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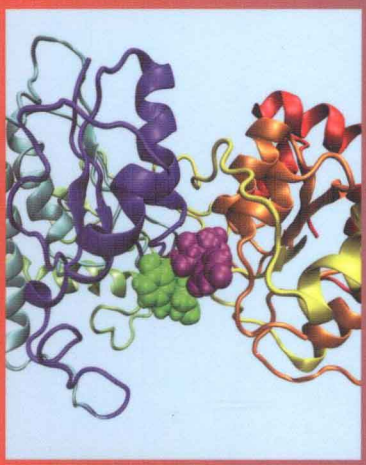
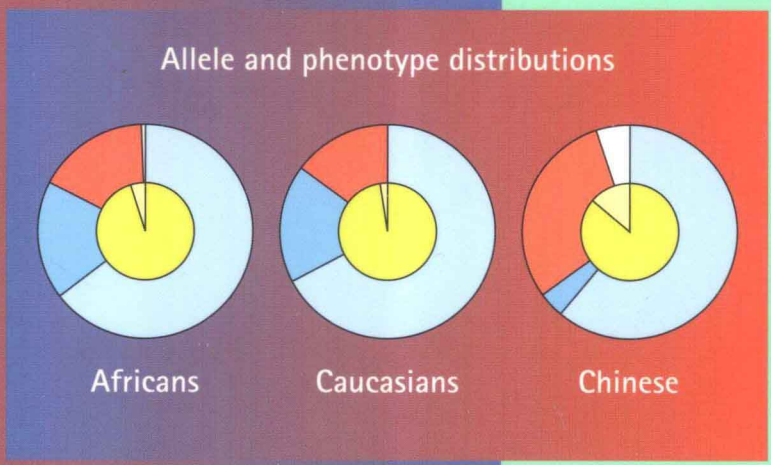
The Biochemistry of Drug Metabolism: Conjugations, Consequences of Metabolism, Influencing Factors





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Complex between human UDP-glucuronyltransferase (UGT2B7), its cofactor UDP-glucuronic acid, and morphine as substrate (see Fig. 4.41 for details; courtesy of Dr. Giulio Vistoli, University of Milan).

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We dedicate this book to all scientists who share our fascination with the endless marvels and complexity of the living world. May it continue to burgeon!

I also dedicate this work to the fond memory of Prof. *William F. Trager* (1937–2009), a quiet scholar and a dear friend. His groundbreaking contributions have opened our eyes to the catalytic intricacies of xenobiotic metabolism and their consequences.

B. T.

Foreword

When I was a lad, drug metabolism was a collection of interesting but disparate and often dissonant facts. Integration of this information and understanding of the associated issues is now an essential component of successful drug discovery and development and promises, through pharmacogenetics, metabolomics, and informatics, to have an increasingly direct impact on health care. As the 'song' has become more complex, there is a real need for great 'singers' to render it in an intelligible, inspiring yet concise manner.

Bernard Testa and *Stefanie Krämer* are a highly accomplished duo, and the second volume of their *magnus opus* takes the score forward to include conjugation reactions and enzymes, the various manifestations of the bioactivity of drug metabolites, and inter- and intra-individual variability (the effects of 'nature' and 'nurture', respectively, on drug metabolism). The piece is characterized by a continuous counterpoint between principles and well-chosen, contemporary examples, with careful attention to definitions and orchestration around basic chemistry (and stereochemistry). While the range of the content is enormous, the device of pinning text to *Powerpoint*™ slides is elegantly effective in providing clarity and conciseness. Many of us who attempt to teach drug metabolism will applaud the opportunity to reprise this material in improving our own presentations – with due acknowledgement to the original composers of course. As well as providing the basis for an understanding of the principles of drug metabolism to those new to the subject, this volume together with the first one represents an invaluable reference source for those interested in the challenge of being able to predict drug metabolism from chemical structure and, in a broader context, of modelling and simulating pharmacokinetic behavior in general.

Bravo to *Testa* and *Krämer* for their virtuoso performance!

September 2009

Geoff Tucker
Emeritus Professor, University of Sheffield,
and Chairman, *Simcyp Ltd*, Sheffield, UK

Preface

Our objectives when undertaking this Work were twofold. First and as research scientists, we aimed at offering a broad and structured portrayal of drug and xenobiotic metabolism, a science whose amazing advances in recent decades and years has contributed to a genuine change of paradigm in drug research. These advances are both factual and conceptual, with an ever deepening understanding of the mechanisms operating and regulating drug metabolism at the biomolecular, cellular, and organismic levels. Our second objective derives from our vocation as teachers, which has taught us that in education content cannot be separated from form – that presentation is meaningful in that it conveys information and contributes to the growth and transmission of knowledge.

