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# **DRUG METABOLISM HANDBOOK**

## **CONCEPTS AND APPLICATIONS**

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Edited by

Ala F. Nassar, Paul F. Hollenberg, and JoAnn Scatina

 **WILEY**

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## PREFACE

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Studies on absorption, distribution, metabolism, and elimination-toxicology (ADME-Tox) have progressed over the years to the point where they now play a major role in drug discovery and development. Until the late 1980s, the primary role of drug metabolism groups in the pharmaceutical industry was to provide ADME-Tox information to support the regulatory package.

During the last decade, with the rapid rise in new molecular entities (NMEs) arising from combinatorial chemistry and high-throughput biological screening, an urgent need has arisen for the determination of the ADME properties of these NMEs at very early stages in the drug discovery pipeline in order to facilitate the selection of “ideal” drug candidates for further development. Back integration of key studies into the discovery phase has resulted in earlier identification of potential DM/PK and safety liabilities. This information aids in decision making and in many instances, has been incorporated into criteria for compound advancement into the development phase. Given the need for earlier and more rapid evaluation of a larger number of compounds, drug metabolism scientists have developed and incorporated novel approaches into early drug discovery including “humanized” *in vitro*-based cell systems, sophisticated automation, higher-throughput ADME assays and screens, ultrasensitive analytical technologies, and computational models in order to accelerate the examination of the drug metabolism pathways for their NMEs. The success of this approach is evident, as the number of failures due to DM/PK liabilities has dramatically decreased.

Clearly, there is a growing need for improving and expanding education of students as well as current practitioners involved in investigations in these areas. The needs for continuing education in the rapidly expanding and dynamic area of ADME-Tox studies are not being met at the university level and oftentimes this is being taught in a piecemeal fashion on the job in pharmaceutical industries. The goal of this book is to provide a systematic approach for the education of younger researchers and students at the university level and to improve their knowledge of drug metabolism by presenting in-depth coverage of the drug disposition process, pharmacokinetic drug–drug interactions, theory, and evaluation approaches and improving the decision-making process in structural modification of drug candidates to reduce toxicity.

ADME-Tox experts in the field from both industry and academia have joined forces and offered their time to write this book, introducing students to modern concepts and practices of ADME-Tox. This book provides the basic training in the area of drug metabolism and disposition, including training programs for students and new employees in the pharmaceutical industry. Mastery of the material in this text will allow them to apply state-of-the-art research tools to *in vitro* and *in vivo* metabolism studies and contribute greatly to their abilities to perform pharmaceutical research in support of industrial, academic, and regulatory agency needs. This textbook consists of five parts. Part I provides an introduction to drug metabolism. Part II presents the *in vitro*

and *in vivo* technologies. Part III presents an important area of drug–drug interaction. Part IV discusses the toxicity and Part V presents the regulatory perspectives.

The editors and contributing authors greatly appreciate the commitment of the publishers to make this book available to our scientific colleagues in developing countries to enhance their knowledge in the area of ADME-Tox and to help them in furthering their careers in this very important area of research. Finally, we thank our many colleagues worldwide who have contributed to the development of the knowledge and techniques described in this book. We feel very fortunate to be able to participate in an area of scientific pursuit in which cooperation and collaboration between investigators in industry, academic institutions, and regulatory agencies is so strongly encouraged and highly valued.

