

# **Introduction to Drug Metabolism**

**G. GORDON GIBSON and PAUL SKETT**



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# INTRODUCTION TO DRUG METABOLISM

*G. Gordon Gibson, PhD*

Lecturer in Pharmacological Biochemistry  
University of Surrey

AND

*Paul Skett, Fil. dr.*

Lecturer in Pharmacology  
University of Glasgow

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# INTRODUCTION TO DRUG METABOLISM

# Preface

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Although the scientific literature on drug metabolism is extensive, it suffers from the disadvantage that the material is diffuse and consists largely of specialist monographs dealing with particular aspects of the subject. In addition, although there are a few excellent texts on drug metabolism in print, these tend to be earlier publications and hence do not take into account the many recent advances in this area. Our motivations for writing this book therefore arose from the clear need for a recent and cohesive introductory text on this subject, specifically designed to cater for the needs of undergraduate and postgraduate students. Much of the subject matter in this text is derived from various courses on drug metabolism given at the University of Surrey and the University of Glasgow to basic science students in pharmacology, biochemistry, nutrition and nursing studies, to pre-clinical medical students and to undergraduate and post-graduate students in toxicology. Therefore, it is our intention that this text will serve as a primer in drug metabolism to a variety of students in the life sciences taking courses in this subject.

The term 'drug metabolism' in its broadest sense may be considered as the absorption, distribution, biotransformation and excretion of drugs. To cover all these facets of drug metabolism in a single text is a voluminous task and therefore we have focused primarily on the biotransformation aspects of the subject. Having said this, the text is not solely a list of drug metabolism pathways, but rather it uses biotransformation reactions to rationalize many

pharmacological and toxicological manifestations of drug action, clearly an important consideration in the clinical use of drugs.

The subject of drug metabolism or drug biotransformation is introduced in Chapter 1 by considering the relevant pathways, i.e. the enzyme-catalysed changes in drug structure. Wherever possible, currently used drugs are given as examples. Consideration is also given to the classification and functional role of the enzymes involved in these pathways. This is of importance as many of the drug-metabolizing enzymes are also involved in the metabolism of endogenous compounds and the chapter concludes by drawing attention to the competition between endogenous and exogenous substrates of these enzymes. Chapter 2 continues on the enzyme theme and considers, in detail, the enzymology of drug metabolism reactions including the molecular mechanisms involved. This chapter concludes by considering drug metabolism pathways not as separate events, but rather from the viewpoint of the interaction of the various pathways both with each other and with the pathways of endogenous, intermediary metabolism. Detailed consideration is also given to the overall control and regulation of drug metabolism pathways from various levels of integration.

Having established the basic chemistry and enzymology of drug metabolism, the three following chapters (3, 4 and 5) discuss factors that modulate drug metabolism, including induction and inhibition and external and internal factors. These are important aspects of any consideration of drug metabolism as the enzymes catalysing the various reactions are susceptible to changes in their activity or concentration, and are particularly influenced by other drugs or chemicals. Accordingly, these chapters are designed to rationalize, on an enzymatic or molecular level, why certain drugs, when given in combination, result in a response that could not be easily predicted by considering each drug in isolation. In view of the widespread use of polypharmacy where several drugs are given simultaneously, it is clear that the student should not only be aware of potential drug-drug interactions but should also be able to rationalize and explain why combination drug therapy interactions occur. These chapters conclude with a consideration of some of the internal or physiological factors that influence or regulate drug metabolism. A knowledge of these physiological factors is of utmost importance in clinical pharmacology in that, depending on the age, sex, hormonal status or genetic background of the patient, drug biotransformation pathways may either be switched on or turned off, thus influencing the clinical usage of drugs. In addition, patients on drug therapy for a particular ailment may have additional disorders of the heart, liver or kidney systems, defects which may subsequently influence drug disposition in the original disease, and attention is therefore drawn to the role of disease states in drug metabolism.