As explained in the *Preface* of *Volume 1*, the layout of the present Work lies halfway between a traditional text enhanced with scattered illustrations, and a book of images with minimal text. In fact, it turns out that the elaboration of clearly designed, logically structured, and easily understandable schemes requires an even greater cognitive input than text writing – partly because such schemes are bidimensional and so are able to show explicit connections. The consistently positive reviews *Volume 1* has received have been an encouragement and our best reward. We are now pleased to submit *Volume 2* which completes and concludes our Work. Its *Part 4* (Conjugation Reactions) follows *Part 2* (Redox Reactions) and *Part 3* (Hydrolyses) in *Volume 1*, and gives a logically structured and comprehensive review on conjugation reactions and the involved enzymes. Its *Part 5* (Metabolism and Bioactivity) places drug and xenobiotic metabolism in the broader context of pharmacological and toxicological sciences providing insightful concepts and examples of bioactivation and toxification, respectively, which are fascinating to read and teach. As for *Parts 6* and *7* (Inter-Individual Factors and Intra-Individual Factors, respectively, affecting drug metabolism), their topics including genetic variability in drug metabolism and regulation mechanisms of enzyme expression and activity are presented in unusual depth and breadth.

Please learn from this Work and take pleasure in doing so – as every teacher hopes for their students.

January 2010 *Bernard Testa* and *Stefanie D. Krämer*

Contents

Part 4

Reactions of Conjugation and Their Enzymes

This *Part 4* of our biochemical introduction to drug metabolism [1–4] presents the reactions of conjugation and their enzymes. As we shall see, reactions of conjugation are also a major focus of interest in the metabolism of drugs and other xenobiotics. Books specifically dedicated to conjugation reactions are rare [5], but much recent information can be found in book chapters (*e.g.*, [6][7]).

For a reaction of conjugation to occur, a *suitable functional group* must be present in the substrate, which will serve as the anchoring site for an endogenous molecule or moiety such as CH_3, sulfate, glucuronic acid, or glutathione. Conjugation reactions are thus synthetic (*i.e.*, anabolic) reactions whose products are of modestly to markedly higher molecular weight than the corresponding substrate. As for the anchoring group, it can either be present in a xenobiotic or be created by a functionalization reaction. In other words, reactions of conjugation are able to produce *first-generation* as well as *later-generation metabolites*. We, therefore, consider as unfelicitous the term of 'phase II reactions' commonly used to designate conjugations.

A first issue when discussing reactions of conjugation will be to offer a clear definition. As we shall see, a number of criteria exist, all of which show some degree of fuzziness, and only one of which must necessarily be met. This has indeed led to some confusion with reactions of hydrolysis, which some biochemists have viewed as conjugation. We oppose such a view for reasons previously explained [3]. To repeat what we stated, reactions of hydrolysis are not catalyzed by *transferases* (EC 2) but by hydrolases (EC 3) [8], and water is not an endogenously synthesized molecule or moiety linked covalently to a cofactor.

Reactions of conjugation, like the reactions of functionalization we saw in *Parts 2* and *3*, act on exogenous substrates (*i.e.*, *xenobiotics* [1]) as well as endogenous substrates (*i.e.*, *endobiotics*). This dual functionality may create a potential for metabolic interaction between a drug and an endogenous substrate, a frequently overlooked mechanism of toxicity. Thus, there may be competitive affinity for the catalytic site of an endobiotic-metabolizing enzyme, or there may be competition for the limited supply of a cofactor. A typical example of the latter case is found with *paracetamol*, a high-dose drug undergoing extensive glucuronidation whose administration is forbidden to neonates and babies, since it deprives them of the glucuronic acid they need to detoxify bilirubin.