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**PART I**

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# **INTRODUCTION**



# Historical Perspective

ROBERTA S. KING

## 1.1 CONTROVERSIES SPANNING PAST, PRESENT, AND FUTURE

Two major issues have been debated throughout the history of drug metabolism, and are still disputed to some degree. One is the name itself, the other is the physiological purpose of “drug” metabolism. In the 1800s and early 1900s, the generally agreed purpose of these reactions was reflected in the most widely used name, *detoxication mechanisms*. However, *detoxication* became widely recognized as a misnomer because not all parent compounds were toxic and not all metabolites were less or nontoxic. A better term was not invented until the 1950s when the term “drug metabolism” was coined. While handy, this term was still not entirely valid, and it needed silent agreement that “drug” be not restricted to medicinal compounds (Bachmann and Bickel, 1985–86). Thus, *xenobiotic metabolism* became popular starting in the 1970s, especially in circles studying carcinogens and environmental compounds. Xenobiotic, by definition, included all compounds foreign to the organism, not just medicinal ones. However, even in the early 1900s many examples were already known of metabolism of endogenous compounds, for example, steroids undergoing glucuronidation. These early examples of endogenous substrates were generally dismissed because they typically occurred at much higher concentrations than normally present, so-called “supraphysiological” concentrations. While none of these three terms could be considered ideal, in 1947 R.T. Williams concluded that the field of detoxication included “... all those metabolic processes not specifically covered by the main streams of fat, carbohydrate and protein intermediary metabolism” (Williams, 1947). Williams went on to explain that, “Detoxication is, in fact, the study of the metabolism of organic compounds other than lipids, carbohydrates, proteins and closely related natural compounds, although the lines of demarcation between these two groups is by no means a sharp one ...” (Williams, 1947; Bachmann and Bickel, 1985–86). Thereby, the earliest clear description of the field mainly described what it was not, and later terms were not much more precise.

The physiological purpose of these reactions was also widely debated. Of note is that several of the early theories are still considered at least partially valid. The first theories tried to answer the question of how these transformations were related to

detoxication. For example, in 1917, Berczeller published the first article trying to answer why conjugation would result in detoxication (Berczeller, 1917; Bachmann and Bickel, 1985–86). His theory, later to be disproved, was that conjugations such as glucuronidation and sulfonation led to a change in the surface tension of dilute aqueous solutions. He related this to *in vivo* conditions by indicating that the conjugates, with less surface activity than the parent drug, would be more easily removed from cellular surfaces while the parent drugs would accumulate at the surfaces to toxic concentrations (Bachmann and Bickel, 1985–86). The next hypothesis, proposed in 1922 by Sherwin (Sherwin, 1922), was based on the idea of “chemical defense” against accumulation of foreign compounds. This hypothesis is still considered valid as one role of drug metabolism. Sherwin proposed that the body needed to completely destroy foreign compounds or excrete them in the urine. Thus, for Sherwin, the purpose of oxidation and reduction reactions was to destroy the foreign molecules. If complete destruction was not possible, then conjugation reactions could make the molecule more aqueous soluble and more easily removed through the urine. In 1925, Schüller (Schüller, 1925) proposed a theory also similar to the modern view of the physiological purpose of drug metabolism. Schüller proposed that conjugation reactions led to an increase in the water solubility of compounds leading to a change in the distribution of the compound in the body (Schüller, 1925). The third theory, which resonates somewhat with today’s metabolism scientists, was proposed by Quick in 1932 (Quick, 1932). His view was that conjugations resulted in an increase in acidity, converting a weak acid parent into a strong acid metabolite which could be eliminated more easily. One benefit of this hypothesis was that it included in its rationale the acetylation reaction, which was ignored by Sherwin and Schüller. In his 1947 comprehensive review of metabolism (Williams, 1947), R.T. Williams criticized each of these potential roles of metabolism. Yet rather than proposing his own theory, Williams concluded that “interpretations worthy of the status of theories were lacking at that time” (Bachmann and Bickel, 1985–86).

In 2008, the field is described as an “elaborate defense system against foreign compounds and against the accumulation of potentially toxic endogenous molecules” (Meyer, 2007). However, the same author also adds recognition of the modern “concept of molecular links between xenobiotic metabolism and endogenous pathways of sterol, lipid, bile acid and energy homeostasis.” To complicate matters, the current generation of genetic technologies has revealed that all of the enzyme families contributing to drug metabolism include both members with selectivity for endogenous compounds and members with selectivity for exogenous compounds, and that homologues are present throughout diverse species from bacteria to human. Indeed, gene knockout studies have shown that multiple members of the “drug”-metabolizing enzyme families are essential to life or reproductive processes, as well as serving as a means to defend against xenobiotics (Sheets, 2007). Thus, in 2008, the field has no more specific name than the popular term “drug metabolism,” yet its physiological function has broadened to include endogenous compounds and endogenous regulatory pathways.