No matter how extensive or informative a test in drug metabolism is at the

basic or theoretical level, a major consideration of this subject must include the the *raison d'être* of drug biotransformation pathways. For example, the earlier chapters in this text highlight that one of the roles of drug metabolism is to make lipid-soluble drugs more polar, hence more readily excreted from the body. Thus if an efficient 'removal mechanism' did not exist for drugs, these highly lipid-soluble drugs would remain in the body for long time periods. Providing that the retained drug was maintained at sufficiently high concentrations at its site of action, then a prolonged pharmacological response would result. The majority of drugs have a half-life of only several hours in man and do not, in general, persist in the body; this underlines the important role of drug metabolism in clearing drugs from the body and hence terminating the pharmacological response. However, it should be emphasized that drug metabolism has additional, equally important roles to play in the overall, biological response to drugs. For example, as considered in Chapter 6, drug biotransformation may result in changes in both pharmacological and toxicological responses to drugs, an important consideration in drug therapy. From the pharmacological viewpoint, many drugs are inactivated by drug metabolism, probably as a result of the chemically changed drug metabolite not being recognized by the appropriate receptor system. Conversely, many 'pro-drugs' are inactive *per se*, and absolutely require biotransformation to release the pharmacologically active drug. Drug metabolism also has an important role to play in the side effects or toxicity of drugs. There are many well-documented examples where drug metabolism results in the transformation of a totally innocuous drug to a metabolite that is highly biologically reactive, and by a variety of mechanisms, can result in an overt toxicological response. Therefore in Chapter 6, we have emphasized that drug metabolism not only facilitates drug excretion, but is also an important determinant of pharmacological and toxicological responses to drugs.

One of the goals in the study of drug metabolism is to understand biotransformation pathways and biological responses to drugs in man. Unfortunately, there are many obvious practical and ethical constraints limiting our study of drug metabolism in man. Chapter 7 deals primarily with *in vivo* drug metabolism in man, and draws attention to the problems and advantages of applying *in vitro* drug metabolism data to man. Emphasis is placed on pharmacokinetics and the clinical relevance of drug metabolism in the human situation, drawing on the knowledge and information built up from previous chapters. Pharmacokinetics is not exclusively considered from a mathematical viewpoint but rather from a physiological/clinical stance. In this way, it is our intention to highlight the importance of pharmacokinetics in drug action, without placing an undue demand on the student to struggle with unnecessary mathematical formulae.

The final chapter in this text is concerned with the more practical aspects of drug metabolism and outlines several experiments that may be undertaken by

practical classes. This chapter should not be considered in isolation but rather is designed to demonstrate experimentally some of the concepts developed in the remainder of the text. Rigid experimental practicals are not extensively described and we have devised this chapter on a 'menu' basis. This was achieved by initially detailing the methodology associated with the study of the enzymes and metabolites of drug biotransformation pathways, and subsequently applying these methodologies to highlight some concepts in drug metabolism. In this manner, the organisers of class practicals can devise their own experiments, tailored to their own needs and constraints placed upon them by factors such as class size and availability of facilities (including instrumentation). This chapter should also be of use to postgraduate students who require a detailed laboratory manual of experimental techniques to assay both the content and functional expression of the drug-metabolizing enzymes. The detailed methodology, analytical techniques and class practicals described in this chapter are based on both our own research activities and experience of undergraduate and postgraduate practical classes; and it should be emphasized that these have successfully run in our laboratories for several years.

In compiling this text, we have made liberal use of both tables and figures. We feel this is an important aspect of an introductory text of this nature in that a concept is more readily appreciated and remembered if numerical data or a simple figure can exemplify the point being made. In addition, we have included an extensive further reading section at the end of each chapter. Where possible, we have included all the text books, monographs and symposium proceedings relevant to each chapter, in addition to recent review articles. Where necessary, we have also included references to original articles to highlight a particular point being made in the chapter. In this way, we hope we have provided reference to sufficient additional reading material such that the interested reader can further pursue any particular area of interest in drug metabolism and related subjects.

### Acknowledgements

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# 1

## *Pathways of drug metabolism*

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### 1.1 Introduction

Drug metabolism is an immense area of study and this is reflected in the range of chemical reactions the substrates can undergo during metabolism, e.g. oxidation, reduction, hydrolysis, hydration, conjugation and condensation. Drug metabolism is normally divided into two phases: phase I (or functionalization reactions) and phase II (or conjugative reactions). The division between phase I and II is given in Table 1.1.

Recently the existence of a third phase of metabolism has been postulated involving metabolism of conjugate (excreted in the bile) by the intestinal microflora and subsequent re-absorption and metabolism (see section on phase III metabolism).

There is great interest in the inter-relationship of the various metabolic routes in terms of competing reactions of the substrate for phase II enzymes. There is much evidence to suggest that the phase I reactions create a reactive functional group on the molecule so that it can be attacked by the phase II enzymes. Thus, the phase II reactions are the true 'detoxification' pathways and give products that account for the bulk of the inactive, excreted products of a drug.

This chapter will examine the different types of reactions involved in drug metabolism using the phase I and II classification as a basis. Examples of each type of reaction will be given and, where possible, these will be actual reactions

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**Table 1.1** Reactions classed as phase I or phase II metabolism

Phase I	Phase II
Oxidation	Glucuronidation/glucosidation
Reduction	Sulfation
Hydrolysis	Methylation
Hydration	Acetylation
Dethioacetylation	Amino acid conjugation
Isomerization	Glutathione conjugation
	Fatty acid conjugation
	Condensation

of drug substrates rather than model substrates. This will show the pharmacological, toxicological and clinical relevance of the reactions. Attention will be drawn to competing reactions for the same substrate where appropriate. A brief discussion of the postulated phase III metabolism will be included.

