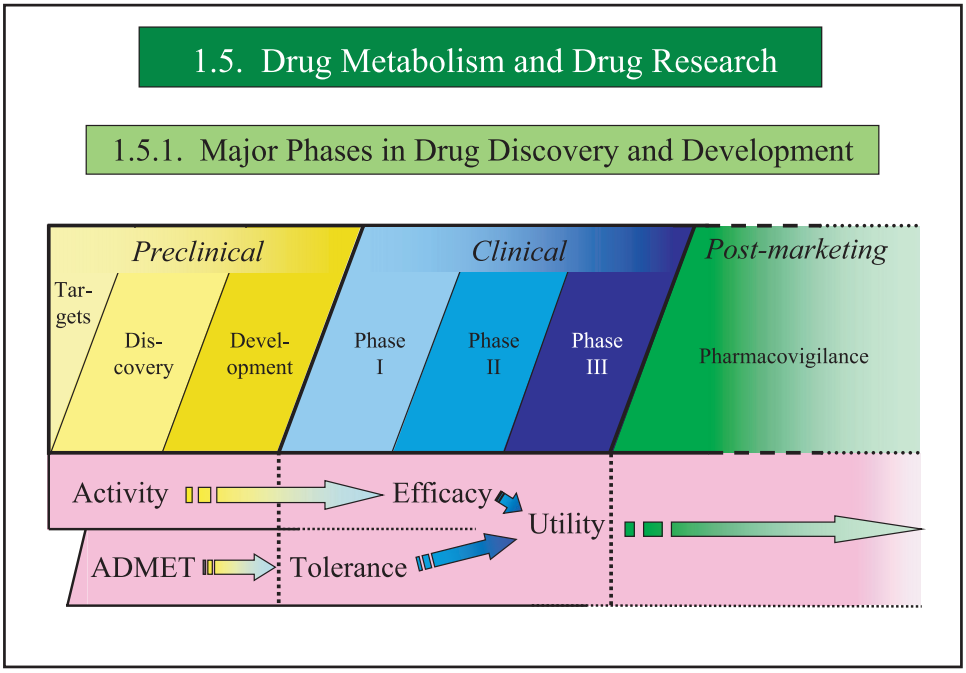


Fig. 1.32. *Chapt. 1.5* is dedicated to the significance of drug metabolism in drug discovery and development [68]. Not so long ago, the metabolism, disposition, and toxicology of selected candidates were studied mainly during preclinical and clinical development. In those days, the mission of medicinal chemistry was to discover and supply very potent compounds, with less interest being given to their behavior in the body. However, the research and development (R&D) paradigm in the pharmaceutical industry has undergone dramatic changes since the 1970s and particularly since the mid-1990s. Rigorous analyses of the root *causes of attrition* during development revealed that lack of efficacy, toxicity, as well as inappropriate absorption, distribution, metabolism, and excretion are among the major determinants of the failure of candidates [69]. A schematic picturing of current drug discovery, development, and clinical assessment is shown in the *Figure*. *Pharmacodynamics* (*i.e.*, bioactivity) is obviously the first object of study, but the new paradigm of drug R&D now has it that ADMET screening must be initiated rapidly. Bioactivity and *ADMET* screening and evaluation thus run in parallel throughout the preclinical phases, and this is when medicinal chemists find themselves in close collaboration with pharmacologists, pharmacists, biologists, biochemists, bioanalysts, physicochemists, computer scientists, and other experts. Assessment of efficacy and tolerance, to merge into utility assessment, then become the objectives of clinical trials.

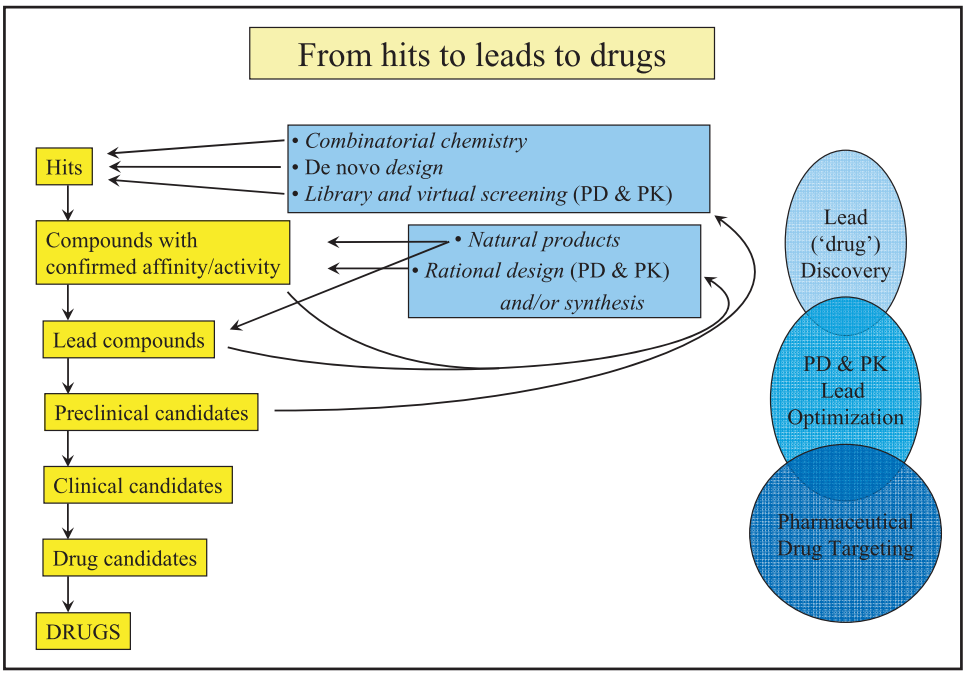


Fig. 1.33. A more detailed picture of the hits-to-leads-to-drugs is presented here. To improve the efficiency of discovery, medicinal chemists have developed new synthetic strategies such as combinatorial chemistry and parallel synthesis. Specialized biotech companies as well as universities also began offering compound collections and focused libraries. As a result, much attention is currently being paid to the design and/or purchase criteria of lead- and drug-like compounds. Increasingly, this includes considerations on ADME-related *physicochemical properties* as well as on the *ADME properties* themselves [70][71]. The concept of *property-based design*, in addition to structure-based design where target structures are available, is now commonly used to address ADME issues as early as possible. High-throughput biological assays were developed which have enabled large series of compounds to be screened, including considerations on ADME properties (see *Fig. 1.38*). In addition, it became reasonable and even essential to develop *in silico* tools (see *Figs. 1.39* to *1.42*) to predict and simulate various physicochemical and ADME properties, and to balance these in decision making processes together with combined *in vivo* and *in vitro* approaches. As shown in *Fig. 1.36*, metabolism-related questions continue to arise throughout the drug development stages.