## **1.2 1800s: DISCOVERY OF MAJOR DRUG METABOLISM PATHWAYS (CONTI AND BICKEL, 1977)**

Many current students of metabolism are surprised to learn that drug metabolism experiments were first conducted and published more than 180 years ago, first in dogs in 1824 and next in humans in 1841 (Wöhler, 1824; Ure, 1841). While these experiments



TABLE 1.1 Early development of the major drug metabolism pathways.<sup>a</sup>

Year	Reaction name	Substrate	Intermediate	Product	Original citation (Conti and Bickel, 1977)
1824, 1841 1842,	Glycine conjugation β-oxidation	Benzoic acid Cinnamic acid	None Benzoic acid	Hippuric acid Hippuric acid	Wöhler (1824), Ure (1841) Erdmann and Marchand (1842a, b)
1848, 1867	Aldehyde oxidation Aromatic and benzylic carbon oxidation	Benzaldehyde Benzene Toluene	None None Benzyl alcohol	Benzoic acid Phenol Benzoic acid (via further oxidation of alcohol to carboxylic acid)	Wöhler and Frerichs (1848) Schultzen and Naunyn (1867)
1844, 1870	Glucuronide conjugation	Mango leaves	None	Euxanthic acid, Euxanthone and oxidized glucose	Erdmann (1844), Baeyer (1870)
1851, 1876	Sulfate conjugation	Benzene Hydroxyindole	Phenol	Conjugated phenol Indole-sulfate	Staedeler (1851), Baumann (1876)
1863	Reduction	Quinic acid Picric acid	Benzoic acid	Hippuric acid Picramic acid	Lautemann (1863) Karpus (1893)
1879, 1884	Glutathione conjugation	Bromobenzene Chlorobenzene	None	Mercapturic acid conjugates Acetylcysteine conjugates	Baumann and Preuss (1879), Jaffe (1879), Baumann (1884)
1887	Methylation	Pyridine acetate	Pyridine	<i>N</i> -methyl pyridinium hydroxide	His (1887)
1887, 1893	Acetylation	Furfural <i>m</i> -nitrobenzaldehyde	<i>N</i> -acetyl furfural	Furfuracrylic acid <i>N</i> -acetyl amino benzoic acid	Jaffe and Cohn (1887), Cohn (1893)

<sup>a</sup>Structures of substrates and products are shown in Fig. 1.1.

of hippuric acid into the urine (Erdmann and Marchand 1842a, b; Wöhler and Frerichs, 1848). We now call this biotransformation of cinnamic acid to benzoic acid,  $\beta$ -oxidation. Wöhler and Frerichs (1848) also discovered aldehyde oxidation when they found that dogs and cats excreted a “considerable amount” of benzoic acid after treatment with benzaldehyde (oil of bitter almonds) (Conti and Bickel, 1977, p. 11).

Nothing of the mechanism of these transformations was yet understood. During this era, the body was considered simply a chemical reaction container. Indeed, the chemists found many transformations “absolutely puzzling” (Conti and Bickel, 1977, p. 8) because, outside the organism, they could only be reproduced only under very harsh conditions, if at all. For example, oxidation of benzene to phenol had never been accomplished when, in 1867, Schultzen and Naunyn published their very clear determination of phenol in the urine of humans and dogs after ingestion of benzene. Even in the twenty-first century, only rather harsh and non-physiological conditions are known to chemically transform benzene to phenol (March, 1992).

In 1844 Erdmann observed that euxanthic acid isolated from urine of cows fed mango leaves could be hydrolyzed to euxanthone, but it took until 1870 to characterize the sugar moiety as an oxidized form of glucose. Similarly, conjugated phenols were observed in 1851, but it took until 1876 to confirm that the conjugate was a sulfate producing sulfuric acid upon hydrolysis (Staedeler, 1851; Baumann, 1876). Baumann is known as the “father of sulfation” and subsequently showed that many other ingested chemicals were also excreted as sulfates in the urine, often after preliminary oxidation to a phenol (Baumann, 1876). Methylation was first described in 1887 after feeding pyridine acetate to dogs and isolating the *N*-methyl product from the urine (His, 1887). Also in 1887, Jaffe and Cohn first observed a product that appeared to be conjugated with acetic acid (Jaffe and Cohn, 1887), although it took until 1893 for Cohn to confirm *N*-acetylation as a major conjugation reaction (Cohn, 1893).