Part 4 Reactions of Conjugation and Their Enzymes

4.1. Introduction
4.2. Methylations
4.3. Sulfonations and Phosphorylations
4.4. Glucuronidations and Other Glycosidations
4.5. Acetylations and Other Acylations
4.6. Formation and Fate of Coenzyme A Conjugates
4.7. Glutathione and Its Reactions
4.8. Reactions of Amines with Carbonyls

Fig. 4.1. The structure of this *Part* follows custom as much as logic. First, an *introductory Chapter* will present an *overview* of the reactions of conjugation, their criteria, and their similarities and differences with functionalization reactions. As for the major reactions to be discussed in the subsequent *Chapters*, there is no overwhelming argument for preferring one order over another. We shall begin with the rather straightforward case of the reactions of *methylation* (*Chapt. 4.2*). Reactions of *sulfonation* (*Chapt. 4.3*) and *glucuronidation* (*Chapt. 4.4*) sometimes compete for the same substrates and will, therefore, be treated in sequence. Together with sulfonation, we will have a few things to say about reactions of *phosphorylation*, whose rarity should not obscure their significance in the activation of some drugs. *Chapt. 4.5* and *4.6* center on *coenzyme A*, but with a difference. Reactions of *acetylation* (*Chapt. 4.5*) follow the usual pattern in having the conjugating moiety carried by the coenzyme, here coenzyme A. In contrast, there is a variety of reactions where the substrate (be it a xenobiotic or an endobiotic) is coupled to coenzyme A prior to being processed along vastly different pathways (*Chapt. 4.6*). *Chapt. 4.7* presents *glutathione* and its reactions, a topic of marked biocomplexity and great toxicological significance. A few unclassifiable reactions will be summarized in *Chapt. 4.8*.

4.1. Introduction

<table>
<tr><th colspan="4">Chemical entities being transferred to or from the substrates</th></tr>
<tr><th colspan="2">**Functionalizations** (Phase I)</th><th colspan="2" rowspan="2">**Conjugations** (Phase II)</th></tr>
<tr><th>Redox reactions</th><th>Hydrolyses</th></tr>
<tr><td>O</td><td>H_2O</td><td colspan="2">Methyl group (Chapt. 4.2)</td></tr>
<tr><td>O_2</td><td>HO^-</td><td colspan="2">Sulfuric or phosphoric acid (Chapt. 4.3)
Glucuronic acid and some sugars (Chapt. 4.4)</td></tr>
<tr><td>e^-</td><td rowspan="3"></td><td colspan="2">Acetyl or other acyl groups (Chapt. 4.5)</td></tr>
<tr><td>2 e^-</td><td rowspan="8">Following conjugation with Coenzyme A (Chapt. 4.6)</td><td>Glycine or other amino acids</td></tr>
<tr><td>H^- (hydride)</td><td>Diglycerides or other lipids</td></tr>
<tr><td rowspan="3"></td><td>Cholesterol or other sterols</td></tr>
<tr><td>Ethyl or other short alkyl groups</td></tr>
<tr><td>β-Oxidation</td></tr>
<tr><td>Chain elongation by C_2 units</td></tr>
<tr><td>Inversion of configuration</td></tr>
<tr><td colspan="2"></td><td colspan="2">Glutathione (Chapt. 4.7)</td></tr>
<tr><td colspan="2"></td><td colspan="2">Carbonyl compounds, CO_2 (Chapt. 4.8)</td></tr>
</table>

Fig. 4.2. This *Figure* is a slightly amended form of *Fig. 1.12* we saw in *Part 1* [1], with a few additional details. The focus here is on the *conjugating moiety* being transferred to the substrate as a result of a conjugation reaction. Reference is made to *Chapt. 4.2–4.8* to help the readers get a better view of the present *Part*. We also have here a first glance at the different pathways which *xenobiotic–coenzyme A conjugates* can follow. Two of these pathways are not conjugations *stricto sensu* and, for this reason, are written in italics. Nevertheless, they will be discussed here, since a coenzyme A conjugate is the indispensable intermediate. These two pathways are the unidirectional inversion of configuration of profens (and a few other xenobiotics) and the β-oxidation of fatty acid analogs. As for the reactions in *Chapt. 4.8*, they represent poorly investigated pathways nevertheless worthy of some attention.