Many of the enzymes involved in drug metabolism are, in fact, principally involved in the metabolism of, or are capable of metabolizing, endogenous compounds. Thus a separate section of the chapter will be devoted to the metabolism of endogenous compounds by 'drug-metabolizing' enzymes. The interaction of drugs and endogenous compound metabolism will be highlighted.

It will be appreciated at this stage that, in a limited space, not every reaction undergone by every drug can be covered and that inevitably there will be omissions. This chapter is designed to give a feel for the possible metabolism of drugs and information regarding specific drugs will be found in specialist publications. A list of further reading material will be found at the end of the chapter, from which further information on specific pathways can be obtained.

### 1.2 Phase I metabolism

Phase I metabolism includes oxidation, reduction, hydrolysis and hydration reactions as well as isomerization and other rarer miscellaneous reactions. The reactions will be discussed in terms of reaction type and, with respect to oxidation, site of enzyme – the classification of phase I reactions can be found in Table 1.2. Oxidation performed by the microsomal mixed-function oxidase system (cytochrome *P*-450-dependent) is considered separately because of the diversity of reactions performed by this enzyme system.

#### 1.2.1 OXIDATIONS INVOLVING THE MICROSOMAL MIXED-FUNCTION OXIDASE

The mixed-function oxidase system found in microsomes (endoplasmic reticulum) of many cells (notably those of liver, kidney, lung and intestine)

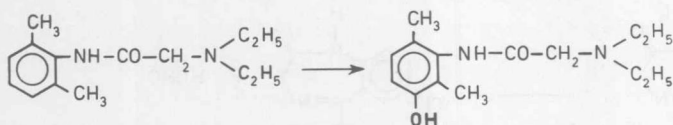
**Table 1.2** Sub-classification of phase I reactions

Oxidation involving cytochrome P-450
Oxidation – others
Reduction
Hydrolysis
Hydration
Isomerization
Miscellaneous

performs many different functionalization reactions (summarized in Table 1.3). An example of each reaction is given below.

#### (a) AROMATIC HYDROXYLATION

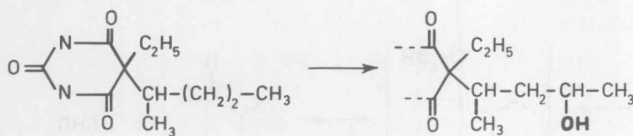
This is a very common reaction for drugs and xenobiotics containing a benzene ring. In this example (Figure 1.1) the local anaesthetic and antidysrhythmic drug lignocaine is converted to its 3-hydroxy derivative.



**Figure 1.1** The 3-hydroxylation of lignocaine.

#### (b) ALIPHATIC HYDROXYLATION

Another very common reaction, e.g. pentobarbitone hydroxylated in the pentyl side chain (Figure 1.2).



**Figure 1.2** The side-chain hydroxylation of pentobarbital.

#### (c) EPOXIDATION

Epoxides are normally unstable intermediates but may be stable enough to be isolated from polycyclic compounds (e.g. the precarcinogenic polycyclic hydrocarbons). Epoxides are substrates of epoxide hydratase (discussed later) forming dihydrodiols; they may also spontaneously decompose to form hydroxylated

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products. It has been suggested that epoxide formation is the first step in aromatic hydroxylation but this remains an area of debate. Figure 1.3 shows the epoxidation of benzo[*a*]pyrene to its 4,5-epoxide.



Figure 1.3 The formation of benzo[*a*]pyrene-4,5-epoxide.

### (d) *N*-DEALKYLATION

This reaction occurs very readily with most drugs containing an alkyl group attached to an amine or ring nitrogen, leading to the formation of a primary or secondary amine. The alkyl group is lost as the corresponding aldehyde. In the example in Figure 1.4 diazepam is converted to *N*-desmethyldiazepam with the loss of formaldehyde.

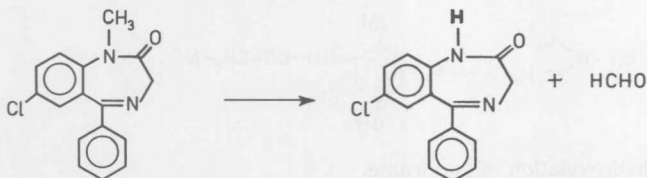


Figure 1.4 The *N*-demethylation of diazepam.

The reaction is considered to occur in two steps, the first being hydroxylation of the methyl group on the nitrogen, and the second a decomposition of this intermediate (see Figure 1.5).