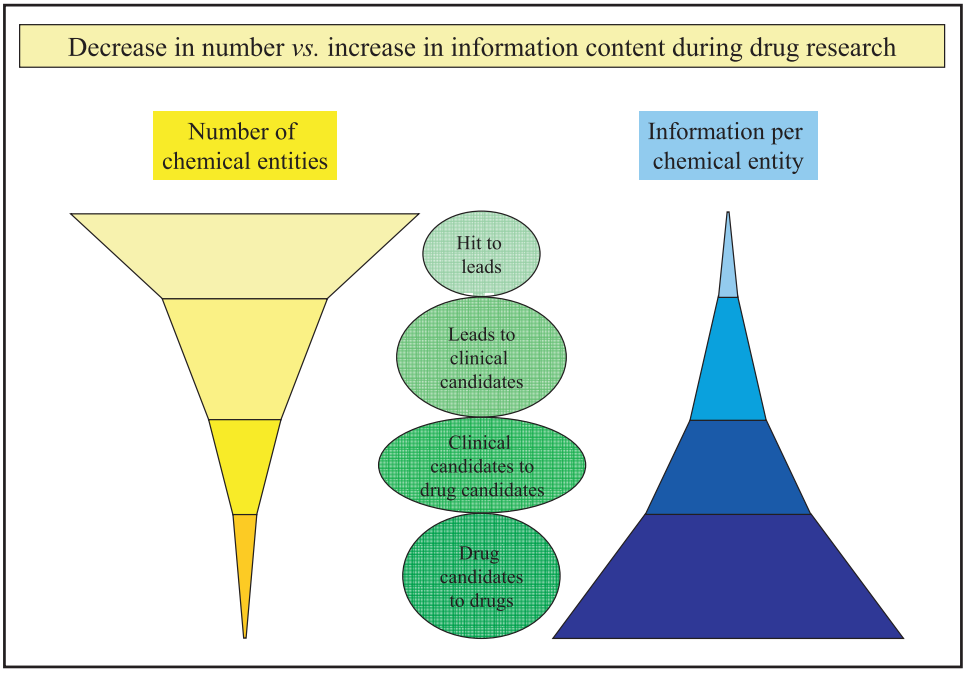


Fig. 1.34. This *Figure* draws a parallel between the number of chemical compounds produced and/or examined at the various stages of drug discovery and development, and how the information available per compound evolves during these stages. A clear if schematic trend is apparent, such that the smaller the number of compounds remaining in the pipeline, the *more information per compound* is needed to advance to further stages. Information on biotransformation is but one facet in the multidisciplinary profiling of candidates. The following *Figures* will show why and how this information is obtained during the discovery and early development stages.

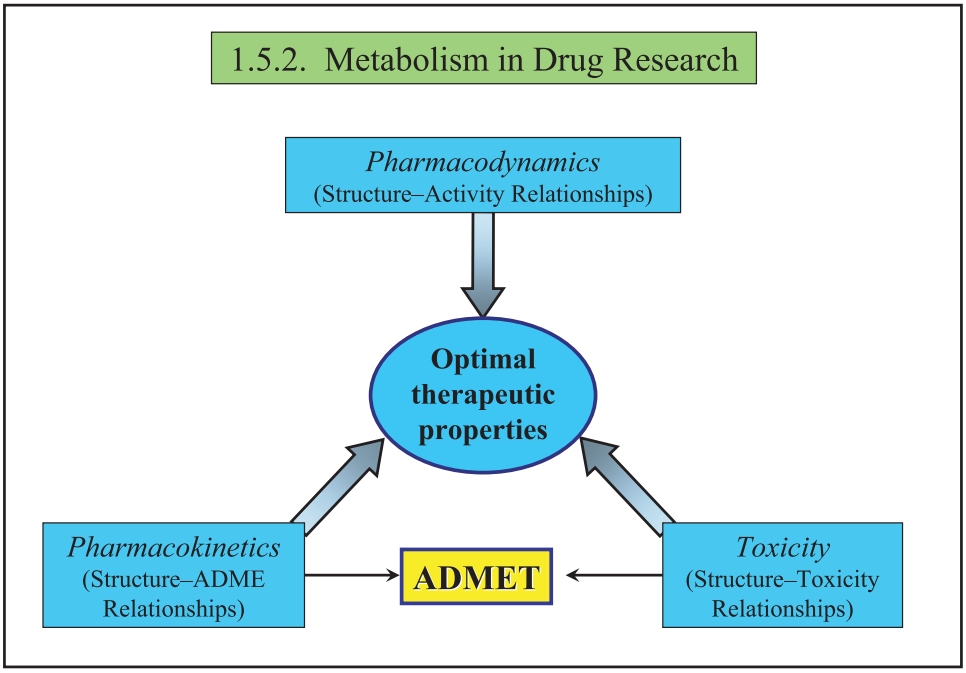


Fig. 1.35. For many years, *structure–activity relationships* (*SARs*) were the dominating paradigm in drug research. This changed when the importance of ADMET properties in developing well-behaved candidates was fully realized. At this point, it was a comparatively simple move to replace the 'activity' component in SARs with a global concept including all drug-like properties to be optimized. And, indeed, *structure–toxicity relationships* and *structure–ADME relationships* could grow in efficiency by using the same *in vitro* techniques (high-, intermediate-, and low-throughput assays) and *in silico* tools (quantitative SARs (QSARs), 3D-QSARs, molecular mechanics, molecular modeling, *etc.*) as used in SARs [72–75]. The ultimate objective now is to optimize all drug-like properties during the preclinical phases so as to minimize attrition during clinical trials. This challenge rests critically on the extrapolation of *in silico*, *in vitro*, and *in vivo* results to humans.

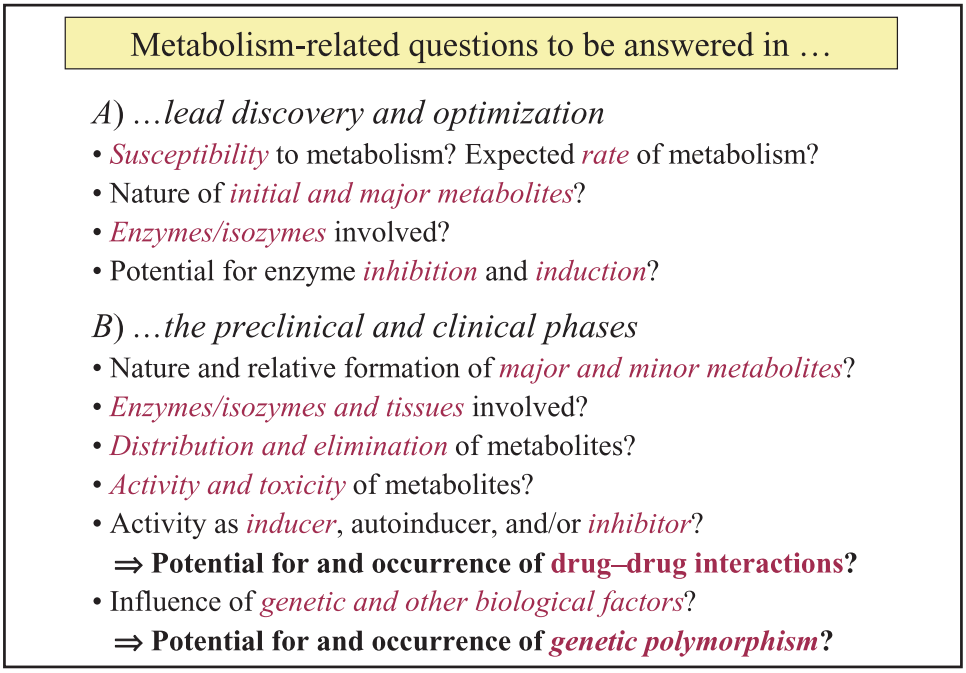


Fig. 1.36. What are the metabolism-related issues to be answered during drug discovery and development? In the *early phases*, susceptibility to metabolism and a first estimate of metabolic stability in humans are required [71][76][77]. The nature of major metabolites and the enzymes involved are investigated. Assessing the potential for metabolic drug–drug interactions has also become of significance [78]. This includes enzyme induction and inhibition [79–81]. In *later phases*, more detailed answers to the above aspects are required. And new queries come to the front and must be answered, such as the activity and toxicity of metabolites [57][82], their distribution and excretion, and the influence of genetic and other biological factors [66][83][84].

