

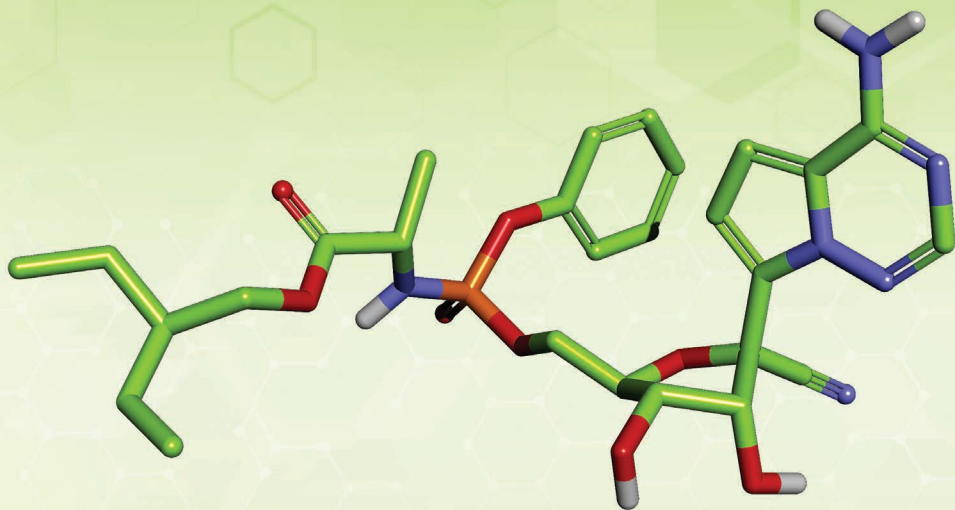
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GLOSSARY AND TUTORIAL OF XENOBIOTIC METABOLISM TERMS USED DURING SMALL- MOLECULE DRUG DISCOVERY AND DEVELOPMENT

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Paul Erhardt*, **Kenneth Bachmann**, **Donald Birkett**, **Michael Boberg**, **Nicholas Bodor**, **Gordon Gibson**, **David Hawkins**, **Gabrielle Hawksworth**, **Jack Hinson**, **Daniel Koehler**, **Brian Kress**, **Amarjit Luniwal**, **Hiroshi Masumoto**, **Raymond Novak**, **Phillip Portoghese**, **Jeffrey Sarver**, **M. Teresa Serafini**, **Christopher Trabbic**, **Nico Vermeulen**, and **Steven Wrighton**

*Corresponding author: **Paul Erhardt**, Center for Drug Design and Development,
University of Toledo, Toledo, Ohio, U.S.A. (TGM and ST)

Kenneth Bachmann, Ceuticare, Inc., Sylvania, Ohio, U.S.A. (TGM and ST)

Donald Birkett, Department of Clinical Pharmacology, Flinders University, Adelaide, Australia
(now Emeritus) (TGM)

Michael Boberg, Metabolism and Isotope Chemistry, Bayer, AG, Germany
(now undetermined) (TGM)

Nicholas Bodor, Center for Drug Discovery, University of Florida, Belle Glade, Florida, U.S.A.
(now Emeritus Grad Res Prof/CEO Bodor Labs) (TGM)

Gordon Gibson, School of Biomedical and Life Sciences, University of Surrey, Surrey, U.K.
(now deceased) (TGM)

David Hawkins, Huntingdon Life Sciences, Huntingdon, U.K. (now retired) (TGM)

Gabrielle Hawksworth, Department of Medicine and Therapeutics, University Aberdeen,
Aberdeen, U.K. (now deceased) (TGM)

Jack Hinson, Division of Toxicology, University Arkansas for Medical Sciences, Little Rock,
Arkansas, U.S.A. (now Emeritus Dist Prof) (TGM)

Daniel Koehler, Department of Pharmacology, University of Toledo, Toledo, Ohio, U.S.A. (ST)

Brian Kress, Department of Medicinal and Biological Chemistry, University of Toledo,
Toledo, Ohio, U.S.A. (ST)

Amarjit Luniwal, NAMSA, Inc., Northwood, Ohio, U.S.A. (ST)

Hiroshi Masumoto, Drug Metabolism, Daiichi Pharm. Corp., Ltd., Chuo, Tokyo, Japan (now
retired) (TGM)

Raymond Novak, Institute of Environmental Health Science, Wayne State University,
Detroit, Michigan, U.S.A. (now undetermined) (TGM)

Phillip Portoghese, Department of Medicinal Chemistry, University of Minnesota,
Minneapolis, Minnesota, U.S.A. (TGM)

Jeffrey Sarver, Department of Pharmacology, University of Toledo, Toledo, Ohio, U.S.A. (ST)

M. Teresa Serafini, Department of Pharmacokinetics and Drug Metabolism,
Laboratories Dr. Esteve, S.A., Barcelona, Spain (now Head Early ADME) (TGM)

Christopher Trabbic, MPI Research, Inc., Mattawan, Michigan, U.S.A. (ST)

Nico Vermeulen, Department of Pharmacochimistry, Vrije University, Amsterdam, Netherlands
(now Emeritus Section Molecular Toxicology) (TGM)

Steven Wrighton, Eli Lilly, Inc., Indianapolis, Indiana, U.S.A. (now retired) (TGM)

TGM: Task Group Member ST: Submission Team

Sponsoring bodies: IUPAC Chemistry and Human Health Division (VII) and Subcommittee on
Drug Discovery and Development. Specific information is provided on page 639.

Our final submission is dedicated to Gordon Gibson and Gabrielle Hawksworth, who were
collegial participants on this project's Founding Working Party. The field of drug metabolism
misses them.

Abstract

This project originated more than 15 years ago with the intent to produce a glossary of drug metabolism terms having definitions especially applicable for use by practicing medicinal chemists. A first-draft version underwent extensive *beta*-testing that, fortuitously, engaged international audiences in a wide range of disciplines involved in drug discovery and development. It became clear that the inclusion of information to enhance discussions among this mix of participants would be even more valuable. The present version retains a chemical structure theme while expanding tutorial comments that aim to bridge the various perspectives that may arise during interdisciplinary communications about a given term. This glossary is intended to be educational for early-stage researchers, as well as useful for investigators at various levels who participate on today's highly multidisciplinary, collaborative small-molecule drug discovery teams.

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ALPHABETICAL LIST OF TERMS

Several of the terms included in this project also appear in other IUPAC glossaries, or "Color Books," such as the Red Book (2005), Blue Book (2013), and Gold Book (2014 PDF plus online version updates), wherein alternative definitions may sometimes be found due to differences in specific context or strictly intended focus. None of the present definitions are meant to displace former IUPAC recommendations, particularly when used within the context of their originating technical disciplines. Instead, the present terms and related tutorial materials are meant to enhance the discourse between technical disciplines. An alphabetical list of terms is provided below. The actual definitions follow in alphabetical order. Readers are encouraged to start with the Introduction section, so as to appreciate the early history and evolution of this list, and to fully relate to the final intent of the definitions and their use within the overall context of today's highly interdisciplinary process for small-molecule drug discovery and development.

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Project Origin

Aside from being a notable calendar event, passage into the new millennium was a significant turning-point for many practitioners involved in drug discovery, because it marked the beginning of major operational changes that soon swept across the entire global pharmaceutical enterprise. Prompted by rapid advances in biotechnology, some of the disciplines that had served as a long-standing foundation for drug discovery were scrambling to relate and to remain relevant as their roles began to change.¹ The IUPAC Medicinal Chemistry Subcommittee's response was highly proactive, and it became one of the leaders in the generation of informational materials pertaining to the potential adoption of new technologies in ways that might improve the overall process of drug discovery. For example, the IUPAC text, *Drug Metabolism: Databases and High-Throughput Testing During Drug Design and Development*,² helped to usher in medicinal chemistry's serious consideration of ADMET parameters, as well as efficacy, during the very early stages of drug design. As part of the effort associated with this text, it was determined that a useful follow-up project would be a glossary of *Drug Metabolism Terms* specifically directed toward providing standardized definitions for use by medicinal chemists.

Historical Development and Reorientation

Getting off to a rapid start, by 2005 the project's working party consisted of 15 highly regarded experts in drug metabolism recruited from around the world (denoted as "Task Group Member" or TGM in the list of authors). They initially identified more than 600 terms that could be included in a comprehensive coverage of this topic, while also providing a healthy overlap with closely allied technical fields. The need to pare this list down to just the most relevant terms for drug discovery was recognized, and a list of about 200 was finally selected by ballot, wherein any candidate term, phrase, or acronym receiving 10 or more votes was retained. With the list divided nearly equally among the team members, preliminary definitions for these terms began to be filled in.

For the next several years the project took advantage of an opportunity to gain "first-hand" input from a variety of drug discovery practitioners, eventually leading to a reorientation of the glossary's intent. This resulted from delivering invited presentations and a short course about "drug metabolism considerations during drug design and development" while on a global lecture circuit that included numerous small, medium, and large pharma organizations, as well as several academic institutions and audiences at international technical meetings. Thus, this period served as a valuable *beta*-test for the still-evolving glossary while deploying its terms among a wide variety of audiences who were quick to provide useful feedback. Two key lessons became clear. The first was that the terms and short definitions would be more useful if they were accompanied by both chemical structure examples and tutorial information applicable to structure-metabolism relationships (SMR) relevant to drug design. The second was that nearly all of the multi-disciplinary technical staff, including non-medicinal chemists, from small pharma would typically attend the short-course and, similarly, more than 25 % of the typical 200-plus attendees during a presentation to big pharma were scientists from disciplines other than medicinal chemistry. Importantly, these interdisciplinary scientists' jobs often involved discussing drug metabolism-related issues with medicinal chemists during collaborative drug discovery and development team meetings. Thus, their attendance reflected a desire to enhance this dialogue by better understanding the context in which their medicinal chemistry counterparts tended to operate.

Returning to the project, the original definitions were adjusted so as to provide structural examples that would be of practical interest to medicinal chemists and informative to non-chemists in a manner useful for interdisciplinary discussions. Additionally, tutorial comments were crafted for many of the terms to enhance cross-communication among the different types, and professional levels, of individuals engaged in the various technical aspects and stages of drug discovery.

Impact of Reorientation

This change represents a significant departure from the long-standing theme that the IUPAC has traditionally adopted for its chemistry-related technical glossaries. Historically, definitions primarily have been devised to clarify and harmonize the use of a given term among different nations while providing what should be considered as a "standard definition" to be adhered to while being deployed across different languages. This clearly remains the preferred orientation for many chemical terms, such as those associated with distinct physical properties, such as a solid compound's melting point. Conversely, this approach may not be optimal for more interdisciplinary networks, where the words used in a given definition can have varying meanings among different technical fields, and where the terms' use may be trying to capture and emphasize differing components or aspects while working within an overarching, collaborative system. Drug metabolism is exactly such a topic, and its participants are represented by a highly interdisciplinary, wide range of investigators who, for this glossary's overarching context, have come together to pursue drug discovery.

As a common example to illustrate this key distinction, even the simple word "drug" within the phrase "drug discovery" can itself become ambiguous. Advocating a "single definition intended to fit all" may not be the best way to enhance communication, as opposed to allowing a group of interdisciplinary scientists to devise a meaning which is suitable for the context that might be needed in their particular setting. In such cases, it will be the common knowledge base potentially garnered from a tutorial about the term that will allow these practitioners to better understand each other's perspectives and then devise a mutually beneficial working definition. This same situation becomes applicable when authoring a publication wherein it may be necessary to provide some explanatory information for a term's definition depending upon its specific context within the technical report

and depending upon the differing perspectives of the envisioned readers. The appended discourse on the seemingly simple term “drug” (see Appendix I) is taken directly from a textbook chapter about “Drug Discovery.”³ This venue was intended for audiences consisting of both pharmacologists and medicinal chemists primarily at the early stages of their careers, and so it became necessary to devise a working definition within that particular context that was also applicable to the chapter’s educational thrust. However, the tutorial discourse leading to the final, rather succinctly stated singular definition, demonstrates how related knowledge can also allow a given term to remain dynamic and thus provide a bridge to whatever other context might be better served across various disciplines having individuals with differing technical perspectives. Finally, this simple example also demonstrates how quickly tutorial material can expand when multiple perspectives are considered for even a very basic term. Although worthwhile for the context of that application within a basic educational text’s chapter entitled “Drug Discovery,” this same level of information would be unwieldy for a list of terms having more than 200 entries. Thus, in the end, revision of this project’s definitions became a careful and judicious exercise of delicately balancing useful tutorial comments with the concise definitions initially laid out by the Founding Working Party for each term. Structural examples were retained or added when relevant.

Submission and Present Intent

Since several years elapsed during *beta*-testing, it became necessary to form a second team to accomplish the last steps leading to the final release of this project’s hybridized product to the public. A new team (labeled “Submission Team” or ST in the authors list) was assembled to polish the revisions and to provide a final review of the definitions while assessing a term’s contributions toward enhancing knowledge in general and improving specific communications among practitioners having various levels of cross-technical savvy. Thus, a mix of early-career, mid-career, and seasoned investigators were recruited within several different disciplines and employment sectors. After completing the refinement of the glossary’s terms, its name was also adjusted to reflect how the final product is now being delivered.

For the reasons discussed above, the product has been slanted toward bridging medicinal chemistry perspectives with those of various other disciplines, while focusing on their common bond as practitioners of

collaborative drug discovery. During this final period, the value of this particular slant has been further underscored by advances in public domain scientific resources, e.g., databases and knowledge bases that are readily accessible via user-oriented interfaces. Typically, these sources provide well-referenced, high-quality information, such as historical notes and concise definitions for terms and acronyms. Likewise, they often recognize the importance of either alerting readers to cases where a given entry may be used differently by different disciplines or actually listing these multiple definitions. However, even when noting such distinctions, these sources may not be as useful for bridging the perspectives different experts bring to the overarching arena or enterprise. This has become critical for interdisciplinary drug discovery discussions, allowing them to be most effective, and ultimately for drug discovery practice to be most fruitful.

In summary, the definitions provided here are not intended to mandate how a given term should always be used by a given discipline, but rather are suggestions for how they might be deployed as a starting point to enhance interdisciplinary communications within the context of drug discovery. Similarly, our use of expressions and symbols, such as percent (%) are not intended as a mandate for how they must be correctly conveyed across different languages. Notably, we have adopted the % format prescribed by the International System of Units (ISO 31-0 standard) and IUPAC where there is a space between the number and the % sign, while the style in English typically deploys this expression without a space. As a closing example, practitioners should be able to use the definition provided here for "Glucuronic Acid Conjugation (Glucuronidation)" as a starting point for a joint discussion. Then, from the accompanying tutorial and the related, cross-referenced definitions/tutorials, all parties should gain a general background/base knowledge level that will allow them to better appreciate the following viewpoints: (1) The *medicinal chemist's* emphasis on the key role of the requisite "nucleophilic functional group" present on the substrate's chemical structure and how it might be masked by incorporating steric features so as to attenuate this particular biotransformation; (2) The *bioanalytical/drug metabolism chemist/biologist's* suggestion about the feasibility for devising an assay to assess such a series of sterically hindered analogs by using LC-MS/MS to detect and sort the substrates and potential glucuronide metabolites from the biological matrix while relying on the glucuronide's distinctive "increases in molecular mass of 175"; and, (3) The *biochemist's/pharmacologist's* comments about the prediction accuracy ("probability") from various *in vitro* and *in vivo* studies, accompanied by a

warning that, while many mammals might be utilized as a model for the latter studies, there would be “one notable exception in that the deployment of cats to monitor glucuronidation would not be recommended when trying to extrapolate the data to human behavior.” The corresponding author looks forward to additional critique of these terms from readers engaged in any aspect of the overall process of drug discovery, and in this same regard, remains prepared to further clarify or evolve their utility as may be helpful for all such disciplines. The author is especially receptive to comments that pertain to the two, related terms noted in the following section and as thoroughly discussed in Appendix II, because a controversy often exists regarding the continued use of these particular terms, even among practitioners within the same discipline.

An Ongoing Terminology Controversy

The discourse covered in Appendix II provides historical background and the differing views on the continued use of the terms “Phase 1” and “Phase 2” in the context of drug metabolism. **None of the definitions provided within the appendix should be taken as the final IUPAC recommendations for this glossary**, but rather as the basis for further consideration and comment related to how these particular terms might best be deployed in the future. Views on this matter range from: (i) “not to be used at all”; (ii) and “to be replaced by less sequential versions, such as Group 1 and Group 2 biotransformations”; to (iii) “Use, as presently still being utilized by many”; and (iv) “Use an even more expanded version” that, although more complex, might become less controversial while potentially having broader appeal for a much wider range of drug metabolism practitioners beyond those involved in drug discovery, e.g., environmentalists and system biology investigators in general.

DEFINITIONS WITH STRUCTURAL EXAMPLES AND TUTORIALS

1. Absorption

Passage of a xenobiotic through physiological membranes, leading to its uptake into the blood circulation.

Note 1: Oral (PO) and intraperitoneal (IP) administrations of pharmaceutical agents initially deliver drugs to the hepatoportal circulation that flows

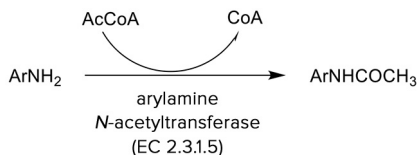
to the liver. Inhaled administration initially delivers drugs to the bronchopulmonary circulation's venous return to the heart. Sublingual (buccal), rectal (intrathecal), topical, subcutaneous (SC), and intramuscular (IM) administrations initially deliver drugs to the peripheral circulation's venous return. Intracerebral administration delivers drugs to the central circulation's venous return. Intravenous (IV) and intra-arterial (IA) administrations do not require an absorption step while directly delivering drugs to the venous return and arterial blood supply, respectively. Administration via the lymph system may allow for localized permeation of tissues with subsequent absorption following one of the above patterns and eventually adding to the venous blood flow.

Note 2: Pharmacokinetic analyses (PK studies) typically obtain samples from the peripheral circulation for the quantification of a drug in order to assess absorption and bioavailability according to various PK models that can accommodate different routes of administration.

See: Permeation/Permeability, Passive Transport, Active Transport, ADME, First-Pass Effect, Extravascular Dosing, Random walk, Pharmacokinetics, and Bioavailability.⁴

2. Acetylation

Conjugation of a xenobiotic's amino, hydroxy, or sulfanyl group with an acetyl moiety donated by acetylcoenzyme A and mediated by an acyltransferase (EC 2.3.1). Primary aromatic amines are the principal substrates (as exemplified below), with sulfonamides being both historically significant⁵ and still important as a major drug class today. The process is also applicable to various other nitrogen compounds, including endogenous amino acids, which become important for the regulation of proteins, and to drugs having hydrazine functional groups. It is a common xenobiotic biotransformation across numerous species, although dogs represent an exception.



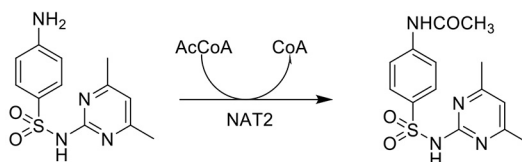
Note 1: Unlike in chemical terminology, in the context of xenobiotic metabolism, the terms "acetylation" and "acylation" characterize two

distinctly different types of biotransformations. Acetylation is closest to the chemical classification system, in that the substrate's (starting material's) nucleophilic center is acylated.

See: Acetylation Phenotype, *N*-Acetylation, *N*-Acetyltransferases and Acylation.⁶

3. Acetylation Phenotype

Different levels of *N*-acetyltransferase (NAT1 and NAT2) activities that lead to individual variation in acetylation rates. Common drug therapies that can be impacted include the half-life for procainamide⁷ and for the sulfonamide antibiotics, wherein the acetylation rate of sulfadimidine (shown below) is often deployed as a standard during phenotyping studies.⁸



Note 1: Variations in NAT2 were one of the first known drug metabolism-related pharmacogenetic traits, namely polymorphic “fast” and “slow” acetylators. About 30 to 45 % of Caucasian, 80 to 90 % of East Asian, and 100 % of Inuit, Eskimo, and Aleut populations are “fast” acetylators.

Note 2: The “fast” NAT polymorphism has been linked with an increased risk for bladder, colorectal, and other cancers associated with certain aromatic amines, which can be bioactivated into carcinogens.

See: Acetylation, *N*-Acetylation, *N*-Acetyltransferases; Extensive (or Rapid) Metabolizer, Poor (or Slow) Metabolizer, Slow (or Poor) Metabolizer, and Phenotype/Phenotyping.⁶

4. Active Metabolite

A metabolic reaction product that exhibits biological activity locally or at more distal sites upon subsequent distribution within an *in vivo* setting.

Note 1: The profile of activity may be the same as, or different from, the parent molecule, including especially the possibility for producing toxicity

at off-target sites. "Whether a metabolite is more or less pharmacologically active than its parent drug depends on the specific compound being studied, the biological properties measured, and the sensitivity of individual tissues or organs."⁹

Note 2: Prodrugs typically rely on bioactivation to produce an active metabolite in order to obtain the desired biological effect. In this case, it is also acceptable to refer to the release or generation of the active form of the drug (aka parent drug) from its inactive precursor or masked prodrug form.

See: Bioactivation, Reactive Metabolite, and Prodrug.

5. Active Transport

The passage of a substrate across a biological membrane by a process that requires energy.

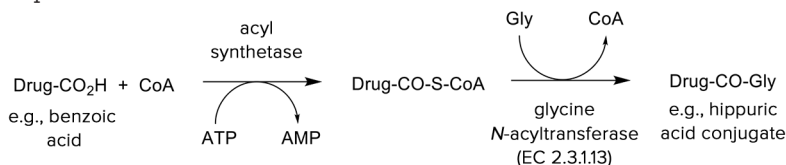
Note 1: Substrates can be moved from sites having a low concentration to sites having higher concentrations, but such gradients are not required and may or may not be important for the distribution and/or metabolism of a given xenobiotic.

Note 2: Several transporter molecules play critical roles during drug metabolism because they influence the uptake or export of xenobiotic compounds by cells possessing various drug metabolism enzymes. For example, within hepatocytes, the Substrate Carrier superfamily of proteins (SLC) are key basolateral (sinusoidal blood) uptake transporters that can serve to prolong the exposure of a given xenobiotic to the enriched array of metabolic possibilities present in the liver. Alternatively, the ATP-Binding Cassette superfamily of proteins (ABC), especially the Multidrug Resistance Protein or *P*-glycoprotein (MDR or Pgp), and the Multiresistance Protein (MRP) subfamilies, represent key basolateral and canalicular (bile) export transporters that can serve to diminish the liver's metabolism of xenobiotics.

See: ADME, Passive Transport, Membrane Permeability, Phases of Drug Metabolism, Transporters, *P*-Glycoprotein (Pgp), Organic Anion Transporters (OATs), Organic Cation Transporters (OCTs), and Random Walk.¹⁰

6. Acylation

Activation of a xenobiotic carboxylic acid's acyl group by attachment to coenzyme A followed by an acyltransferase-mediated (EC 2.3.1) conjugation with an endogenous amine to form an amide. Glycine (Gly) is the most commonly used amino acid. The resulting Gly conjugates are called "hippurates,"¹¹ borrowing historically from this pathway's initial characterization involving the metabolism of benzoic acid in 1842, notably credited as the first biotransformation reaction to be discovered.¹² It is exemplified below:



Note 1: Although the carbonyl moiety undergoes an initial activation step analogous to a chemical synthetic manipulation during a typical acylation procedure, this biotransformation process is notably different from the chemical classification of such reactions because the substrate (starting material) instead undergoes what would be termed an "amidation reaction," while it is the endogenous partner (reagent) that actually becomes acylated. Alternatively, by definition, biochemical acetylation reactions acylate xenobiotic substrates that have a nucleophilic heteroatom moiety.

Note 2: Glutamine is also commonly used as the endogenous amino acid and is the more predominant partner for xenobiotic substrates having an aryl-acetic acid moiety. Taurine and ornithine can also serve as partners, but are less frequently used.

See: Acyltransferase, Acetylation, Amino Acid Conjugation, Glutamine Conjugation, Glycine Conjugation, and Hippurate.¹³

7. Acyltransferase

The broad family of enzymes (EC 2.3.1) that transfer various acyl moieties, other than amino-acyl functionality, from acylated coenzyme A to substrates having receptive heteroatoms, such as O, S, and especially N, including for the latter the endogenous amino acid partners incorporated during the conjugation of xenobiotic acids.

Note 1: For drug metabolism, the most significant members include glycine *N*-acyltransferase (EC 2.3.1.13), depicted above, and the two arylamine *N*-acetyltransferase (EC 2.3.1.5) genetic polymorphs, NAT1 and NAT2.

See: Acylation, Acetylation, Acetylation Phenotype, *N*-Acetylation, and *N*-Acetyltransferases (NATs).^{6,13}

8. ADME; ADMET

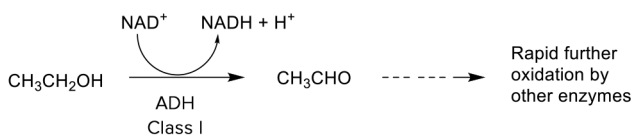
Acronym for the series of absorption, distribution, metabolism, and excretion processes that can occur when a living system is exposed to a xenobiotic, the most common context being the delivery of a drug to humans and *in vivo* animal models. The extended acronym ADMET is also often used when toxicity is included as an additional parameter within the overall profile conveyed by this term.

Note 1: Because they often become hurdles to overcome during drug development, ADME and ADMET processes are typically characterized and potentially optimized among efficacious analogs during the early stages of drug discovery by deploying *in vitro* models and *in vivo* PK studies that attempt to predict such behaviors within the clinic (e.g., see reference 14).

See: Absorption, Distribution, Metabolism, Excretion, Membrane Permeation, Phases of Drug Metabolism, Random Walk, DMPK, Pharmacokinetics (PK), and Toxicity.^{3,15}

9. Alcohol Dehydrogenase (ADH)

A family of cytosolic enzymes (EC 1.1.1.1) existing in multiple dimer forms having five distinct Classes (I-V). Each Class oxidizes various aliphatic alcohols, typically with a preference for primary over secondary and with specific selectivities for differing chain lengths. ADH is important in the processing of the retinoids, while Class III readily oxidizes formaldehyde to formic acid, so as to avoid toxicity.



Note 1: The Class I forms are among the most relevant for drug metabolism. They are expressed at high levels in the liver and stomach wall as three subunits (α , β and γ), which can form multiple dimers. They prefer shorter-chain alcohols, as shown above for a commonly ingested xenobiotic.

Note 2: Overall these enzymes are zinc-containing dimeric proteins having two 40 kDa subunits that utilize the oxidized form of nicotinamide adenine dinucleotide (NAD^+) as a cofactor to capture a hydride ion. In humans, the subunits are encoded by seven gene loci for which some transcribe to multiple allelic variants. All subunits can combine as homodimers and a smaller set can form heterodimers. They are divided into five major classes: Class I, as described above, is encoded by ADH1, 2, and 3; Class II is encoded by ADH4 and prefers longer aliphatic alcohols and aralkyl alcohols as substrates; Class III, encoded by ADH5, has been shown to be identical to the ubiquitous formaldehyde dehydrogenase (EC 1.2.1.46) that readily oxidizes formaldehyde, as well as longer-chain (aliphatic and aralkyl alcohols, particularly those with five carbons or more; Class IV, encoded by ADH7; and Class V, which is encoded by ADH6 and has representation in the upper GI tract for oxidizing medium-chain alcohols, with a particularly high capacity to oxidize retinol (vitamin A_1).

See: Atypical Alcohol Dehydrogenase.¹⁶

10. Aldehyde Dehydrogenase (NAD) (ALDH)

A family of cytosolic and membrane-bound enzymes (EC 1.2.1.3) existing in multiple dimer and tetramer forms and having three distinct Classes (I-III). A wide range of aldehydes are substrates for these enzymes, which also play a role in retinoid processing.



Note 1: The Class 1 and 2 forms are generally the more important for xenobiotic metabolism. They are found in many tissues of the body and have high concentrations in the liver. They can oxidize aldehydes, having a range of chain lengths and, as exemplified above, are important in detoxifying the acetaldehyde produced after the consumption of alcohol. As noted above, formaldehyde dehydrogenase does not belong to the ALDH family, but is instead a Class III ADH enzyme.

Note 2: In general, these enzymes' active sites utilize distinct amino acid residues to bind substrates, with magnesium sometimes also playing a role during the catalytic process. Oxidized nicotinamide adenine dinucleotide (NAD⁺) functions as a cofactor to capture a hydride ion. All three Classes have constitutive and inducible forms: Class 1 with low K_m is cytosolic, Class 2 with low K_m is mitochondrial, while Class 3 can have high K_m when expressed in the stomach, the cornea, and in certain tumors. The first two Classes, ALDH1 and ALDH2, are tetrameric enzymes having 54 kDa subunits.

See: Oxidation/Oxidase/Oxidoreductase, Aldehyde Dehydrogenase Polymorphism or Deficiency, Disulfiram Reaction, Alcohol Dehydrogenase, and Atypical Alcohol Dehydrogenase.¹⁷

11. Aldehyde Dehydrogenase Polymorphism or Deficiency

A genetic polymorphism that leads to an inactive allelic variant of ALDH termed ALDH2*2. This phenotype is present in ca. 30 to 50 % of various Asian populations around the Pacific Rim and affects their ability to metabolize alcohol.

Note 1: When coupled with the ALDH2*2 phenotype, the initial production of acetaldehyde from ethanol is no longer subsequently oxidized to acetic acid at a pace to prevent accumulation of this toxic electrophile notorious for its chemical reactions with amines to form Schiff bases. Its bioaccumulation leads to the classic "flushing" syndrome and hangover effects that can then occur at even lower amounts of consumption for this population when drinking alcohol.

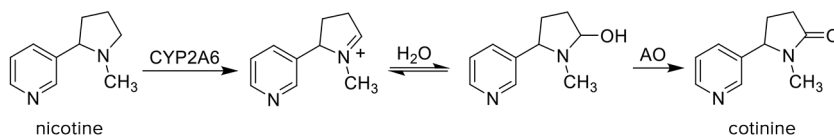
Note 2: Small-molecule inhibitors of ALDH2 can cause this same effect and are deployed to treat alcoholism because they result in an unpleasant drinking experience. Disulfiram was the first of such agents, and this drug-induced metabolic effect is thus called a "disulfiram-like reaction."

See: Aldehyde Dehydrogenase, Alcohol Dehydrogenase, Atypical Alcohol Dehydrogenase, and Disulfiram Reaction.¹⁸

12. Aldehyde Oxidase (AO)

One of two molybdenum-containing enzymes, aldehyde oxidase (AO; EC 1.2.3.1) plays an important role in xenobiotic metabolism, namely the oxidation of carbon atoms within nitrogen-containing heterocyclic

systems.^{19,20} As its name implies, AO can also oxidize certain aldehydes to carboxylic acids. The example below shows AO performing the second step in the oxidation of the common xenobiotic present in cigarettes.²¹ The iminium species is thought to be the substrate for AO.



Note 1: AO is expressed primarily in the liver, where it can play a role in ethanol metabolism and have an influence upon alcohol-induced liver damage. However, xenobiotic aromatic aldehydes are markedly preferred over aliphatic aldehydes. Endogenously, a number of important substrates include homovanillyl-aldehyde and 5-hydroxyindol-3-ylethanal, derived from cytochrome *P*-450-mediated catabolism of dopamine and serotonin, respectively, and catabolism of retinal as the activated form of retinol (vitamin A_1).

Note 2: Working in concert with the CYP enzymes, which generally prefer to oxidize electron-rich carbon atoms, AO prefers carbon atoms with lowered electron density. For example, naphthalene is a good substrate for CYPs and a poor substrate for AO, quinazoline is a substrate for both, and pteridine is a good substrate for AO while a poor substrate for the CYP enzymes.