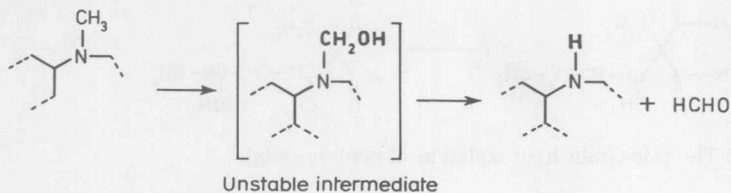


Figure 1.5 The mechanism of *N*-demethylation of diazepam.

### (e) *O*-DEALKYLATION

A very similar reaction to (d) above. Figure 1.6 shows the *O*-demethylation of codeine to yield morphine.

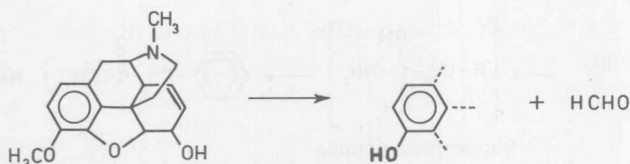


Figure 1.6 The O-demethylation of codeine.

The reaction proceeds via a hydroxy intermediate as *N*-dealkylation.

#### (f) *S*-DEALKYLATION

Various *S*-methyl compounds can be *S*-demethylated by hepatic microsomes but a soluble factor appears to be necessary as well. This reaction may not, therefore, be a true microsomal one. The *S*-demethylation of *S*-methylthiopurine is illustrated in Figure 1.7.

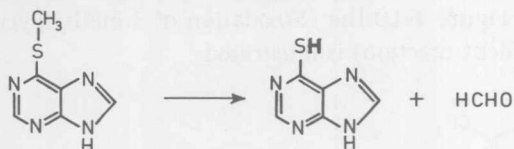


Figure 1.7 The *S*-demethylation of *S*-methylthiopurine.

#### (g) OXIDATIVE DEAMINATION

Amines containing the structure  $\begin{array}{c} \text{—CH—NH}_2 \\ | \\ \text{CH}_3 \end{array}$  are metabolized by the micro-

somal mixed-function oxidase system to release ammonia and leave the corresponding ketone. This is a different substrate specificity to the other amine metabolizing enzymes, namely monoamine oxidase (MAO – see later) and the two enzymes do not compete for the same substrates. Figure 1.8 shows the deamination of amphetamine.

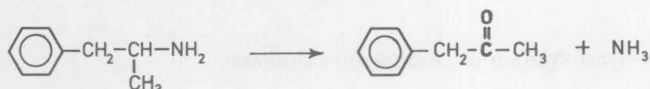


Figure 1.8 The oxidative deamination of amphetamine.

The ketone formed in this case is phenylacetone. As with dealkylation, oxidative deamination involves an intermediate hydroxylation step (Figure 1.9) with subsequent decomposition to yield the final products.



## 6 Introduction to Drug Metabolism

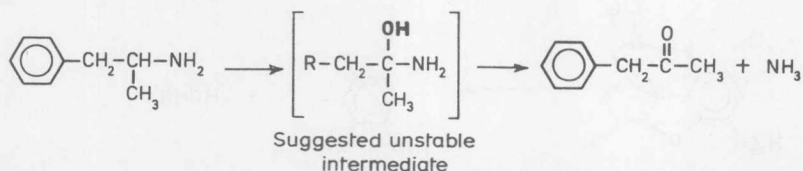


Figure 1.9 The mechanism of oxidative deamination of amphetamine.

### (h) N-OXIDATION

Hepatic microsomes in the presence of oxygen and NADPH can form *N*-oxides. These oxidation products may be formed by the mixed-function oxidase system or by separate flavoprotein *N*-oxidases (see later). The enzyme involved in *N*-oxidation depends on the substrate under study. Many different chemical groups can be *N*-oxidized including amines, amides, imines, hydrazines and heterocyclic compounds. In Figure 1.10 the *N*-oxidation of 3-methylpyridine (a cytochrome *P*-450-dependent reaction) is illustrated.



Figure 1.10 The *N*-oxidation of 3-methylpyridine.

*N*-Oxidation may manifest itself as the formation of a hydroxylamine as in the metabolism of 2-acetylaminofluorene (2-AAF) (Figure 1.11). This is of interest as the hydroxylamine of 2-AAF is thought to be a proximate carcinogen giving the toxicity of 2-AAF.



Figure 1.11 The *N*-hydroxylation of 2-acetylaminofluorene.

### (i) S-OXIDATION

Phenothiazines can be converted to their *S*-oxides by the microsomal mixed-function oxidase system. As an example the *S*-oxidation of chlorpromazine is shown in Figure. 1.12.