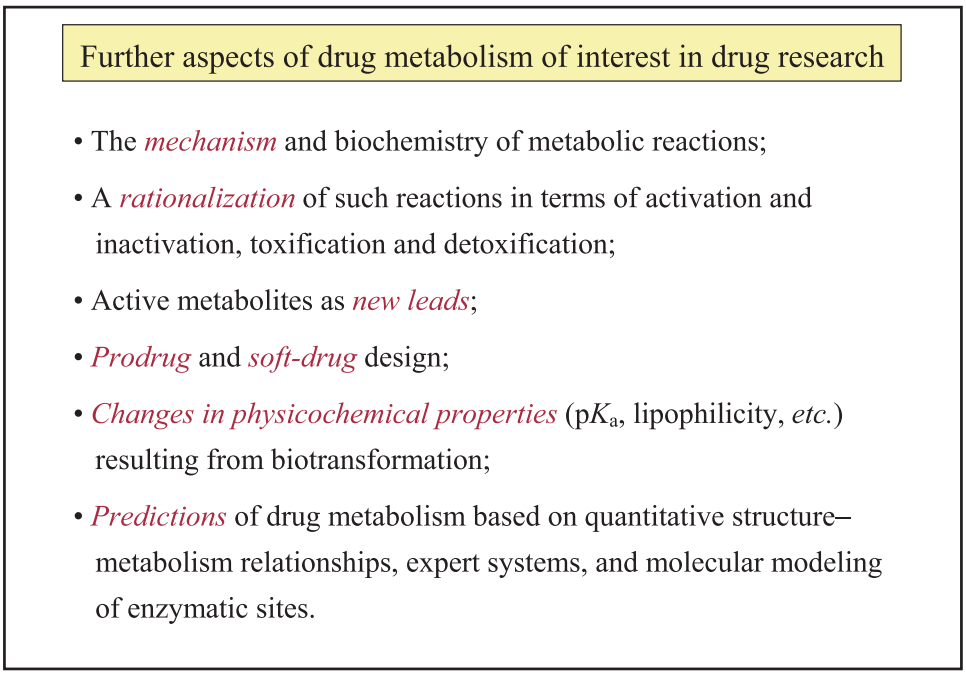


Fig. 1.37. The previous *Figure* is specifically oriented toward drug discovery and development, and it does not cover all metabolism-related aspects of drug research. Further aspects of interest in drug research are listed here. Some of these aspects are also of high interest in drug discovery, *e.g.*, active metabolites as lead compounds [57], prodrug and soft-drug design [28][30][58][59][85], and *in silico* predictions of drug metabolism [15][86][87]. Other aspects are of a more fundamental nature in drug research, *e.g.*, the mechanisms and biochemistry of metabolic reactions [13][25][28][88][89], a rationalization of such reactions in terms of bioactivity and toxicity [60][61], and the changes in physicochemical properties resulting from biotransformation (*e.g.*, [90]).

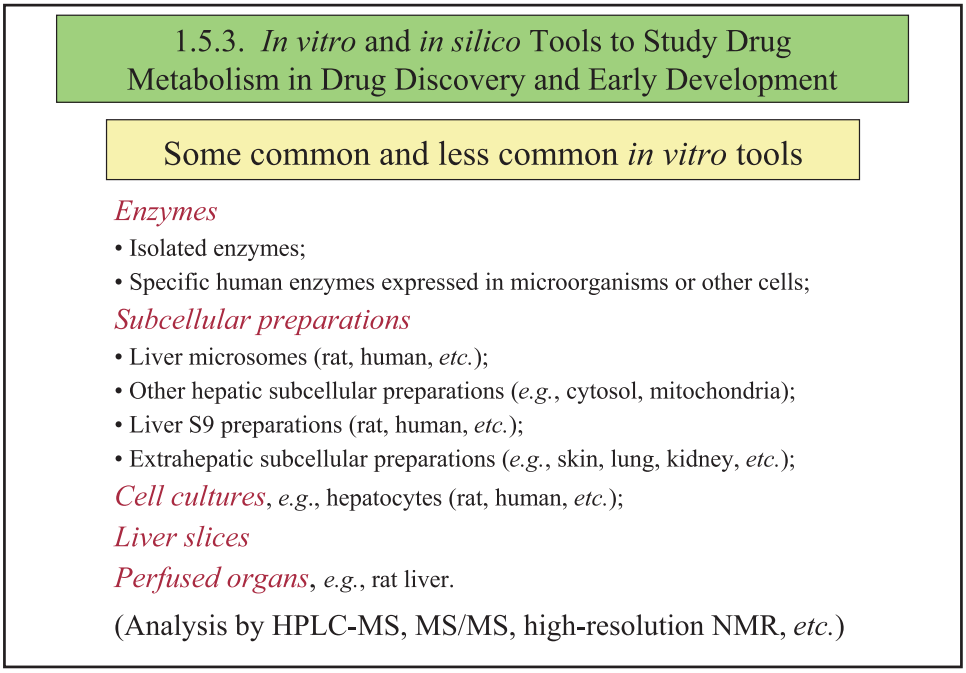


Fig. 1.38. The tools used to study drug metabolism during drug discovery and early development must ensure good throughput and be as relevant as possible to metabolism in human subjects [91]. *In vitro* tools are listed here in a classification that goes from the simplest to more complex ones [20][75]. The simplest systems are isolated *enzymes* or human enzymes expressed in genetically engineered micro-organisms or multicellular organisms (insects, plants, ...). At a higher level of biological complexity, we find the *subcellular preparations* obtained by homogenization and centrifugation of cells or tissues (see *Fig. 1.22*). More than often, such subcellular preparations are hepatic ones obtained from hepatocytes. Human microsomes and hepatocytes, despite their cost, are of particular interest given their relevance. In some special cases, other tissues are used, *e.g.*, lung or skin. *Liver slices*, particularly of human origin, are also of value. *Cell cultures*, mainly primary cultures of *hepatocytes*, afford a level of information unequaled by subcellular preparations [92][93]. First, the integrity of the cellular organization preserves the functional interactions between enzymes. Second, the viability and functionality of intact cells is maintained over longer durations (several hours), allowing longer experiments to be carried out. And finally, there is a permeation component in cell experiments which better reflects the *in vivo* situation where substrates must cross a membrane before reaching intracellular enzymes. *Perfused organs* are labor-intensive and difficult to carry out. They are often performed *in situ*, namely, with the animal alive under deep anesthesia, and are, therefore, *in vivo* investigations which will not be discussed here.