Note 3: Further demonstrating the importance of AO's role in oxidizing xenobiotic heterocycles, some medicinal chemistry efforts have been specifically directed toward avoiding this type of biotransformation (e.g., see reference 22).

See: Alcohol Dehydrogenase (ADH), Aldehyde Dehydrogenase (ALDH), and Xanthine Dehydrogenase/Xanthine Oxidase (XD/XO).²³

13. Allometric Scaling

Extrapolation of xenobiotic activities (including drug metabolism events and PK profiles) between different species as a function of body weight or surface area. Assumes that physiologic variables, such as metabolic processing and clearance, are related to body weight or surface area according to the following general allometric equation:

$$y = aW^b$$

wherein y represents the physiological parameter, W represents the weight or surface area, and a and b represent allometric constants expressed as a coefficient and as an exponent, respectively. The logarithmic transformation of this relationship is linear.

See: PBPK (Physiologically Based Pharmacokinetic Model) and Pharmacokinetics (PK).²⁴⁻²⁶

14. Allosteric Regulation

A biochemical process wherein the function of a protein's receptor or enzyme active site can be influenced by interaction with another compound (ligand) whose association occurs at a location different from the receptor or active site. The influence can be either positive, so as to enhance functional activity, or inhibitory. Today, it is recognized that an ensemble of proteins can often interact in this manner, expanding the context for the word "ligand" and for the overall process, so as to include cell signaling systems. In general, this type of regulation has not previously appeared to be significant for the biotransformation of xenobiotics. However, studies using the wider dimensions of today's definition for this type of regulation remain ongoing for the area of drug metabolism and are key to understanding signal transduction.

Note 1: Cofactors and prosthetic groups needed to form a functional holoenzyme are not regarded as components operating by a process of allosteric regulation.

Note 2: In some systems, a receptor's ligand or an enzyme's substrate is also the ligand that can interact with a distal, allosteric site.

See: Enzyme, Apoenzyme, Cofactor, Holoenzyme, Ligand, Nonlinear Kinetics, Prosthetic Group, Substrate/Substrate Specificity, Substrate Inhibition/Product Inhibition, and Uncompetitive Inhibition.²⁷

15. Amino Acid Conjugation

Biotransformation wherein a xenobiotic's carboxylic acid moiety forms an amide bond with the amino group of an endogenous amino acid, usually glycine or glutamine, and sometimes taurine, via an acylation reaction accomplished by an acyltransferase enzyme.

Note 1: In this distinctive mechanism, the xenobiotic's carboxylic acid group is first activated by coupling with coenzyme A (CoA), so as to produce an acyl-CoA thioester that then reacts with the amino group of the endogenous amino acid.

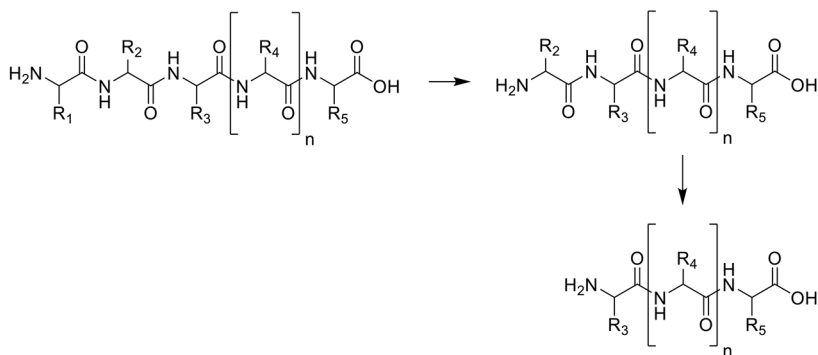
Note 2: Xenobiotics or initial metabolites having an aromatic hydroxylamine are also considered to be undergoing "amino acid conjugation," although their activation engages aminoacyl-tRNA synthetase and typically uses serine or proline for the amino acid partner.

Note 3: Alternatively, the conjugation of xenobiotics having electrophilic centers by the tripeptide glutathione is classified as a separate pathway for metabolism, because its chemistry and associated enzymes are distinctly different.

See: Acylation, Acyltransferase, Hippurate, Glutathione Conjugation, and Glycine Conjugation.¹³

16. Aminopeptidases

An important class within the protease family (EC 3.4), many of which are Zinc metalloenzymes, aminopeptidases readily catalyze amide bond hydrolysis at the amino-terminus of a protein or peptide, sometimes in rapid succession along the peptide's backbone. The latter is depicted generally below. Because they play key roles in the processing and catabolism of many endogenous proteins, avoiding such hydrolyses becomes a critical ADME hurdle for short peptide and peptidomimetic drug candidates.



Note 1: There are several types of aminopeptidases and various biochemical formats for their assembly, the majority having widespread distributions

within the *in vivo* setting. Cell-based assays can display considerable activity as well.

Note 2: *N*-substitution, inversion of stereochemistry, or other peptidomimetic alterations made near the amino-terminus can remedy this type of ADME-related bioinactivation hurdle. Alternatively, simple prodrug approaches that attempt to use the amino-terminus are generally complicated in this case, because the rapid rate of deactivation after bioactivation to an active amine can still remain problematic.

Note 3: When an initial peptide lead compound is converted to a peptidomimetic structure and finally to a small-molecule drug scaffold (with little peptide character remaining), the overall metabolic fate of the series of analogs can generally be expected to follow the pattern shown below.

Metabolism by	Peptide Drug →	Peptidomimetic Drug →	Small-Molecule Drug
Peptidases:	100%	Mix of Both	0%
Phase1 and/or 2 Processes:	0%	Mix of Both	100%

See: Carboxypeptidases.²⁸⁻³⁰

17. Anabolism

Composite of biochemical pathways that construct or liberate endogenous molecules intended for distinct functions.

Note 1: This term is not used within the context of xenobiotic metabolism, even though many enzymes important for this process also have specific roles in various anabolic pathways. Likewise, the metabolic conjugation of a xenobiotic or the liberation of an active metabolite from a xenobiotic after a hydrolytic biotransformation are not regarded as anabolic processes.

See: Catabolism.³¹

18. Antibody-Drug Conjugate (ADC)

The chemical connection (conjugation) of a monoclonal antibody (mAb) with a small-molecule drug, wherein: the mAb serves to selectively deliver the conjugate to a target cell via its specific recognition of a protein expressed on the target cell's surface; the chemical connection uses a prodrug strategy

to release the drug from the mAb, typically also having chemical linkages to prevent the prodrug's metabolically labile connection from becoming sterically hindered by the mAb; and, upon its release, the small molecule exhibits a therapeutic action. At least one such connection for a single mAb biomolecule is present and, in general, several can occur, so that several molecules of the drug may be liberated. The ADCs are becoming very useful in the treatment of cancers, where the latter often over-express distinct cell surface proteins and where the highly toxic nature of small-molecule, natural product toxins can then remain masked until their selective release at the cancer cells.

See: Prodrugs.^{32,33}

19. Apoenzyme

The protein portion of an enzyme complex without any cofactors or prosthetic groups that may be required for activity. Many of the phase 1 and all of the phase 2 drug metabolizing enzymes require such complexes in order to catalyze their biotransformations.

Note 1: Although they may be able to associate with substrates, apoenzymes are inactive until they combine with their cofactors and prosthetic groups to form an intact complex called a holoenzyme.

Note 2: Cofactors are small organic or inorganic molecules that are typically easy to remove by laboratory manipulations. Prosthetic groups are tightly bound partners that are often difficult to remove, such as the heme molecule present in a CYP complex.

See: Cofactor, Prosthetic Group, and Holoenzyme.³⁴

20. Aromatic Hydrocarbon Receptor (AHR)

Cytosolic transcription factor component having high affinity for polycyclic aryl hydrocarbons that activate the signaling pathway to eventually upregulate genes coding for several cytochrome *P*-450 drug metabolizing enzymes.

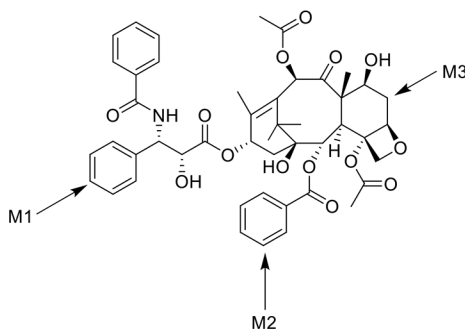
Note 1: Upon binding with polycyclic aryl hydrocarbons, AHR loses its association with heat shock protein (hsp) 90 and forms a heterodimer

complex with a nuclear translocator protein (ARNT). Serving as a transcription factor, this complex moves to nuclear DNA and binds with the xenobiotic response element (XRE) sequences that upregulate genes encoding for CYP 1A1, 1A2 and 1B1.

See: Peroxisome Proliferator Activator Receptor (PPAR), Pregnane X Receptor (PXR), Glucocorticoid Responsive Element (GRE), and Xenobiotic Responsive Element (XRE).³⁵

21. Aromatic Hydroxylation

Frequent biotransformation mediated by various members of the CYP family (see specific nomenclature separate from the EC system) wherein a hydroxy group replaces a hydrogen atom (see **Note 2** below), typically at the most electron-rich site within an aromatic system, while also accounting for steric accessibility.



Note 1: The example above depicts some of the CYP-mediated metabolites observed for paclitaxel in rodents and humans.³⁶ In rodents, aromatic hydroxylation is predominately at M1, followed by M2, with no observation of an aliphatic oxidation at M3. In humans, aliphatic hydroxylation by CYP 2C8 at M3 becomes most prevalent, followed by CYP 3A4 aromatic hydroxylation at M1. The four esters are remarkably stable due to the large steric bulk of the central scaffold.

Note 2: The resulting aryl-hydroxy groups often undergo subsequent biotransformation to form either the glucuronide or sulfate conjugates, which are good candidates for some of the excretory pathways. This sequence follows the classical phase 1, phase 2 pattern of biotransformation (see discussion in the Introduction section).

Note 3: After CYP-mediated insertion of an oxygen atom, re-aromatization and formation of the hydroxy group is thought to occur via an intermediate stabilized in complexation with the CYP's prosthetic heme group, or as a bound epoxide, either of which can then undergo a hydride shift called the "NIH Shift." Thus, mechanistically, in addition to replacing a hydrogen atom, halide atoms and other groups capable of undergoing such a shift can also serve as substrates for an aromatic hydroxylation reaction. Although their electronegative character will diminish the overall reactivity of the aryl-system for these types of biotransformations in general, this effect has been observed experimentally, initially by the U.S. National Institutes of Health (NIH) laboratories, which gives the effect its name.

Note 4: Reactive epoxides failing to collapse to the re-aromatized hydroxyl-containing metabolites can become a source of localized toxicity, such cases then representing one example of an enzyme "suicide inhibitor." Moderately stable epoxides can sometimes bypass re-aromatization and dissociate from the enzyme complex, so as to become active metabolites that undergo further distribution and eventually cause distal toxicity. Epoxide hydrolase and glutathione-S-transferase (GST) serve to intercept these types of active metabolites and to detoxify them by forming non-aromatic diols and glutathione conjugates, respectively. Benzo(a)pyrene is a classic example of an exogenous material, found in soot and charcoal, that forms a moderately reactive epoxide metabolite able to evade epoxide hydrolase and GST to ultimately become a carcinogen by reacting with RNA via covalent bond formation at the C-2-amino group of guanosine. Highly stable epoxides can dissociate from the enzyme complex and potentially be eliminated without further metabolic processing or incidents of toxicity.

See: NIH Shift, Phases of Drug Metabolism, Glucuronide Conjugation, Sulfate Conjugation, Reactive Metabolite, Active Metabolite, Epoxide Hydrolase, and Glutathione Conjugation.³⁷

22. Atypical Alcohol Dehydrogenase

A member of the ADH2 isozymes within Class I of the alcohol dehydrogenase family (EC 1.1.1.1) for which the human phenotype results in the rapid oxidative conversion of ethanol to acetaldehyde.

Note 1: This phenotype is present in ca. 90 % of the Pacific Rim Asian population, whereas it is either not present or is much less prevalent among

different Caucasian populations (below 5 % in Americans of Caucasian descent and ca. 10 to 20 % among various European populations).

Note 2: When this population also has an aldehyde dehydrogenase genetic polymorphism (ALDH2*2), such that subsequent oxidation to acetic acid is slowed, individuals are highly susceptible to the flushing syndrome and hangover effects associated with the consumption of alcohol.

See: Alcohol Dehydrogenase (ADH), Aldehyde Dehydrogenase (ALDH), and Aldehyde Dehydrogenase Polymorphism.¹⁶⁻¹⁸

23. AUC (Area Under the Curve)

Common acronym for the area under the curve, *A*. Applicable to xenobiotic (drug) and metabolite pharmacokinetic (PK) studies, it is defined to be the integral of the given analyte's (most often drug's) concentration measurement in a given biological media (typically plasma, serum, whole blood or urine) with respect to time. This area can be derived from the formula:

$$A = \int_0^{\infty} C \, dt$$

Note 1: Analyte concentration *C* is typically measured and quantified by either HPLC (UV/Visible wavelength) or tandem mass spectrometry (LC-MS/MS).

Note 2: AUC is commonly used in the measurement of bioavailability and to determine if a drug's presence follows linear kinetics in a given media or compartment with time.

See: Plasma Concentration, Serum Concentration, Whole Blood Concentration, Pharmacokinetics, Linear Kinetics, Nonlinear Kinetics, Bioavailability, Absolute Bioavailability, and Relative Bioavailability.³⁸⁻⁴⁰

24. Autoinduction

Substrate mediated induction (up-regulation) of an enzyme that is involved with the metabolism of that substrate. In its most common usage, this

definition is taken to mean that more of the enzyme is produced due to enhanced genetic signaling for its biosynthesis (anabolism). In a broader sense, however, the same physiological observation can occur if less of the enzyme is degraded due to diminished genetic signaling for its catabolic enzyme or enhanced genetic signaling of an enzyme that degrades the catabolic enzyme. These latter possibilities would need to be specified while using this term if they were suspected of being operative. Even though the endpoints are essentially the same, these alternate possibilities represent distinctly different drug design targets for conducting structural modification of their associated ligands.

Note 1: When it occurs for xenobiotics or their metabolites undergoing subsequent biotransformations, it is generally observed after repeated exposure to the xenobiotic. While it can be applicable to a given metabolite, it most typically pertains to the parent xenobiotic compound and leads to a nonlinear PK profile, characterized by its lower-than-expected concentration *in vivo*. For certain cases, it may be able to be detected at the cell-culture level when studies have been designed to assess this possibility.⁴¹

See: Induction, Autoinhibition.

25. Autoinhibition

Both historically and today, substrate-mediated inhibition of an enzyme that is involved with the metabolism of that substrate. Unlike receptors, which can undergo gene-signaled down-regulation upon over-stimulation by an agonist ligand, enzymes are generally not subject to such negative feedback control upon exposure to their substrate ligands and, instead, may undergo up-regulation (autoinduction). Also see **Note 2** below for another, more recent usage of this term.

Note 1: When autoinhibition occurs, it is most often due to product inhibition from the substrate's biotransformation, such that it can be observed upon the first, as well as on repeated, exposures of the xenobiotic. While it can be applicable to a given metabolite, it most typically pertains to the parent xenobiotic compound and leads to a nonlinear PK profile characterized by a higher-than-expected concentration *in vivo*.

Note 2: A related usage of this term is now being applied to enzyme systems that can equilibrate between functional conformations and non-functional

("autoinhibited") conformations, either as a single protein species acting independently of a substrate's presence, or in response to a substrate, such as by an allosteric interaction or as protein complexes of dimers, trimers, etc., wherein one or more of the members is non-functional and, in turn, modulates the activity of the overall complex (again either independently or in an interactive manner with a potential substrate). The multiple protein complex has become important for understanding the many systems wherein the genome produces one or more additional, but inactive, versions of a functional enzyme, sometimes by utilizing duplicate paralog genes that read to analogous proteins having altered enzymatic activity.⁴²

See: Inhibition, Autoinduction.

26. Bioactivation

The biotransformation of a xenobiotic to a metabolite that is more biologically active or is active in a manner different than that of the parent compound.

Note 1: Activity may be efficacious or toxic. For drugs, bioactivation is generally unwanted, since the metabolites typically interact with "off-target" sites that can lead to undesired side-effects and toxicity. Prodrugs are an exception, because they have been designed to rely upon a metabolic step in order to unmask a desired activity from the inactive parent molecule.

Note 2: Biotransformation to an active metabolite that is equivalent to that of the parent compound does not constitute bioactivation.

See: Active Metabolites and Prodrugs.³

27. Bioavailability (Absolute and Relative)

For xenobiotics in general (not having formal PK studies) and for dietary supplements, herbs, and other nutrients, bioavailability is defined as the fraction that is absorbed, i.e., the per cent of the mass unaccounted for at the site after an exposure. More specifically for drug administration, this is the fraction of the dose that reaches its site of action or that reaches a biological fluid from which the drug is presumed to gain access to its site of action. Generally depicted by the term "F," which ranges from 0 to 1, where unity represents 100 % bioavailability.

Note 1: Typically, the biological fluid from which drugs are presumed to gain access to their sites of action is the blood of the general circulation. For a drug taken orally (PO), examples of factors that can limit bioavailability include formulation issues, such as slow dissolution rate; drug stability, such as degradation under the acidic conditions of the stomach; metabolic transformation by the gut microflora, intestinal epithelial cells, or liver; efflux by transporters within epithelial cells, such as P-glycoprotein (Pgp); or direct biliary excretion.

Note 2: For determination of absolute bioavailability, F is measured experimentally during PK studies as the ratio of the AUC from plasma samples taken for a dose given by a non-intravenous route (e.g., PO), divided by those taken for a dose given intravenously, with the comparison normalized to account for different doses or varying weights of the subjects.

Note 3: Relative bioavailability compares the AUC between different formulations of a drug using the same route of administration, or between different routes of administration for the same drug formulation when neither route is an intravenous injection.

See: Absorption, Gut Microflora, P-Glycoprotein (Pgp), First Pass Effect, Pharmacokinetics, AUC, and Bioequivalence.³⁹

28. Bioequivalence

Different formulations or generic products for a given active agent are regarded as bioequivalent when the active agent is absorbed, metabolized, and excreted in approximately the same amount over approximately the same time period as the active agent in the original product.

Note 1: Studies directed toward demonstrating bioequivalence typically include *in vitro* dissolution testing and relative bioavailability after administration to humans, emphasizing blood concentration, how fast it is achieved, and how long it is sustained.

Note 2: For example, in the U.S., regulatory approval requires a generic to demonstrate a 90 % confidence interval for the ratio of the mean responses of its product to that of the original drug while remaining within limits of 80 % to 125 % for maximum achieved concentration ($C_{\max}$), the time it takes to reach $C_{\max}$ ($T_{\max}$), and the overall duration of a measurable concentration

(AUC profile). According to the Hatch-Waxman Act, when these conditions are met, a generic brand-name product may be able to take advantage of an abbreviated new drug application (ANDA) to obtain approval for marketing.

See: Absorption, Bioavailability, Pharmacokinetics, and AUC.^{43,44}

29. Bioinactivation

Biotransformation of a xenobiotic or an active metabolite to a metabolite that is significantly less active.

Note 1: Virtually all tissues possess at least some degree of inactivating enzymes, but the greatest xenobiotic metabolizing activities are present in the liver, gastrointestinal tract, kidneys, and lungs. Alternatively, metabolic activity that can afford protection from xenobiotics is not significantly enhanced in the blood-brain barrier (BBB), mammary glands, or placenta, although the latter does have some added capabilities.

Note 2: In general, bioinactivation is not a pre-programmed feature of a drug, but typically something to be avoided or attenuated during ADME optimization, so as to prolong the efficacious half-life of clinical candidate compounds. However, there is one strategy for drug design, namely that of soft drugs, that intentionally deploys bioinactivation mechanisms as the central theme of their design process.

See: Detoxification, Bioactivation, Active Metabolites, and Soft Drugs.^{3,45}

30. Biotransformation

Enzyme-mediated alteration of a xenobiotic or its metabolites by living systems or isolated enzyme preparations.

Note 1: By strict definition, the hydrolysis of an ester due to the basic nature of physiological pH (i.e., 7.4), or the acidic nature of the stomach or the lysosome within a cell, are not examples of a biotransformation. Instead, these are chemical events afforded by the specific environments present within various compartments of a living system.

See: Bioinactivation, Bioactivation, Active Metabolites, Prodrugs, and Soft Drugs.⁴⁶

31. Blood-Brain Barrier (BBB)

A highly selective barrier between the circulating blood and the brain's interstitial (extracellular) fluid.

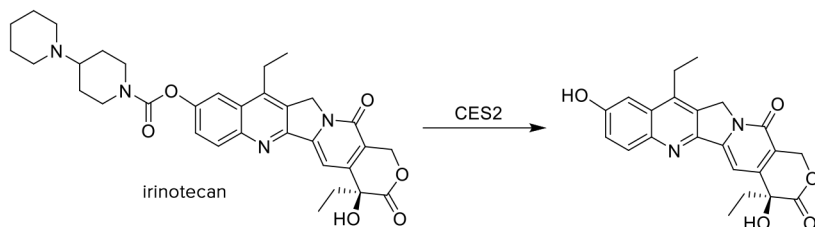
Note 1: Physically, the BBB is formed by tight junctions of the brain's endothelial cells arranged in numerous, partially overlapping zones contained in a highly anisotropic lipid bilayer that is additionally supported biochemically by astrocytes (star-shaped brain glial cells). The tight junctions are key for precluding the paracellular diffusion that is normally operative across other membrane structures. Likewise, brain endothelial cells have comparatively few pinocytotic vesicles, diminishing that mode of crossing membranes. The BBB allows the passage of water, oxygen and some other gasses, as well as lipid-soluble small molecules, by passive diffusion. Essential nutrients that are polar, such as glucose and amino acids, are afforded passage by specific, active transport systems. Similar to the gut epithelium, brain endothelial cells are enriched with certain metabolizing capabilities and express high levels of the efflux transporter *P*-Glycoprotein (Pgp), which is well suited for excluding lipophilic molecules across a wide range of structures. Thus, both polar and non-polar xenobiotics will have diminished distribution into the Central Nervous System (CNS). Large-molecule therapeutics are not able to traverse the BBB, and estimates suggest that less than 5 % of all small-molecule drugs may be able to do so.

Note 2: Drugs or prodrugs targeting the CNS after oral or peripheral administration are typically designed to have: (i) a structural scaffold modeled after a prototypical agent already known to traverse the BBB; (ii) Log*P* values optimized to be near 2.0 to 2.5, so as take advantage of potential lipoidal entry, while minimizing Pgp's affinity for the same physicochemical profile and also considering that Pgp inhibitors might be additionally deployed as adjunct therapeutic agents; (iii) an overall pharmacophore that can prompt efficacy while also being suitable for active transport; or (iv) further attachment via a stable or prodrug-type linker to a separate structural moiety that is a known substrate for an active transport system. Alternatively, drugs can be delivered directly to the brain in such a way that traversal of the BBB is not necessary. Given the invasive nature of the latter method, less frequent administration becomes imperative and prolonged dosage forms or surgical implants, such as drug-containing wafers, become important ADME-related elements during drug design.

See: Absorption, Passive Transport, Active Transport, Bioinactivation, P-Glycoprotein (Pgp), Bioactivation, and Prodrugs.⁴⁷⁻⁴⁹

32. Carboxyl Esterases (CESs)

An important class (EC 3.1.1) within the esterase family that can hydrolyze a range of xenobiotic ester and ester-like moieties. These enzymes are often exploited as a bioactivating process during the design of prodrugs. CESs are divided into five major groups (CES1-5) according to sequence homology to human CES1A1. Most members belong to either CES1 or CES2, with the former being further classified into eight subfamilies. CES1 prefers esters having small alcohol groups, while CES2 is more tolerant in that regard. An example for these preferences is shown below for the bioactivation of the prodrug irinotecan.



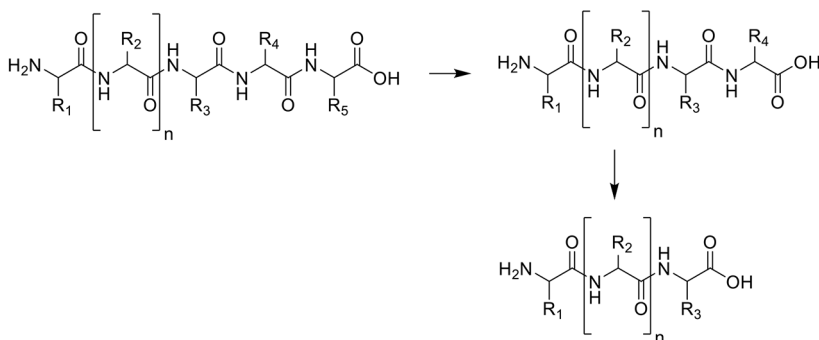
Note 1: The more relevant human CESs are about 60 kDa glycoproteins. While CES1 can exist in monomer, dimer, trimer, and hexamer arrangements, CES2 is typically only assembled as the monomer. CES1 and 2 are found in a wide variety of tissues, including serum, with high levels being present within the liver, where they are mostly in association with the endoplasmic reticulum, followed by lower amounts in lysosomes and the cytosol. As members of the broader class of hydrolases, however, the overall composite for this type of biotransformation can be considered as being ubiquitous throughout the body. Likewise, together the hydrolases constitute a very aggressive metabolic capability toward a wide range of ester-related groups when present in xenobiotics, unless the latter are sterically sheltered from such a barrage.

Note 2: As is the case for esterases in general, the catalytic triad consists of a Ser, Glu, and His imbedded at the bottom of a cleft that has a pocket on one side for the alcohol portion and one on the other side for the acyl moiety present in an ester or ester-like linkage.

See: Hydrolases, Prodrugs, and Bioactivation.^{50,51}

33. Carboxypeptidases

An important class (EC 3.4.16-3.4.18) within the protease family that readily catalyzes amide bond hydrolysis at the carboxy-terminus of a protein or peptide, sometimes in rapid succession along the peptide's backbone. The latter instance is depicted in a general way below. Because they play key roles in the processing and catabolism of many endogenous proteins, avoiding such hydrolyses becomes a critical ADME hurdle for short peptide and peptidomimetic drug candidates.



Note 1: There are several types of carboxypeptidases. The majority have widespread distributions within the *in vivo* setting, and even cell-based assays can display considerable activity. In addition to the standard peptidase nomenclature based upon reaction mechanism, they can be classified by their substrate preferences. Most important for xenobiotics are carboxypeptidase A and B, the former preferring terminal amino acids having neutral aromatic or aliphatic sidechains and the latter preferring basic amino acids, like Arg and Lys, that tend to be charged at physiological pH.

Note 2: Inversion of stereochemistry or other peptidomimetic alterations made near the carboxy-terminus often remedy this type of ADME-related bioinactivation hurdle. Alternatively, simple prodrug approaches are generally complicated in this case because the rapid rate of deactivation after bioactivation to an active acid can still remain problematic.

See: Peptidases/Proteases, Aminopeptidases, Peptidomimetic Drug Metabolism, and Prodrugs.^{51,52}

34. Catabolism

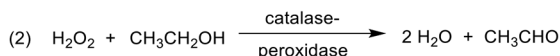
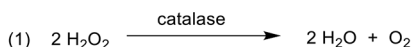
Composite of biochemical pathways that break down endogenous and specific xenobiotic molecules, such as food and vitamin components, to either release energy that can be captured for functional purposes or to provide building blocks for anabolism.

Note 1: This term can be applicable within the context of xenobiotic metabolism when the latter pertains to nutritional compounds, but is otherwise generally not used to describe xenobiotic biotransformations, even though many enzymes important for the latter also have specific roles in various catabolic pathways.

See: Anabolism.^{31,53}

35. Catalase and Catalase-Peroxidase

Catalase is a specific enzyme (EC 1.11.1.6) that converts hydrogen peroxide to water and molecular oxygen, for which its stoichiometry is shown in reaction (1). A closely related enzyme, catalase-peroxidase (EC1.11.1.21), further participates in the oxidation of certain xenobiotics and their metabolites, such as for ethanol, as shown in reaction (2).



Note 1: Regarded as a detoxifying enzyme, catalase prevents cell damage associated with reactive oxygen species (ROS) and oxidative stress in general. These enzymes are found in all living systems exposed to oxygen.^{54,55} In humans, it is present in all tissues, with particularly high levels being present in the liver. Its subcellular location is largely confined to organelles called peroxisomes.

Note 2: Catalase is a tetramer having four polypeptide chains of over 500 amino acids, each accompanied by a prosthetic heme-porphyrin moiety that plays a role during reaction with hydrogen peroxide.

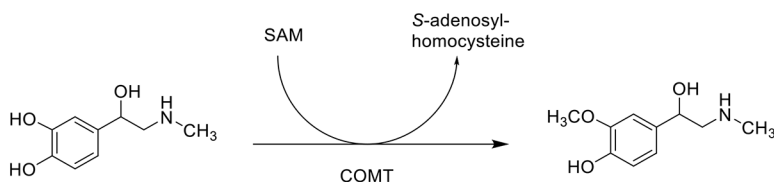
Note 3: Several genetic variants have been identified, some of which can give rise to catalase deficiency, a syndrome called "acatalasia" (homozygotes) or

"hypocatalasia" (heterozygotes). Although there may be some association with an increased likelihood of developing type 2 diabetes, these deficiencies do not otherwise appear to lead to any significant ill effects, perhaps owing to the major role that the peroxiredoxins play in also scavenging hydrogen peroxide in mammalian cells.

See: Alcohol Dehydrogenase (ADH), Aldehyde Dehydrogenase (ALDH) and Aldehyde Oxidase (AO), Reactive Oxygen Species.⁵⁶

36. Catechol-O-Methyl Transferase (COMT)

An enzyme (EC 2.1.1.6) that conjugates a methyl group to one of the hydroxyl moieties present in a catechol arrangement. *S*-adenosylmethionine (SAM) serves as a cofactor. While applicable xenobiotics are ready substrates, COMT plays a major role in the bioinactivation of the endogenous catecholamine neurotransmitters, wherein it demonstrates exquisite regiospecificity for the meta-hydroxy group of the parent compounds and their deaminated metabolites (shown below for epinephrine).⁵⁷



Note 1: Although low concentrations of extracellular enzyme have been noted, COMT is essentially located in the cytoplasm of all tissues, with the highest concentrations being found in the liver, followed by the kidney. As for other methyltransferases, COMT's active holoenzyme takes advantage of a complex with SAM imbedded deep within the active site to supply the methyl group.

Note 2: The human COMT gene possesses alleles coding for high and low activity forms (COMT^H and COMT^L), with the latter reflecting a valine to methionine mutation at position 158, (Val¹⁵⁸Met)rs4680, that reduces catabolic rate by greater than 4-fold. There is an even distribution of these allelic variants in the Caucasian population (25 % homozygous for high, 25 % for low, and 50 % heterozygous becoming intermediate metabolizers), while the African-American population has a higher frequency of the

COMT^H allele. High COMT can be associated with more difficult therapeutic management of Parkinson's disease (see Note 3 below).

Note 3: Levodopa is a catechol-containing prodrug used to treat Parkinson's disease by virtue of its passage into the CNS and subsequent bioactivation by dopa decarboxylase to dopamine, which cannot itself traverse the BBB. Because both levodopa can be metabolized and dopamine can be bioinactivated by COMT, an inhibitor of this enzyme (e.g., entacapone) is used as an adjunct agent during levodopa therapy, generally with an inhibitor of peripheral dopa decarboxylase (e.g., carbidopa) to prevent premature conversion to dopamine outside of the CNS, and sometimes with an inhibitor of monoamine oxidase B (e.g., selegiline) to additionally attenuate the bioinactivation of dopamine by deamination.⁵⁸

See: Methylation, Methyltransferases, Bioinactivation, Monoamine Oxidase (MAO), Deamination, Absorption, Blood-Brain Barrier (BBB), Prodrugs, and Bioactivation.