While biochemists, chemists, physiologists, and pharmacologists contributed to these discoveries, the emphasis was on studying the metabolism of “foreign” compounds. More importantly, the emphasis was the fate of the chemical compound, rather than the organism that transformed it. Indeed, Bachmann and Bickel concluded that before circa 1870, “the whole matter was a biochemical curiosity rather than a physiologically meaningful process” (Bachmann and Bickel, 1985–86, p. 213). Evidence that this view began to change comes from Nencki’s thesis of 1870, foreseeing that, “... one will on the one hand be able to establish laws allowing predictions on the fate of new compounds, and on the other hand gain increasing insight into the organism as a ‘chemical agent’.” (Nencki, 1870) Beginning in about 1876 with Baumann’s phenyl sulfate (Baumann, 1876), it was often found that the excreted compounds were much less toxic than their parent compounds. And by 1893, enough evidence existed for introduction of the term “detoxication” in a textbook of physiological chemistry (Neumeister, 1893; Bachmann and Bickel, 1985–86, p. 213). Detoxication still stands as one major physiological role of metabolism, but is no longer recognized as the only role.

### 1.3 1900–1950s: CONFIRMATION OF MAJOR PATHWAYS AND MECHANISTIC STUDIES

As better techniques for identification of compounds were developed, the major reactions were confirmed and put upon somewhat stronger structural foundations; and in two cases the active cofactors for the reactions were elucidated. For example, Lipmann

earned the 1953 Nobel Prize in Medicine, in part, for his 1945 publication of the role of coenzyme A in acetylation of sulfanilamides (Lipmann, 1945). In 1953, Cantoni published evidence for *S*-adenosyl methionine as the active cofactor for methylation reactions (Cantoni, 1953). The biosynthetic source of glycine was elucidated in 1946 (Shemin, 1946), but it was not yet understood how the conjugation reaction occurred under physiological conditions.

Nencki's (1870) prediction of gaining insight into the organism as a chemical agent was also developed in significant ways. In the 1800s, the blood was often considered the localized source of the transformations, and the emphasis was on animal or human ingestion of a compound and isolation of excretion products from the urine. In the early 1900s, techniques such as hepatectomy and perfusion of livers and kidneys of laboratory animals proved the alternative paradigm of organ-based metabolism. One novel approach (Hemingway, Pryde and Williams, 1934) used serially perfused dog organs (such as liver or spleen) in combination with the kidney to demonstrate that the liver was the main site of glucuronic acid conjugation. In 1936, Potter and Elvehjem described an improved alternative to the commonly used tissue slice and tissue mince methods (Potter and Elvehjem, 1936). Their device used a glass test tube as mortar with a motor-driven blown-glass pestle for nondestructive tissue homogenization. Claude added differential centrifugation to the improved homogenization technique, and made possible subcellular localization of metabolism via isolation of individual tissue organelles (Claude, 1940). He also coined the still-popular term "microsome" (Claude, 1943).

Of note is that during the early twentieth century, most metabolism studies were conducted within biochemistry departments of university medical schools. Also during this time, because of (or in spite of) the World Wars, metabolism research expanded across Europe to England and North America, and by 1950 the United States had replaced Germany as the dominant origin of metabolism publications.

Approximately 100 years after the first published discoveries, "Modern" metabolism science was founded by the Welshman R.T. Williams, who in 1947 published the first text devoted to metabolism entitled *Detoxication Mechanisms: The Metabolism of Drugs and Allied Organic Compounds*. This text was expanded in a second edition in 1959 with a modified title, *The Metabolism and Detoxication of Drugs, Toxic Substances, and Other Organic Compounds*. In these texts, Williams brought systematic organization and clarity to what had previously been broad and disconnected research, "so that working hypotheses (could) be advanced" (Williams, 1947, quoted from Caldwell, 2006). It was Williams who proposed that metabolism occurred through reactions representing two distinct phases leading to the still popular terms, "phase I" and "phase II" metabolism reactions. Another revolutionary, B.B. Brodie of the United States National Institutes of Health, first published in 1948 and "led the field into its modern phase" (Bachmann and Bickel, 1985–86, p. 188). As foreseen by Nencki in 1870 (Nencki, 1870), predictive rules for functional group transformations began to emerge, and the enzymology of the reactions began to be elucidated.