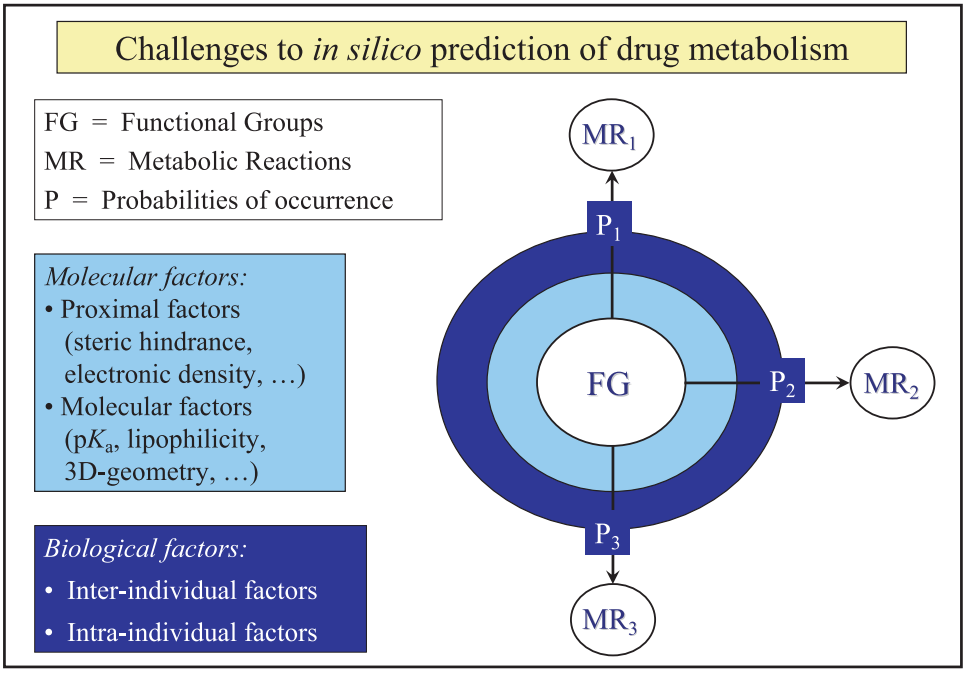


Fig. 1.39. Metabolic prediction in itself is a fuzzy and broad concept which calls for definition and clarification [30][86][94]. Schematically, a number of *objectives* toward predicting the metabolism of a given compound can be listed, namely, *a*) a list of all reasonable phase I and phase II metabolites, taking molecular factors into account; *b*) the metabolites organized in a metabolic tree; *c*) a warning for reactive/adduct-forming metabolites; *d*) a prioritization of pathways depending on biological conditions. What is explicit in this list of objectives are the factors that influence the transformation of a substrate into metabolites [95][96], as schematized in the *Figure*. A given functional group in the substrate (FG_i, also labeled a target site) will enter a given metabolic reaction (MR_i) with a probability of occurrence P_i, which depends on a number of factors conveniently subdivided into molecular and biological ones. *Proximal molecular factors* are features near the target site which will influence the catalytic reaction, for example, steric hindrance and electronic properties (densities, delocalizations, *etc.*). *Global molecular factors* (ionization, lipophilicity, 3D geometry) influence the penetration of the substrate into the enzyme compartment, the recognition of the substrate by the enzyme, and the catalytically productive binding mode of the substrate in the catalytic pocket. The *biological factors* are so many, so varied, and of such immense importance that they will need two Parts to be discussed in this Work, namely *Parts 6* and *7*. Suffice it to say here that they represent at present an apparently unsurmountable challenge to entirely successful predictions of drug metabolism.

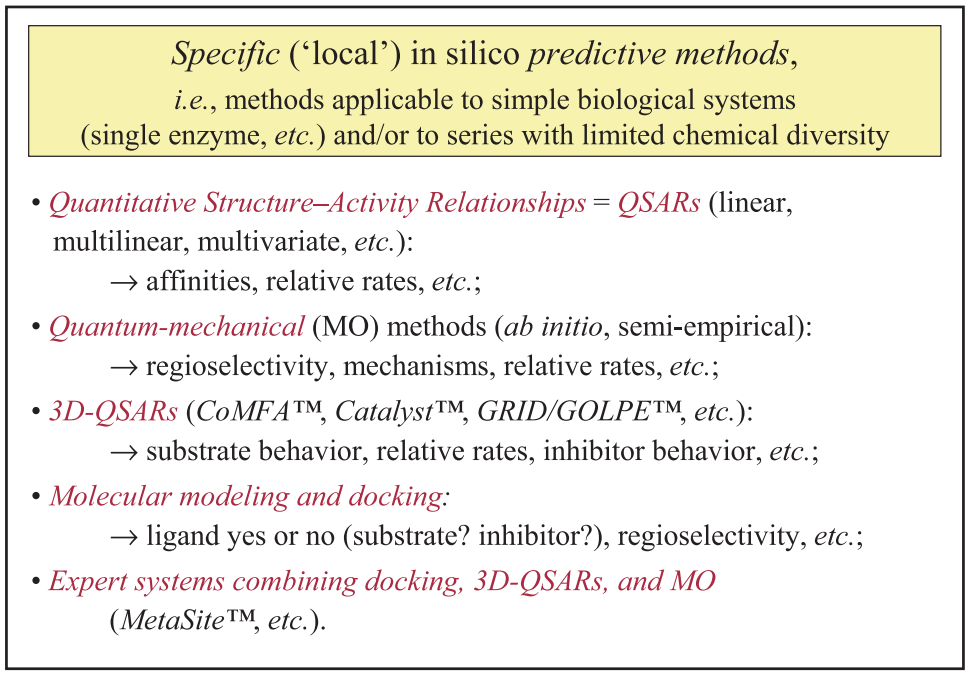


Fig. 1.40. In a simplified manner, one can distinguish between two types of algorithms to predict drug and xenobiotic metabolism, namely, specific ('local') systems and comprehensive ('global') systems. *Specific systems* apply to simple biological (*e.g.*, single enzymes) and/or to single metabolic reactions, and they may or may not be restricted to rather narrow chemical series. Such systems include *quantitative structure–metabolism relationships* (QSMRs) based on structural and physicochemical properties [96][97]. *Quantum mechanical calculations* may also shed light on SMRs and generate parameters to be used as independent variables in QSMRs, revealing, for example, correlations between rates of metabolic oxidation and energy barrier in H-atom abstraction [98][99]. *Three-dimensional QSMRs* (3D-QSMRs) 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). 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 [100][101]. The *molecular modeling* of xenobiotic-metabolizing enzymes affords another approach to rationalize and predict drug–enzyme interactions [102][103]. Its application to drug metabolism was made possible by the crystallization and X-ray structural determination of cytochromes P450, first bacterial, and now human ones. While 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 other xenobiotic-metabolizing enzymes such as DT-diaphorase and various transferases. The last approaches mentioned in this *Figure* are *expert systems*

combining several methods, for example, pharmacophore models (obtained by 3D-QSAR), protein models (obtained by molecular modeling), and docking [104][105]. Another powerful combination are *a*) 3D models obtained by molecular modeling, and *b*) sophisticated QSAR approaches based on multivariate analyses of parameters obtained from molecular interaction fields (MIFs), as found in the *MetaSite* algorithm [106]. *MetaSite* is a specific system 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.

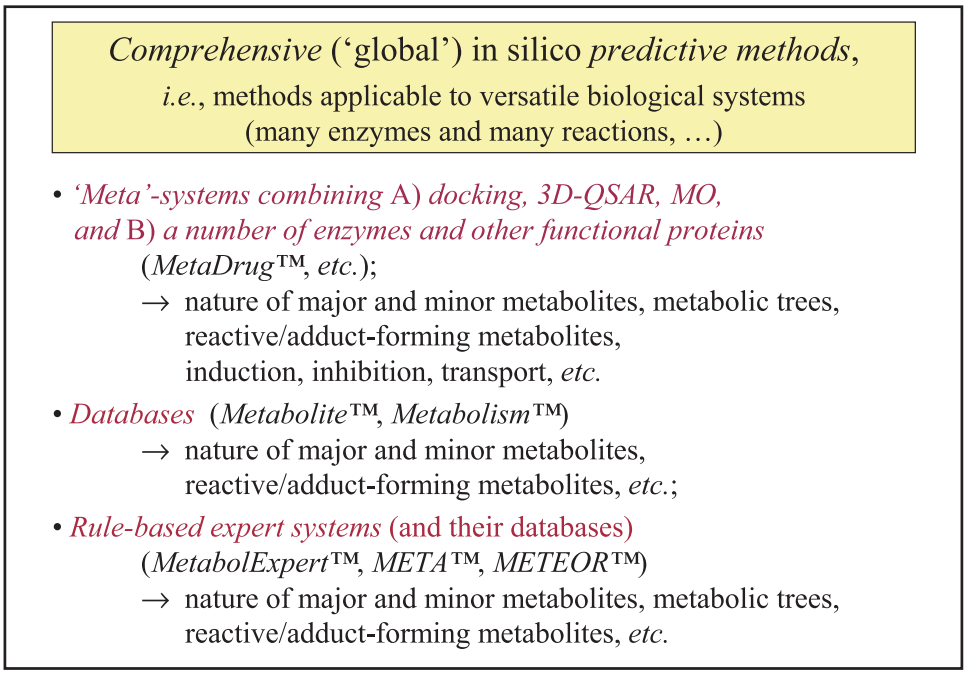


Fig. 1.41. Comprehensive expert systems are, in principle, applicable to versatile biological systems (*i.e.*, to any enzyme and reaction) and to any chemical compound. As shown in the *Figure*, this is the final goal of *'meta'-systems* combining docking, 3D-QSAR, and MO method not for a single enzyme, but for a number of them (ultimately, all!). 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 before such approaches can be seen as genuinely comprehensive. To the best of our knowledge, the release of *MetaDrug* is currently the most significant and promising step in this direction [107]. As reviewed by *Hawkins* [87], one approach to global prediction of metabolism is to use *databases* in