37. Clearance (CL)

The rate of elimination of a xenobiotic, normalized to its concentration in a given biological fluid, usually blood, serum, or plasma. CL has units of volume per time because it represents the volume of biological fluid, per unit of time, from which the xenobiotic would have to be completely removed in order to account for the observed elimination rate. The total systemic clearance (CL_{tot}) is the sum of the clearances of each organ serving to remove the xenobiotic from the biological fluid, e.g., blood. The equation below expresses this relationship for a drug that is cleared from the body by both the liver and kidneys.

$$CL_{\text{tot}} = CL_{\text{hepatic}} + CL_{\text{renal}}$$

Note 1: At steady-state concentrations, CL_{tot} will equal the dose rate (DR), such that it can be calculated as follows, where C_{ss} is the steady-state concentration of the drug:

$$CL_{\text{tot}} = DR / C_{\text{ss}}$$

See: Elimination, Excretion, Intrinsic Clearance, and Pharmacokinetics.³⁸⁻⁴⁰

38. Cocktail Study

Study of two or more simultaneously administered xenobiotics, either to assess their own metabolism, metabolism-related interactions or PK or ADMET properties.

Note 1: Multiple enzyme activities can be simultaneously evaluated *in vivo* through the combined administration of several selective enzyme probes, i.e., a metabolic or phenotypic probe cocktail study. Such studies can characterize the subject's metabolic phenotype.

Note 2: The metabolism of multiple drug candidates (drug cocktail) can be simultaneously assessed within a single animal, providing that cross-over metabolic induction or inhibition (drug-drug interactions) do not occur and that there are no cross-over interferences of the assay method. It is not uncommon for more than five "hit compounds" to be tested in this simultaneous manner when supporting large compound screening campaigns.

See: N-in-One Dosing, Drug-Drug Interactions, Induction, Inhibition, Competitive Inhibition, and Non-competitive Inhibition.⁵⁹⁻⁶⁰

39. Cofactor

A non-protein substance whose loosely bound association with an enzyme is required for the latter's function.

Note 1: Cofactors may be ions, such as those derived from various oxidation states of copper, iron, or zinc, or they may be small organic molecules, such as vitamins, NADH, or NADPH.

Note 2: Cofactors may initially facilitate or enhance an enzyme's ability to bind with its substrates, and may subsequently be required for the specific enzymatic reaction to occur.

See: Apoenzyme, Holoenzyme, and Prosthetic Group.^{34,61}

40. Compartment Model

Pharmacokinetic (PK) model where the body is represented by one or more inter-accessible compartments that a xenobiotic may enter. Absorption,

distribution, and excretion are defined by rate terms for the movement of the xenobiotic into, out of, or between different compartments, while metabolism is defined by a rate term for the biotransformation of the agent. When all of these terms follow linear kinetics, the compartment model for plasma, serum, or whole blood concentration (C) versus time predictions (t) can be expressed as a sum of exponential terms having the form shown below, where the A terms are coefficient constants and the k terms are linear kinetic rate constants.

$$C = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + \dots + A_n e^{-k_n t}$$

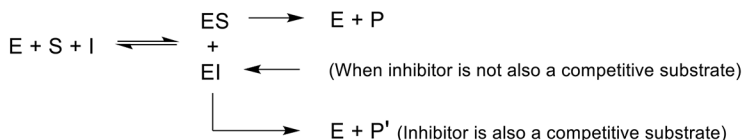
Note 1: Each compartment is taken to be internally homogeneous and well-mixed, thus representing a collection of fluids and tissues that have similar concentration versus time profiles. While inter-accessible, a compartment's components need not be directly interconnected, e.g., a two-compartment model categorized as all extracellular aqueous media versus all fat tissue would separately encompass all of the appropriate components for each of these two categories, regardless of their locations and lack of direct physical connections.

Note 2: The number of exponential terms for a linear kinetics model without absorption (intravascular administration) is equal to the number of compartments, while that requiring an absorption phase (extravascular administration) is equal to one more than the number of compartments.

See: Absorption, ADME, Clearance, Pharmacokinetics, PBPK Model, and Non-compartment Model.³⁸⁻⁴⁰

41. Competitive Inhibition

Reversible inhibition of an enzyme or transporter by a xenobiotic (inhibitor) that competes with the natural substrate, or with another intended substrate, for the enzyme's binding domain in a non-covalent manner. The interaction between a non-reversible enzyme (E), its intended substrate (S) leading to product (P), and an inhibitor (I), which itself may or may not serve as a secondary substrate leading to an alternative product (P'), can be depicted as follows:



Note 1: Depending upon conditions, many enzymes can also operate in reverse, i.e., $E + P \leftrightarrow EP \leftrightarrow E + S$. Likewise, in some cases the product can serve as an inhibitor whether it is a substrate or not. These processes will lead to non-Michaelis-Menten kinetics.

Note 2: Competitive inhibition is a mass-action phenomenon that can be overcome with a sufficiently high concentration of substrate. For enzymes that metabolize substrates in accordance with Michaelis-Menten kinetics, competitive inhibition will raise the enzyme's K_m , but will not alter its V_{max} .

See: Inhibition, Non-Competitive Inhibition, Uncompetitive Inhibition, Michaelis-Menten Kinetics.⁶²

42. Conjugate

Within the context of xenobiotic metabolism,⁶³ the metabolite resulting from a biotransformation that forms a covalent linkage with an endogenous reactant and a functional group present on a xenobiotic or its metabolite.

Note 1: This is different from the term "drug conjugate," or "DC," which derives from the practice of drug design and involves connecting an efficacious drug molecule to another molecule that can home in on (or preferentially distribute/concentrate in) a desired biological target (physiologic compartment). For example, one strategy is to link an anticancer agent with an antibody that can recognize proteins being over-expressed by a particular cancer cell, the resulting complex then called an "antibody-drug conjugate," or "ADC."⁶⁴ The DC/ADC strategies are currently so popular that several technical journals, emphasizing such subject matter, presently bear some aspect of this phraseology as part of their name.

Note 2: Though conjugates frequently possess diminished biological activity and are generally more polar and subject to rapid excretion (e.g., the very common formation of a glucuronide on a phenolic hydroxyl-group), in some cases conjugation leads to less polar metabolites (e.g., acetylation of an amino-group or methylation of a sulfhydryl-group), which can extend the duration of activity, including potential toxicity.

Note 3: Certain conjugates are subject to further metabolic processing (e.g., reaction of glutathione with xenobiotics having an electrophilic group followed by a specific sequence of biotransformations that lead to formation

of mercapturic acids), and in some cases they can be converted back to their starting material (e.g., hydrolysis of glucuronides by the gut microflora).

See: Conjugation Reactions and Phases of Drug Metabolism.⁶³

43. Conjugation Reactions

Biotransformations that form a covalent bond between an endogenous molecule that is programmed biochemically to serve as a partner during metabolic processing and a functional group present on a xenobiotic or its metabolite. A special path occurs for a xenobiotic carboxylic acid moiety, because in this singular case the xenobiotic itself becomes biochemically programmed to undergo a conjugation reaction with an endogenous amino acid partner.

Note 1: Conjugation reactions are often referred to as “Phase 2” biotransformations because they so frequently follow introduction of a functional group by a preceding metabolic event that, in turn, is referred to as a “Phase 1” biotransformation. However, this is not a required sequence for using this terminology and, whenever a parent xenobiotic already has a suitable functional group, Phase 2 metabolism can occur directly and without the need for a preceding Phase 1 metabolic event. See thorough discussion of this topic in the introduction.

Note 2: Examples include: (1) glucuronidation reactions that combine glucuronic acid quite commonly with phenols, sometimes with alcohols, occasionally with amines, and more rarely with carboxylic acids; (2) sulfation reactions that combine sulfate with phenols and, to a lesser extent, with alcohols, including hydroxylamines; (3) acetylation reactions that combine an acetyl moiety with amines and sometimes with hydroxy and sulfanyl groups; (4) rapid reactions that combine glutathione with xenobiotics or their metabolites when they happen to contain an electrophile that might lead to toxicity via their reaction with other endogenous nucleophiles; (5) methylation reactions commonly involving sulfhydryl-groups and catechols, and to a lesser extent other amine and alcohol moieties; and (6) amino acid reactions typically utilizing glycine or taurine that combine with carboxylic acid groups.

Note 3: Among the six conjugation reactions listed above, their relative contributions during drug metabolism are approximated in the following

pie chart, adapted from reference 65. This summary reflects the types of structures that have become deployed as small-molecule drugs (namely the specific suitability of their functional groups), as well as the distribution accessibility, compartment abundance, and specific reaction rates for each of the processing systems associated with a given type of conjugation pathway. Note that category (6) encompasses all remaining conjugation reactions, as well as amide formation with specific endogenous amino acids.

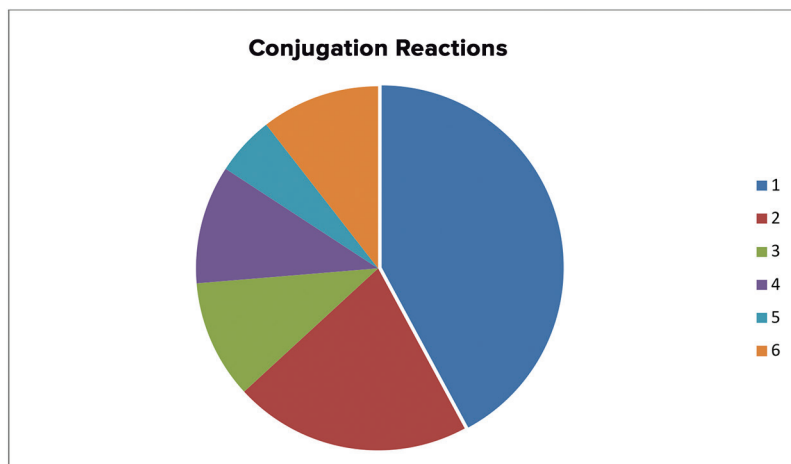


Figure of relative contributions for the various types of conjugation reactions toward overall human metabolism of small-molecule xenobiotics. Types: (1) Glucuronidation; (2) Sulfation; (3) Acetylation; (4) Reaction with glutathione; (5) Methylation; and (6) Reaction with an endogenous amino acid such as glycine or taurine.

See: Acetylation, Acylation, Acyltransferase, *N*-Acetyltransferases, Catechol-*O*-Methyl Transferase (COMT), Conjugate, Covalent Binding, Glucuronidation, Glucuronosyltransferases, Glutathione Conjugation, Glutathione-*S*-Transferase, Methylations, Phases of Drug Metabolism, Sulfation, and Sulfotransferases.⁶⁶

44. Covalent Binding

A biochemical process where a xenobiotic or metabolite forms a chemical bond with an endogenous molecule by sharing a pair of electrons between the two bonding atoms.

Note 1: Relative to various aspects of drug metabolism, covalent binding can occur as a requisite feature of conjugation or phase 2 biotransformations;

within enzyme active sites as an inherent but reversible mechanistic step (as described below) or by either intentional (drug design) or inadvertent (from production of a reactive species) reaction that alters the enzyme's function; or, for reactive metabolites, spuriously, with off-target protein sites that can lead to unwanted side effects and toxicity.

Note 2: In general, when the endogenous molecule's bonding atom resides on the functional surface of a protein, such as within an active site of an enzyme, this type of interaction can lead to permanent inhibition of that function, i.e., non-competitive, non-Michaelis-Menten type of kinetics for which the inhibition cannot be overcome by increasing the concentration of the normal substrate. Alternatively, some enzymes, such as certain members of the Hydrolases and Proteases, utilize a covalent bond-forming reaction as the first step toward hydrolysis of their ester or amide containing substrates. In these specific cases, other functional groups are also present in the active site to assist in re-establishing the enzyme's non-covalent bound "ground state." Lacking specific biochemical machinery for recovery, covalently inhibited systems must rely upon protein turnover to re-establish function.

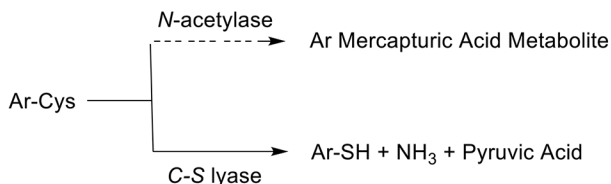
Note 3: For drug molecules, this term is most frequently associated with undesirable interactions, i.e., inhibition of metabolizing enzymes leading to drug-drug interaction issues, or interaction with off-target proteins whose functions are not meant to be inhibited, such that toxic side-effects may occur. Thus, in the selection of "drug-like" molecules among compound libraries, structures having reactive functional groups capable of forming covalent bonds, such as an *alpha-beta* unsaturated ketone, are often removed from further consideration.

See: Competitive Inhibition, Non-Competitive Inhibition, Michaelis-Menten Kinetics, Conjugation Reactions, Hydrolases, Esterases, Proteases, Drug-Drug Interactions, Drug-Like Properties/Profile.⁶²

45. Cysteine-S-Conjugate β -Lyase (C-S Lyase)

A pyridoxal phosphate-dependent β -lyase enzyme (EC 4.4.1.13) that can hydrolyze a cysteine conjugated xenobiotic intermediate otherwise on route to becoming a mercapturic acid metabolite. This process is shown below for a general aryl-containing xenobiotics (Ar) metabolic intermediate

(Ar-Cys) in a manner that depicts this key departure from the more typical route toward becoming a mercapturate (dotted line).



Note 1: While the parent xenobiotic, Ar, may have started as a highly reactive and potentially toxic electrophile, instead of progressing to the innocuous mercapturate, that center has now been masked with a less reactive (but still reactive) sulfanyl group. As a result, the latter is often quickly further metabolized by a methylation reaction, so as to produce the much less reactive thioether metabolite.

See: Glutathione and Mercapturic Acid.⁶⁷

46. Cytochrome b_5

A protein which can donate the second of two electrons required by the CYPs during their catalytic cycle associated with the metabolism of xenobiotics. In combination with the flavoprotein cytochrome b_5 reductase (EC 1.6.2.2), this pair normally functions as a non-phosphorylating electron-transport chain associated with the endoplasmic reticulum, where it plays a key role in the desaturation of endogenous fatty acids.

Note 1: In addition to increasing the catalytic rate, cytochrome b_5 increases the apparent affinity with which certain of the CYPs appear to bind their substrates. Thus, it may be increasing $V_{\max}$ and/or decreasing the apparent K_m , since it is the ratio $V_{\max}/K_m$ that relates to catalytic efficiency and intrinsic clearance.

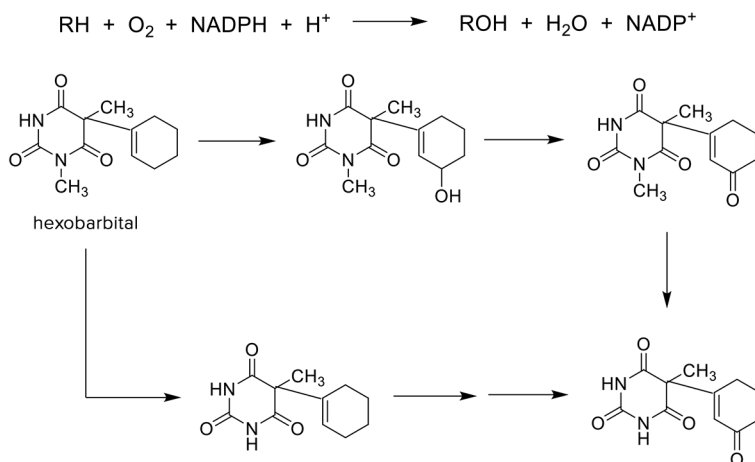
Note 2: While there are numerous isoforms of the various CYPs within liver microsomes, there appears to be only one form of NADPH-cytochrome P-450 oxidoreductase (accepted name: NADPH-hemoprotein reductase; EC 1.6.2.4) and only one form of cytochrome b_5 that serve as the principal partners for the CYPs.

Note 3: Since the reductase is important for reducing heme iron from Fe^{+3} to the oxygen-carrying Fe^{+2} state, disruption of this system can lead to methemoglobinemia.⁶⁸

See: Cytochrome *P*-450 Enzymes (CYPs), NADH-Cytochrome *b*₅ Reductase, Microsomes, Reconstitution System, Regenerating System, and NADPH-Cytochrome *c* Reductase/NADPH-Cytochrome *P*-450 Oxidoreductase/Cytochrome *P*-450 Reductase (CYPR).

47. Cytochrome *P*-450 Enzymes (CYPs)

The CYPs belong to the superfamily of heme proteins that have a heme (Fe^{+3}) cofactor, the P450 phrase in this broad term actually deriving from these enzymes' diagnostic spectrophotometric peak shift to 450 nm when the reduced heme iron (Fe^{+2}) is complexed with carbon monoxide and enzymatic activity is poisoned. In addition to having numerous roles in mammalian biochemical pathways involving the processing of endogenous materials, the CYPs are a major contributor toward a variety of xenobiotic oxidative metabolic events. The overall process is shown below for a general xenobiotic (R). Due to rearrangements, however, products are not limited to just aliphatic alcohols and phenols. A specific case that exemplifies several of hexobarbital's CYP-mediated sequential biotransformations is also shown below. In certain cases, most often prompted by anaerobic conditions, the CYPs can also perform reductive biotransformations. The CYPs have a distinct nomenclature based upon their protein homology, rather than substrate preferences. These designations are elaborated in Note 1. Note 3 provides additional mechanistic details.

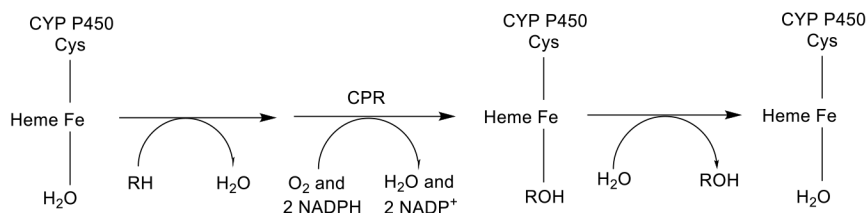


Note 1: CYP Family: a subset of the cytochrome *P*-450 superfamily of enzymes that shares at least 40 % amino acid sequence homology. A specific family is denoted by an Arabic numeral following the uppercase letters "CYP," e.g., CYP 2. **CYP Subfamily:** a subset of the CYP 450 superfamily that

shares at least 55 % amino acid sequence homology. A specific subfamily is denoted by adding an Arabic uppercase letter after the numerical family designation, e.g., CYP 2D. **CYP Isoform:** an individual form (distinct amino acid sequence) of the CYP 450 superfamily. A specific isoform is denoted by adding an Arabic numeral after the designation of its family and subfamily, e.g., CYP 2D6.

Note 2: More than 20,000 CYP isoforms have been identified in nature. However, for human drug metabolism, only three major families, about one-half-dozen subfamilies, and less than a dozen specific isoforms seem to be the most important. Just six isoforms account for 90 to 95 % of the metabolism of marketed drugs. The top four of these can be ranked: CYP 3A4 > CYP 2D6 > CYP 2C9 > CYP 1A2, with several others contributing considerably less (and nearly equally to each other) to human drug metabolism. Common examples of xenobiotic biotransformations mediated by the CYPs include: aromatic hydroxylation; *N*- and *O*-Dealkylations, especially demethylations; and aliphatic and alicyclic oxidations and ring scissions.

Note 3: The most prevalent mechanism utilized by various CYPs during metabolism of xenobiotics is a monooxygenase, hence they are often referred to as "mixed-function oxidases" in relation to drug metabolism discussions. NADPH is a requisite cofactor from which electrons are transferred by a required coenzyme, generally NADPH cytochrome *P*-450 oxidoreductase (CPR), so as to balance the reduction of molecular oxygen, while the CYP simultaneously utilizes the latter as its source to introduce an oxygen atom into a xenobiotic substrate (RH). This overall process is summarized below. It should be noted that the depiction of the various enzyme-bound oxygen species has been simplified to just a single oxygen atom, and the changes in the iron's oxidation state are not shown where $\text{Fe}^{+3} \rightarrow \text{Fe}^{+2} \rightarrow \text{Fe}^{+3}$ so as to maintain balance of the net charge throughout the biotransformation.



Note 4: While the CYPs can be found in nearly every tissue, their expression in the liver, the lungs, and the gut wall is especially noteworthy for drug metabolism and distribution in general, as is their acceptance toward the biotransformation of a wide range of xenobiotic structural types. Largely localized within the endoplasmic reticulum (ER) of cells and to a lesser extent within the mitochondria, their membrane-bound nature within that locale allows for their functional isolation by disruption of the cells and centrifugation to form lipid sacs called “microsomes” or the “microsomal fraction.” Microsomes are highly enriched with the CYPs and are used to conduct *in vitro* drug metabolism studies from various mammalian donors, particularly rodents. Expression and isolation of the key humanized isoforms has also allowed for biochemical studies with the distinct human enzymes.

Note 5: CYP-mediated biotransformations are often referred to in a general manner as “Phase 1” metabolic processes because they frequently instill or unmask a functional group that subsequently lends itself to one of the metabolic conjugation reactions, which, in turn, are referred to as “Phase 2” biotransformations. However, this is not a required sequence for using this terminology and, even after a conjugation reaction has occurred, there are numerous cases where additional CYP-mediated “Phase 1” biotransformations are observed. A thorough discussion of this topic can be found in the Introduction and the Appendix.

Note 6: Genetic polymorphism in CYP expression can lead to considerable individual variation. There are two general phenotypes based on how quickly individuals metabolize debrisoquine. These are commonly referred to as: (1) debrisoquine-related extensive metabolizers (DR EMs); and (2) debrisoquine-related poor metabolizers (DR PMs). The variations caused by these phenotypes extend to well over 20 other types of marketed drugs. DR PM reflects an inherited defect in CYP 2D6 expression that affects 5 to 10 % of the Caucasian, 2 % of the East Asian, and 1 % of the Arabic populations. Due to the potential for this variation, developers of new drugs often try to avoid relying on the CYP 2D6 pathway for the metabolic clearance of their candidate compounds. Another phenotype is associated with phenytoin-related metabolism and likewise extends to numerous other types of drugs. Phenytoin-related poor metabolizers (PR PMs) have an inherited defect in CYP 2C18, with an occurrence of 15 to 20 % in East Asian and 2 to 6 % in Caucasian populations. For this reason, the CYP 2C18 pathway is often also avoided during the development of new drug candidates.

See: Aromatic Hydroxylation, Dealkylation, *N*-Dealkylation, *O*-Dealkylation, Oxidation, Mixed-Function Oxidase, Monooxygenase, Phases of Drug Metabolism, NADPH-Cytochrome c Reductase/NADPH-Cytochrome *P*-450 Oxidoreductase/Cytochrome *P*-450 Reductase (CYP_R), Cytochrome *b*₅, NADH-Cytochrome *b*₅ Reductase (CB_R)/Methemoglobin Reductase, Microsomal Fraction/Enzyme; Microsomes, Regenerating System, Hexobarbital Sleeping Time, Induction, and Drug-Like Properties/Profile.^{45,69-73}

48. Deacetylation

Typically used within the context of xenobiotic metabolism to specifically refer to the hydrolysis of *N*-acetyl groups.



Note 1: This special designation likely derives from the commonality of this particular functionality in drug molecules coupled with the resulting frequency for this type of deacetylation reaction. Reactions that remove an acetyl group, as well as other acyl groups, from other heteroatoms, such as oxygen are, instead, generally referred to as “hydrolyses” or “hydrolytic reactions.”

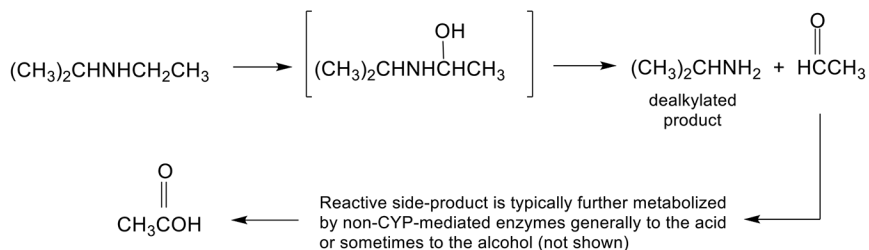
Note 2: Within the context of DNA packaging, histone acetylation versus deacetylation plays a key role in the extent of chromatin organization. Inhibitors of histone deacetylase (HDAC inhibitors) prevent cleavage of some of the acylated amines on the histone protein complex, which in turn decreases their organization with DNA, because the latter’s negatively charged phosphate groups cannot associate with as many protonated ammonium partners. Because some HDAC inhibitors have been found to be effective anticancer agents, this endogenous pathway involving key acetylation/deacetylation processes has become a popular area for usage of this term.

See: Hydrolysis and Hydrolases.⁵¹

49. Dealkylation

Common biotransformation wherein an alkyl group is removed from a heteroatom. Most often mediated by the CYPs, the alkyl group first becomes oxidized to an alcohol *alpha* to the heteroatom and then collapses to leave as

an aldehyde or ketone. Since the aldehyde side-products can be toxic, they are generally quickly processed by further, non-CYP-mediated oxidation to the carboxylic acid or, sometimes, by reduction to an alcohol. These pathways are illustrated below.



Note 1: For CYP-mediated dealkylations, substrates must possess at least one *alpha*-hydrogen, which becomes extracted during the early stages of the biotransformation. While *N*-dealkylations are quite general, *O*- and *S*-examples tend not to be nearly as prominent. In these latter dealkylations, only demethylation remains important, and especially so when an arylmethoxy-group is present.

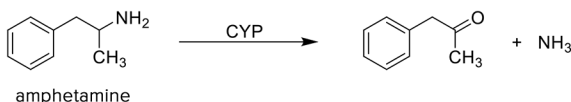
Note 2: The overall process is readily subject to steric hindrance: tertiary amines are less subject to dealkylation than secondary amines, which are similar to primary amines; isopropyl groups undergo less reaction than propyl groups or other non-branched longer chains; and as indicated above, simple methyl groups are typically the quickest substrates.

Note 3: Sterically hindered tertiary amines typically become prone to *N*-oxidation, rather than serving as substrates for dealkylations.

See: Demethylation, Deamination, Monoamine Oxidase (MAO), Diamine Oxidase (DAO), Polyamine Oxidase, *N*-Dealkylation, *O*-Dealkylation, *N*-Oxidation, Aldehyde Dehydrogenase, and Aldehyde Reductase.⁷⁴

50. Deamination/Oxidative Deamination

For xenobiotics, this represents a special case of *N*-dealkylation, mostly applicable to primary amines that can be mediated by CYPs, monoamine oxidase, diamine oxidase, or polyamine oxidase. Analogous mechanistically, after formation of the *alpha*-hydroxy intermediate, further collapse liberates ammonia and either an aldehyde or ketone moiety. The biotransformation of amphetamine is representative:



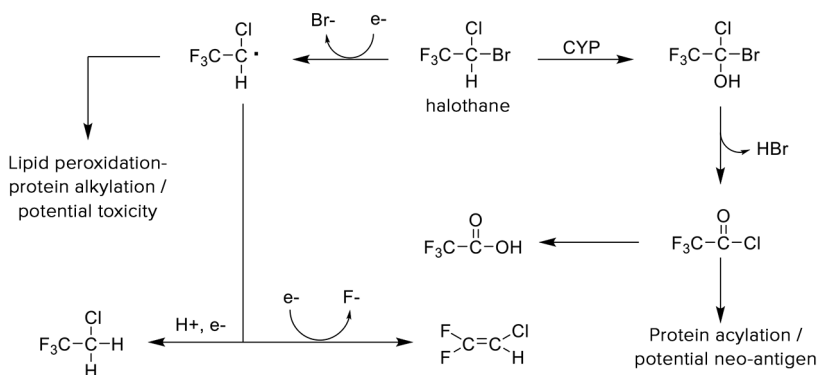
Note 1: This example is mediated by a CYP because the other applicable oxidase enzymes are far less tolerant of substrates whose *alpha*-carbon bears two substituents, which results in a steric impediment.

Note 2: For endogenous biochemistry, this term is used similarly in several other contexts. Important examples include: catabolism of amino acids, such as the conversion of glutamic acid to α -ketoglutaric acid (now 2-oxopentanedioic acid), wherein the liberated ammonia joins the urea cycle and the keto-acid participates in subsequent transamination reactions, so as to also deaminate other amino acids; similar catabolism of excess proteins within the body; and analogous reactions involving nucleosides for which some spontaneous (hydrolytic driven) deaminations can lead to mutations that prompt repair mechanisms.

See: Dealkylation, *N*-Dealkylation, Monoamine Oxidase (MAO), Diamine Oxidase (DAO), and Polyamine Oxidase.⁷⁴

51. Dehalogenation

Removal of one or more aliphatic halogens from a xenobiotic. This can be accomplished by any combination of three mechanisms (see Note below), all of which traverse reactive intermediates that can lead to potential toxicity. The metabolism of halothane is representative:



Note 1: The three most prominent mechanisms for this type of biotransformation are: reductive dehalogenation, in which a halide is replaced by a hydrogen; oxidative dehalogenation, in which a halide and a hydrogen on the same carbon are replaced by an oxygen; and removal of two halides or of one halide and one hydrogen (also called "dehydrohalogenation") on adjacent carbon atoms, so as to produce a double bond. Each of these pathways is evident in the metabolic composite that is undertaken by halothane.

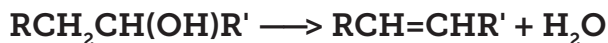
Note 2: All three types of dehalogenation are catalyzed by the CYPs. Formation of the double bond can also be catalyzed by glutathione-*S*-transferase.

Note 3: The halothane example also illustrates the different sources of potential toxicity during these mechanisms. In general, when a carbon atom contains two halides and a hydrogen, the dihalohydrin intermediate that is formed during oxidative dehalogenation collapses to an acylhalide, which, unless it is quickly hydrolyzed to a carboxylic acid, can acylate proteins that then become neo-antigens (antigens not present in the normal genome, generally resulting from an aberrant metabolic step) and ultimately lead to immune hepatitis, as is sometimes seen after repeated exposure of humans to halothane and other structurally related volatile anesthetics.

See: Bioactivation and Bioinactivation.⁷⁵

52. Dehydration

For xenobiotics, dehydration most frequently pertains to the formation of unsaturated metabolites by the elimination of water from hydroxy-containing compounds, typically those having an acidic hydrogen at an adjacent carbon atom.



Note 1: In addition to being enzymatically catalyzed,⁷⁶ the dehydration of certain alcohols can occur non-enzymatically as a one-step acid-catalyzed reaction or as a two-step process involving the metabolic activation of the hydroxyl group, e.g., by sulfation or glucuronidation and the subsequent elimination of sulfuric acid or glucuronic acid, respectively. Dehydration of aldoximes to nitriles can be catalyzed by the CYPs in their reduced state.

See: Dehydrogenation.

53. Dehydrogenase

General term for a large family of enzymes that oxidize a substrate by removing one or more hydrides and transferring them to an endogenous acceptor, such as $\text{NAD}^+/\text{NADP}^+$ or FAD/FMN , which in turn becomes reduced. They are further designated by the type of substrate involved. Common examples include alcohol dehydrogenase (ADH, EC 1.1.1.1) and aldehyde dehydrogenase (ALDH, EC 1.2.1.3).

See: Alcohol Dehydrogenase, Aldehyde Dehydrogenase, and Dehydrogenation.⁷⁷

54. Dehydrogenation

This term is used to designate biotransformations that convert single bonds to double bonds without undergoing an equivalent loss of water and especially for the specific production of unsaturated aliphatic compounds from saturated compounds. These conversions are typically catalyzed by the dehydrogenases and occasionally by the CYPs (see Notes).