#### **1.4 1950s–1980: MODERN DRUG METABOLISM EMERGES, WITH ENZYMATIC BASIS**

Because of advances in analytical technologies and biochemical methods, metabolism studies took off starting in about 1950. For example, partition chromatography improved

separations and allowed differentiation of drug versus metabolites. Isotope-tracer methods (mostly  $^{14}\text{C}$  and  $^{15}\text{N}$ ) allowed metabolites of foreign compounds to be detected and quantified at nontoxic doses. Absorption spectrophotometry also improved both quantification and identification of drug versus metabolites. For the first time, the cofactors of the enzymatic reactions were fully elucidated, and the enzymatic biosynthesis pathways of the cofactors were established. For example, the molecular mechanism of glucuronidation utilizing the reactive cofactor, uridine-3',5'-diphosphate glucuronic acid (UDPGA) was published in 1954 (Dutton and Storey, 1954). Advances in tissue fractionation methods provided the means to prove the enzymatic basis of metabolism. Indeed, Brodie and coworkers published the first review of this emphasis in 1958 entitled, "Enzymatic metabolism of drugs and other foreign compounds" (Brodie, Gillette and La Du, 1958). In the late 1950s and early 1960s, the cytochrome P450 enzyme system was discovered and characterized as the source of microsomal oxidations of several drugs and steroid hormones (Mason, 1957a, b; Klingenberg, 1958; Omura and Sato, 1962; Omura *et al.*, 1965). By the end of this period, the role of cytochrome P450 in drug metabolism was well established and the important modifying factors (inhibition, induction, and polymorphisms) were beginning to be understood (Conney *et al.*, 1980; Netter, 1980; Orrenius, Thor and Jernström, 1980; Ritchie *et al.*, 1980).

## 1.5 1980–2005: FIELD DRIVEN BY IMPROVED TECHNOLOGIES

Major advances during this 25-year period were driven by improved technologies. The major human cytochrome P450 members were identified, purified, and characterized from human liver tissue starting in 1983 (Wang *et al.*, 1983). Shortly after, development of cloning and heterologous expression techniques led to single isoform preparation with relative ease. Robotics for liquid handling and improvements in fluorescent technologies have driven the development of successful high-throughput screening procedures. Advanced separation systems linked to mass spectrometry or NMR detection systems have driven metabolite structure analysis and quantitation. Advances in viable hepatocyte isolation have improved the ability to characterize inducers of specific enzymes. Other advances include *in silico* modeling of all stages of metabolism including *in vitro* to *in vivo* modeling and computational prediction of human drug metabolism. Improvements in protein crystallization techniques, availability of powerful synchrotron sources, and improvements in 3D-structure generation software have led to the generation of multiple structures of each enzyme family providing an appreciation of structure variability and invariability. More recently, the study of transporters and transport systems has driven a paradigm shift toward the recognition of the important contributions of transport to drug development and drug safety.

## 1.6 2005+: HIGH TECHNOLOGY

While some would describe the metabolism field as "mature," technological advances still push the field forward in dramatic ways. We are now in an age of high technology in metabolism science, as evidenced by the emphasis in Part II of this text. Indeed, most of the knowledge described in this text would not be possible without the technological advances of the recent 30 years. Much of this modern innovative technology has been

driven by the pharmaceutical industry, including highly advanced analytical instrumentation and high-throughput technologies. It has also been influenced by great advances of the “genomics era,” through recognition of the genetic basis of variation in drug metabolism among individuals and populations. The future of the “drug” metabolism field will be driven by further technological advances toward the purposes of elucidating and understanding the impact of metabolic “cross talk”: the complex three-way interactions among endogenous compounds and their regulatory pathways, exogenous compounds, and disease states.

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