the form of either knowledge-based systems or predictive, rule-based systems [15]. Existing *knowledge-based systems* include the *MDL Metabolite Database* [108] and the *Accelrys Metabolism* database [109] originally established using data compiled in the book series *Biotransformations* [110]. These databases can be searched to retrieve information on the known metabolism of compounds with similar structures or containing specific moieties. *Predictive, rule-based systems* attempt to portray the metabolites of a compound based on knowledge rules, defining the most likely products [111]. Existing systems of this type are *MetabolExpert* [112], *META* [113], and *METEOR* [114].

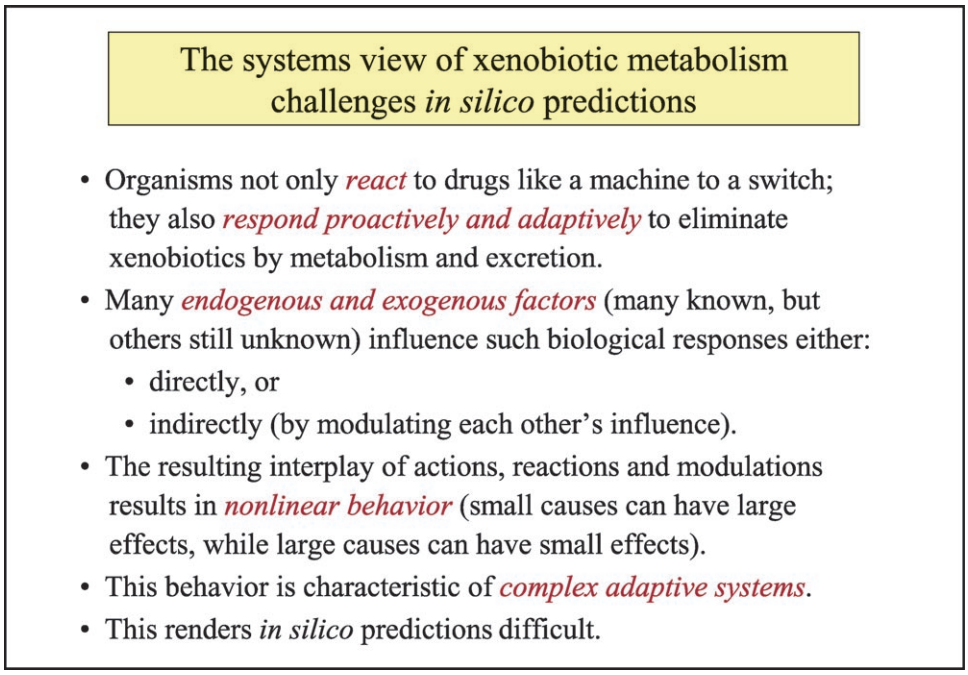


Fig. 1.42. This *Figure* concludes *Part 1* and brings some forward-looking words about metabolism predictions in particular, and the complexity of xenobiotic metabolism in general. First, biological systems are not machines; they respond to xenobiotic invasion by an array of defensive strategies (*Fig. 1.7*) such as prevented absorption, facilitated excretion, and chemical breakdown reactions (*Parts 2–4*). Rather than being rigidly fixed, such responses are adaptable and can be adjusted within limits to the nature and magnitude of the invasion. Many endogenous factors are involved in these adjustements and are themselves influenced by exogenous factors. While many factors influencing xenobiotic metabolism have now been uncovered (*Parts 6* and *7*), their interdepend-

character of the responses, such that small causes can have small or large effects, and large causes can have large or small effects. This chaotic behavior is the characteristic of complex adaptive systems; it is also the source of the apparently unsurmountable difficulty of making close-to-perfect *in silico* predictions, and above all it is the source of the endless satisfactions one obtains when studying drug metabolism.

REFERENCES

[1] A. Conti, M. H. Bickel, 'History of drug metabolism: discoveries of the major pathways in the 19th century', *Drug Metab. Rev.* **1977**, *6*, 1–50; C. Bachmann, M. H. Bickel, 'History of drug metabolism: the first half of the 20th century', *Drug Metab. Rev.* **1986**, *16*, 185–253.
[2] International Society for the Study of Xenobiotics, www.issx.org.
[3] R. T. Williams, 'Detoxication Mechanisms', Chapman & Hall, London, 1947.
[4] R. T. Williams, 'Detoxication Mechanisms', 2nd edn., Chapman & Hall, London, 1959.
[5] B. Testa, P. Jenner, 'Drug Metabolism: Chemical and Biochemical Aspects', Dekker, New York, 1976.
[6] B. Testa, P. Jenner, 'The coming of age of drug metabolism', *Curr. Contents Life Sci.* **1990**, *33*, 17.
[7] 'Concepts in Drug Metabolism', Eds. P. Jenner, B. Testa, Dekker, New York, 1980 and 1981, Part A and Part B.
[8] 'Drug Metabolism – from Molecules to Man', Eds. D. J. Benford, J. W. Bridges, G. G. Gibson, Taylor & Francis, London, 1987.
[9] 'Conjugation Reactions in Drug Metabolism', Ed. G. J. Mulder, Taylor & Francis, London, 1990.
[10] 'Pharmacokinetics of Drugs', Eds. P. G. Welling, L. P. Balant, Springer, Heidelberg, 1994.
[11] 'Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity', Ed. F. C. Kauffman, Springer Verlag, Berlin, 1994.
[12] 'Conjugation-Dependent Carcinogenicity and Toxicity of Foreign Compounds', Eds. M. W. Anders, W. Dekant, Academic Press, San Diego, 1994.
[13] 'Cytochrome P450. Structure, Mechanism, and Biochemistry', 2nd edn., Ed. P. R. Ortiz de Montellano, Plenum Press, New York, 1996.
[14] 'Handbook of Drug Metabolism', Ed. T. F. Woolf, Dekker, New York, 1999.
[15] 'Drug Metabolism: Databases and High-Throughput Testing during Drug Design and Development', Ed. P. W. Erhardt, International Union of Pure and Applied Chemistry and Blackwell Science, London, 1999.
[16] 'Enzyme Systems that Metabolise Drugs and Other Xenobiotics', Ed. C. Ioannides, John Wiley & Sons, Chichester, 2002.
[17] 'Drug-Drug Interactions', Ed. A. D. Rodrigues, Dekker, New York, 2002.
[18] 'Handbook of Drug-Nutrient Interactions', Eds. J. Boullata, V. T. Armenti, Humana Press, Totowa, 2004.
[19] 'Drug Metabolism and Transport', Ed. L. H. Lash, Humana Press, Totowa, 2005.
[20] 'Cytochrome P450 Protocols', Eds. I. R. Phillips, E. A. Shephard, Humana Press, Totowa, 2006.
[21] A. Albert, 'Selective Toxicity', Chapman & Hall, London, 1985.
[22] A. Albert, 'Xenobiosis', Chapman & Hall, London, 1987.
[23] J. A. Timbrell, 'Principles of Biochemical Toxicology', 2nd edn., Taylor & Francis, London, 1991.
[24] R. B. Silverman, 'The Organic Chemistry of Drug Design and Drug Action', Academic Press, San Diego, 1992.
[25] B. Testa, 'The Metabolism of Drugs and Other Xenobiotics – Biochemistry of Redox Reactions', Academic Press, London, 1995.
[26] W. N. Aldridge, 'Mechanisms and Concepts in Toxicology', Taylor & Francis, London, 1996.