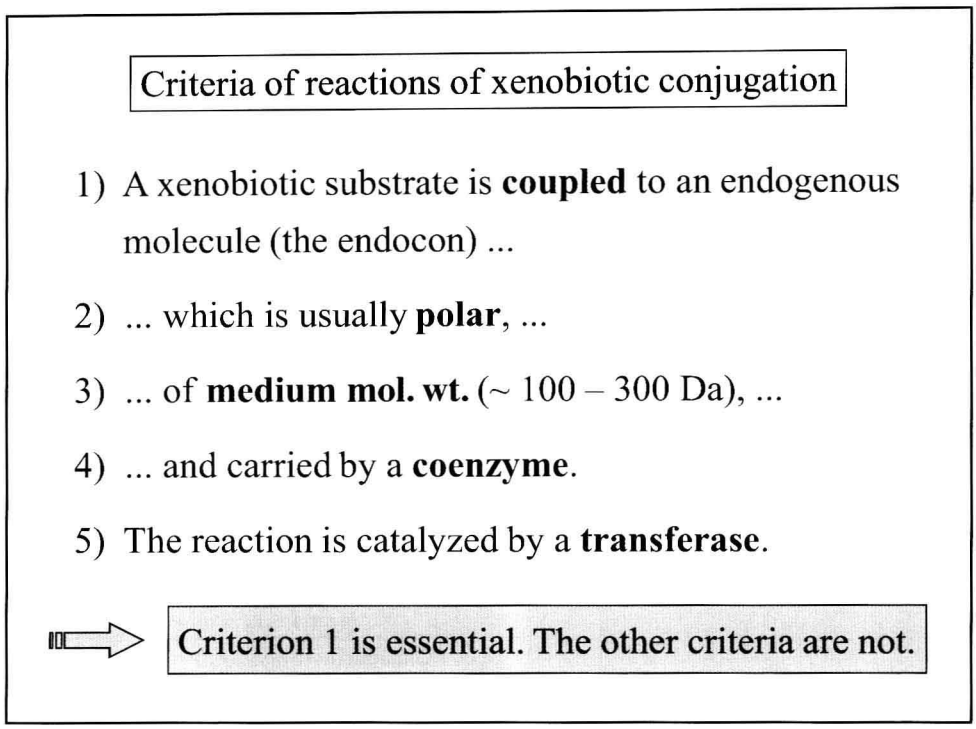

Fig. 4.3. Conjugation reactions are characterized by a number of *criteria* which are presented here [5][6][9]. *First* and above all, they involve an endogenous molecule (called the *endogenous conjugating moiety*, and sometimes abbreviated as the '*endocon*') with which the substrate is coupled. This is the *absolute criterion* of conjugation reactions, although, as we shall see, there may be arbitrariness in deciding whether a conjugating moiety such as CO_2 is endogenous. *Second*, this endogenous molecule or moiety is generally polar (*hydrophilic*) or even highly polar, but there are exceptions. *Third*, the *size* of the endocon is generally in the range of *100–300 Da*. *Fourth*, the endogenous conjugating moiety is usually carried by a *cofactor*, with the chemical bond linking the cofactor and the endocon being a high-energy one such that the *Gibbs* energy released upon its cleavage drives the transfer of the endocon to the substrate. *Fifth*, conjugation reactions are catalyzed by enzymes known as *transferases* (EC 2) which bind the substrate and the cofactor in such a manner that their close proximity allows the reaction to proceed. The metaphor of transferases being a '*nuptial bed*' has not escaped some biochemists. It is important from a biochemical and practical viewpoint to note that *Criteria 2–5* considered separately are neither sufficient nor necessary to define conjugation reactions. They are not sufficient, since, in hydrogenation reactions (*i.e.*, typical reactions of oxidoreduction), the hydride is also transferred from a cofactor (NADPH or NADH). And they are not necessary, since they all suffer from some important *exceptions* (see next *Figure*).

How are the conjugation criteria satisfied?

Reactions \ Criteria	1 (endocon)	2 (polar)	3 (medium mol. wt.)	4 (co-enzyme)	5 (trans-ferase)
Methylations	+	**0**	**0**	+	+
N-Methylations of aromatic azaheterocycles	+	+	**0**	+	+
Sulfonations, phosphorylations	+	+	+	+	+
Glucuronidations	+	+	+	+	+
Acetylations	+	(+)	**0**	+	+
Amino acid conjugations	+	+	+	**0**	+
Conjugations with lipids or sterols	+	**0**	**0**	**0**	+
C_2 Elongation	+	**0**	**0**	+	+
Glutathione conjugations	+	+	**0**	**0**	+ or **0**
Conjugations with carbonyl compounds, CO_2	+	+	+ or **0**	**0**	**0**