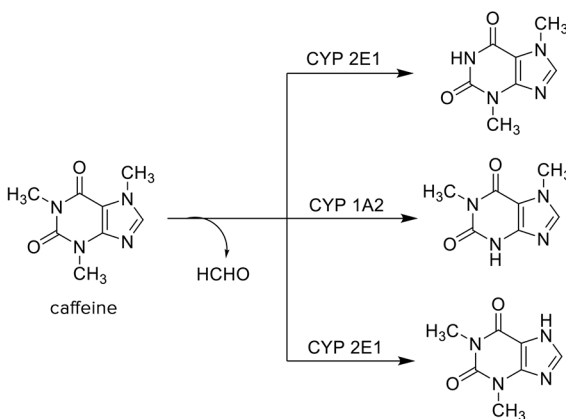
Note 1: While ADH and ALDH perform this type of reaction, they are more typically thought of as causing oxidations.

Note 2: The CYPs occasionally catalyze the de-saturation of hydrocarbons by a mechanism that does not proceed via hydroxylated intermediates. The oxidation of dihydropyridines to pyridines is likewise CYP-mediated, as is the formation of quinones from hydroquinones and the oxidation of secondary alcohols to ketones.

See: Dehydrogenase, Cytochrome P-450 Enzymes (CYPs), Alcohol Dehydrogenase, and Aldehyde Dehydrogenase.⁷⁸

55. Demethylation

This is a very common biotransformation, wherein a methyl-group is removed from arrangements associated with an amine, ether (especially phenolic methoxy ethers), or a thioether when they are present on a xenobiotic or its metabolite. Typically mediated by the CYPs, the process begins by oxidation of the methyl group. The metabolism of caffeine represents an interesting case of various *N*-demethylation possibilities, including the regio-promiscuity that can be demonstrated by the same CYP isoform:



Note 1: This is a special case of the more general dealkylation reactions typically catalyzed by the CYPs which involves an oxidative cleavage mechanism. CYP 2E1 is EC 1.14.13.178 (methylxanthine N^1 -demethylase), and CYP 1A2 is EC 1.14.13.179 (methylxanthine N^5 -demethylase).

Note 2: The formaldehyde side product is quickly metabolized further by other enzymes (e.g., ALDH), so as to avoid its potential toxicity.

See: Dealkylation, N -Dealkylation, O -Dealkylation, and Cytochrome P -450 Enzymes (CYPs).^{79,80}

56. Detoxification

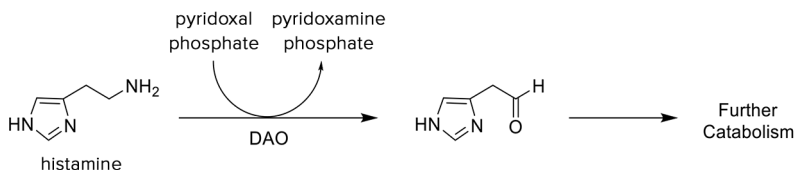
A biotransformation that eliminates a toxicant or prevents formation of a toxicant.

Note 1: Includes all metabolic pathways of the toxic substance that lead to non-toxic metabolites. In principle, these processes compete with those forming toxic metabolites and reactive intermediates.

See: Bioinactivation, Bioactivation, Active Metabolites, Reactive Intermediates, Soft Drugs, Glutathione, and Hydrolases.^{81,82}

57. Diamine Oxidases (DAO)

A pair of copper-containing amine oxidases that catalyze the oxidative deamination of short chain diamines: primary amine oxidase (EC 1.4.3.21) and diamine oxidase (EC 1.4.3.22). The catabolism of histamine is exemplary:



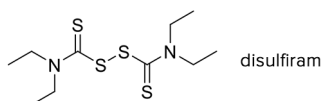
Note 1: In terms of xenobiotic structure-metabolism relationships (SMRs), any compound having a basic center with more than two but less than seven carbon atoms' distance away from a primary amine or secondary methylamine center is a candidate for deamination of the latter by DAO.

Note 2: DAO catabolizes short chain diamines, such as putrescine (4 C spacer) and cadaverine (5 C spacer), while longer, more complicated polyamines, such as spermine, are catabolized by spermine oxidase or by monoamine oxidase (MAO).

See: Catabolism, Deamination, Dealkylation, *N*-Dealkylation, and Monoamine Oxidase (MAO).⁸³

58. Disulfiram Reaction

Disulfiram inhibits aldehyde dehydrogenase (ALDH), causing a buildup of its aldehyde substrates, which in turn leads to toxicity and what is referred to as the "disulfiram (physiological) reaction."



Note 1: Disulfiram is used to treat alcoholism because the buildup of acetaldehyde after the ingestion of ethanol leads to an acute sensitivity, much like the unpleasant toxicity associated with a "hangover."⁸⁴

See: Alcohol Dehydrogenase (ADH), Aldehyde Dehydrogenase (ALDH), Aldehyde Dehydrogenase Polymorphism or Deficiency, Aldehyde Oxidase (AO), and Atypical Alcohol Dehydrogenase.

59. Disposition

All processes that occur to a xenobiotic (and its metabolites) within the living recipient after it has been absorbed, especially as they relate to the pharmacokinetic profile.

Note 1: While distribution (to and from tissues/compartments) and elimination (by metabolism and excretion) represent the major dispositional processes, additional examples include binding to proteins, wherein the latter may prompt desirable biological activity (e.g., efficacy at the desired target site of a drug), as well as toxicity (e.g., off-target drug interactions) or a passive association (e.g., plasma protein binding).

Note 2: While the term is generally meant to be all-encompassing, in some uses one or more of the ADME processes may be further distinguished, such as is the case for the journal entitled *Drug Metabolism and Disposition*.

Note 3: After the excretion of a xenobiotic and/or its metabolites, these compounds enter the environment, where their further dispositions are repeated by continued exposures to other living systems. Each of the latter can be separately tracked using this same terminology and a composite chart can be composed to represent the overall environmental fate for all of the eventual components. A discussion about the phases of drug metabolism is provided in the Introduction.

See: Absorption, First Pass Effect, Distribution, Plasma Protein Binding, Metabolism, Random Walk, Excretion, ADME/ADMET, Pharmacokinetics, and Phases of Drug Metabolism.³⁸⁻⁴⁰

60. Distribution

Generally taken as the pharmacokinetic-related parameter for the movement of a xenobiotic and its metabolites between the blood circulation and other tissues or fluids in the body. The term can also be used to designate more localized parameters, such as an uneven distribution of a xenobiotic or metabolite among the organelles within a given cell where, for example, drug metabolizing enzyme concentrations are known to be significantly different.

Note 1: After excretion of a xenobiotic and/or its metabolites, these compounds enter the environment, where their environmental distribution is impacted by physical factors and their further dispositions can be repeated by continued exposures to other living systems. Each of the latter can be separately tracked using this same terminology and a composite chart can be composed to represent the overall environmental fate for all of the eventual components. See also the discussion about the phases of drug metabolism provided in the Introduction.

See: Absorption, Disposition, First Pass Effect, Plasma Protein Binding, Metabolism, Random Walk, Excretion, ADME/ADMET, Pharmacokinetics, and Phases of Drug Metabolism.³⁸⁻⁴⁰

61. DMPK

Acronym for drug metabolism and pharmacokinetic processes, typically used in conjunction with their study, with their related profile for a given molecule, or to designate a lab or group of investigators involved with such studies.

Note 1: The same acronym is used for dystrophin myotonic protein kinase, the latter being important within the context of protein coding genes for a family of associated diseases.

See: ADME, Drug Metabolism, and Pharmacokinetics.⁴⁵

62. Dose

Amount of xenobiotic delivered to the site of an intentional administration or an unintentional exposure.

See: Absorption.⁸⁵

63. Dose-Dependent Kinetics or Metabolism

In this context, dose-dependency is the alteration of pharmacokinetic parameters that occurs with changing dose levels, where the result leads to nonlinear relationships.

Note 1: A system is linear when all pharmacokinetic parameters are independent of a xenobiotic's concentration. Linearity is demonstrated by superimposition of the data when normalized for dose. A system is nonlinear when one or more pharmacokinetic parameters changes with the xenobiotic concentration or dose. Lack of linearity is demonstrated by the absence of superimposable data when normalized for dose.

Note 2: Dose-dependent kinetics can be due to saturable processes encountered during any of the ADME parameters, e.g., transport across membranes, protein binding, metabolic enzyme activity, or active excretion pathways. While autoinhibition can likewise cause this effect, autoinduction

is generally observed only with repeated administration or exposure to the xenobiotic.

See: Pharmacokinetics, ADME/ADMET, Autoinduction, and Autoinhibition.³⁸⁻⁴⁰

64. Drug Delivery Formulations

Historically, these have been created by the inclusion of non-active excipients that have little impact on drug metabolism and by encapsulations that enhance the release of the active agents for absorption by specified segments of the gut across specified (slow release) timeframes. The field has now moved on to biocompatible polymeric assemblies, which can provide for delayed release from selected sites of (often implanted) administration and into micro-particle and nanotechnology-derived carriers, which can target specific sites for release of the active agents.⁸⁶ These new types of formulations can have profound effects on drug pharmacokinetics and biotransformations, sometimes even relying on the latter as part of their end-point design strategies, much like prodrugs and soft drugs rely on programmed drug metabolism to control the pharmacokinetic profiles of their active forms.

See: Prodrugs and Soft Drugs.

65. Drug-Drug Interactions

These interactions occur when one drug alters the efficacy, toxicity, or the pharmacokinetic profile observed for another drug, either upon their overlapping administration or from previous administrations of the drug that can cause the alteration.

Note 1: In general, these interactions occur at the pharmacokinetic level, particularly with regard to drug metabolism, and can, in turn, often cause undesirable variations in efficacy and/or toxicity. Because of this, the reclinical testing of candidate compounds typically includes co-administration with other established drugs that are likely to also be present in the intended patient population to *in vitro* models of metabolism, as well as to appropriate *in vivo* models. Criteria are generally then set so that there are no (or only minimal) interactions. Alternatively, these interactions may be intentionally utilized: the use of disulfiram represents such an example of an intentional drug-drug interaction.

Note 2: If an inhibitory mechanism is responsible for the interaction, then the effect can be observed immediately, whereas if a common ADME process is enhanced due to induction, then a prior and often repeated exposure of the altering drug is required.

Note 3: The phrase is sometimes used in a general manner to include cases where a xenobiotic or food component demonstrates similar interactions with a given drug or, more specifically, a food-drug interaction.⁸⁷ A classic example of the latter is the ability of the furanocoumarins present in grapefruit juice to inhibit CYP 3A4, such that the PK half-life may become prolonged for a drug which engages this metabolic pathway, e.g., atorvastatin.⁸⁸

See: ADME/ADMET, Aromatic Hydrocarbon Receptor (AHR), Autoinduction, Autoinhibition, Cocktail Study, Competitive Inhibition, Covalent Binding, Disulfiram Reaction, Dose-Dependent Kinetics or Metabolism, Induction, Inhibition, Phenotype/Phenotyping, Pregnane X Receptor (PXR), and Suicide Inhibitor.^{89,90}

66. Drug-Like Properties or Profile

The range of physicochemical properties and functional groups typically displayed by small-molecule compounds that are being used in the clinic. The profile can be selectively refined depending on the route of administration and the clinical indication, e.g., CNS drugs are generally different from systemic drugs,^{47,48} and oral agents are nearly always different from injectable drugs.⁴ Notably, the concept is often deployed in the reverse manner, since it also delineates chemical groups that should be excluded from compounds if they are to be regarded as being “drug-like.” Groups that are known to cause toxicity are especially likely to be excluded. An example pertinent to drug metabolism would be the exclusion of a 1*H*-imidazole group, which is known to chelate with the iron of the CYPs, causing them to be poisoned and potentially leading to hepatotoxicity.

Note 1: Differing versions of the range of desirable properties and the list of groups to be avoided are often used as criteria that new drug candidates must meet during the early stages of the drug discovery process. Different participants will emphasize different degrees of adherence at different stages of drug development, additionally refining the ranges based on the anticipated progression of molecular changes that may be needed for any given program.

Note 2: The “rule of five” represents a historical landmark for adopting such considerations during drug design and development.

See: Rule of Five.^{1,3,15}

67. Drug Metabolism

The general term for all aspects of the biotransformation of xenobiotics upon interaction with biological systems.

Note 1: Biological systems include isolated enzymes, cellular fractions, intact cells, and tissues and organs, as well as intact organisms and various species, including humans. Aspects include: the structural modifications that xenobiotics and their metabolites undergo; the anabolism, function, homeostasis, induction, inhibition, and catabolism of the enzymes and their prosthetic groups and cofactors involved in metabolic reactions; and, in turn, the effects that xenobiotics and their metabolites exert on the systems.

See: Metabolism, Enzyme, Prosthetic Groups, Cofactors, and Phases of Drug Metabolism.^{2,45,79,80,91-93}

68. EC Number (Enzyme Commission Number)

A distinct identity number assigned to a given enzyme, where the letters “EC” are followed by four sets of digits, separated by periods, that are used to convey classification-related information. The acronym is often taken to mean “enzyme classification number.” Truncated versions of the EC numbers are also sometimes used to convey groups of enzymes according to the same classification system.

Note 1: The first digit conveys a general reaction type from among seven classification groups: 1 Oxidoreductases; 2 Transferases; 3 Hydrolases; 4 Lyases; 5 Isomerases; 6 Ligases; and 7 Translocases. Subsequent digits convey sequentially more specific information about the types of substrates that the enzyme will transform.

Note 2: Presently, newly discovered enzymes are submitted to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB), which meets with the Joint Commission on Biochemical Nomenclature (JCBN) for classification. EC numbers are then assigned by the IUBMB.

See: Enzyme.^{94,95}

69. Elimination

The processes that cause reductions in the concentration of a xenobiotic and its metabolites.

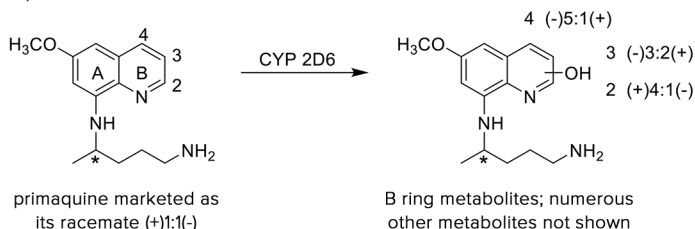
Note 1: Processes include biotransformation reactions, spontaneous degradation, and excretion.

Note 2: This term should not be used for the “E” portion of the “ADME” acronym, wherein “E” is meant to signify only excretion.

See: ADME, Clearance, Excretion, Extraction Ratio, and Phases of Drug Metabolism.^{45,92}

70. Enantioselective and Enantiospecific Metabolism

This biochemical process occurs when a biotransformation proceeds to a greater extent along a preferred stereochemical pathway. “Enantiospecific” further indicates that the preference is exclusive to just one of the enantiomers. The example shown below demonstrates this effect for several of primaquine’s aromatic hydroxylation reactions on its “B ring,” which can occur with varying regiochemistry and multiplicities (di- and tri-hydroxylations are not depicted, nor are the oxidative biotransformations that occur on the “A ring” or *N*-dealkylation followed by reduction of the aldehyde).⁹⁶



Note 1: The inherent stereochemical bias present in all living systems is often manifest during xenobiotic metabolism, and this term is meant to encompass all diastereomeric consequences that can result when a biochemical surface interacts with a xenobiotic having one or more chiral or prochiral centers. Within the context of drug metabolism, this becomes most interesting when enantiomeric species exhibit preferential

biotransformation even when the enzymes are quite promiscuous relative to their array of substrates and to their display of regiochemistry variation for a given substrate (as demonstrated by the example shown above).

Note 2: Based on chiral enzymic discrimination between enantiomeric substrates or between two sites of enantiotopic groups, substrate, and product selectivity are distinguished. An example of substrate selectivity involves the formation of different amounts of single metabolites from two enantiomers of a racemic xenobiotic. An example of product selectivity involves the metabolism of an achiral substrate at a prochiral center where the two types of enantiomeric metabolites are formed in different amounts.

Note 3: Both substrate and product selectivity are common across a wide range of biotransformation reactions. They vary considerably between different species and within humans can impact both a drug's efficacious and toxicological profiles.

See: Substrate Specificity, Regioselective Metabolism, and Stereoselective Metabolism.

71. Endoplasmic Reticulum (ER)

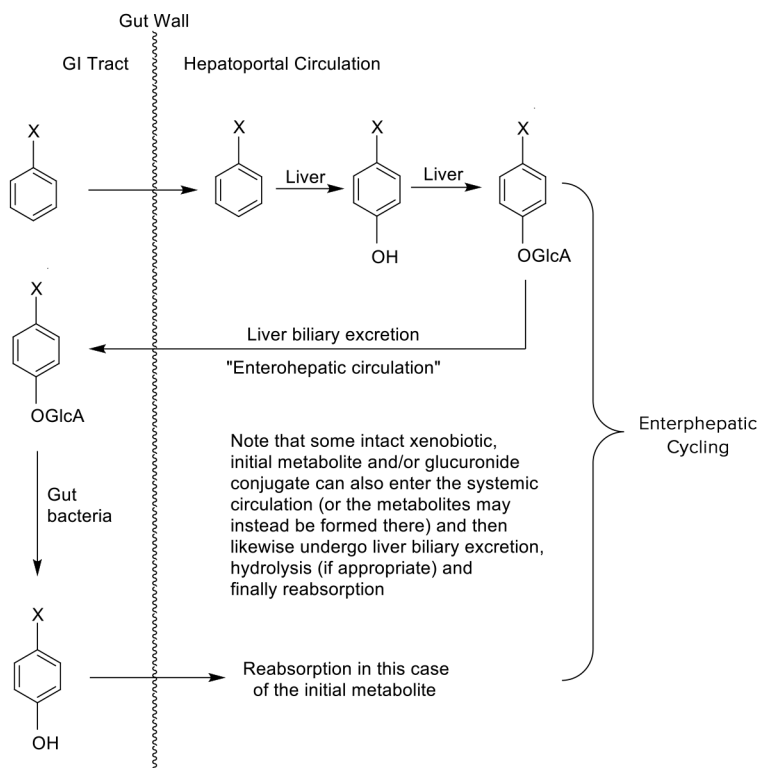
A network of tube-like structures in nearly all eukaryotic cells, called cisternae, that extend throughout the cytosol from the outer nuclear membrane. Ribosomes are attached to certain portions of the ER in clusters, giving them a studded appearance and their further classification as "rough ER," compared to the non-ribosome portions, which are called "smooth ER." The CYP enzymes are intimately associated, anatomically and functionally, with the ER lipid bilayer, while certain of the enzymes responsible for conjugation reactions, such as glucuronide formation, are present within the ER lumen. Since only the smooth ER is noticeably responsive to induction of the CYPs by the barbiturates, it is thought to be more important than the rough ER for xenobiotic metabolism in general.

Note 1: The ER membranes, along with the CYPs and their other associated enzymes, are captured within the microsomal fractions isolated to perform drug metabolism studies in an *in vitro* setting.

See: Cytochrome P450 Enzymes (CYPs), Glucuronic Acid Conjugation, Phases of Drug Metabolism, Microsomes, Microsomal Enzyme, and Hexobarbital Sleeping Time.⁹⁷

72. Enterohepatic Cycling

This physiological process occurs when a xenobiotic is absorbed from the gastrointestinal tract (GI), excreted into the bile, and then reabsorbed from the GI. It can also be applied to the metabolites from a xenobiotic, either directly as such or upon their reversion to either the original xenobiotic or to any of its metabolites after their excretion into the GI via the bile. An example of the latter is portrayed below for a xenobiotic material "X" undergoing: aromatic hydroxylation; conjugation with glucuronic acid (GlcA); biliary excretion; hydrolysis by β -glucuronidase enzymes present in the GI bacterial flora; and finally, reabsorption of the initial metabolite. Note that the involvement of gut bacteria is not a requisite step for enterohepatic cycling, but in this case is needed to remove the glucuronic acid conjugate, since these types of compounds typically have very low absorption and bioavailability.



Note 1: The enterohepatic cycling of drugs can have a major impact on the distribution and PK profile, either directly or indirectly through one or more metabolites. For example, it can increase the systemic exposure to a drug or metabolite even when the hepatic extraction of the drug or metabolite is high. Likewise, if passage through the systemic circulation is a step that precedes liver biliary excretion, then a pulsating increase and decrease in systemic exposure may result after just a single dose of compound. Repeated exposures and prolonged elevated blood levels due to this process are thought to contribute to the toxicity observed for arsenic.⁹⁸

Note 2: Some of the endogenous steroids take advantage of this pathway both in order to influence systemic concentration with time and as a catabolic salvage pathway to recoup larger building blocks for steroid anabolism. Likewise, the biliary acids themselves utilize this cycling pathway while assisting in both the digestion/absorption of nutrients and in the excretion of xenobiotics and the latter's metabolites.

See: Hepatoportal Circulation, First Pass Metabolism, and Extraction Ratio.^{74,79-82,85,91,93}

73. Enzyme

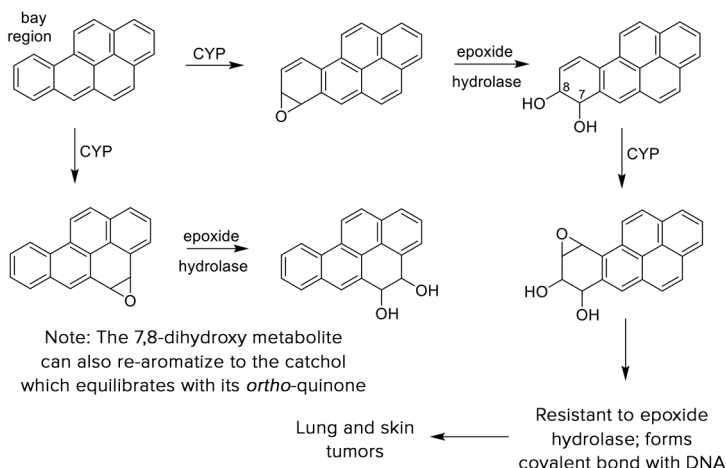
A macromolecule (typically protein) that functions as a biocatalyst (often complexed with cofactors and prosthetic groups) by increasing the reaction rate. In general, a given enzyme catalyzes only one reaction type (reaction selectivity) and operates on only one type of substrate (substrate selectivity), which are transformed at the same site (regioselectivity), where only one chiral substrate or racemate is preferentially transformed (stereoselectivity or enantioselectivity, respectively). However, see note below.

Note 1: Within the context of xenobiotic metabolism, many of the participating enzymes are very promiscuous in terms of their ability to act on a wide range of substrate types and sometimes in terms of their differing action upon a given substrate. An example of the latter is the differing *N*-dealkylations that CYPs can perform on caffeine.

See: Apoenzyme, Haloenzyme, Cofactors, Prosthetic Groups, Enantioselective and Enantiospecific Metabolism, Substrate Specificity, Regioselectivity, Dealkylation, and Demethylation (caffeine *N*-dealkylations).^{62,99}

74. Epoxidation

Oxidation of an aromatic or olefin group within a xenobiotic to form an epoxide (oxirane) metabolite. Epoxidation is typically accomplished by members of the CYP family. These metabolites are often toxic, but can also be non-toxic or even serve to prevent toxicity,¹⁰⁰ depending on the reactivity of the product. The metabolism of benzo[a]pyrene is shown below. It represents a classic example of how the epoxidation of certain aryl systems can lead to toxicity.



Note 1: Within the context of drug metabolism, these biotransformations are typically done by the CYPs. An oxygenated biradical intermediate is thought to form first and rearrange to the epoxide as one of several possible products.

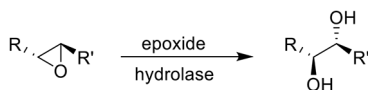
Note 2: For aryl systems, aromaticity is lost: the arene oxide products generally are unstable electrophiles that can undergo ring opening and rearrangement to phenols. In addition, and as shown in the example, epoxide hydrolase can usually convert the reactive epoxides to diols that are less toxic. Conjugation with glutathione likewise serves to detoxify these types of metabolites. The polycyclic hydrocarbons (PAHs) are known for their insidious carcinogenicity because they form epoxides having transient stability while retaining their reactivity, so as to form covalent bonds with distinct biological systems, such as DNA. As shown in the example, epoxides formed in the "bay region" of the PAHs are resistant to detoxification by epoxide hydrolase.

Note 3: Epoxides formed from olefins are usually less reactive and more stable. However, they can also undergo similar intramolecular rearrangement reactions to form ketones or become hydrolyzed or trapped by nucleophiles.

See: Aromatic Hydroxylation, Active Metabolite, Bioactivation, Covalent Binding, Epoxide Hydrolase, and Glutathione Conjugation.

75. Epoxide Hydrolases

A pair of enzymes that add water to three-membered cyclic ethers, forming products that are usually 1,2-diols: microsomal epoxide hydrolase (EC 3.3.2.9) and soluble epoxide hydrolase (EC 3.3.2.10). Examples for arene oxides are shown under the term "Epoxidation." A generalized example for an alkene-derived epoxide is provided below with the specific stereoselectivity also designated.



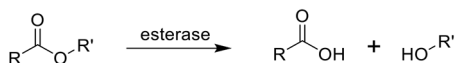
Note 1: These are key participants in the common xenobiotic metabolism process where lipophilic materials are converted to more polar metabolites.

Note 2: Most epoxides demonstrate moderate reactivity, but some are highly reactive, the latter being responsible for the electrophilic insults associated with many known carcinogens, mutagens, and toxins. Thus, along with glutathione, the epoxide hydrolases play an important role in the detoxification of such species.

See: Epoxidation, Active Metabolite, Bioinactivation, and Glutathione Conjugation.¹⁰¹

76. Esterases

A ubiquitous family of enzymes (EC 3.1) that catalyze the hydrolysis of ester-containing xenobiotics into their respective free acids and alcohol components. A generalized example is depicted below.



Note 1: Numerous biomolecules are also processed by these enzymes into active forms or are catabolized into inactive components, the hydrolysis of the neurotransmitter acetylcholine by acetylcholine esterase representing a common example for the latter.

Note 2: The esterases are further subdivided according to their principal types of substrates: carboxyl esterases (CESS; EC 3.1.1) as specifically shown above; thiol esterases (EC 3.1.2); the various phosphatases (ECs 3.1.3, 3.1.4, 3.1.5, 3.1.7 and 3.1.8); sulfatases (EC 3.1.6); the various exonucleases (ECs 3.1.11 to 3.1.16); and the various endonucleases (EC 3.1.21 to 3.1.31), for which this list continues to be refined. However, many of these enzymes exhibit broad and overlapping substrate specificity, such that their distinct classification can be difficult.

Note 3: Amid such overlapping participation during the metabolism of xenobiotics and their different expression levels in various compartments, the carboxyl esterases appear to play the most important roles during drug metabolism. This subdivision is specifically described as a distinct term.

Note 4: Given their ubiquitous and often high levels of distribution as an overall enzymatic class, coupled with their promiscuous and aggressive activity toward a wide array of substrates, it can be generally assumed that if a xenobiotic contains an ester group that is not sterically encumbered, then it will likely become rapidly metabolized at that site at an initial probability that is highest among all of the other biotransformation possibilities.

See: Carboxyl Esterases, Prodrugs, Soft Drugs Rule of One (Metabolism's Rule of One), and Metabolic Probabilities versus Possibilities.^{45,50,51,79,80,93,102}

77. Excretion

Removal of xenobiotics or their metabolites from the systemic circulation by transfer into bile, saliva/nasal fluid, perspiration, or body wastes, such as urine, feces, or exhaled gases. This is the "E" in "ADME."

Note 1: Biliary excretion generally prefers substrates having molecular masses greater than 500 Da, while renal excretion generally prefers those under 500 Da.

See: ADME and Elimination.^{38-40,91}

78. Extensive (or Rapid) Metabolizer

A subpopulation within a given species that metabolizes a xenobiotic via a pathway under polymorphic influence at a significantly higher rate compared to other subpopulations. The slower rate groups, in turn, can be classified as "poor metabolizers." The terms "intermediate" and "ultra-extensive" metabolizers are also sometimes used to provide further delineations.

Note 1: As an example, individual variations in *N*-acetyltransferase (NAT) activity that result in phenotypically "fast" and "slow acetylators" were one of the first pharmacogenetic traits recognized in humans. Similarly, 7 % of the Caucasian population can be regarded as poor metabolizers for drugs that primarily rely upon CYP 2D6.¹⁰³ Due to the potential to cause individual variation in the response to drugs, companies often try to have their lead compound candidates either avoid this pathway altogether, or they try to also engage several alternative pathways for the compound's clearance.

Note 2: Genetic testing has now been firmly adopted as part of the drug selection and development process, as well as part of the process for determining clinical study populations. The growing list of genetic tests already includes CYP 2D6, 2C9, 2C19, and 1A2.

See: Acetylation, Acetylation Phenotype, Aldehyde Dehydrogenase Polymorphism or Deficiency, Atypical Alcohol Dehydrogenase, Genetic Polymorphism, Genotype/Genotyping, *N*-Acetylation, *N*-Acetyltransferases, and Poor Metabolizer.

79. Extraction Ratio

This ratio represents the fraction of a xenobiotic or given metabolite's blood concentration that enters an organ of elimination and is extracted or metabolized by that organ during a single pass. Symbolized as "*E*," the pharmacokinetic (PK) representation for this is shown below:

$$E = (C_{\text{in}} - C_{\text{out}}) / C_{\text{in}}$$

where C_{in} is the concentration in the blood entering on the arterial side and C_{out} is the concentration leaving on the venous side. By definition, *E* is always greater or equal to 0 and less than or equal to 1.

Note 1: It follows that “clearance” by the organ, CL , is proportional to E and the amount of blood flow, Q , according to $CL = Q \times E$. Thus, the clearance of drugs with a high extraction ratio is flow limited, $CL \propto Q$.

$$Q = (C_{in}) \rightarrow \text{Elimination Organ} \rightarrow C_{out}$$

See: Pharmacokinetics (PK), ADME, Elimination and Excretion, First Pass Effect/Metabolism, and Second Pass Metabolism (ψ First Pass Metabolism).^{38-40,104}

80. Extravascular Dosing

Drug administration to any body site other than the systemic blood circulation.

See: Absorption and Intravascular Dosing.^{38-40,85,91,104}

81. First Pass Effect/Metabolism (Pseudo-First Pass Effect)

Strictly intended as the metabolism of a xenobiotic that occurs after its administration or exposure, before it reaches the general systemic circulation and becomes distributed to all tissues. Most frequently taken as the metabolism of a drug that occurs after oral delivery until it passes the liver on route to the heart, thus including the metabolic capability of the gut microflora and emphasizing capabilities in the gut lining, hepatoportal circulation, and liver, while typically excluding the metabolic capabilities present in the bronchopulmonary circulation and lungs (see **Note 2**).

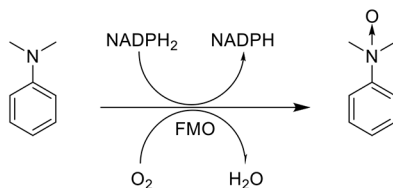
Note 1: For drugs that can serve as good substrates for metabolism, the “first pass effect” can significantly limit oral bioavailability, because the hepatoportal circulation delivers all of its blood flow (100 % Q) to the liver, which is the most aggressive drug “elimination organ” in terms of overall xenobiotic metabolizing capability.

Note 2: Drugs administered intravenously bypass the ‘first pass effect’ as the phrase is most commonly used. However, such drugs initially flow to the heart and through the bronchopulmonary circulation prior to being distributed to the general circulation. Because the lungs are also an aggressive drug metabolizing organ, these cases can then be thought of as undergoing a “pseudo-first pass effect.”

See: Absorption, ADME, Elimination, Extraction Ratio, Bioavailability, and Oral Bioavailability.^{105,106}

82. Flavin Monooxygenase (FMO)

A flavoprotein enzyme (EC 1.14.13.8) that can act, for example, on xenobiotic dialkylarylamines to form *N*-oxides.