[27] G. G. Gibson, P. Skett, 'Introduction to Drug Metabolism', 3rd edn., Nelson Thornes, Cheltenham UK, 2001.
[28] B. Testa, J. M. Mayer, ' Hydrolysis in Drug and Prodrug Metabolism – Chemistry, Biochemistry, and Enzymology', Verlag Helvetica Chimica Acta, Zurich, and Wiley-VCH, Weinheim, 2003.
[29] F. J. Di Carlo, 'Metabolism, pharmacokinetics, and toxicokinetics defined', *Drug Metab. Rev.* **1982**, *13*, 1–4.
[30] B. Testa, W. Soine, 'Principles of drug metabolism', in 'Burger's Medicinal Chemistry and Drug Discovery', 6th edn., Vol. 2, Ed. D. J. Abraham, Wiley-Interscience, Hoboken, 2003, p. 431–498.
[31] A. Albert, 'The behaviour of foreign substances in the human body', *Trends Pharmacol. Sci.* **1987**, *8*, 258–261.
[32] P. Jenner, B. Testa, F. J. Di Carlo, 'Xenobiotic and endobiotic metabolizing enzymes: an overstretched discrimination?', *Trends Pharmacol. Sci.* **1981**, *2*, 135–137.
[33] W. B. Jakoby, D. M. Ziegler, 'The enzymes of detoxication', *J. Biol. Chem.* **1990**, *265*, 20715–20718.
[34] D. W. Nebert, 'Proposed role of drug-metabolizing enzymes: Regulation of steady state levels of the ligands that effect growth, homeostasis, differentiation, and neuroendocrine functions', *Mol. Endocrinol.* **1991**, *5*, 1203–1214.
[35] F. J. Gonzalez, D. W. Nebert, 'Evolution of the P450 gene superfamily: animal-plant 'warfare', molecular drive and human genetic differences in drug oxidation', *Trends Genet.* **1990**, *6*, 182–186.
[36] B. N. Ames, M. Profet, 'Nature's pesticides', *Nat. Toxins* **1992**, *1*, 2–3.
[37] W. R. Jondorf, 'Drug metabolism and drug toxicity: Some evolutionary considerations', in 'Concepts in Drug Metabolism', Part B, Eds. P. Jenner, B. Testa, Dekker, New York, 1981, p. 305–376.
[38] J. R. Gillette, 'The use of theoretical pharmacokinetic concepts in studies of the mechanisms of formation of chemically reactive metabolites in vitro and in vivo', *Drug Metab. Rev.* **1983**, *14*, 9–33.
[39] T. J. Monks, S. S. Lau, 'Reactive intermediates and their toxicological significance', *Toxicology* **1988**, *52*, 1–54.
[40] B. Testa, 'Pharmacokinetic and pharmacodynamic events: can they always be distinguished?', *Trends Pharmacol. Sci.* **1987**, *8*, 381–383.
[41] 'ADME-Tox: The Fate of Drugs in the Body', Eds. B. Testa, H. van de Waterbeemd, in 'Comprehensive Medicinal Chemistry', 2nd edn., Eds. D. J. Triggle, J. Taylor, Elsevier, Oxford, Vol. 5, in press.
[42] B. Testa, P. Jenner, 'Novel metabolites produced by functionalization reactions: Chemistry and toxicology', *Drug Metab. Rev.* **1978**, *7*, 325–369.
[43] J. Caldwell, 'Conjugation reactions in foreign-compound metabolism: definition, consequences, and species variations', *Drug Metab. Rev.* **1982**, *13*, 745–777.
[44] B. Testa, 'Nonenzymatic contributions to xenobiotic metabolism', *Drug Metab. Rev.* **1982**, *13*, 25–50.
[45] T. Walle, U. K. Walle, L. S. Olanoff, 'Quantitative account of propranolol metabolism in urine of normal man', *Drug Metab. Dispos.* **1985**, *13*, 204–209.
[46] B. Testa, P. Jenner, 'The concept of regioselectivity in drug metabolism', *J. Pharm. Pharmacol.* **1976**, *28*, 731–744.
[47] 'Enzyme Nomenclature', Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB), www.chem.qmul.ac.uk/iubmb/enzyme.
[48] T. Rodgers, D. Leahy, M. Rowland, 'Tissue distribution of basic drugs: Accounting for enantiomeric, compound and regional differences amongst β-blocking drugs in rat', *J. Pharm. Sci.* **2005**, *94*, 1237–1248; L. Z. Benet, B. Y. T. Perotti, L. Hardy, 'Drug absorption, distribution, and elimination', in 'Burger's Medicinal Chemistry and Drug Discovery', 6th edn., Vol. 2, Ed. D. J. Abraham, Wiley-Interscience, Hoboken, 2003, p. 633–647; B. Fichtl, A. von Nieciecki, K. Walter, 'Tissue binding versus plasma binding of drugs: General principles and pharmacokinetic consequences', in 'Advances in Drug Research', Vol. 20, Ed. B. Testa, Academic Press, London, 1991, p. 117–166.
[49] P. J. McNamara, M. Abbassi, 'Neonatal exposure to drugs in breast milk', *Pharm. Res.* **2004**, *21*, 555–566.
[50] I. Mahmood, 'Interspecies scaling of biliary excreted drugs: A comparison of several methods', *J. Pharm. Sci.* **2005**, *94*, 883–892.