Fig. 4.4. This *Figure* summarizes in tabular form the cases of *compliance and non-compliance* to the five conjugation criteria. As can be seen, *Criterion 1* is indeed the only one that knows no exception, since all conjugating moieties involved are indeed endogenous. Thus, the C_2 unit in chain elongation is derived from acetyl-coenzyme A (*Chapt. 4.6*). Most of the CO_2 used in the formation of carbamic acids (*Chapt. 4.8*) is clearly also produced *in vivo*. The criterion of polarity of the endocon (*Criterion 2*) knows only two major exceptions, namely the coupling of xenobiotic carboxylic acids to sterols or to diglycerides (to yield mixed triglycerides), and the C_2 chain elongation (*Chapt. 4.6*). The transfer of a CH_3 group is special, since it adds a hydrophobic moiety except when forming quaternary ammonium metabolites (*Chapt. 4.2*). The CH_3 group, being small, is also an exception to *Criterion 3* as is the acetyl moiety. But we also have relatively large endocons such as sterols (*Chapt. 4.6*) and glutathione (*Chapt. 4.7*). As for the conjugating moiety being carried by a coenzyme (*Criterion 4*), exceptions are glutathione (*Chapt. 4.7*), carbonyl compounds (*Chapt. 4.8*), and all reactions in *Chapt. 4.6*, since here and as stated it is the substrate rather than the endocon that is attached to coenzyme A. Finally, catalysis by a transferase (*Criterion 5*) is almost always the case, the few exceptions being the coupling of hydrazines with carbonyl compounds (*Chapt. 4.8*) and some nonenzymatic conjugations with glutathione (*Chapt. 4.7*).