Note 1: FMO (*N,N*-dimethylaniline, NADPH₂:oxygen oxidoreductase) is part of the oxidoreductase family (EC 1.14). Its holoenzyme composite serves as a paired donor for the incorporation and reduction of molecular oxygen.

Note 2: Alterations in the gene which transcribes to FMO3, an important liver version that metabolizes trimethylamine to the *N*-oxide, is suspected to be the cause of several types of trimethylaminuria, which is a metabolic disorder where individuals excrete a fishy body odor in their urine, sweat, breath, and other body excretions.

See: Holoenzyme, and *N*-Oxidation.^{107,108}

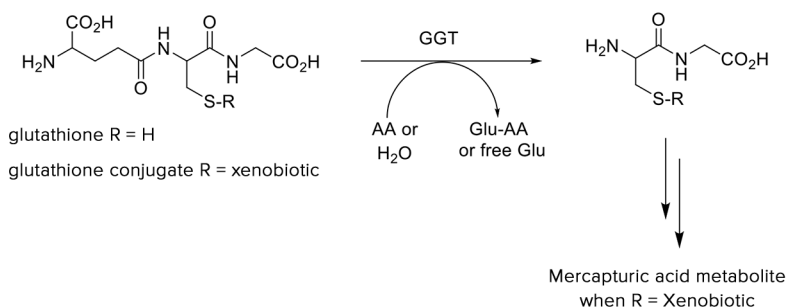
83. Futile Metabolism

Initial metabolic events that are ineffective because they rapidly revert back to the parent compound by non-enzymatic, chemical stability-driven mechanisms. Although difficult to detect, one example is the first, one-electron reduction step for nitro-imidazoles that generates an extremely reactive radical anion which, under well-oxygenated conditions, is rapidly oxidized back to the parent compound, leaving no intermediate for the second step of the overall pathway.¹⁰⁹ These events are different from "reversible metabolism."

See: Reversible Metabolism.

84. Gamma-Glutamyl Transpeptidase (γ -Glutamyltransferase; GGT)

Distinct from the acyltransferases (EC 2.3.1), this family of transpeptidases (EC 2.3.2) transfers specific aminoacyl functionality from a peptide to another peptide, amino acid, or water, the latter liberating the aminoacyl group as its free amino acid. The family member most significant to drug metabolism is GGT (EC 2.3.2.2), which transfers a glutam-5-yl moiety that is important for: (i) the "gamma-glutamyl cycle" and its role in the synthesis and degradation of glutathione; and, (ii) the initial step in the secondary processing of xenobiotic-glutathione-conjugates eventually leading to 'mercapturic acid' metabolites. GGT's degradative step involving glutathione is shown below for the parent compound (R = H) as part of the gamma-glutamyl cycle, and for a xenobiotic conjugate (R = X).



Note 1: The family is ubiquitous in humans, with GGT having especially high levels in the renal tubules, liver, and pancreas.

Note 2: The level of these enzymes can be elevated by a variety of diseases and especially so for liver disease and hepatic biliary obstructions. GGT is particularly sensitive to alcohol. Levels can be elevated from moderate intake as well as chronic alcoholism, even without clinical evidence of hepatic injury.

See: Acylation, Acyltransferases, Amino Acid Conjugation, Glutathione Conjugation, and Mercapturic Acid Metabolites.¹¹⁰⁻¹¹³

85. Genetic Polymorphism

A trait determined by a single gene that results in at least two phenotypes within a normal population, neither of which is rare, i.e., they are observed

for larger than 1 %. It is an important contributor to the large inter-individual variations that exist in the biotransformation of xenobiotics by humans, where different subpopulations metabolize the same compound with significantly different rates because of genetic polymorphism among at least one of the enzymes contributing to the associated metabolic pathway.

Note 1: Individuals can be classified as either “extensive” (rapid) or “poor (slow) “metabolizers” on the basis of phenotypic differences, assessed by the amount of drug or metabolic standard compound that is excreted through any given metabolic pathway that is subject to genetic polymorphism.

Note 2: In addition to classifying the populations recruited for clinical studies, it is important to study species during preclinical and even early-stage drug development that are most representative for the anticipated metabolic pathways, including the latter’s potential variation. Given such complexity during development and the variation that can still result when approved drugs eventually become available to the general public, it is a common strategy not to advance candidate compounds through development that are suspected to become subject to metabolic pathways known to have genetic polymorphism that can be difficult to characterize for an individual within the population at large. CYP 2D6 is one example of such a metabolic pathway to try to avoid.

Note 3: Historically, the first genetic polymorphism described was for the *N*-acetylation of isoniazide. In this case, the slow acetylator phenotype is homozygous for the mutant allele. Early genetic polymorphisms pertaining to the common oxidative pathways were uncovered by the phenotypic differences observed for debrisoquine and mephenytoin metabolism.

See: Genotype/Genotyping, Phenotype/Phenotyping, Acetylation, Acetylation Phenotype, Alcohol Dehydrogenase (ADH), Atypical Alcohol Dehydrogenase, Aldehyde Dehydrogenase (ALDH), Aldehyde Dehydrogenase Polymorphism or Deficiency, Cytochrome P-450 Enzymes (CYPs), Drug-Like Properties or Profile, Extensive (or Rapid) Metabolizer, Poor (or Slow) Metabolizer, Cocktail Study, Single Nucleotide Polymorphism (SNP), and ADME. Specific Enzyme Refs.: 103,114-129.

86. Genotype/Genotyping

The term “genotype” can take on various meanings in different contexts, applicable to different aspects of xenobiotic metabolism considerations. For example, starting from the more global level and proceeding to a more localized context: (i) the type species of a genus; (ii) the entire genetic constitution of an individual; and (iii) the alleles present at one or more distinct gene loci. Genotyping, within the specific context of human disease and drug therapy, is the process of reading selected components of the sequence in selected strands of DNA in order to understand how mutations influence the onset, progression, and treatment of some diseases, including an individual’s drug metabolism profile.

Note 1: Because the genotypes responsible for the observed phenotypes associated with certain xenobiotic metabolism events have become very well established, it is possible to do some metabolic “genotyping” in an indirect manner for an individual by assessing the phenotypic response to administered standard probe compounds.

See: Genetic Polymorphism, Phenotype/Phenotyping, Cocktail Study, and ADME. Specific Enzyme Refs.: 103,114-129.

87. Glucocorticoid Responsive Element (GRE)

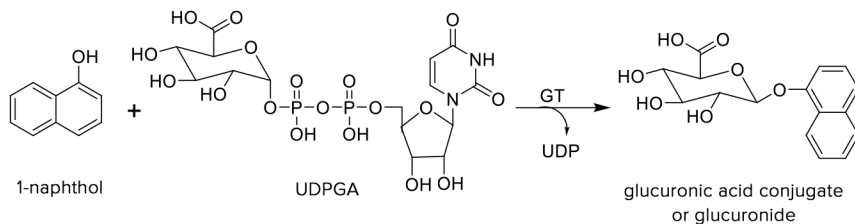
Short DNA sequence within the regulatory regions of genes that are recognized by the activated glucocorticoid receptor and provide specificity to induction of gene transcription by glucocorticoids. This control mechanism, in turn, impacts the status/phenotype for various components of the overall biotransformation machinery associated with the metabolism of xenobiotics, as well as several endogenous processes.

See: Aromatic Hydrocarbon Receptor (AHR), Peroxisome Proliferator Activator Receptor (PPAR), Pregnane X Receptor (PXR), and Xenobiotic Responsive Element (XRE).³⁵

88. Glucuronic Acid Conjugation (Glucuronidation)

A biotransformation where a substrate having a nucleophilic group is coupled to endogenous glucuronic acid. The latter is donated by uridine-5'-diphospho- α -D-glucuronic acid (UDPGA) serving as a cofactor, while the S_N2 -like coupling is enzymatically catalyzed via glucuronosyltransferase

(GT; EC 2.4.1.17). In addition to endogenous compounds, such as estrogen, xenobiotics having hydroxyl, sulfanyl, carboxylic acid, or primary amino groups can serve as substrates, with phenolic hydroxyl groups being particularly prone to this metabolic pathway. The latter is exemplified below for 1-naphthol.



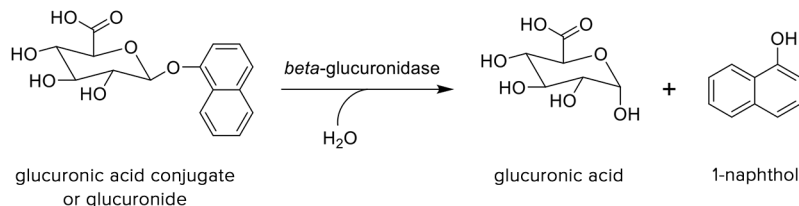
Note 1: This is a major pathway for the biotransformation of phenolic compounds. Because GT is abundantly expressed in both the gut wall and liver, as well as other locations, xenobiotics having such functionality are typically subject to a high clearance rate during first pass metabolism and thus have very poor bioavailability after oral administration or ingestion. GT's predominant intracellular location is within the lumen of the endoplasmic reticulum, where it resides in close proximity to the CYPs (see discussion in the Introduction section pertaining to the phases of drug metabolism).

Note 2: Both enzyme polymorphism and differing levels of the cellular UDPGA pools can lead to individual variation, but the human population on average remains well-equipped to accomplish this biotransformation and no significantly varying phenotypes have risen to merit further classification. Alternatively, in any individual case, the sustained exposure of this pathway to a substrate can lead to localized depletion of UDPGA and result in a shift to another, closely related conjugation pathway, also present in a significant functional capacity, namely that of sulfate conjugation or sulfation. The latter occurs on the same types of nucleophilic groups and is actually a competitive pathway. Although glucuronidation typically predominates, in some cases sulfation is the preferred pathway. Among mammalian models, the cat is distinct in its higher utilization of the sulfation pathway compared to glucuronidation.

See: Uridine-diphospho-glucuronic Acid (UDPGA), Glucuronosyl Transferase (GT), Conjugation Reactions, Phases of Drug Metabolism, Aromatic Hydroxylation, Glucuronide, Glucuronidase (β -Glucuronidase), and Sulfate Conjugation (Sulfation).^{45,130,131}

89. Glucuronidase (β -Glucuronidase)

This enzyme (EC 3.2.1.31) can catalyze the hydrolysis of glucuronic acid conjugates of xenobiotics, as well as endogenous materials, such as the mucopolysaccharides. A xenobiotic example is depicted below for the glucuronide of 1-naphthol.



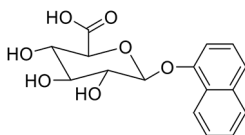
Note 1: Found in lysosomes, this enzyme is important for the catabolism of several endogenous materials. An inherited deficiency can lead to toxic accumulation of mucopolysaccharides, known as "Sly syndrome."

Note 2: Its presence in the gut wall and bacterial flora of the human GI tract can lead to enterohepatic cycling of glucuronide metabolites by reverting them back to their aglycone precursors, which can be reabsorbed. Repetition of this cycle can significantly prolong the residence time (half-life) of the aglycone in the body. This pathway may also be an energy conserving pathway that serves to recycle certain endogenous steroid hormones, rather than to rely only on their re-syntheses.

See: Glucuronic Acid Conjugation (Glucuronidation), Glucuronide, and Enterohepatic Cycling. Chemistry Ref.: 132.

90. Glucuronide

A common name for the metabolite derived from the conjugation of glucuronic acid to a xenobiotic. The term *beta-* or β -glucuronide is synonymous and is also frequently used. The β -glucuronide of 1-naphthol is depicted below.



Note 1: The original α -configuration of UDPGA, which serves as the donor for glucuronic acid, is inverted to a β -configuration for the latter during its S_N2 conjugation reaction with the xenobiotic.

Note 2: Glucuronides have an increase in molecular mass of about 175 Da, which, depending on the mass of the initial substrate, can result in relatively more biliary excretion compared to renal excretion, since the latter tends to prefer substrates having molecular masses below 500 Da, whereas the liver route continues to process compounds having masses above 500 Da.

Note 3: Glucuronides generally have a significant increase in polarity due to the additional hydroxyl groups, with the free carboxylic acid moiety, especially, being largely ionized at physiological pH. The acid moiety can also serve to enhance the suitability for excretion via the organic anion transporter (OAT) family. The OATs are particularly active in the liver, where they can transport such substrates into the bile.

Note 4: Glucuronides generally are inactive or have significantly reduced activity, although certain morphine conjugates represent an important exception. In addition, they are sometimes subject to hydrolysis by β -glucuronidase, which, in turn, may restore activity to the non-conjugated xenobiotic.

See: Glucuronide Conjugation (Glucuronidation), Uridine-diphosphoglucuronic Acid (UDPGA), Uridine-diphosphoglucuronosyl Transferase (UGT), Conjugation Reactions, Phases of Drug Metabolism, Excretion, Glucuronidase (β -Glucuronidase), and Enterohepatic Cycling.^{45,130,131}

91. Glucuronosyltransferase (GT) (Previously Uridinediphosphoglucuronosyl-transferase and UGT or UDPGT)

A family of enzymes (EC 2.4.1.17) that catalyze the conjugation of the glucuronic acid component of uridinediphosphoglucuronic acid to an appropriate nucleophilic functional group on small-molecule substrates.

Note 1: The GTs are 50 to 60 kDa integral membrane proteins where ca. 15 to 20 of the C-terminal amino acids form a transmembrane span of the endoplasmic reticulum (ER) lipid bilayer, with the remainder of the enzyme, including its catalytic domain, located within the lumen of the ER.

Note 2: These specific transferase enzymes, and their requisite glucuronic acid pools, are anatomically situated close to the many CYPs also residing along the ER, such that they seemingly can join in sequential metabolic reactions as part of a two-step enzymatic cascade involving a phase 1 and phase 2 biotransformation.

Note 3: About 25 human GT genes have been identified and divided into two families, based on at least 50 % homology of their amino acid sequences: TG 1 and TG 2. The GT 1A and 2B subfamilies appear to be the most important for drug metabolism, with several of their isoforms being highly expressed in the liver: GT 1A1, 1A3, 1A4, 1A6, 1A9 and UGT 2B4, 2B7, 2B10, 2B11 and 2B15.

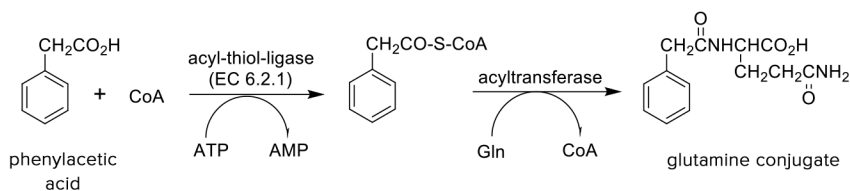
Note 4: Maturation of the GT pathways can lag during early life: neonates with underdeveloped GTs exhibit decreased clearance of bilirubin, a fairly common syndrome that can be alleviated by exposure to fluorescent light until enzymatic maturation normalizes, generally within just a few days.

Note 5: Several other inter-individual variations in GT expression levels have also been identified. At least six polymorphisms are known: GT 1A1, 1A6, 1A7, 2B4, 2B7 and 2B15. In addition, certain cancer cells can enhance their expression of the UGTs, thus promoting a phenotype with greater resistance to treatment by certain chemotherapeutic agents.

See: Uridinediphosphoglucuronic Acid (UDPGA), Conjugation Reactions, Phases of Drug Metabolism, Aromatic Hydroxylation, Glucuronic Acid Conjugation (Glucuronidation), Glucuronide, Glucuronidase (β -Glucuronidase), Sulfate Conjugation (Sulfation), Genetic Polymorphism, Genotype/Genotyping, and Phenotype/Phenotyping.^{45,63,66,130,131}

92. Glutamine Conjugation

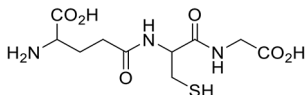
Conjugation of a xenobiotic's carboxylic acid group with endogenous glutamine according to the acylation pathway, where the acid moiety is first activated by attachment to coenzyme A. It is most applicable to xenobiotics having an aryl-acetic acid group (shown below), while glycine more generally serves as the endogenous partner across a much wider range of acid-containing compounds.



See: Acylation, Acyltransferase, Acetylation, Amino Acid Conjugation, Glycine Conjugation, Hippuric Acid Conjugate (Hippurate), and Serine Conjugation.¹³

93. Glutathione (GSH)

An endogenous tripeptide consisting of γ -Glu, Cys and Gly that serves as the major antioxidant produced by cells. It is also an important partner in biotransformation reactions catalyzed by glutathione transferase (EC 2.5.1.18) that can conjugate xenobiotics and metabolites, which contain potentially toxic electrophilic centers. Because of the latter, it is often referred to as “the body’s defensive nucleophile.” The structure of GSH is shown below in its reduced form.

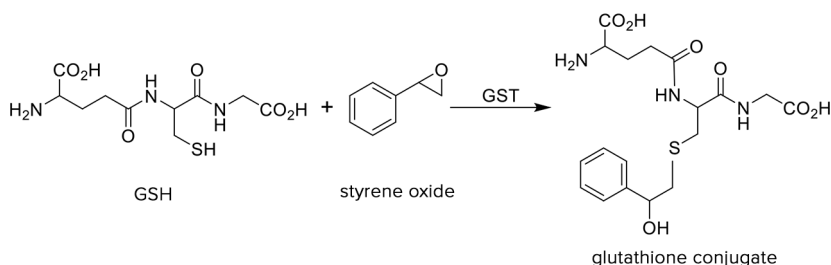


Note 1: GSH exists largely in the reduced form in cells (ca. 90 %), but is in equilibrium with its oxidized form (ca. 10 %), which is the disulfide linked dimer or “GSSG.” Higher levels of GSSG are indicative of oxidative stress.

See: Glutathione Conjugation, Glutathione Transferase, Cysteine Conjugate β -Lyase (C-S Lyase), Mercapturate/Mercapturic Acid Conjugation, and Phases of Drug Metabolism.^{45,133-138}

94. Glutathione Conjugation

A biotransformation where a xenobiotic substrate having a reactive electrophilic center is coupled to endogenous glutathione (γ -Glu-Cys-Gly or GSH). The coupling is catalyzed by a family of predominantly liver cytosolic enzymes called the glutathione transferases (GSTs; EC 2.5.1.18). The example below depicts the reactive xenobiotic styrene oxide undergoing bioinactivation due to conjugation by GSH.



Note 1: Serving as a “defensive” pathway to inactivate xenobiotics having electrophilic centers that might otherwise prove toxic, this biotransformation is applicable to a broad range of substrates, including: alkyl-, alkenyl-, aryl- and aralkyl-halides; carbons having sulfate or nitro leaving groups; small ring systems such as epoxides and β -lactones; and activated β -carbons of α,β -unsaturated carbonyl compounds (Michael acceptors).

Note 2: The initial GSH adduct has an increase in molecular mass of 307 Da, which, in turn, often enhances biliary excretion, since the latter tends to prefer substrates having molecular masses above 500 Da, whereas the renal route prefers molecular masses below 500 Da. However, these initial adducts frequently become subject to further metabolic processing by a specific pathway that sequentially removes the Glu and Gly and then acetylates the remaining Cys amino-group. The conjugate resulting from this catabolic process is called a “mercapturic acid” metabolite, and its molecular mass is only 162 Da. Not too dissimilar from the mass gained when a xenobiotic forms a conjugate with glucuronic acid (i.e., 170), this smaller increase leaves either the renal or the biliary route as viable options for excretion, depending on the mass of the initial xenobiotic compound.

See: Glutathione (GSH), Glutathione Transferase (GST), Bioinactivation, Catabolism, Mercapturate/Mercapturic Acid Conjugation, Cysteine Conjugate β -Lyase (C-S Lyase), ADME; ADMET, and Excretion.^{45,133-138}

95. Glutathione Transferase (GST)

A family of enzymes (EC 2.5.1.18) that catalyze the conjugation of endogenous glutathione (GSH) with xenobiotics having reactive electrophilic centers that could otherwise cause toxicity.

Note 1: Most GSTs are cytosolic homo- or heterodimers composed of ca. 25 kDa subunits from one of four major protein structural classes called *Alpha*,

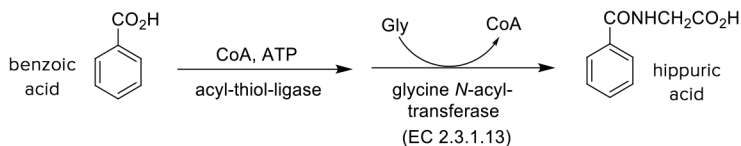
Mu, *Pi*, or *Theta*. There are multiple isoforms and allelic variants. Their nomenclature has adopted some of this structural description, e.g., GST A1, GST M1, etc.

Note 2: GST A1 is found in the kidney, intestine, lung, and liver. GST M1 is found mainly in the liver. GST P1 is widely distributed, except for in the liver, and is the most abundant in many types of tumor cells. A null GST M1 genotype has been identified, and these individuals may be more susceptible to lung cancer.

See: Glutathione (GSH), Glutathione Conjugation, Bioinactivation, and Mercapturic Acid.^{45,133-138}

96. Glycine Conjugation

Conjugation of a xenobiotic's carboxylic acid group with endogenous glycine according to the acylation pathway where the acid moiety is first activated by attachment to coenzyme A. This biotransformation is used to test human liver function by administering benzoic acid, which is converted to hippuric acid (exemplified below).



See: Acylation, Amino Acid Conjugation, Acyltransferase, Hippuric Acid Conjugate (Hippurate), Glutamine Conjugation, and Serine Conjugation.¹³

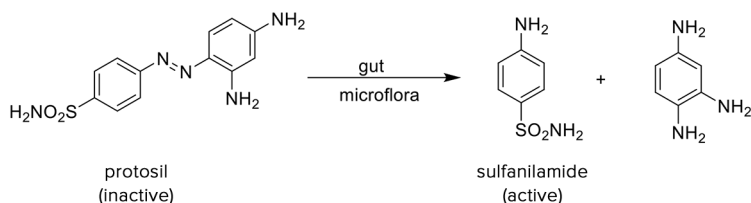
97. Glycosylation (or Glycosidation)

Enzymatic conjugation of an endogenous sugar with a substrate having an OH, NH, or SH group. While glucose is the most common partner for endogenous substrates, glucuronic acid is by far the most important partner for xenobiotic substrates. The latter is typically referred to by the more specific term "glucuronidation."

See: AGlucuronide and Glucuronic Acid Conjugation (Glucuronidation). Therapeutic Ref.: 139.

98. Gut Microflora

The community of mutualistic microorganisms living within the digestive tract of animals. They can impact drug metabolism in humans after oral administration, particularly by performing anaerobic biotransformations that are less prevalent among the various human pathways. An example would be the reduction of various types of double bonds. The sulfonamide antibiotics were discovered as a result of this type of microbial biotransformation (shown below) after oral administration of the azo dye protosil, which became recognized as an effective antibiotic prodrug.



Note 1: In some cases, the gut microflora can hydrolyze glucuronide conjugates excreted via the bile. When the parent compound is liberated, this can lead to enterohepatic cycling.

Note 2: Oral administration of antibiotics leads to an alteration of the various individual populations of bacteria within the overall gut microflora, which, in turn, can lead to altered metabolic profiles and clearance of other orally administered drugs and ingested xenobiotics.

See: Drug Metabolism, First Pass Metabolism, Bioactivation, Prodrug, Glucuronide, Glucuronic Acid Conjugation (Glucuronidation), and Enterohepatic Cycling. Therapeutic Ref.: 140.

99. Half-Life ($t_{1/2}$)

Used in xenobiotics in general, the time required to decrease the concentration of the solute of interest by one-half from a specified matrix. Most typically used in relation to the elimination of a pharmaceutical agent, in which case it is called the "elimination half-life" and refers to the time it takes for 50 % of the agent to be cleared from the body by metabolism and/or excretion. Half-life values are only constant (always the same and independent of the concentration and dose) for linear kinetic processes.

The half-life for any linear kinetic process can be related to a corresponding rate constant (k) by the natural logarithm ($\ln$) relationship:

$$t_{1/2} = \ln(2) / k \approx 0.693 / k$$

Note 1: Matrices can range in complexity from a whole animal, as indicated above for the elimination half-life, to various compartments within or taken from a whole animal, as well as to specified cells or *in vitro* systems, including simple aqueous media, where decreasing concentrations of the solute can still result from spontaneous decomposition or precipitation.

Note 2: For pharmacokinetic studies (PK analyses), the term can be applied to any such PK processes, e.g., in addition to the elimination half-life, absorption and distribution half-lives can also be defined. When compartmental or non-compartmental PK models have several exponential terms, the half-life value that is generally of the most interest and most frequently reported is called the “terminal half-life” or “terminal elimination half-life,” which can be derived from the exponential term with the smallest rate constant and hence the longest half-life. As such, it then constitutes the slowest and final removal process.

Note 3: It is also possible to cite an “effect” or “pharmacodynamic half-life” where no measurement of the xenobiotic’s concentration has been obtained.

See: Disposition, Distribution, Clearance, Pharmacokinetics, Compartment Model, Non-Compartment Model, and Pharmacodynamics.^{38-40,104}

100. Hepatic Clearance/Extraction

The ability of the liver to remove substances from the blood. Clearance is dependent on hepatic blood flow in terms of percentage (100 % directed by the hepatoportal circulation; 25 % of the cardiac output/systemic circulation) and rate, hepatic uptake of the substance by passive and active transport versus efflux by active transport, excretion of the substance into the bile, and the liver’s rich complement of metabolizing processes.

See: Hepatoportal Circulation, Bioavailability, Clearance (CL), Excretion, Drug Metabolism, and Extraction Ratio.^{38-40,104}

101. Hepatocytes

The predominant cell type in the liver, taking up about 90 % of this organ's volume. Their primary function is associated with the intermediary metabolism of food-related nutrients and endogenous materials and in the biotransformation and clearance of xenobiotic substances.

Note 1: These cells, and thus the liver, are the richest source of the overall CYP family members expressed in humans. They also have high levels of the metabolic components associated with glucuronidation.

Note 2: Hepatocytes from various mammalian species are often harvested, in a form called the microsomal fraction or microsomes, for use in conducting *in vitro* drug metabolism studies.

See: Hepatoportal Circulation, Hepatic Clearance/Extraction, Bioavailability, Drug Metabolism, First Pass Metabolism, Bioinactivation, Bioactivation, Cytochrome *P*-450 Enzymes (CYPs), Conjugation Reactions, Glucuronic Acid Conjugation (Glucuronidation), and Microsomes.¹⁴¹⁻¹⁴³

102. Hepatoportal Circulation

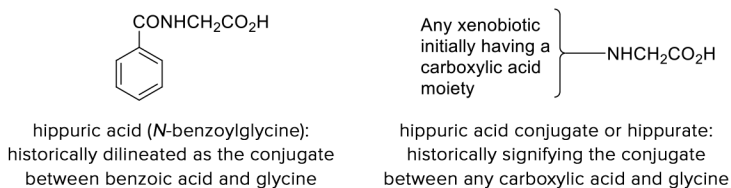
The vasculature system that provides venous drainage from the capillaries in the spleen, stomach, small and large intestines, pancreas, and gallbladder and delivers all of its flow to the liver. This localized uptake and delivery forces all materials absorbed after oral ingestion (food and xenobiotics) to enter liver hepatocytes, where they have the potential to be metabolized, before flowing to the heart/lungs and into the general circulation.

See: Absorption, Hepatic Clearance/Extraction, Bioavailability, Hepatocytes, Drug Metabolism, First Pass Metabolism, Bioinactivation, Bioactivation, and Enterohepatic Cycling.¹⁴⁴

103. Hippuric Acid Conjugate (Hippurate)

Historically, this phrase has been used to designate the metabolite obtained from the conjugation of a xenobiotic having a carboxylic acid moiety, with a glycine residue via an acylation type of biotransformation. This process was first described for benzoic acid, which forms actual "hippuric acid,"¹² and the phrase was then extended to include any glycine conjugate with a

carboxylic acid group that is present on a xenobiotic. Today, this phrase is instead falling in usage, and it is no longer officially recognized by IUPAC. The pathway is itself part of the even broader classification noted as amino acid conjugation. Regarded as the first biotransformation discovered, this "first metabolite" is depicted below, along with how the term has historically been used more broadly in practice.



Note 1: The molecular mass of the initial carboxyl-containing xenobiotic will be increased by 57 Da, while the glycine residue adds a new carboxylic acid group, thus retaining the potential to be excreted by the organic anion transporter (OAT), which is rich in the liver and is directed toward bile formation.

See: Conjugation Reactions, Acylation, Amino Acid Conjugations, Glycine Conjugation, and Serine Conjugation.¹³

104. Holoenzyme

The complete and generally functional complex of an enzyme plus all of its requisite prosthetic groups and cofactors, assuming a source of energy is likewise available.

Note 1: By definition, this complex will be active in its native environment. However, since it is additionally necessary to adopt a functional conformation within an exogenous setting, the complex may not be active in an *in vitro* matrix. Membrane-bound enzymes and transporters are particularly subject to this situation.

Note 2: Alternatively, it is also possible to achieve "functional" activity, even in an *in vitro* setting, using specifically modified and truncated protein forms of the enzyme, as long as the key residues of the active site are preserved, appropriate conformational arrangements remain accessible, and all of its requisite prosthetic groups, cofactors, and an energy supply

are provided. This strategy is often deployed to render the enzymes more amenable toward crystallization followed by X-ray diffraction studies.

See: Enzyme, Apoenzyme, Cofactor, and Prosthetic Group.³⁴

105. Hydrolases

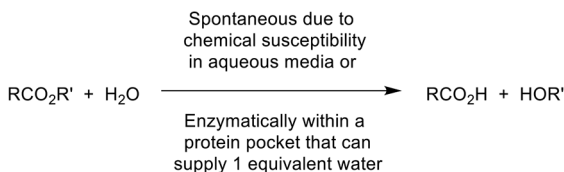
Within the context of drug metabolism, this term generally refers to hydrolysis enzymes that specifically catalyze the *trans*-addition of water to alkene epoxides and arene oxides, e.g., the microsomal and soluble epoxide hydrolases (EC 3.3.2.9 and EC 3.3.2.10). Epoxides and arene oxides are sometimes formed during CYP-mediated oxidation of aliphatic alkenes and aromatic hydrocarbons, respectively. Because they can be reactive, the hydrolases thus serve as important detoxification enzymes.

Note 1: In mammals, two major forms of epoxide hydrolase (EH) handle the majority of xenobiotic-related biotransformations, one being membrane-bound (microsomal), mEH, and the other present in the soluble fraction, sEH.

See: Hydrolysis, Epoxidation, Epoxide Hydrolases, and Detoxification.¹⁰¹

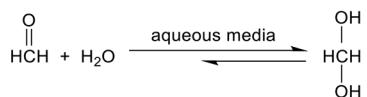
106. Hydrolysis

A reaction involving the addition of water to a functional group so as to permanently form one or more new species prompted by either spontaneous chemical behavior or an enzymatically catalyzed biotransformation. The case of a simple ester hydrolysis is shown below in a general manner where two new compounds are produced.



Note 1: Within the context of xenobiotic metabolism (but not necessarily that of chemical terminology), this definition becomes distinguishable from "hydration," which designates the formation of new species in rapid chemical equilibrium with water, e.g., hydrogen-bonded alcohols and

amines in an aqueous media, and likewise for even the gem-diol forms of hydrated ketones or aldehydes, as shown below for formaldehyde, whose equilibrium favors the "water-added" species.¹⁴⁵



Note 2: Common metabolizing enzymes that catalyze the hydrolysis of both endogenous and xenobiotic compounds include: peptidases; carboxylesterases, such as the cholinesterases and pseudocholinesterases, organophosphatases; and epoxide hydrolases.