[51] A. E. Cribb, M. Peyrou, S. Muruganandan, 'The endoplasmic reticulum in xenobiotic toxicity', *Drug Metab. Rev.* **2005**, *37*, 405–442.
[52] S. A. Robert, 'High-throughput screening approaches for investigating drug metabolism and pharmacokinetics', *Xenobiotics* **2001**, *31*, 557–589.
[53] M. Recanatini, E. Poluzzi, M. Masetti, A. Cavalli, F. De Ponti, 'QT prolongation through hERG K^+ channel blockade: Current knowledge and strategies for the early prediction during drug development', *Med. Res. Rev.* **2005**, *25*, 133–166.
[54] W. J. Watkins, T. E. Renau, 'Antifungal agents', in 'Burger's Medicinal Chemistry and Drug Discovery', 6th edn., Vol. 5, Ed. D. J. Abraham, Wiley-Interscience, Hoboken, 2003, p. 881–918.
[55] M. Strolin Benedetti, M. Bani, 'Metabolism-based drug interactions involving oral azole antifungals in humans', *Drug Metab. Rev.* **1999**, *31*, 665–717.
[56] D. E. Drayer, 'Pharmacologically active metabolites of drugs and other foreign compounds. Clinical, pharmacological, therapeutic and toxicological considerations', *Drugs* **1982**, *24*, 519–542.
[57] A. Fura, Y. Z. Shu, M. Zhu, R. L. Hanson, V. Roongta, W. G. Humphreys, 'Discovering drugs though biological transformation: Role of pharmacologically active metabolites in drug discovery', *J. Med. Chem.* **2004**, *47*, 4339–4351.
[58] P. Ettmayer, G. Amidon, B. Clement, B. Testa, 'Lessons learned from marketed and investigational prodrugs', *J. Med. Chem.* **2004**, *47*, 2393–2404.
[59] B. Testa, 'Prodrug research: Futile or fertile?', *Biochem. Pharmacol.* **2004**, *68*, 2097–2106.
[60] D. P. Williams, D. J. Naisbitt, 'Toxicophores: Groups and metabolic routes associated with increased safety risk', *Curr. Opin. Drug Discov. Dev.* **2002**, *5*, 104–115.
[61] A. S. Kalgutkar, I. Gardner, R. S. Obach, C. L. Schaffer, E. Callegari, K. R. Henne, A. E. Mutlib, D. K. Dalvie, J. S. Lee, Y. Nakai, J. P. O'Donnell, J. Boer, S. P. Harriman, 'A comprehensive listing of bioactivation pathways of organic functional groups', *Curr. Drug Metab.* **2005**, *6*, 161–225.
[62] M. H. Bickel, 'Factors affecting the storage of drugs and other xenobiotics in adipose tissue', in 'Advances in Drug Research', Vol. 25, Eds. B. Testa, U. A. Meyer, Academic Press, London, 1994, p. 56–86.
[63] E. Stokstad, 'Pollution gets personal', *Science* **2004**, *304*, 1892–1894; P. Webster, 'Exposure to flame retardants on the rise', *Science* **2004**, *304*, 1730.
[64] K. R. Scott, 'Anticonvulsants', in 'Burger's Medicinal Chemistry and Drug Discovery', 6th edn., Vol. 6, Ed. D. J. Abraham, Wiley-Interscience, Hoboken, 2003, p. 263–328.
[65] J. Lazarou, B. H. Pomeranz, P. N. Corey, 'Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies', *J. Am. Med. Assoc.* **1998**, *279*, 1200–1205.
[66] R. Bullingham, 'Pharmacogenomics: how gene variants can ruin good drugs', *Curr. Drug Discov.* **2001** (March), 17–20.
[67] C. Esser, 'Immunotoxicology', in 'ADME-Tox: The Fate of Drugs in the Body', Eds. B. Testa, H. van de Waterbeemd, in 'Comprehensive Medicinal Chemistry', 2nd edn., Eds. D. J. Triggle, J. Taylor, Elsevier, Oxford, Vol. 5, in press.
[68] H. van de Waterbeemd, B. Testa, 'The Why and How of ADMET Research', in 'ADME-Tox: The Fate of Drugs in the Body', Eds. B. Testa, H. van de Waterbeemd, in 'Comprehensive Medicinal Chemistry', 2nd edn., Eds. D. J. Triggle, J. Taylor, Elsevier, Oxford, Vol. 5, in press; 'Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical, and Computational Strategies', Eds. B. Testa, H. van de Waterbeemd, G. Folkers, R. Guy, Verlag Helvetica Chimica Acta, Zurich, and Wiley-VCH, Weinheim, 2001; 'Pharmacokinetic Profiling in Drug Research: Biological, Physicochemical, and Computational Strategies', Eds. B. Testa, S. Krämer, H. Wunderli-Allenspach, G. Folkers, Verlag Helvetica Chimica Acta, Zurich, and Wiley-VCH, Weinheim, 2006.
[69] D. A. Smith, E. F. Schmid, 'Drug withdrawals and the lessons within', *Curr. Opin. Drug Discov. Dev.* **2006**, *9*, 38–46.
[70] D. A. Smith, E. F. Schmid, B. Jones, 'Do drug metabolism and pharmacokinetic departments make any contribution to drug discovery?', *Clin. Pharmacokin.* **2002**, *41*, 1005–1019.
[71] T. N. Thompson, 'Optimization of metabolic stability as a goal of modern drug design', *Med. Res. Rev.* **2001**, *21*, 412–449.

[72] C. Helma, 'In silico predictive toxicology: The state-of-the-art and strategies to predict human health effects', *Curr. Opin. Drug Discov. Dev.* **2005**, *8*, 27–31.
[73] N. Greene, 'Computational models to predict toxicity', in 'ADME-Tox: The Fate of Drugs in the Body', Eds. B. Testa, H. van de Waterbeemd, in 'Comprehensive Medicinal Chemistry', 2nd edn., Eds. D. J. Triggle, J. Taylor, Elsevier, Oxford, Vol. 5, in press.
[74] M. Cronin, 'In silico models to predict passage through the skin and other biological barriers', in 'ADME-Tox: The Fate of Drugs in the Body', Eds. B. Testa, H. van de Waterbeemd, in 'Comprehensive Medicinal Chemistry', 2nd edn., Eds. D. J. Triggle, J. Taylor, Elsevier, Oxford, Vol. 5, in press.
[75] Y. Parmentier, M. J. Bossant, M. Bertrand, B. Walther, 'In vitro studies of drug metabolism', in 'ADME-Tox: The Fate of Drugs in the Body', Eds. B. Testa, H. van de Waterbeemd, in 'Comprehensive Medicinal Chemistry', 2nd edn., Eds. D. J. Triggle, J. Taylor, Elsevier, Oxford, Vol. 5, in press.
[76] I. A. M. De Graaf, C. E. Van Meijeren, F. Pektas, H. J. Koster, 'Comparison of in vitro preparations for semi-quantitative prediction of in vivo drug metabolism', *Drug Metab. Dispos.* **2002**, *30*, 1129–1136.
[77] H. C. Rawden, D. J. Carlile, A. Tindall, D. Hallifax, A. Galetin, K. Ito, J. B. Houston, 'Microsomal prediction of in vivo clearance and associated interindividual variability of six benzodiazepines in humans', *Xenobiotica* **2005**, *35*, 603–625.
[78] R. J. Weaver, 'Assessment of drug-drug interactions: concepts and approaches', *Xenobiotica* **2001**, *31*, 499–538.
[79] D. C. Evans, D. P. Hartley, R. Evers, 'Chapter 31. Enzyme Induction – Mechanisms, Assays, and Relevance to Drug Discovery and Development', *Annu. Rep. Med. Chem.* **2003**, *38*, 315–331.
[80] D. A. Smith, 'Induction and drug development', *Eur. J. Pharm. Sci.* **2000**, *11*, 185–189.
[81] C. Yao, R. H. Levy, 'Inhibition-based metabolic drug-drug interactions: predictions from in vitro data', *J. Pharm. Sci.* **2002**, *91*, 1923–1935.
[82] B. Oesch-Bartlomowicz, F. Oesch, 'Mechanisms of toxification and detoxification which challenge drug candidates and drugs', in 'ADME-Tox: The Fate of Drugs in the Body', Eds. B. Testa, H. van de Waterbeemd, in 'Comprehensive Medicinal Chemistry', 2nd edn., Eds. D. J. Triggle, J. Taylor, Elsevier, Oxford, Vol. 5, in press.
[83] M. Ingelman-Sundberg, 'Implications of polymorphic cytochrome P450-dependent drug metabolism for drug development', *Drug Metab. Dispos.* **2001**, *29*, 570–573.
[84] H. Reiser, 'Pharmacogenetics and drug development', *Annu. Rep. Med. Chem.* **2005**, *40*, 414–427.
[85] B. Testa, 'Prodrug objectives and design', in 'ADME-Tox: The Fate of Drugs in the Body', Eds. B. Testa, H. van de Waterbeemd, in 'Comprehensive Medicinal Chemistry', 2nd edn., Eds. D. J. Triggle, J. Taylor, Elsevier, Oxford, Vol. 5, in press.
[86] B. Testa, A.-L. Balmat, A. Long, 'Predicting drug metabolism – Concepts and challenges', *Pure Appl. Chem.* **2004**, *76*, 907–914.
[87] D. R. Hawkins, 'Comprehensive expert systems to predict drug metabolism', in 'ADME-Tox: The Fate of Drugs in the Body', Eds. B. Testa, H. van de Waterbeemd, in 'Comprehensive Medicinal Chemistry', 2nd edn., Eds. D. J. Triggle, J. Taylor, Elsevier, Oxford, Vol. 5, in press.
[88] W. F. Trager, 'Principles of drug metabolism 1: Redox reactions', in 'ADME-Tox: The Fate of Drugs in the Body', Eds. B. Testa, H. van de Waterbeemd, in 'Comprehensive Medicinal Chemistry', 2nd edn., Eds. D. J. Triggle, J. Taylor, Elsevier, Oxford, Vol. 5, in press.
[89] B. Testa, 'Principles of drug metabolism 2: Hydrolysis and conjugation reactions', in 'ADME-Tox: The Fate of Drugs in the Body', Eds. B. Testa, H. van de Waterbeemd, in 'Comprehensive Medicinal Chemistry', 2nd edn., Eds. D. J. Triggle, J. Taylor, Elsevier, Oxford, Vol. 5, in press.
[90] Y. Giroud, P. A. Carrupt, A. Pagliara, B. Testa, R. G. Dickinson, 'Intrinsic and intramolecular lipophilicity effects in O-glucuronides', *Helv. Chim. Acta* **1998**, *81*, 330–341.
[91] R. DeWitte, 'Overcoming the quantity-quality trade-off in preclinical profiling', *DrugPlus Intern.* **2006** (April/May), 16–19.
[92] K. P. Persson, S. Ekehed, C. Otter, E. S. M. Lutz, J. McPheat, C. M. Masimirembwa, T. B. Andersson, 'Evaluation of human liver slices and reporter gene assays as systems for predicting the cytochrome P450 induction potential of drugs in vivo in humans', *Pharm. Res.* **2006**, *23*, 56–69.