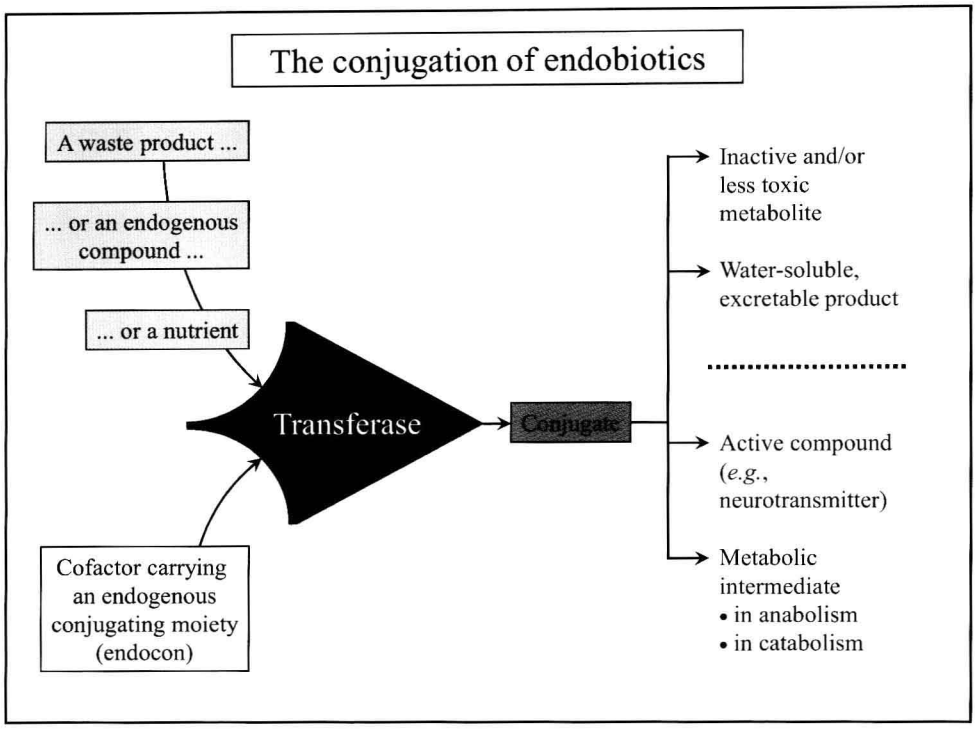

Fig. 4.5. This *Figure* and the next one illustrate the deep analogy between the physiological reactions of *endobiotic conjugation* and the conjugation of xenobiotics. Their graphical similarity with *Fig. 1.23* in *Part 1* [1] is not fortuitous. As shown here, a *waste product* of physiological metabolism (*e.g.*, bilirubin, a toxic breakdown product of hemoglobin), an *endogenous compound* (*e.g.*, the neurotransmitter noradrenaline), or a *nutrient* (*e.g.*, a fatty acid) is captured by a transferase. The latter catalyzes the transfer of the adequate conjugating moiety from the cofactor to the substrate, yielding a *conjugate*. These reactions have evolved to fulfill a variety of functions, as classified in the *Figure*. Thus, the toxic *bilirubin* is detoxified by conjugation with glucuronic acid, the resulting glucuronide being excreted in the bile. The case of noradrenaline is different, being *N*-methylated to the neurotransmitter adrenaline or *O*-methylated to an inactive metabolite. The case of metabolic intermediates in *anabolism* (synthetic metabolism) and *catabolism* (breakdown metabolism) is illustrated with fatty acids, whose coenzyme A conjugates can undergo anabolism by C_2 chain elongation, or catabolism by β-oxidation.

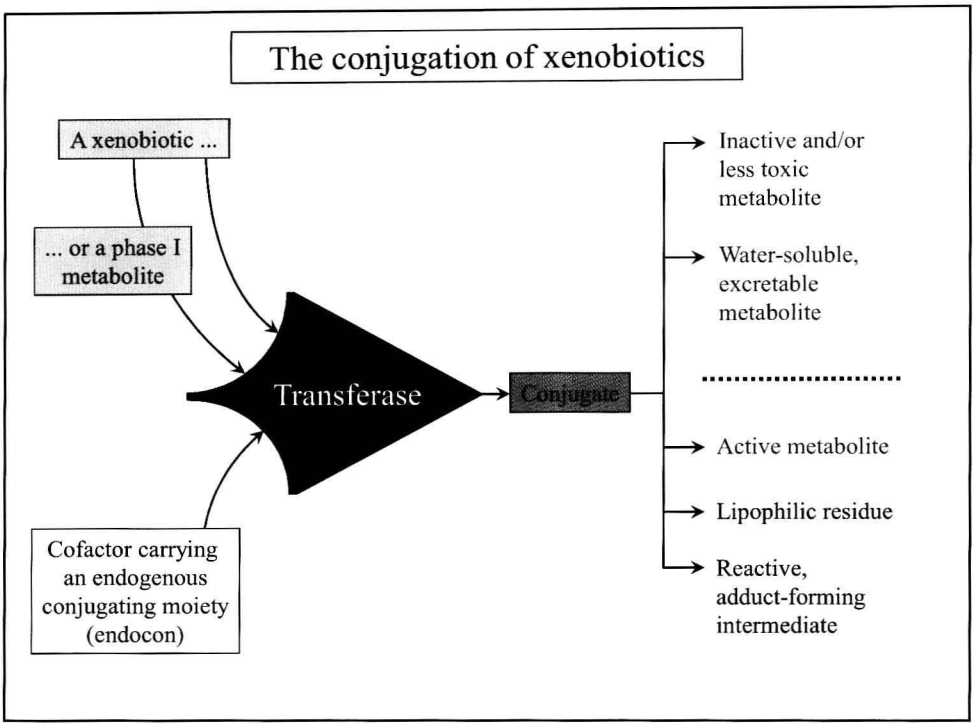

Fig. 4.6. Reactions of xenobiotic conjugation have evolved from physiological conjugations to fulfill *protective functions* [10]. Thus, a xenobiotic containing an adequate target group, or a phase I metabolite, is captured and metabolized by a transferase. As we shall see, some transferases recognize endobiotics and xenobiotics alike (*e.g.*, catechol *O*-methyltransferase), while others have diversified and are specialized to some extent toward endobiotics or xenobiotics (*e.g.*, UDP-glucuronyltransferases). As a rule, drug conjugation *inactivates* the substrate, but there are only few noteworthy exceptions such as the highly active morphine 6-*O*-glucuronide. Similarly, *toxicity* is usually greatly decreased by conjugation (*e.g.*, *N*-methylpyridinium), but, as we shall see, there are numerous examples of conjugations leading to *toxification*. Some conjugates may indeed be reactive (*e.g.*, some *acyl glucuronides*), whereas others are highly lipophilic and may accumulate in tissues as *residues* (*e.g.*, some mixed triglycerides). Such exceptions should not hide the fact that the greatly increased hydrophilicity of many conjugates relative to their parent compound facilitates their excretion. What is more, a *co-evolution of transferases and transporters* is believed to have occurred, such that the formation of polar conjugates (*e.g.*, glucuronides and glutathione derivatives) is coupled to their *active excretion* [11].