See: Hydrolases, Epoxide Hydrolase, Esterases, Carboxyl Esterases, Carboxypeptidases, and Biotransformation.⁵¹

107. Hydroxylation

A biotransformation where a hydrogen atom on a xenobiotic is replaced by a hydroxyl-group. The most common type occurs on an aryl system, namely "aromatic hydroxylation."

See: Aromatic Hydroxylation.³⁷

108. Inducing Agent

A substance that causes enzyme induction and an enhancement of metabolizing activity. For xenobiotics, this is most often observed following repeated exposure to such a substance.

See: Induction and Autoinduction.¹⁴⁶⁻¹⁴⁸

109. Induction

An increase in enzyme activity above its normal, homeostatic level. Induction can result from increased expression of the enzyme or proenzyme, increased conversion of a proenzyme, increased liberation from a storage complex, decreased inactivation by negative control systems, such as the kinases, or decreased catabolism of the enzyme. In cases where levels of a cofactor or prosthetic group become the rate limiting factor for a holoenzyme's activity, their induction by any of these same mechanisms can likewise result in enzymatic induction. The most common mechanism

associated with the metabolism of xenobiotics is increased expression of the active enzyme.

Note 1: Mutations that enhance activity can occur, and these are then typically also referred to as having been “induced” when prompted by a xenobiotic. While prominent in microorganisms for both induction and inhibition, this type of induction is not a common occurrence within the mammalian drug metabolizing enzymes.

See: Inducing Agent, Autoinduction, Enzyme, Apoenzyme, Holoenzyme, Cofactor, Prosthetic Group, Ah Receptor, Xenobiotic Response Element (XRE), Pregnane X Receptor (PXR), Anabolism, Catabolism, Drug-Drug Interactions, and Phenobarbital Sleeping Time.¹⁴⁶⁻¹⁴⁸

110. Inhibition

A reduction in enzyme activity below its normal, homeostatic level relative to a given substrate that results from the interaction of the enzyme with an inhibiting substance. For xenobiotics, this can be observed upon the first exposure to substances capable of inhibiting the system.

Note 1: In some cases, the substance may be the substrate itself or a substrate’s enzymatic product; these events are also referred to as “autoinhibition” or “product inhibition,” respectively.

Note 2: The inhibiting substance may interact with the enzymes’ active (functional) site in either a competitive (reversible binding and also itself serving as a substrate) or non-competitive (typically covalent binding and itself not serving as a substrate) manner. Alternatively, the inhibiting substance may interact with a regulatory site on the enzyme, in which case the event is also referred to as “allosteric inhibition.”

Note 3: Exogenous inhibitors can be present in dietary materials, as well as drugs. The furanocoumarins present in grapefruit juice represent a classical example for inhibition of CYP 3A4.¹⁴⁹

Note 4: In addition to these drug metabolism related comments, it can be mentioned in a very general manner in relation to drug discovery aimed at enzymatic targets or specific receptor systems that it is typically far easier to

inhibit a given enzyme or receptor than it is to design a superior substrate or agonist for that system.

See: Autoinhibition, Competitive Inhibition, Noncompetitive Inhibition, Substrate Inhibitor, Mechanism-Based inhibitor, and Suicide Inhibitor. Drug Metabolism Related Refs.: 146-148.

111. Intraperitoneal Dosing

Administration of a pharmaceutical agent into the body cavity (peritoneum) surrounding the lower stomach/upper GI tract, such that its absorption is dependent on the hepatoportal circulation but is not dependent on "oral absorption" across the lining of the stomach and intestines. By definition, this venous return circulation proceeds to the liver, thus still retaining this particular component of the "first pass effect."

Note 1: While intraperitoneal ("IP") dosing is not a common route in humans, it is frequently deployed when testing substances during *in vivo* studies in small animals to eliminate some of the variables associated with oral bioavailability.

See: Absorption, Hepatic Clearance/Extraction, Bioavailability, Drug Metabolism, First Pass Metabolism, Bioinactivation, Bioactivation, and Intravascular Dosing.¹⁴⁴

112. Intravascular Dosing

Administration of a pharmaceutical agent directly into the systemic blood circulation, intravenously (commonly abbreviated as "IV") utilizing the venous return to the heart or intra-arterially utilizing arteries delivering blood to the body. By definition, there is no "absorption" step when a drug is given in this manner.

Note 1: Intravenous administration is a common route for agents where: (i) an immediate drug effect is needed, such as in emergency or critical care situations; (ii) the drug has poor or erratic oral bioavailability, and particularly so when it does not need to be frequently taken in a repetitive manner for prolonged periods; or (iii) the drug can be highly toxic to normal cells, as is the case for certain chemotherapeutic agents, particularly several of the anticancer drugs.

Note 2: For the purposes of PK studies, the timeframe for absorption can either be dropped or taken as instantaneous when devising mathematical models.

Note 3: While this route eliminates the entire “first pass effect” by bypassing the GI tract, its membrane linings, and the liver, the IV route will still pass through the bronchopulmonary circulation prior to arterial delivery to the entire systemic blood circulation. Since the lungs represent a significant source of drug metabolizing capability and provide a “second pass effect” after oral absorption of xenobiotics, this type of passage after IV administration can be thought of as a “pseudo first pass effect” where the pharmaceutical agents are instead first exposed to the lungs.

See: Absorption, Extravascular Dosing, Bioavailability, First Pass Effect, and Pharmacokinetics (PK).⁹¹

113. Intrinsic Clearance

The relationship between a xenobiotic's rate of metabolism and its concentration at the enzyme site.

Note 1: In biochemical terms, it is derived from the ratio of the Michaelis-Menten parameters for maximum rate of metabolism ($V_{\max}$) and the drug interaction constant (K_m).

Note 2: Conceptually important, in that no matter how fast a xenobiotic may be determined to be metabolized during an *in vitro* study involving its incubation with selected enzymes, it may undergo little metabolism and essentially no intrinsic clearance by those same enzymes unless it also becomes exposed to them in adequate concentration and time periods *in vivo*. Alternatively, if the *in vitro* study demonstrates essentially no intrinsic clearance of the xenobiotic by a given enzyme, then it becomes unlikely that this particular biotransformation will take place no matter how high and long the exposure of the xenobiotic to that same enzyme *in vivo* (assuming no other enzymes are able to cause the identical biotransformation).

See: Clearance, Michaelis-Menten Kinetics, and ADME.^{38-40,45,104}

114. Isoform or Isozyme (Isoenzyme)

A member of an enzyme family that shares comparable catalytic sites, prosthetic groups, cofactors, and reaction mechanism but where the isoforms exhibit different substrate specificities or reaction rates due to genetically determined differences in the primary structure of their protein components. Although certainly relevant historically, there has been a recent trend to abandon these distinctions and simply call them “enzymes,” accompanied by whatever distinct informational content may then be needed.

Note 1: Individual variation in drug metabolizing capabilities can result from expression of different isoforms and from differential expression levels of the same isoform, i.e., individuals having the same genetic makeup in terms of drug metabolizing enzymes can still differ in their drug metabolizing capabilities due to differences in their expression levels.

See: Acetylation Phenotype, Aldehyde Dehydrogenase Polymorphism or Deficiency, Atypical Alcohol Dehydrogenase, Cytochrome P450 Enzymes (CYPs), Extensive (or Rapid) Metabolizers, and Genetic Polymorphism.^{103,114-129}

115. IVIVE (Sometimes IVIV)

Acronym designating the use of *in vitro* to *in vivo* extrapolation to predict pharmacokinetics and metabolic drug-drug interactions for the *in vivo* case based upon results obtained from *in vitro* studies.

See: ADME; ADMET, Pharmacokinetics, and Metabolic Prediction/Possibilities versus Probabilities.^{150,151}

116. Ligand

Within the context of xenobiotic metabolism, this term can be used to describe two different physical settings. The first refers to the metal ions that often play a role as requisite cofactors in many of the holoenzyme complexes, the latter usually having a distinct chelation arrangement with preference for a specific metal ligand species. The second refers quite generally to any compound that can bind to an enzyme's active or allosteric regulatory sites in any capacity, whether or not they behave as substrates or regulators.

See: Allosteric Regulation, Cofactors, Enzymes, Holoenzymes, and Prosthetic Groups.¹⁵²

117. Linear Kinetics

Any kinetic process for which the rate (R) of a xenobiotic's or metabolite's appearance (e.g., absorption or production) or disappearance (e.g., clearance) is directly proportional to the local concentration (C) or amount of the xenobiotic or parent agent (A). This relationship can be expressed mathematically as

$$R = kC \text{ or } R = kA$$

where, in both cases, " k " is a constant called the "rate constant." By convention, the rate of appearance of the substance is taken to be positive, while the rate of disappearance is taken to be negative.

Note 1: Nonlinear kinetic processes that follow Michaelis-Menten kinetics (e.g., saturable enzymes or transporters) are often approximated by linear kinetics, as long as the local concentration is much less than the Michaelis-Menten constant, K_m . This relationship can be expressed as

$$R = V_{\max} C / (K_m + C) \approx (V_{\max} / K_m) C$$

when $C \ll K_m$, because $V_{\max}/K_m$ is a constant, such that the rate equation approximates the linear kinetic equation.

See: Half-Life ($t_{1/2}$), Enzyme, Nonlinear Kinetics, and Michaelis-Menten Kinetics.^{38-40,104}

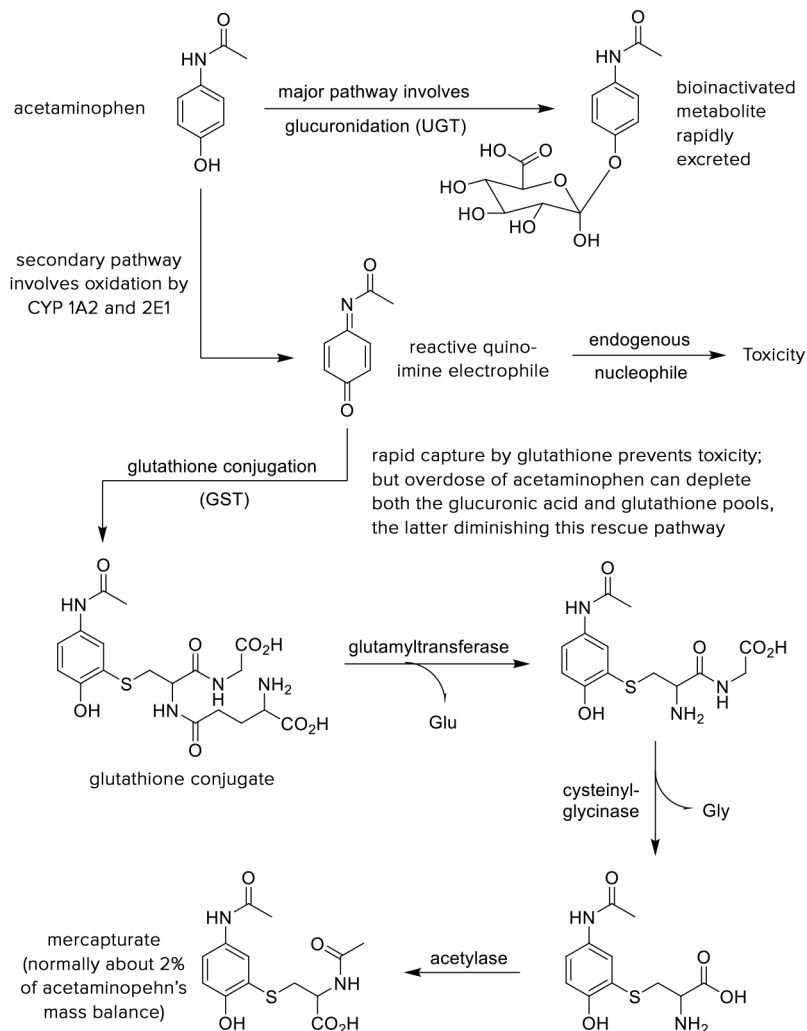
118. Mechanism-Based, Product and Transition State Inhibitors

Mechanism-based inhibition of an enzyme results from the interaction of an intermediate or product (aka "product inhibition") formed during the metabolic reaction. Typically occurring within the enzyme's active site, the interaction can become either competitive or noncompetitive. When the intermediate is a transient species representative of the reaction's transition state, such compounds are also referred to as "transition state inhibitors." The latter term additionally encompasses agents designed to directly mimic the transition state without the need for any mechanism-based conversion to occur.

See: Autoinhibition, Competitive Inhibition, Noncompetitive Inhibition, Substrate Inhibitor, and Suicide Inhibitor.⁶²

119. Mercapturate/Mercapturic Acid Conjugation

The *N*-acetylcysteine metabolite of a xenobiotic after the latter has first undergone a conjugation reaction with glutathione and then been further metabolized by a three-step process that modifies the initial glutathione adduct. The overall process of glutathione conjugation followed by the sequence of three metabolic events is referred to as "mercapturic acid conjugation." The example below depicts a reactive quino-imine electrophile being captured and detoxified by this overall pathway.



Note 1: The initial glutathione adduct of a xenobiotic parent or the latter's metabolite has an increase in molecular mass of 307 Da, which, in turn, often enhances relative biliary excretion, since it tends to handle substrates having molecular masses above 500 Da, compared to the renal route, which prefers molecular masses below 500 Da. After further metabolic processing by this specific catabolic pathway that sequentially removes the Glu and Gly and then acetylates the remaining Cys amino-group, the resulting mercapturate's molecular mass is only increased by 162 Da compared to the parent xenobiotic. This smaller increase, which is not too dissimilar from the mass gained when a xenobiotic forms a conjugate with glucuronic acid (i.e., 175), leaves either the renal or the biliary route as viable options for excretion, depending on the mass of the initial xenobiotic compound or its metabolite.

See: Glutathione (GSH), Glutathione Conjugation, Glutathione-S-Transferase (GST), Bioinactivation, Bioactivation, Catabolism, Cysteine Conjugate β -Lyase (C-S Lyase), ADME; ADMET, and Excretion.^{45,133-138}

120. Metabolic Capacity

This term finds wide use in the context of nutrition and exercise, where it includes all of the body's anabolic and catabolic processes across all endogenous and exogenous substances. It takes on two, much narrower, definitions when used in the context of drug metabolism, where it becomes either the maximum rate at which a given compound can be metabolized by the sum of the participating enzymes, whether identified or unknown, or, alternatively, the maximum catalytic rate that a specified (and thus known) enzyme can achieve under optimal conditions for an ideal substrate.

Note 1: Excretion is excluded from this biotransformation-dependent definition, just as it is also not encompassed by the phrase "metabolic clearance." Alternatively, the phrase "overall clearance" encompasses both metabolism and excretion considerations.

See: Drug Metabolism, Anabolism, Catabolism, Clearance, and Metabolic Clearance.^{38-40,104}

121. Metabolic Clearance

The volume of blood cleared of a specified compound by the organism's metabolism per unit of time.

See: Metabolic Capacity, Drug Metabolism, Anabolism, Catabolism, and Clearance.^{38-40,104}

122. Metabolic Fate

Typically, the delineation of all metabolic events that contribute to, and only to, a parent xenobiotic's complete clearance. In a less common but broader sense, the delineation of all metabolic events and pathways that occur on a xenobiotic plus all of its metabolites until the levels for all of these materials are no longer detectable anywhere in the body.

See: Drug Metabolism, Metabolic Clearance, Clearance, and Metabolic Pathway/Pattern/Profile.^{38-40,45,91,104}

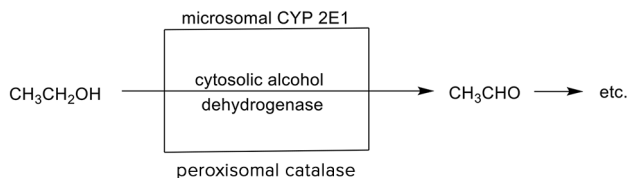
123. Metabolic Pathway/Pattern/Profile

Starting from a parent, xenobiotic compound, a metabolic pathway is either a single, end-point metabolic event or, more frequently, a series of metabolic events that proceed sequentially in a step-wise manner. The latter is also referred to as "sequential metabolism." The parent compound is most often subject to multiple metabolic pathways (also referred to as "competing metabolism"), which can each, in turn, also divide into multiple pathways, some of which can be designated as "complementary" or "overlapping metabolism." An accounting of the relative formation of metabolites along all of the pathways engaged by a given xenobiotic constitutes the latter's metabolic pattern or profile. Examples are provided below in a general manner for a xenobiotic "X" and its various metabolites M#. One example specifically uses ethanol.

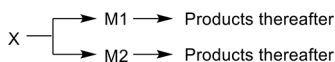
Single step pathway: $X \rightarrow M1 \rightarrow \text{Excretion}$.

Sequential pathway/metabolism: $X \rightarrow M1 \rightarrow M2 \rightarrow \text{etc.} \rightarrow \text{Excretion}$.

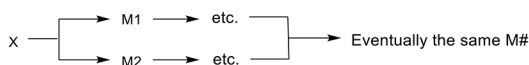
Complementary pathways/metabolism:



Competing pathways/metabolism:



Overlapping pathways/metabolism:



Note 1: A common theme in drug metabolism involves the sequential pathway wherein a xenobiotic compound is first “functionalized” in an appropriate manner by an oxidative (“Phase I”) metabolic step so as to subsequently become a good substrate for a conjugation (“Phase 2”) biotransformation. An example of this would be aromatic hydroxylation to insert a phenolic hydroxyl-group that then undergoes rapid glucuronidation, which, in turn, would likely be followed by excretion. A thorough discussion of this topic can be found in the introduction.

See: Drug Metabolism, Aromatic Hydroxylation, Conjugation, Glucuronic Acid Conjugation (Glucuronidation), Phases of Drug Metabolism, Alcohol Dehydrogenase (ADH), Catalase, and Metabolic Ratio.^{45,82,91}

124. Metabolic Prediction/Possibilities versus Probabilities

A prediction of possible metabolites and metabolic pathways after the administration of a clinical candidate compound to humans can be obtained by software programs associated with either expert systems or user-friendly databases. These programs typically convey all of the metabolic possibilities that could happen to a given query molecule across an “averaged mammalian” (and thus hypothetical) species. As such, they are generally extremely exhaustive and are unlikely to miss something that is eventually observed experimentally. However, their ability to consider relative rate data between the potentially competing possibilities, and thus provide a ranked list of only the metabolic probabilities that are most likely to actually occur, has yet to be successful.

Note 1: Even the software packages having expert systems which assign a probability ranking to a suggested metabolic possibility typically designate a number between four and six for a given possibility, while using a ranking scale that runs from 1 to 10, and thus lack adequate discriminatory power.

See: Drug Metabolism, Biotransformation, ADME, Metabolic Fate, Metabolic Pathway/Pattern/Profile, and Metabolic Ratio.^{2,90,115,153}

125. Metabolic Probe

A substance whose rate of metabolism or metabolic clearance can be used as a marker for the activity of a specific enzyme or for a distinct enzyme system associated with a specific metabolic pattern. Similarly, a selective inhibitor or inducer of a specific enzyme that can be used to alter the role that the enzyme might be playing in another compound's metabolism.

Note 1: Tables are available of substances that have at least one metabolite known to be reproducibly formed after interaction with a specific enzyme, which allows monitoring of that enzyme by measuring the probe's metabolite. Likewise, tables are available of substances known to be selectively cleared by a single metabolic pathway, and these can be used to directly monitor the status of that pathway. Such data can be used to profile an individual's genotype/phenotype and/or help characterize the metabolic pathways undertaken by a new drug candidate. Tables of selective inhibitors and inducers of specific enzymes are available, and these can also be deployed to study their effect on the metabolic fate of new drug candidates, so as to implicate pathways likely to be operative for the new compound.

See: Drug Metabolism, Pharmacogenetics and Pharmacogenomics, Metabolic Fate, and Metabolic Pathway/Pattern/Profile.^{115,119,126,128,150,151}

126. Metabolic Profiling/Fingerprinting

The process of experimentally assessing the metabolic fate or metabolic pattern/profile for a given xenobiotic, while assigning as much structural detail as possible among the metabolites, along with their metabolic ratios. When structural information cannot be ascertained for a given metabolite or for several metabolites, their presence can be said to be "fingerprinted" in a qualitative manner if they are detectable in a reproducible fashion from one experimental run to another. Quantitation for the fingerprint of a group

of unidentified metabolites may still be possible when a radiolabel is being deployed as part of the experiment.

Note 1: These types of experiments are often undertaken by deploying liquid chromatography-dual mass spectrometry (LC-MS/MS) so that both the molecular weight and distinct fragmentation pattern for a given metabolite can be ascertained after its separation by chromatography. Software is available to predict metabolites based on this type of data relative to the known structure of the parent compound.

See: Drug Metabolism, Pharmacogenetics and Pharmacogenomics, Metabolic Fate, Metabolic Pathway/Pattern/Profile and Metabolic Ratio.^{115,119,126,128,150,151}

127. Metabolic Ratio

The mass ratio of a given metabolite to the parent compound or to another metabolite, most often accounted for by tracking an initial radiolabel-to-mass relationship present in the parent compound. When only a mass balance is pursued, it is necessary to account for increases in molecular weights due to the formation of conjugates, assuming that such structural information can be discerned. Alternatively, when structural detail allows, this ratio is sometimes accounted for by mole percent, which inherently adjusts for changes in molecular weights in either direction from the parent compound.

See: Metabolic Pathway/Pattern/Profile.^{38-40,91,104}

128. Metabolic Switching

In relation to drug discovery, today's common use of this phrase pertains to when a derivative of an initial xenobiotic undergoes a different biotransformation pathway after a specific metabolic event becomes avoided or diminished due to a synthetic modification of the initial xenobiotic's structure. Differences in metabolic profile due to the same xenobiotic's administration by different routes does not constitute metabolic switching.

Note 1: It is a standard strategy during drug development to alter a candidate compound's metabophore if it is responsible for too short a duration of action, so as to avoid the implicated metabolic pathway and potentially

prolong the duration. This becomes a challenge, however, when the modified drug candidate then switches to another metabolic pathway that is still rapid enough to cause the new analog to retain too short a duration of action.

Note 2: Historical use of this phrase has pertained to such switching relative to isotope exchange effects during biochemistry studies¹⁵⁴ and to various enzyme modifications during molecular pharmacology studies,¹⁵⁵ as well as to the dynamics of a living system's gross metabolic/catabolic state relative to changes in diet, etc.¹⁵⁶

See: Drug Metabolism, Biotransformation, Metabolic Pathway/Pattern/Profile, and Metabophore.¹⁵⁷

129. Metabolism

A general term that encompasses all of the physical and chemical processes involved in the maintenance and reproduction of life in which nutrients and endogenous materials are broken down (catabolism) to liberate energy that can be captured and used by the organism (heterotrophic organisms), as well as to generate simpler molecules that may be used in turn as building blocks to form more complex molecules (anabolism) that can perform specific functions at the molecular level. To convey a more specific context relative to xenobiotics or drugs, the phrase "xenobiotic or drug metabolism" should be used.

See: Drug Metabolism, Biotransformation, Anabolism, Catabolism, and Metabolomics.^{53,79,80,82,158}

130. Metabolite versus Decomposition and Degradation Products

Metabolites are any intermediate or product resulting from an enzymatically catalyzed event. Materials resulting from autoxidation and spontaneous chemical reactions are not regarded as metabolites, even when prompted within select anatomical compartments or under specific physiological conditions, such as pH 7.4 and 37°C. Such materials would be called decomposition products. The interrelated term "degradation products" can encompass both metabolic degradation and spontaneous decomposition.

Note 1: Autoxidation can complicate the study of a xenobiotic's metabolism. In some cases, this can be remedied by the use of antioxidants, including examination of the roles of the CYP-450s, despite the latter's inherent requirement for oxygen.¹⁵⁹⁻¹⁶¹

See: Drug Metabolism and Biotransformation.^{2,45,46,79,80,91-93}

131. Metabolomics/Metabonomics

The study of all endogenous biomolecules, metabolites, and degradation materials within a cell or organism under both normal and other conditions, including exposure to xenobiotics, as well as various disease states.

Note 1: The focus is on qualitative, and when possible quantitative, analyses of the endogenous materials, rather than on the xenobiotic and its metabolites, so as to ascertain the endogenous materials' functions, interconnections, and regulation, while adopting a systems biology approach. In relation to xenobiotic exposures, this can include alterations that perturb normal ratios and concentrations, or binding and fluxes, of endogenous biomolecules, either by the xenobiotic's direct chemical reaction or by its binding to regulatory proteins or nucleic acids that control metabolism.

See: Metabolism, Drug Metabolism, and Pharmacogenetics and Pharmacogenomics. Refs. associated with xenobiotics: 162-166.

132. Metabophore

The specific spatial arrangement of the key structural features within an overall molecule that prompt the compound to be a substrate for a given metabolizing enzyme.

Note 1: The features can also be thought of in terms of their distinct electronic surface potentials, which match corresponding regions for recognition, binding, and catalysis by the enzyme, either simultaneously or in a dynamic fashion involving mutual molding across a sequence of associations.

Note 2: Generally taken to encompass the specific set of molecular features on a xenobiotic that can lead to a distinct metabolic reaction, such as the specific structure-metabolism relationships applicable to *N*-dealkylation by CYP 2D6.

Note 3: The term is analogous to the more commonly used terms “pharmacophore” and “toxiphore,” which apply to pharmacological efficacy and toxicology, respectively.

See: Drug Metabolism, Biotransformation, Enzyme, Metabophore Probe, and Structure-Metabolism Relationship (SMR).^{1-3,15,62,79,80,82,85,90,91,153} Refs. associated with prodrugs and soft drugs: 167-170.

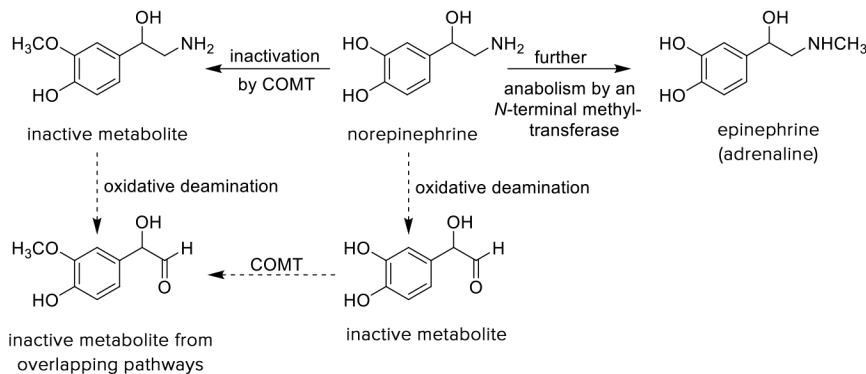
133. Metabophore Probe

A molecule with defined chemical features that is used to assess the relevance of those features for recognition, binding, and/or metabolism by a distinct enzyme or by a select enzyme system associated with a given biotransformation. Deployment of a series of such probes (metabolism-related directed-library) can be used to assemble a database of structure-metabolism relationships relevant for the enzyme/system under investigation.

See: Metabophore and Structure-Metabolism Relationship (SMR).^{1-3,15,62,79,80,82,85,90,91,153} Refs. associated with prodrugs and soft drugs: 167-170.

134. Methylation

Biotransformation of a xenobiotic involving conjugation of catechols, *N*-heterocyclic systems, and some sulfanyl- and phenolic hydroxy-groups, with a methyl-group donated in nearly all cases by *S*-adenosyl-L-methionine. These metabolites are less water soluble than their parent molecules, but generally are less active/toxic, particularly for a catechol group. Numerous endogenous materials also undergo this reaction as part of anabolism or as a regulatory step that can cause either activation or inhibition. The example below shows how methylation of norepinephrine can serve as both an anabolic-related event and a bioinactivation event.



Note 1: Several methyltransferase enzymes (EC 2.1.1) form an overall family that can accommodate a wide range of endogenous and exogenous structural types. Class I members have a protein fold that specifically binds *S*-adenosyl-*L*-methionine as a cofactor, and these are the most prominent for methyl conjugation of xenobiotics. Common examples include catechol *O*-methyltransferase (COMT), as shown above, and thiol methyltransferase, which adds a methyl-group to captopril.

See: Conjugation Reactions, Conjugate, Deamination/Oxidative Deamination; Methyltransferase, *N*-Methyltransferases, and Catechol *O*-Methyltransferase (COMT). General Ref: 171.

135. Methyltransferase

A heterogeneous family of enzymes (EC 2.1.1) that catalyze the transfer of a methyl-group to several endogenous materials and to xenobiotic compounds having a catechol, *N*-heterocyclic system, or a sulfhydryl- or phenolic hydroxyl-group. *S*-adenosyl-*L*-methionine (SAM) serves as the source of the methyl-group for the biotransformations that are most relevant for drug metabolism in humans.

Note 1: A practical categorization for this broad family's members can be arranged according to general substrate types: protein methyltransferases, which are often important for functional regulation of the proteins; DNA methyltransferases; xenobiotic methyltransferases; and non-SAM-dependent methyltransferases.

See: Conjugation Reactions, Conjugate, Methylation, *N*-Methyltransferases, and Catechol *O*-Methyltransferase (COMT). Refs. associated with xenobiotics: 172.

136. Michaelis-Menten Equation/Kinetics

A mathematical description of the relationship between a substrate's reaction rate and its responsible enzyme. It is written as

$$V = V_{\max} [S] / (K_m + [S]),$$

where *V* is the reaction rate, [*S*] is the concentration of the substrate, and, for a fixed amount of enzyme, *V*_{max} is the maximum rate at infinite substrate

concentration, while the rate constant K_m becomes equivalent to the substrate concentration at one-half the maximum velocity.

Note 1: The equation is representative for most enzymes, including those where an intermediate enzyme-substrate complex is formed. It describes the typical situation, where rate is dependent on the concentration of both enzyme and substrate.

Note 2: When product formation exhibits a hyperbolic relationship to substrate concentration, the reaction is said to follow Michaelis-Menten kinetics. There are a number of linear and nonlinear regression methods, as well as classical graphical analyses, that can be used to derive V_{max} and K_m values from experimental data obtained by measuring the biotransformation of a substrate to its product.

See: Enzyme, Half-Life ($t_{1/2}$), and Linear Kinetics.^{38-40,104}

137. Microdosing; Phase 0 Clinical Study

The common definition for microdosing is administration of a drug, generally to humans, at significantly lower dose than what is normally used to prompt an efficacious response (e.g., 100 times less), so that the lower dose circumvents toxicity while potentially demonstrating some other beneficial effect that may be achievable at this level. This approach is also undertaken with drug candidates to study their disposition and pharmacokinetic profile as a first step in clinical testing, where it is then also referred to as a "Phase 0 clinical study."

Note 1: Phase 0 clinical studies usually deploy a radio-labeled compound with exposure levels low enough to be considered non-radioactive by regulatory agencies. Analytical sensitivity is typically achieved by using accelerator mass spectrometry (AMS) methods originally developed to accomplish radiocarbon dating of precious samples within the field of archaeology.

See: ADME, ADMET, Disposition, Distribution, Pharmacokinetics (PK), Random Walk, Phases of Drug Clinical Testing, and Phases of Drug Metabolism.¹⁷³

138. Microsomal Fraction/Enzyme; Microsomes

A fraction of the cellular homogenate that contains the endoplasmic reticulum (ER) and thus many drug-metabolizing enzymes. It is typically reformed as lipoidal spheres called "microsomes."

Note 1: This fraction is generally obtained while operating at 2 to 3°C throughout by: (i) homogenizing cells, such as from extracted liver tissue; (ii) centrifugation of the homogenate at 9,000 G to pellet mitochondria and other heavy cellular components; (iii) ultracentrifugation of the supernatant at 100,000 G for ca. 1 hour; and (iv) reconstituting the pellet in physiological saline, where they spontaneously form "microsomes" that contain the ribosomes and are filled with numerous drug-metabolizing enzymes. Microsomes can be stored for several days at low temperature and are typically used to study drug metabolism *in vitro* upon the addition of the required enzymatic cofactors and warming to 37°C, after which enzymatic functional activity is then typically robust for periods up to 15-30 minutes.