[93] D. M. Cross, M. K. Bayliss, 'A commentary on the use of hepatocytes in drug metabolism studies during drug discovery and development', *Drug Metab. Rev.* **2000**, *32*, 219–240.
[94] D. S. Wishart, 'Bioinformatics in drug development and assessment', *Drug Metab. Rev.* **2005**, *37*, 279–310.
[95] B. Testa, G. Cruciani, '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, R. Guy, Verlag Helvetica Chimica Acta, Zurich, and Wiley-VCH, Weinheim, 2001, p. 65–84.
[96] B. Testa, P. Crivori, M. Reist, P. A. Carrupt, 'The influence of lipophilicity on the pharmacokinetic behavior of drugs: Concepts and examples', *Perspect. Drug Discov. Des.* **2000**, *19*, 179–211.
[97] C. Hansch, S. B. Mekapati, A. Kurup, R. P. Verma, 'QSAR of cytochrome P450', *Drug Metab. Rev.* **2004**, *36*, 105–156.
[98] J. P. Jones, M. Mysinger, K. R. Korzekwa, 'Computational models for cytochrome P450: A predictive electronic model for aromatic oxidation and hydrogen atom abstraction', *Drug Metab. Dispos.* **2002**, *30*, 7–12.
[99] D. L. Harris, '*In silico* predictive metabolism: A structural/electronic filter method', *Curr. Opin. Drug Discov. Dev.* **2004**, *7*, 43–48.
[100] L. E. Korhonen, M. Rahnasto, N. J. Mähönen, C. Wittekindt, A. Poso, R. O. Juvonen, H. Raunio, 'Predictive three-dimensional quantitative structure-activity relationship of cytochrome P450 1A2 inhibitors', *J. Med. Chem.* **2005**, *48*, 3808–3815.
[101] M. J. Sorich, J. O. Miners, R. A. McKinnon, P. A. Smith, 'Multiple pharmacophores for the investigation of human UDP-glucuronosyltransferase isoform substrate selectivity', *Mol. Pharmacol.* **2004**, *65*, 301–308.
[102] D. F. V. Lewis, 'Molecular modeling of human cytochrome P450-substrate interactions', *Drug Metab. Rev.* **2002**, *34*, 55–67.
[103] D. F. V. Lewis, ' Homology modelling of human CYP2 family enzymes based on the CYP2C5 crystal structure', *Xenobiotica* **2002**, *32*, 305–323.
[104] M. J. de Groot, A. A. Alex, B. C. Jones, 'Development of a combined protein and pharmacophore model for cytochrome P450 2C9', *J. Med. Chem.* **2002**, *45*, 1983–1993.
[105] C. de Graaf, N. P. E. Vermeulen, K. A. Feenstra, 'Cytochrome P450 in silico: An integrative modeling approach', *J. Med. Chem.* **2005**, *48*, 2725–2755.
[106] L. Afzelius, I. Zamora, C. M. Misimirembwa, A. Karlén, T. B. Andersson, S. Mecucci, M. Baroni, G. Cruciani, 'Conformer- and alignment-independent model for predicting structurally diverse competitive CYP2C9 inhibitors', *J. Med. Chem* **2004**, *47*, 907–914; G. Cruciani, E. Carosati, B. De Boeck, K. Ethirajulu, C. Mackie, T. Howe, R. Vianello, 'MetaSite: Understanding Metabolism in Human Cytochromes from the Perspective of the Chemist', *J. Med. Chem.* **2005**, *48*, 6970–6979; www.moldiscovery.com.
[107] S. Ekins, S. Andreyev, A. Ryabov, E. Kirillov, E. A. Rakhmatulin, A. Bugrim, T. Nikolskaya, 'Computational prediction of human drug metabolism', *Exp. Opin. Drug Metab. Toxicol.* **2005**, *1*, 303–324; www.geneco.com.
[108] *MDL Information Systems Inc.*, www.mdl.com.
[109] *Accelyrs Ltd.*, www.accelrys.com.
[110] 'Biotransformations: A Survey of the Biotransformations of Drugs and Chemicals in Animals', Vols. 1–7, Ed. D. R. Hawkins, Royal Society of Chemistry, Cambridge, UK, 1988–1996.
[111] S. A. Kulkarni, J. Zhu, S. Blechinger, 'In silico techniques for the study and prediction of xenobiotic metabolism: A review', *Xenobiotica* **2005**, *35*, 955–973.
[112] P. Darvas, S. Marokházi, P. Kormos, G. Kulkarni, H. Kalász, A. Papp, 'MetabolExpert: Its use in metabolism research and in combinatorial chemistry', in 'Drug Metabolism: Databases and High-Throughput Testing During Drug Design and Development', Ed. P. W. Erhardt, International Union of Pure and Applied Chemistry and Blackwell Science, London, 1999, p. 237–270; www.compudrug.com.
[113] G. Klopman, M. Tu, 'META: A program for the prediction of the products of mammal metabolism of xenobiotics', in 'Drug Metabolism: Databases and High-Throughput Testing During Drug Design

and Development', Ed. P. W. Erhardt, International Union of Pure and Applied Chemistry and Blackwell Science, London, 1999, p. 271–276; www.multicase.com.

[114] J. J. Langowski, A. Long, 'Computer systems for the prediction of xenobiotic metabolism', *Adv. Drug Delivery Rev.* **2002**, *54*, 407–415; B. Testa, A. L. Balmat, A. Long, P. Judson, 'Predicting drug metabolism – An evaluation of the expert system METEOR', *Chem. Biodiv.* **2005**, *2*, 872–885; www.lhasalimited.org.

Received June 10, 2006