Note 2: Associated enzymes include the entire family of CYPs, Flavin Monooxygenase, UDP Glucuronosyltransferase, and Epoxide Hydrolase.

Note 3: When isolating liver tissue from rodents, it is common practice to first treat the animals with a standard, enzyme-inducing agent, such as phenobarbital, so as to provide increased functional capacity for the various biotransformation reactions residing in the eventual microsomes.

See: Metabolism, Biotransformation, Enzyme, Apoenzyme, Holoenzyme, Cofactor, Prosthetic Group, Regenerating System, Cytochrome *P*-450s (CYPs), Glucuronic Acid Conjugation (Glucuronidation), Epoxide Hydrolase, Flavin Monooxygenase (FMO), Induction, and Phenobarbital Sleeping Time.⁶⁹

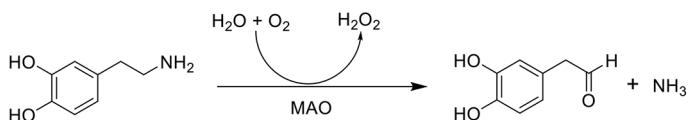
139. Mixed-Function Oxidase

Older terminology that is still widely used today in a general manner for enzyme systems that oxidize xenobiotics. It was initially derived from the oxygenating enzymes' requirements for both molecular oxygen and reducing agent cofactors. The isolation and purification of distinct forms of the CYP and FMO enzymes has progressed considerably, while accompanied by the elaboration of more specific and descriptive nomenclatures, all of which can be used in a more specific manner.

See: Cytochrome P450 Enzymes (CYPs) and Flavin Monooxygenase (FMO).⁶⁹

140. Monoamine Oxidase (MAO)

A family (EC 1.4.3.4) of flavin-containing amine oxidases that catalyze the oxidative deamination of monoamine substrates and diamines when the latter's second basic center is located more than six atoms away from the primary amine (shorter separations instead being catalyzed by diamine oxidase). The catabolism of dopamine is exemplary and demonstrates the importance of this pathway with regard to endogenous neurotransmitters:



Note 1: The mechanism involves the initial formation of an imine intermediate, which is hydrolyzed to the aldehyde and ammonia. The reactive and potentially toxic aldehyde undergoes rapid bioinactivation by other enzymes to either a carboxylic acid or an alcohol, the former being the more typical path for the endogenous neurotransmitters.

Note 2: In humans, MAO occurs as two forms, called MAO-A and MAO-B, which are separate gene products having about 70 % sequence homology. Both forms are present throughout the brain and in neurons, where they reside on the outer surface of mitochondrial membranes. MAO-A also has high levels in the liver, lung, kidney, intestine, and placenta, while MAO-B is found in blood platelets.

Note 3: MAO function has been implicated as playing a role in certain CNS disorders. For example, inhibitors (MAOIs) have been deployed clinically to treat depression. However, their use has largely become limited to secondary therapy because of side effects and the development of alternative agents that can serve as front-line therapy.

See: Catabolism, Deamination, Dealkylation, *N*-Dealkylation, and Diamine Oxidase (DAO).¹⁷⁴

141. Monooxygenase/Monooxygenation

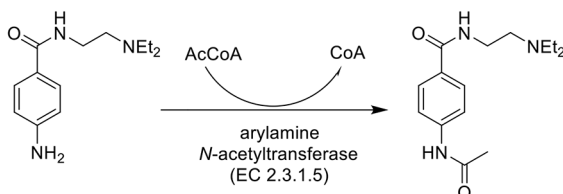
A specific term for the general class of oxygenase enzymes (EC 1.13 and EC 1.14) that occurs when one oxygen atom of molecular oxygen is added to a substrate while the other atom becomes reduced by NADPH to form water. Common examples of monooxygenases important to the metabolism of xenobiotics include the CYPs and FMOs.

Note 1: Alternatively, the term dioxygenation specifies a biotransformation where both atoms of molecular oxygen are incorporated together into one substrate or sometimes separately into two.

See: Cytochrome P-450 enzymes (CYPs), Flavin Monooxygenase (FMO), and Oxidoreductase.¹⁷⁵

142. *N*-Acetylation

Conjugation of a xenobiotic's amino group with an acetyl moiety donated by acetylcoenzyme A and catalyzed by an *N*-acetyltransferase (NAT). Aryl primary amines and hydrazines are particularly susceptible. Bioactivation of the local anesthetic procaine to the antiarrhythmic agent procainamide serves as an example:



Note 1: Primary aromatic amines are the principal substrates, followed by hydrazines, although several other nucleophiles can sometimes undergo this reaction, such as primary aliphatic amines (including amino acids), sulfonamides, and hydroxylamines at the chemically reactive oxygen atom.

Note 2: Two isoforms are especially important for drug metabolism in humans, namely the arylamine-*N*-acetyltransferases NAT1 and NAT2. Their polymorphisms lead to distinct metabolic phenotypes.

See: Acetylation, Acetylation Phenotype, *N*-Acetyltransferases (NATs), Acylation (which is notably a different metabolic pathway), and Bioactivation.⁶

143. *N*-Acetyltransferases (NATs)

The family of enzymes which catalyze the conjugation of amino groups with an acetyl moiety donated by acetylcoenzyme A. Two arylamine-*N*-acetyltransferase (EC 2.3.1.5) isoforms (NAT1 and NAT2) play an important role in humans to acetylate primary arylamines.

Note 1: Both NAT1 and NAT2 are 33 kDa cytosolic proteins having a high degree of polymorphism, which leads to two distinct phenotypes, namely "fast" and "slow acetylators." NAT1 is expressed in numerous tissues, while NAT2 is primarily expressed in the liver and gut wall. The two isoforms have considerable overlap in their acceptance for substrates, although certain trends in the preference for selected materials are also apparent.

See: Acetylation, Acetylation Phenotype, *N*-Acetylation, and Acylation (which is notably a different metabolic pathway).^{6,176} Specific forms Refs.: 177-179.

144. NADH-Cytochrome b_5 Reductase (CBR)/ Methemoglobin Reductase

This enzyme (EC 1.6.2.2) mediates an electron transfer from NADH to cytochrome b_5 using FAD as a cofactor. Cytochrome b_5 , in turn, serves as a ubiquitous electron carrier that participates in numerous biochemical pathways. For xenobiotic metabolism, a membrane-bound form of CBR located on the cytosolic side of the endoplasmic reticulum is particularly important because it participates in cytochrome *P*-450 (CYP) related biotransformations.

Note 1: A soluble form of CBR is present in erythrocytes, where it participates in the conversion of methemoglobin (Fe^{+3}) to hemoglobin (Fe^{+2}). Because of this, CBR is also referred to as "methemoglobin reductase." Deficiencies of this enzyme can lead to methemoglobinemia, where the Fe^{+3} form does not bind and carry oxygen as well as the Fe^{+2} form.

See: Cytochrome b_5 , Cytochrome *P*-450 Enzymes (CYPs), NADPH-Cytochrome *c* Reductase/NADPH-Cytochrome *P*-450 Oxidoreductase/Cytochrome *P*-450 Reductase (CYPR), Microsomal Fraction/Enzyme, Microsomes, and Regenerating System.^{43,64-69}

145. NADPH-Cytochrome *P*-450 Reductase (preferably)/ NADPH-Cytochrome *c* Reductase/NADPH-Cytochrome *P*-450 Oxidoreductase/Cytochrome *P*-450 Reductase (CYPR)

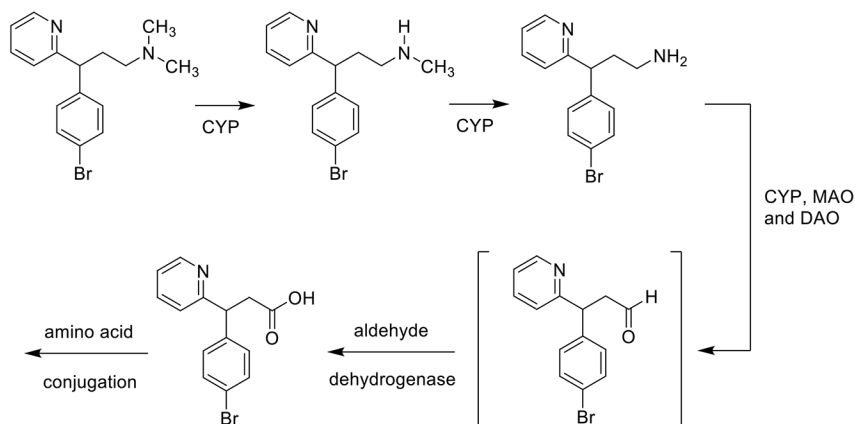
This enzyme (EC 1.6.99.1) mediates an electron transfer from NADPH to cytochrome *c* using FAD and FMN as cofactors. After the preferred term, the sequence of still acceptable names follows historically along the progression of its more detailed functional characterization. CYPR, along with other microsomal enzymes, depending upon their oxidation status, readily interacts with NADPH. It can also interact with NADH, but has less affinity for this interaction and also partners with CBR in that regard. In this context, CYPR is critical for certain of the cytochrome *P*-450 (CYP) catalyzed biotransformations of xenobiotics.

Note 1: The CYPs require two electrons to perform their typical monooxygenation reactions. The first is mainly delivered by CYPR to the substrate-cytochrome *P*-450⁺³ complex, while the second electron, which subsequently reduces the substrate-cytochrome *P*-450⁺²-O₂ complex, can be supplied by either CYPR or cytochrome *b*₅ via CBR.

See: Cytochrome *b*₅, NADH-Cytochrome *b*₅ Reductase (CBR)/Methemoglobin Reductase, Cytochrome *P*-450 Enzymes (CYPs), Microsomal Fraction/Enzyme, Microsomes, and Regenerating System. General Refs.: 45,68-73.

146. *N*-Dealkylation

A biotransformation catalyzed by the CYPs, where a nitrogen-alkyl-carbon bond (most typically in a xenobiotic's secondary or tertiary *N*-alkyl amine or amide) is oxidatively cleaved, resulting in an aldehyde derived from the alkyl group (and for the typical substrates, in the corresponding primary or secondary amine or amide, respectively). *N*-demethylation of alkylamines is a frequently observed pathway owing, in part, to how common this structural motif is within drug molecules. In addition to alkyl amines and amides, a diverse range of nitrogen compounds can be substrates, e.g., carbamates, ureas, and sulfonamides. One of the metabolic pathways for brompheniramine is illustrative, where its first two steps are classified as *N*-dealkylations, while the third is classified as a deamination:



Note 1: In this example, the deamination step can involve catalysis by monoamine oxidase (MAO) and diamine oxidase (DAO), as well as by a cytochrome *P*-450 (CYP). Analogous to an *N*-dealkylation, all of these enzymes will produce the aldehyde from cleavage of the *N*-alkyl group, which then becomes quickly oxidized to the carboxylic acid that, in this particular case, is both excreted into the urine and further conjugated to an amide using glycine as an endogenous amino acid partner.

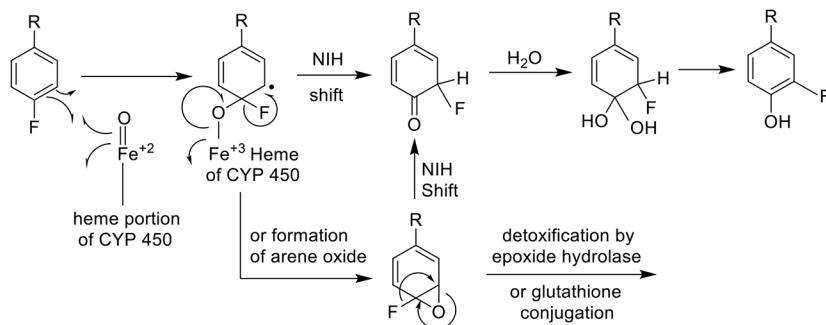
Note 2: CYP-mediated *N*-dealkylation of aliphatic amines occurs by a stepwise, electron transfer process that is initiated by a rate-limiting, one-electron *N*-oxidation reaction to form an aminium radical cation. Abstraction of a hydrogen atom from the α -carbon of the alkyl ligand is then rapid and forms an iminium intermediate that undergoes hydrolysis via a carbinolamine. Alternatively, the process for alkyl-amides is initiated by abstraction of an α -carbon hydrogen atom without a preceding electron abstraction.

See: Dealkylation, Demethylation, Deamination, Monoamine Oxidase (MAO), Diamine Oxidase (DAO), Polyamine Oxidase, and Cytochrome *P*-450 Enzymes.^{74,79,80,83,174}

147. NIH Shift

An intramolecular rearrangement of the transient intermediates produced during CYP-mediated aromatic hydroxylation. The rearrangement involves a radical or hydride shift and is also applicable to the migration of

other groups capable of undergoing these types of 1,2-transfers. The two mechanistic pathways are depicted below for the case of a fluorine atom as the radical (top line) or as the fluoride anion (lower line):



Note 1: This reaction was named after the U.S. National Institutes of Health (NIH), where it was first observed and characterized. It is more prominent when R = OMe, NMe₂, SO₂Ph, Ph, CN, NO₂, CONH₂, and halides; and less so when R = OH, NH₂, NHAc, NHCOPh, NHSO₂Ph, and NHCHO.

Note 2: The common strategy of adding substituents, such as one or more fluorine atoms, to block or attenuate metabolic degradation at a given locale on a drug candidate's structure can be impacted by the NIH shift when dealing with these specific molecular arrangements and their related metabolic pathway. It may work, because halide atoms are electron withdrawing, such that their addition to the phenyl-ring will decrease the latter's overall electron density or "richness." That, in turn, will decrease the overall susceptibility of the phenyl-ring to undergoing an aromatic hydroxylation biotransformation at any location. The strategy may not work, however, because halide atoms cannot be relied on to block a given site for an aromatic hydroxylation because of the possibility for an NIH shift.

See: Aromatic Hydroxylation and Epoxidation.¹⁸⁰

148. N-in-One Dosing

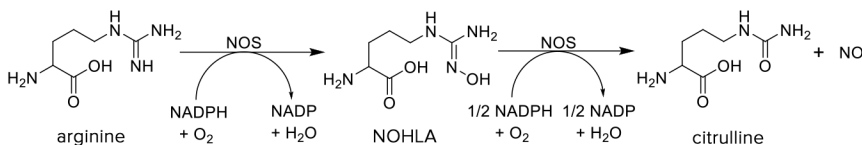
Study of two or more simultaneously administered xenobiotics, either to assess their own metabolism or to assess metabolism-related interactions, PK, or ADMET properties.

Note 1: Generally used more often during *in vivo* studies in order to increase throughput for the assessment of interesting compounds while reducing the number of animals that might be needed. Thus, within the context of drug metabolism studies, this is distinguishable from the practice of high-throughput screening for efficacy and toxicity at the *in vitro* level, where it is common to test mixtures having large numbers of compound candidates followed by various methods to de-convolute the resulting dataset.

See: Cocktail Study.^{59,60}

149. Nitric Oxide Synthases (NOSs)/Nitrogen Monoxide/Nitrogen (II) Oxide/Oxidonitrogen

A family of CYP-related enzymes (EC 1.14.13.39) that generate nitric oxide (NO) and citrulline from arginine, oxygen, and NADPH. Because of their high specificity, they do not appear to play a role in the metabolism of xenobiotics having a guanidine moiety. Alternatively, they can be mechanistic targets for drug design.



Note 1: There are three major isoforms: neuronal (nNOS), endothelial (eNOS), and inducible (iNOS).

See: Cytochrome *P*-450 Enzymes (CYPs).¹⁸¹ Inhib. Ref.: 182.

150. *N*-Methyltransferases

Members within the family of methyltransferase enzymes (EC 2.1.1) that can mediate the conjugation of amino groups with a reactive methyl group provided by *S*-adenosyl-L-methionine (SAM)

Note 1: Histamine-*N*-methyltransferase and indolethylamine-*N*-methyltransferase are commonly known for their roles with endogenous partners. However, the latter has a broader range for substrates, including the potential to catalyze reactions with xenobiotics.

See: Methylation and Methyltransferase.^{171,172}

151. Non-Compartment PK Analysis

Pharmacokinetic (PK) model where the body is considered as an unknown "black box," rather than a series of interconnected compartments, as in a compartment or PBPK model. Measured drug concentrations (C), generally in plasma, serum, or blood, are fitted to a series of exponential terms of the form shown below. The form of this equation and the definition for its terms' symbols are the same as those provided for the equation pertaining to *Compartment Model*.

$$C = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + \dots + A_n e^{-k_n t}$$

Without requiring any type of assumed model structure, the terms of this fitted empirical equation can then be used to estimate useful PK parameters numerically, e.g., AUC, bioavailability, clearance, terminal half-life, and the mean residence time of a drug in the body.

See: Absorption, ADME, Clearance, Pharmacokinetics (PK), Compartment Model, and PBPK Model.^{38-40,104}

152. Non-Competitive Inhibition

Can occur by two different mechanisms: (i) the inhibitor binds irreversibly within an enzyme or transporter's active site for a substrate, e.g., by forming a covalent bond with one of the protein's amino acids; or (ii) the inhibitor binds either reversibly or irreversibly to another site on the enzyme or transporter that is not subject to substrate binding, but when bound by the inhibitor has a negative influence on the substrate's binding within the active site.

Note 1: Unlike competitive inhibition, non-competitive inhibition cannot be overcome by raising the concentration of substrate. For enzymes that metabolize substrates in accordance with Michaelis-Menten kinetics, non-competitive inhibition will lower the enzyme's $V_{\max}$ but will not alter its K_m .

See: Autoinhibition, Competitive Inhibition, Uncompetitive Inhibition, Mechanism-Based Inhibition, Covalent Binding, and Michaelis-Menten Kinetics.^{62,146-148}

153. Nonlinear Kinetics

Any kinetic process for which the rate of the analyte's appearance or disappearance is not directly proportional to the local concentration or amount of the agent.

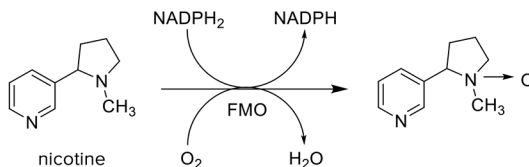
Note 1: Nonlinear kinetics are typically encountered for enzymes or transporters that follow Michaelis-Menten kinetics or some other type of saturable or rate-limited process.

Note 2: PK models that attempt to address nonlinear kinetic processes are more difficult to solve than those with only linear kinetic parameters and generally require the use of computer-assisted numerical methods rather than direct algebraic solution.

See: Linear Kinetics, Michaelis-Menten Kinetics, and Pharmacokinetics (PK).^{38-40,104}

154. N-Oxidation

In the broadest sense, this phrase can be used for any biotransformation of an organic nitrogen-containing xenobiotic where the oxidation state of the nitrogen atom is increased. Most commonly, however, it is taken to refer to the formation of an *N*-oxide, particularly with regard to tertiary amines (see Notes). The more common usage is exemplified below.



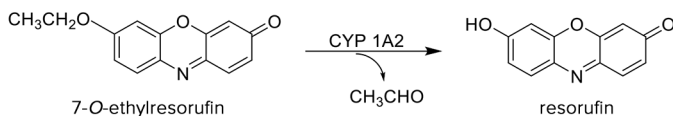
Note 1: The process is catalyzed by either an FMO or a CYP and involves electron transfer from nitrogen to the enzyme. Most primary amines can potentially undergo three successive two-electron oxidations that can give rise to hydroxylamines (or imines) (-2), nitroso groups (or oximes, cyanos) (-4), or nitro groups (-6). In all cases the indicated valencies represent the combined change in oxidation state for nitrogen and its *alpha*-carbon atoms. Secondary amines can undergo a maximum of two, two-electron oxidations that give rise to hydroxylamines (or imines) (-2) or nitrones (-4). Tertiary amines can only undergo a single, two-electron oxidation that gives rise to their *N*-oxides.

Note 2: Initial one-electron *N*-oxidation of amines affords highly unstable aminium cation radicals (-1), which can give rise to a variety of end products. Tertiary amines have a much greater propensity to form stable *N*-oxide metabolites than either secondary or primary amines. As a result, tertiary amines, which are sterically hindered from the common *N*-dealkylation pathways, are often metabolized to observable *N*-oxides.

See: Oxidation, Dealkylation, *N*-Dealkylation, Demethylation, Deamination, Cytochrome *P*-450 Enzymes (CYPs), Flavin Monooxygenase (FMO), Monoamine Oxidase (MAO), Diamine Oxidase (DAO), and Polyamine Oxidase.¹⁸³

155. O-Dealkylation

A biotransformation catalyzed by the CYPs where an oxygen-alkyl-carbon bond in a xenobiotic's ether linkage is oxidatively cleaved, resulting in the corresponding alcohol and an aldehyde derived from the alkyl group. Most commonly observed for aralkylethers, as exemplified by the fluorescent CYP functional probe shown below.



Note 1: *O*-dealkylation is not as prominent as *N*-dealkylation. It is readily subject to steric hindrance (branching) and is also attenuated by increasing the length of the alkyl group. *O*-demethylation, particularly of an arylmethoxy group, however, presents itself as a highly probable metabolic possibility.

Note 2: Because of their reactivity and potential toxicity, other metabolic pathways are in place to rapidly biotransform the initial aldehyde metabolites into less reactive species, mainly aldehyde dehydrogenase (ALDH). This is a well-established sequential pathway/pattern/profile observed in drug metabolism.

Note 3: Alternatively, when an arylhydroxy group becomes exposed in the metabolite, it is often a good candidate for a subsequent conjugation reaction, mainly glucuronidation. This is a common sequential pathway/pattern/profile observed in drug metabolism.

See: Oxidation, Dealkylation, *N*-Dealkylation, Cytochrome *P*-450 Enzymes (CYPs), Metabolic Possibilities versus Probabilities, Metabolic Pathway/Pattern/Profile, Aldehyde Dehydrogenase (ALDH), and Glucuronidation.^{74,184}

156. Oral Bioavailability

The ratio of the AUC from plasma samples taken for a dose of drug given orally (PO) divided by those for a dose of the drug given intravenously, with the comparison normalized for different doses or varying weights of the subjects.

Note 1: In this context, the blood of the general circulation (or its plasma component as a practical matrix for analytical measurements) is always utilized, whereas for the term “bioavailability” the compartment under consideration may vary and so must be specified by the user, e.g., “bioavailability within the cerebrospinal fluid” for drugs intended to have effects in the CNS.

Note 2: For a drug taken PO, examples of factors that can limit oral bioavailability include: formulation issues, such as slow dissolution rate; drug stability, such as degradation under the acidic conditions of the stomach; metabolic transformation by the gut microflora, intestinal epithelial cells, or liver; efflux by transporters within epithelial cells, such as P-glycoprotein (Pgp); or direct biliary excretion. Many of these factors work together to limit the access of xenobiotics into the circulation, a process called the “first pass effect or metabolism.”

See: Absorption, Bioavailability (Absolute and Relative), Gut Microflora, P-Glycoprotein (Pgp), First Pass Effect/Metabolism, Pharmacokinetics, AUC, and Bioequivalence.³⁹

157. Organic Anion Transporters (OATs)

A family of transmembrane proteins that actively transport substrates having organic anion groups, such as a carboxylic acid moiety, when ionized at physiologic pH. More than a dozen members have been identified at significant expression levels in various locations throughout the body.

Note 1: In addition to their influence on the distribution profile of endogenous and xenobiotic compounds, of particular relevance for metabolism is the

OATs' facilitation of drug and metabolite excretion. For example, OAT1, OAT3, OATP4C1, MDR1, MRP2, MRP4, and URAT1 are expressed in cells forming the S_2 segment of the proximal tubules of the kidneys, where the first three are thought to transport from the plasma into the cells and the latter four from the cells into the lumen of the proximal tubules, such that their substrates are then excreted into the urine. OAT2, with only low levels in the kidneys, is highly expressed in the liver, where it and other family members ultimately deliver substrates to the bile in an analogously tandem manner.

Note 2: Notably, both the common glucuronide and glutathione/mercapturate metabolites add a carboxylic acid group to their substrates as part of their conjugation molecules.

Note 3: Various of the OATs can also be classified according to their systematic names, associated with either the solute carrier (SLC) or ATP-binding cassette (ABC) transporter super families. For example, OAT1 is also SLC₂₂A6, while MDR1 is also ABCB1.

See: Active Transport, Organic Cation Transporters (OCTs), Distribution, Excretion, Glucuronic Acid Conjugation (Glucuronidation), Glutathione Conjugation, Mercapturate/Mercapturic Acid Conjugation, Hippuric Acid Conjugate (Hippurate). Renal-related Refs.: 185,186.

158. Organic Cation Transporters (OCTs)

A family of transmembrane proteins that actively transport substrates having positively charged groups, such as basic amines, which become highly ionized at physiologic pH. However, some of their family members can also transport non-charged molecules, such as paclitaxel, and even certain negatively charged compounds. Several members have been identified at high expression levels in various locations throughout the body.

Note 1: In addition to their influence on the distribution profile of endogenous and xenobiotic compounds, of particular relevance for metabolism is the OCTs' facilitation of drug and metabolite excretion. For example, their expression at specific subcellular sites in renal proximal tubular cells and in hepatocytes becomes sequentially engaged like that of the OATs, so as to promote excretion into the urine and into the bile, respectively. Over-expression of the P-glycoprotein member, PgP, within cancer cells can result in multidrug resistance.

Note 2: Various of the OCTs can also be classified according to their systematic names, associated with the solute carrier (SLC) or ATP-binding cassette (ABC) transporter super families.

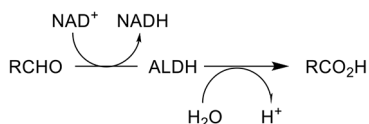
See: Active Transport, Organic Anion Transporters (OATs), Distribution, Excretion, P-Glycoprotein (Pgp)/MDR1/ABCB1.¹⁸⁷ Renal Ref.: 185

159. Oxidation/Oxidase/Oxidoreductase

Oxidation is an encompassing term used within the context of drug metabolism to generally categorize biotransformations that ultimately increase the oxidation state of the substrate, often incorporating one or more oxygen atoms donated from either molecular oxygen or from a water molecule. They can be catalyzed by numerous types of enzymes (e.g., ALD, ALDH, AO, MAO, DAO, FMO, and CYPs) that remove one or more electrons or hydrides and/or directly add one or more oxygens to a substrate or its metabolized fragments (e.g., further conversion of aldehyde products from metabolic dealkylation reactions into carboxylic acids). Similarly, the general terms used for the variety of enzymes/enzyme systems that can perform oxidation reactions can be either oxidases or oxidoreductases, depending on how much of the biochemical system's functional components are being incorporated into the intended description for a given process. The broadness of the term "oxidoreductase" is reflected by its EC number, namely EC 1, which is then further delineated into more than 20 subclasses.

Note 1: Oxidation, as opposed to reduction, reactions constitute the vast majority of xenobiotic biotransformations within mammals. Conversely, after oral ingestion the gut microflora can contribute to a xenobiotic's metabolism by performing a considerable array of reductive biotransformations. Metabolic enzymes that normally catalyze oxidations may sometimes perform a reduction, e.g., certain of the CYPs have this capability under anaerobic conditions.

Note 2: The following example intends to clarify the variety of terminology that can be used in relation to the oxidation of a xenobiotic by an oxidoreductase pathway as typically catalyzed by an enzyme with its cofactors:



Catalyzed by aldehyde dehydrogenase (ALDH), which serves as an "oxidoreductase," the aldehyde-containing xenobiotic RCHO becomes an "electron donor" or "reductant" when it transfers a "hydride ion" to cofactor NAD⁺, which, in turn, becomes an "electron acceptor" or "oxidant." At this point bound to a cysteine within the ALDH active site as its thioester intermediate, the xenobiotic can already be considered to have been "oxidized," while the NAD⁺ cofactor has been "reduced" to NADH. Continuing its role as an "oxidase," ALDH then catalyzes hydrolysis of the thioester, so as to produce the now formally "oxygenated," carboxylic acid-containing "oxidized" product that results from this particular "oxidation" reaction, wherein a water molecule has provided its "oxygen atom" in the form of a hydroxy group after being polarized and positioned for the transfer by hydrogen bonding as a donor to a glutamate within the ALDH active site.

See: Aldehyde Dehydrogenase (ALDH) and Cytochrome *P*-450 Enzymes (CYPs), among many other examples. Thorough Ref.: 188; Chem. Mechanisms Ref.: 189.

160. Oxidative Stress

In general, an imbalance between the level of reactive oxygen species (ROS), such as superoxide and hydroxyl radicals (O₂⁻ and ·OH) or hydrogen peroxide (H₂O₂), compared to a biological system's ability to detoxify the ROS or repair their damage. There are several interrelationships with the metabolism of xenobiotics. For example: (i) many of these biotransformations engage oxidoreductase enzymes, which are dependent on or sensitive to localized oxygen tension (balance); (ii) some reactive xenobiotics or metabolites also draw from (and can thus contribute toward the depletion of) the pool of systems in place to detoxify normal levels of reactive oxygen species (e.g., glutathione conjugation of reactive electrophiles derived from exogenous materials or catalase, which converts hydrogen peroxide to water and molecular oxygen); and (iii) alternatively, oxidative stress can deplete glutathione stores and thus alter the pathways available for xenobiotic metabolism.

See: Oxidation/Oxidase/Oxidoreductase, Mixed-Function Oxidase, Catalase, Glutathion (GSH), Glutathione Conjugation, Cytochrome *P*-450 Enzymes (CYPs), Flavin Monooxygenase (FMO), and Reactive Oxygen Species (ROS).¹⁹⁰⁻¹⁹² Research Topic Refs.: 193,194.

161. Parent Compound

Within the context of xenobiotic metabolism, the molecule (drug) initially presented to the biological system, which may then undergo biotransformation.

Note 1: In drug design, this phrase typically refers to an initial molecule that serves as a structural template for the production of new analogs or scaffold-hopping strategies. This alternative usage is particularly prevalent during the design of prodrugs and soft drugs, and this can lead to confusion. Even though both are correct in the following example, the context is crucial for appreciating their meaning: A prodrug designed/modified from a parent drug becomes the parent compound upon its administration to the biological system in terms of assessing and discussing its ADME behavior.

See: Metabolite versus Degradation and Decomposition Products, Metabolism, Drug Metabolism, and Biotransformation.³

162. Partition Coefficient (P ; $\log_a P$ and $\epsilon \log_a P$); Distribution Coefficient (D ; $\log_a D$)

For drug metabolism, a partition coefficient (P) is a property of a xenobiotic that reflects the ratio of this solute's concentration as a neutral (uncharged) species in a non-polar phase (generally defined to be octan-1-ol), compared to that in an aqueous phase, after the two phases are equilibrated by mixing and allowed to separate. This property is generally expressed as a $\log_a$ value ($\log_a P$, where a signifies the basis of the logarithm), as calculated below, where α reflects the degree of dissociation for compounds capable of ionizing in an aqueous environment according to their ionization constants and thus accounts for their enhanced solubility in that phase. In case of base 10 (decadic logarithm), the expression reads

$$\text{Log}_{10} P = \log_{10} ([S]_o / [S]_w) - (1 - \alpha)$$

where $[S]$ is the concentration of the solute in octanol (subscript o) and water (subscript w).

Note 1: Neutral or weakly ionizing compounds ($\alpha \approx 1$) generally fit this equation well, but those that significantly ionize in water, such as xenobiotics having basic amine or acidic carboxylic acid groups, can be

problematic. Thus, another convention for these compounds is to drop the $(1 - \alpha)$ term and allow the enhanced aqueous solubility to be maintained in the calculation while stipulating that this is now a distribution coefficient (D) having $\log_a D$ values. Because the contribution from ionization will be pH-dependent, it then becomes important in $\log_a D$ determinations to specify what specific pH was utilized during the partition experiment, e.g., pH 7.4. Similarly, temperature changes can also affect the degree of ionization and so these are also reported, e.g., 37°C.

Note 2: Compounds having greater solubility in water have P values less than 1, while those having greater solubility in octan-1-ol have values greater than 1. Studies have demonstrated that there is a bell-shaped-curve relationship between the $\log_a P$ values for various compounds and their rate of passive transport across biological membranes. Relevant for oral bioavailability, the optimal $\log_a P$ for many drug-like molecules tends to be less than 5, because too much lipophilicity can cause them to remain/concentrate within the membrane. Conversely, compounds having significantly lower values tend to stay in the aqueous phase, rather than entering the membrane as a prelude to passive transport. A $\log_a P$ near 2 is thought to be relevant for penetration into the brain.

Note 3: Since P derives from the summation of the properties for each of a xenobiotic's molecular components/substituents as displayed across its overall scaffold, various computational methods have been devised to calculate this net property for a given molecule, such results then being designated as $\log P_c$ values, as opposed to experimentally determined values. Comparisons between $\log_a P$ and $\log_a P_c$ values for the same compounds are generally acceptable, but become most reliable when a given computational method has been refined with the aid of experimental data to best reflect specific classes of chemical structures.

Note 4: Historically, $\log_a P_c$ has been symbolized as simply $\text{clog } P$, and while it has remained customary for the latter to represent $\log_a P_c$, no mathematical symbol clog exists; the calculated property is the partition coefficient P_c . Specification of the basis is now mandatory wherein other symbols exist, for example $\lg$ for $\log_{10}$, $\ln$ for $\log_e$, or ld for $\log_2$.

See: Permeation/Permeability, Passive Transport, Rule-of-Five, Absorption, Oral Bioavailability, ADME, Distribution, Pharmacokinetics (PK), Active Transport, and Blood-Brain Barrier (BBB).^{195,196}

163. Passive Transport

The passage of a solute across a biological membrane by a process that does not require the expenditure of energy.

Note 1: This process is driven by the solute's concentration gradient. Thus, solutes cannot move from sites or compartments having lower concentrations into sites or compartments having higher concentrations.

See: Absorption, Membrane Permeation, Active Transport, ADME, First-pass Effect, Extravascular Dosing, Random Walk, Pharmacokinetics, and Bioavailability.^{4,10,195,196}

164. PBPK (Physiologically Based Pharmacokinetic Model)

Acronym for physiologically based pharmacokinetic model, in which compartments represent specific tissues, and the rates into and out of a compartment represent transport by blood flow, permeation across a physiological barrier, or metabolism within a given tissue.

Note 1: Many of the numerical values for the parameters in PBPK models can be taken directly from physiological tables, such as those for species specific tissue volumes and blood flow rates. Metabolism data from *in vitro* studies is also sometimes incorporated into these models.

Note 2: PBPK models generally provide a more reliable means of PK-related extrapolations between species than allometric scaling, because they can be modified to represent the actual physiological differences between species, rather than an empirical scaling to body weight or surface area.

See: Pharmacokinetics (PK), Allometric Scaling, Blood Brain Barrier (BBB), Compartment Model, ADME; ADMET, Clearance (CL), Distribution, DMPK, Disposition, Elimination, Excretion, Extraction Ratio, Gut Microflora, Half-Life ($t_{1/2}$), Hepatoportal Circulation, Hepatic Clearance/Extraction, IVIVE, Linear Kinetics, Metabolic Pathway/Pattern/Profile, Michaelis-Menten Equation/Kinetics, Non-Compartment PK Analysis, and Nonlinear Kinetics.^{38-40,104}

165. Permeation/Permeability

Within the context of drug metabolism, the movement/capability of a xenobiotic to enter a given compartment. The latter most often refers to lipid membranes, where this term includes passage and exit to the opposite side, such as for the gastro-intestinal tract epithelial lining. This property is important for a xenobiotic's absorption and oral bioavailability or for the blood-brain barrier (BBB), where it is important for penetration of a drug into the central nervous system (CNS).

Note 1: Under static conditions, a xenobiotic's permeability is simply reflected by its physicochemical properties, e.g., its solubility in a given compartment, such as the lipid/hydrophobic nature of a membrane *versus* its aqueous/hydrophilic solubility. This makes aqueous-lipophilic solvent (generally octan-1-ol) partitioning experiments valuable to determine partition coefficient values for a given compound or series of compounds, where this data can then be directly proportional to passive transport and distribution profiles.

Note 2: However, within the dynamic conditions of a living system, other factors can additionally influence a xenobiotic's permeability, such as a drug's ability to serve as a substrate for active (influx or efflux) transporters. Since the transporters are, in turn, subject to up- and down-regulation, substrate xenobiotics that can also interact with the transporters' associated translation and signaling pathways may exhibit a differing permeability profile upon their continued exposure to the system.

See: Partition Coefficient, Passive Transport, Rule-of-Five, Absorption, Oral Bioavailability, ADME, Distribution, Pharmacokinetics (PK), Active Transport, and Blood-Brain Barrier (BBB).^{4,10,195-197}

166. Peroxisome Proliferator-Activated Receptors (PPARs)

The PPARs are a family of nuclear receptor proteins that serve as transcription factors to regulate the expression of selected genes. In addition to their regulation of cellular differentiation, development, and basal-level endogenous metabolism (anabolism and catabolism), some of these family members can interact with xenobiotic metabolism. This interaction generally results in gene overexpression and induction of the biotransformation pathway. For example, the binary complex of PPAR and

retinoid X receptor (RXR) acts as a transcription factor that increases the expression of CYP 4A genes by binding peroxisome proliferator responsive element.

Note 1: The family is divided into three subfamilies: (i) PPAR-alpha; (ii) -beta or -delta; and (iii) -gamma, with this last group being further divided into $\gamma 1$, $\gamma 2$, or $\gamma 3$ as a result of alternative splicing.

Note 2: This type of overlapping interaction (between gene regulation factors associated with basal-level endogenous signaling pathways and certain xenobiotic metabolism pathways) is also observed for the hormone nuclear receptors. For example, regulation of CYP 3A genes can occur through a receptor-mediated mechanism where the complex of ligand and steroid receptor binds to the glucocorticoid responsive element (GRE). The PPAR and steroid receptor pathways are thus similar to each other while being significantly different from the aromatic hydrocarbon receptor (AHR) pathway that can upregulate certain of the CYP 1A and 1B subfamily members by relying on the xenobiotic response element (XRE) without playing a fundamental role in any other endogenous processes. In this regard, the pregnane X receptor (PXR) sits between these two classification types, because it behaves more like a promiscuous nuclear receptor protein that interacts with both a variety of endogenous and xenobiotic compounds, ultimately being capable of upregulating some of the CYP 2B, 2C, and 3A members, as well as components for the glutathione drug-conjugation pathway and P-glycoprotein (Pgp) drug-efflux transporter system.

See: Aromatic Hydrocarbon Receptor (AHR), Pregnane X Receptor (PXR), Glucocorticoid Responsive Element (GRE), Retinoid X Receptor (RXR), Constitutive Androstane Receptor (CAR) and CAR/RXR Heterodimers, and Xenobiotic Responsive Element (XRE).³⁵ Res. Topic Ref.: 198.

167. P-Glycoprotein (Pgp/MDR1/ABCB1)

Pgp is an ATP-dependent efflux transporter protein that spans the cell membrane and serves to pump a broad range of compounds out of cells, including, especially, xenobiotics that present themselves as suitable substrates. The importance of the relationship that transporters have with drug metabolism events, as well as with pharmacokinetic profiles in general, cannot be emphasized enough (see Note 4).

Note 1: Borrowing from enzyme-related terminology, a transporter's cargo is acceptably referred to as a "substrate," even though no chemical change is imparted to the structure.

Note 2: Pgp was first identified from cancer cells that had become resistant to chemotherapeutic agents during prolonged treatment by its overexpression, as well as by several other possible mechanisms. Since the observed resistance included a broad range of drug structural types, the term first adopted for Pgp was "multidrug resistance protein (MDR1)," which is still in use today. It was later determined that there is a family of these types of transporters (some being uptake as well as additional efflux members), where Pgp falls into the category of an ATP-binding cassette sub-family B group and is designated as member number 1. Thus, the acronym "ABCB1" is also utilized to designate Pgp. Among this family, Pgp remains one of the foremost and most significant of the transporters.

Note 3: Especially high levels of Pgp are normally expressed in: the intestinal epithelium, where it can serve to pump xenobiotics back into the lumen and thus limit the latter's oral bioavailability; liver cells, where it pumps its substrates (e.g., parent drugs and their metabolites) into the bile duct and thus serves to excrete them from the body by the biliary route; the proximal tubule cells of the kidney, where it pumps its substrates into the urine-forming ducts as a prelude to their excretion by the renal route; and the capillary endothelial cells associated with the blood-brain barrier (BBB), where it serves to limit the access of many endogenous compounds and xenobiotics into the brain.¹⁹⁹⁻²⁰¹

Note 4: Any possible metabolic event for a given xenobiotic at any locale within the body depends on: (i) the suitability of the xenobiotic's structure to serve as a substrate for the enzyme(s) associated with the event; (ii) the level(s) of the relevant enzyme(s) and competing enzymes present at that locale; and (iii) the concentration and residency time of the xenobiotic at that locale. Component (iii) is a function of the inherent physical properties of the substrate (e.g., $\log_a D$) and its suitability to serve as a substrate for transporters, where the latter's importance grows exponentially with the levels of the various transporters that may or may not be present and operative at that locale. While all three components represent a challenge for prediction of a drug's metabolism *in vivo*, component (iii) arguably presents itself as the largest hurdle due to the presence of the transporters that additionally complicate the variables that need to be taken into account.

See: Absorption, Permeation/Permeability, Partition Coefficient (P ; $\log_a P$ and $\text{clog}_a P$); Distribution Coefficient (D ; $\log_a D$), Passive Transport, Active Transport, Blood-Brain Barrier (BBB), and Metabolic Prediction/Possibilities versus Probabilities. Structure/Mechanism Refs.: 202-205.

168. Pharmacodynamics

In one usage, this is the time course for a drug's effects (therapeutic or undesired), such that it is possible to cite a "pharmacodynamic onset" and, importantly, a "pharmacodynamic half-life" subsequent to a given dose (various routes of administration are possible, but should be indicated in a specific manner) without ever determining the drug's concentration in any particular tissue/compartiment. In another usage, this is the relationship between drug concentration and the magnitude of its effects. While the concentration at the site of action is the most appropriate for this latter relationship, most often a more readily sampled tissue or fluid is used, such as plasma, serum, or blood.

See: Pharmacokinetics (PK) and Half-life ($t_{1/2}$).^{3,38-40,104}

169. Pharmacogenetics and Pharmacogenomics

Pharmacogenetics is the study of inherited genetic differences that impact directly on an individual's responses to drugs in terms of efficacy and ADMET profile. Pharmacogenomics encompasses pharmacogenetics and further includes an individual's acquired genetic differences. Both fields track germline single-nucleotide polymorphisms (SNPs), with pharmacogenomics also studying somatic mutations, e.g., within diseased cells, such as cancer. Finally, pharmacogenomics also considers an individual's ongoing exposure to xenobiotics and other circumstances that can alter the expression of one's inherited and acquired genetic code (that composite leading to the observed phenotype).

Note 1: The characterization of rapid versus slow metabolizers and various other phenotypes associated with xenobiotic metabolism preceded the "omics" field and can be considered to have established a solid base of interest to pursue individual variation toward drug responses well before the genetic code and the study of its variation were unraveled.

See: Acetylation Phenotype, Aldehyde Dehydrogenase Polymorphism or Deficiency, Atypical Alcohol Dehydrogenase, Drug-Drug Interactions, Extensive (or Rapid) Metabolizer, Genetic Polymorphism, Genotype/Genotyping, Inducing Agent, Induction, Inhibition, Isoform or Isozyme (Isoenzyme), Metabolic Prediction/Possibilities versus Probabilities, Metabolic Probe, Metabolic Profiling/Metabolic Fingerprinting, Metabolomics/Metabonomics, Phenotype, Polymorphism, Single Nucleotide Polymorphisms (SNPs), and Ultra-Rapid Metabolizer. Representative Refs. (across this topic's history as it connects drug metabolism to clinical practice): 206-215.

170. Pharmacokinetics (PK)

Either the study of, or the resulting data/profile reflecting, the time course for a pharmaceutical agent's absorption, distribution, metabolism, and excretion (ADME) from the body, assessed in a quantitative manner by tracking the agent's concentration with time. These assessments most commonly focus on plasma, serum, or whole blood concentration versus time after administration of the test agent. However, any of the various tissues (physiological compartments) may be additionally assayed, such as the use of cerebrospinal fluid so as to further assess penetration into the brain for agents designed to be potential CNS drugs.

Note 1: Historically, radiolabeling was typically relied on as a sensitive method to detect and track the test agent. While still useful today, most of these studies are now able to deploy liquid chromatography-mass spectrometry (LC-MS) to sufficiently generate a PK profile. In addition, LC-MS/MS can now be used to also gain a preliminary, if not final, determination of what metabolites may have been produced from the parent compound within the given test subjects/species.

See: ADME, ADMET, Allometric Scaling, Allosteric Scaling, AUC, Bioavailability (Absolute and Relative), Clearance (CL), Compartment Model, Disposition, DMPK, Dose-Dependent Kinetics or Metabolism, Extraction Ratio, Half-Life ($t_{1/2}$), Intrinsic Clearance, IVIVE, Linear Kinetics, Metabolic Clearance, Metabolic Pathway/Pattern/Profile, Michaelis-Menten Equation/Kinetics, Non-Compartment PK Analysis, Nonlinear Kinetics, Oral Bioavailability, Partition Coefficient (P ; $\log_a P$ and $\text{clog}_a P$); Distribution Coefficient (D ; $\log_a D$), PBPK, Pharmacodynamics, and Pharmacogenetics and Pharmacogenomics. ^{3,38-40,104}

171. Phases of (Drug) Clinical Testing

Investigations pertaining to the first time that a new drug is administered to humans are undergoing global harmonization. The following terms have been taken from the system presently used by the U.S. Federal Drug Administration (FDA) and are generally representative. Phase 0, also often called "human microdosing" (HMD), is not a mandatory first step, but can be used to gain a preliminary assessment about a new agent's anticipated PK profile after administering doses that are too small to prompt efficacy and, importantly, toxicity. A Phase I trial involves testing in healthy human volunteers to establish the agent's short-term safety/tolerability; PK profile, including likely metabolites, and a dosing paradigm that may be able to be used in subsequent efficacy studies (whenever possible, assays for relevant biomarkers of toxicity and physiological mechanistic parameters leading to anticipated efficacy are now also being added, where the latter is often considered to be a practical/investment-worthy validation of the therapeutic concept). Phase II testing/trials utilize volunteer patients to see if the drug can treat the intended disease in a beneficial manner, thus firmly establishing efficacy while further assessing possible side-effects and continuing the refinement of administration protocols toward an eventual recommended dosing paradigm. Phase III trials recruit large numbers of volunteer patients, so as to establish a broader knowledge base about the efficacy, side-effect profile, and general safety of the agent relative to a recommended dosing paradigm that can include considerably longer exposure cycles. Finally, Phase IV involves post-marketing surveillance to watch the new drug's reports from longer-term use across the general public where patients have sought treatment from a physician.

Note 1: Drug metabolism is a critical component of the ADMET profile in humans that often has direct relationships with the desired efficacious activity and any undesired off-target or side-effect toxicity. Its thorough characterization, including the possibility of metabolism-related drug-drug interactions as appropriate for anticipated future usage, is mandated for a new drug candidate to successfully proceed to the marketplace. Numerous types of preclinical studies at the *in vitro* level and several *in vivo* studies in various animal models, as well as computer program databases and expert systems, attempt to discern what is likely to occur upon the administration of a drug to humans, but the above series of clinical trials are ultimately intended to provide the final answer before a new drug can be released for sale to the general public.

Note 2: In the U.S., the composite of chemical production information; analytical quality control measures for the drug substance and its measurement in various biological matrices; and preclinical efficacy, PK, and ADMET testing studies, with a special emphasis on the evaluation of toxicity across several animal species for durations longer than those to be deployed in humans; is all assembled into an Investigational New Drug (IND) document that is an FDA-mandated application needed to enter into human testing. A similar New Drug Application (NDA) must be submitted before entering the marketplace—it additionally includes all of the information gathered during the Phase I to III clinical trials.

Note 3: The terms used to designate clinical trials and those used for the phases of drug metabolism are very similar. They should be distinguished by the use of a capital “P” and by using a Roman numeral for each of the clinical phase testing/trial designations, whereas the phases in drug metabolism are not capitalized and are denoted by Arabic numerals.

See: Microdosing, Phase 0 Clinical Study, Drug Metabolism, Biotransformation, ADME, Metabolic Fate, Metabolic Pathway/Pattern/Profile, Metabolic Ratio, Metabolic Prediction/Possibilities versus Probabilities, and Phases of Drug Metabolism.^{173,216–219}

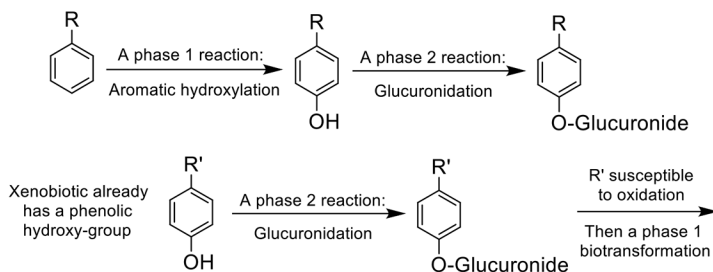
172. Phases of Drug Metabolism

Historically, the now controversial terms “phase 1” and “phase 2” metabolism have been used for many years to designate xenobiotic biotransformations involving hydrolytic, oxidative, and reductive processes (“phase 1”) versus conjugation reactions (“phase 2”).⁸¹ Subsequently, the term “phase 3” has sometimes been used to designate the excretion of xenobiotics and their metabolites from the body.²²⁰ More recently, the term “phase 0” has seen some use in order to convey events, such as a xenobiotic’s absorption and distribution, generally in the context where these take place prior to a biotransformation event.¹³⁰ Some experts want to move away from using any of these types of terms (for the reasons indicated in Note 2 below),²²¹ while others suggest an even broader expansion of the terminology to incorporate systems biology and cross-disciplinary concerns relative to a xenobiotic’s metabolic fate within both the individual that it is initially administered to, plus its subsequent appearance in the environment (Note 3). **Readers are encouraged to see the sections within the glossary’s Introduction and detailed Appendix II that discuss the “pros and cons” of preserving the**

use of these historical terms and their potential expansion, as well as considering the notes provided below.

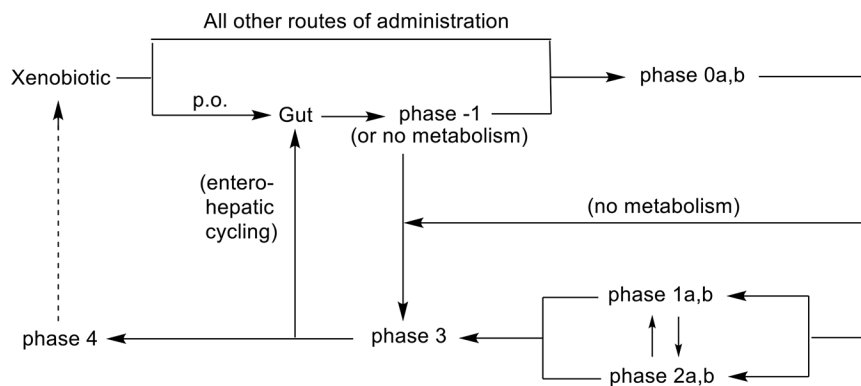
Note 1: The clinical testing phases use very similar nomenclature. However, they can be distinguished by the use of a capital "P" and a Roman numeral for each of their sequential steps, whereas the phases in drug metabolism are not capitalized and are denoted by Arabic numerals.

Note 2: The early identification of a very common metabolic pathway, where a xenobiotic having a susceptible aryl-group undergoes aromatic hydroxylation followed by glucuronide conjugation to the instilled hydroxyl-group, grew into the general theme where lipophilic compounds are first metabolized (especially by an oxidative process) so as to gain a functional-group handle that allows the body to couple (conjugate) one of its own, highly polar adducts so as to dramatically increase the initial xenobiotic's water solubility and enhance its excretion from the body. This theme, in turn, became the basis for the phase 1 and 2 designations. But when a xenobiotic already has an appropriate functional group present, then conjugation can occur without the need for a preceding phase 1 event. Likewise, in some cases further oxidative metabolic events will follow a conjugation reaction. This has led to the confusion implied by this terminology, which has led some experts to suggest moving away from its usage altogether. The historical and confusing aspects about this nomenclature are illustrated below by the upper and lower pathways.



Note 3: Appreciating that these designations are more like process classifications, rather than strictly sequential pathways, allows for their continued usage. Alternatively, retaining their specific phraseology as a "phase" recognizes the historical note conveyed above, while underscoring that there are specific sequences of events often required by the substrate to

traverse an overall metabolic pathway. In this context, a potential expansion of this nomenclature is illustrated below for the human case of exposure to a xenobiotic. This is not intended to be a recommended “final” definition, but rather the basis for further discussion. It encompasses a systems biology approach that could provide a useful context for investigators interested in the “phase -1” and “phase 4” regions of xenobiotic metabolism, areas that reflect distinctive, cutting-edge directions for the field in general.



This nomenclature begins by illustrating the potentially important difference in the metabolic fate of a xenobiotic contributed by the gut microflora after oral ingestion (PO) versus other routes of administration. Thus, phase -1 (minus one) designates all of the possible biotransformations (some being quite unique to the microorganisms) that can be catalyzed by the gut microflora. As such, phase -1 also encompasses the role that these microorganisms can sometimes play during enterohepatic cycling, e.g., hydrolysis of a phenolic-glucuronide metabolite that has undergone biliary excretion back to the phenol, which can then be reabsorbed rather than proceed toward fecal elimination. Phase 0 designates all of the non-metabolic dispositional processes to which a xenobiotic or metabolite will traverse, such as absorption passively or by transport across membranes and association with biomolecules not involved in drug metabolism, while undergoing fluid-driven distribution through the living system. The important relationships of a drug's transport coupled to its metabolism, and a metabolite's transport coupled to its excretion, are thus recognized by this nomenclature. For this term, the “a” designates that these phenomena occur prior to a xenobiotic's first mammalian metabolic event (as shown in the scheme), while the “b” intends to further

convey that these processes continue throughout the entire timeframe after a xenobiotic's arrival until it and all of its metabolites are cleared from the living system. In line with the historical definition, phase 1 includes all hydrolytic, oxidative, and reductive biotransformations. The "a" then provides a sub-classification for such events occurring at a given point on the substrate for the first time, while the "b" indicates that the reaction event occurs at a point on the substrate which has already participated in a preceding biotransformation. Typical phase 1a examples include ester hydrolysis, aromatic hydroxylation, arylether *O*-demethylation, and the first of possible *N*-dealkylations. Aside from the hydrolytic reactions, the most common phase 1a biotransformations are generally catalyzed by the CYP family. The phase 1b events utilize a broader range of enzymes to catalyze the same types of chemical reactions. Typical phase 1b examples include: the rapid reduction or further oxidation of the aldehyde metabolites resulting from CYP-mediated *N*- or *O*-dealkylations, further processing of an initial glutathione conjugate so as to form a mercapturic acid metabolite, and successive β -oxidations of aliphatic carboxylic acid chains. It can be noted that phase 1b metabolism is often critical for avoiding metabolite toxicity (i.e., further processing of aldehydes) or for reducing a metabolite's molecular mass as a prelude to the latter's excretion (i.e., further processing of a glutathione conjugate reduces its initial molecular mass increase of 307 Da to only 162 Da, which is then similar to the increase of 175 Da that results from the formation of a common glucuronide conjugate). Also in line with the historical definition, while additionally incorporating similar sub-classifications, phase 2 metabolism includes all of the conjugation reactions that can take place, either as the first metabolic event at a given point on the substrate ("a") or as a subsequent reaction at a point on the substrate having already undergone a biotransformation. Typical examples include: glucuronidation and sulfation of aromatic hydroxyl groups either present on the parent xenobiotic (phase 2a) or having been instilled there (phase 2b) by a preceding phase 1a, CYP-mediated aromatic hydroxylation or an aryl-*O*-demethylation biotransformation; glutathione entrapment of highly reactive electrophilic centers present in the substrate; *N*-acetylation or *N*-, *O*-, and *S*-methylation reactions; and glycine conjugation of carboxylic acid moieties either present on the parent xenobiotic (phase 2a) or having resulted (phase 2b) from a preceding phase 1a, esterase-mediated hydrolysis reaction. The term phase 3 encompasses the various excretion-associated events that either the un-metabolized xenobiotic (note first line immediately after the phase 0a,b processes) or its metabolites will eventually engage. Key

examples include: physicochemical-driven partitioning behavior between biological milieu associated with excretion, such as the loop of Henle within the kidneys' nephrons; transport into the bile canaliculi at the hepatobiliary interface, especially for negatively charged metabolites by the organic anion transporters (OATs) present within the liver's hepatocytes; and transport across the GI epithelium into the gut lumen, especially for metabolites which are good substrates for P-glycoprotein (Pgp). Also accounted for in this expanded nomenclature's diagram is the possibility for enterohepatic cycling, a key example afforded by the steroids that, upon glucuronide conjugation and excretion into the gut, can be hydrolyzed by the microflora and thus subject to reabsorption. Finally, phase 4 encompasses all of the processes that can continue to contribute toward an excreted compound's environmental fate. Typical examples include: the possibility for both continued biodegradation (catabolism) or potential anabolism by a variety of microbial, plant or animal species; as well as "distribution" by waterways or by edible plants and animals so as to enter the nutritional cycles of mammals, where ingestion by humans, as suggested by the dotted line in the nomenclature's accompanying scheme, then comes full circle along a systems biology theme.

See: Phases of (Drug) Clinical Testing; Drug Metabolism, Biotransformation, ADME, Metabolic Fate, Metabolic Pathway/Pattern/Profile, Metabolic Ratio, Cytochrome P450s Enzymes (CYPs), Aldehyde Dehydrogenase (ALDH), Aldehyde Oxidase (AO), Aromatic Hydroxylation, Conjugation Reactions, Conjugate, Glucuronidation, Glutathione Conjugation, Mercapturate/Mercapturic Acid Conjugation, Enterohepatic Cycling, and Phenobarbital Induction and Sleeping Time.^{81,130,158,220-227}

173. Phenobarbital Induction and Sleeping Time

In addition to its clinical indications, phenobarbital is often deployed to induce the over-expression of liver enzymes within rodents destined to serve as donors for the preparation of liver fractions (e.g., microsomal) that are to be used during *in vitro* drug metabolism studies. It induces many members of the CYP enzyme family, with CYP 2B6 being particularly responsive via the CAR/RXR nuclear receptor signaling pathway.

Note 1: A typical protocol involves intraperitoneal injection of the donor group daily for three days. Starting with a dose that can cause all animals to sleep for ca. an hour, even the second dose will have an observably weakened

pharmacodynamic effect due to the agent's more rapid metabolism, e.g., perhaps 50 % will sleep and then only for 0.5 hours. By day three, that same dose is likely to cause less than 25 % of the study group to sleep, and those for only a few minutes. A detectable increase in body weight can occur, the latter resulting from a very observable and pronounced increase in liver weight when compared to untreated control animals.

Note 2: Phenobarbital itself largely undergoes the classical pattern of CYP-mediated aromatic hydroxylation followed by glucuronide conjugation; this metabolite is then primarily excreted by the renal route; this is historically called the phase 1 and phase 2 metabolism sequence, or, according to certain proposed newer nomenclature, a phase 1a, phase 2b, phase 3 and phase 4 sequence, with the latter's details remaining unspecified.

See: Induction, Inducing Agent, Microsomal Fraction/Enzymes; Microsomes, Phenotype, RXR and CAR/RXR, Pharmacodynamics, and Phases of Drug Metabolism.²²⁸ Applied Research Refs.: 159,229.

174. Phenotype/Phenotyping

Within the context of drug metabolism, a phenotype is the observed manner in which an individual handles the disposition of an administered agent. It begins with the individual's inherited genotype, which can then become influenced by environmental factors through interactions at the genetic level (e.g., induction or inhibition of expression) or at the immediate functional level for any of the processes associated with each of the ADME parameters (e.g., agonist or antagonist effects in play at any given process). A common environmental factor would be prior and/or simultaneous exposure to one or more other drugs (e.g., drug-drug interactions) or to xenobiotics present in one's diet. Equally common is when continued exposure to the same drug causes an alteration of one or more of the ADME processes with time, thus causing an alteration in the agent's observed disposition (i.e., the person's phenotype). Compounds with well-defined disposition patterns across normal and/or various genotype-defined individuals can be deployed for both genotyping and phenotyping of an individual.

See: Genotype/Genotyping, Genetic Polymorphism, Phenobarbital Induction and Sleeping Time, ADME, Drug-Drug Interactions, and Cocktail Study.^{116,119,125,127,230}

175. Plasma and Serum; Plasma and Serum Concentration

Pertaining to drug metabolism, plasma is the fluid portion of whole blood. It can be separated from the latter by adding an anticoagulant (e.g., heparin or EDTA) and centrifuging to remove cellular components. If no anticoagulant is added and clotting is allowed to occur, then the centrifugation's separated fluid is called serum (plasma that also lacks the majority of the clotting factors, such as the fibrinogens).

Note 1: Plasma/serum represents more than 50 % of the total blood volume. They are more than 90 % water and, in addition to a plethora of small inorganic and organic molecules, contain numerous proteins, including various enzymes, the most important of which for drug metabolism probably belong to the Esterase family. A family of highly lipophilic plasma proteins unique to plasma/serum (hence their name) can sometimes have a significant impact on the disposition of a xenobiotic, even when there is no susceptible ester group present. This is especially the case when non-polar xenobiotics bind tightly to such proteins, which can then serve as a reservoir while decreasing the immediate plasma/serum concentration of unbound agent that would otherwise be free to undergo metabolism or to potentially cause toxicity.

Note 2: Because blood is the primary means of transporting xenobiotics throughout the body and provides for readily accessible sampling, it and/or its subsequently separated plasma or serum is commonly used during PK studies to track the distribution and disposition of parent compounds and their metabolites, such as, for example, their plasma concentrations.

Note 3: Total blood volume can be ascertained by the following steps: 1) injection of a standard dye (e.g., Evans blue); 2) allowing for its distribution throughout the body; 3) removal of a blood sample, which is treated with an anticoagulant and centrifuged; and 4) measurement of the dye's plasma concentration. The concentration of a test drug can be determined similarly, while also following a protocol that instead specifies several measurements to be taken across a continuing timeline (typically for several hours after a single oral dose) so as to deduce a half-life within this compartment that, in turn, is generally taken as an estimate of the terminal half-life for the agent relative to the entire body.

Note 4: Serum measurements should not be used when there is a suspicion that significant amounts of the test agent may bind to blood clotting factors or that the agent may be susceptible to entrapment during the blood clotting process. Similarly, plasma protein binding should be assessed for either fluid during analytical method development so as to account for how it might impact the agent's recovery from the matrix and its ultimate response in the assay. Finally, although plasma can be relied upon as a preliminary assessment of an agent's susceptibility to Esterases, the use of whole blood in follow-up studies is additionally recommended, because the surface and interior of red blood cells also contain considerable Esterase activity.

See: Pharmacokinetics (PK), ADME, *AUC*, Clearance (*CL*), Compartment Model, Disposition, DMPK, Dose-Dependent Kinetics or Metabolism, Half-Life ($t_{1/2}$), Intrinsic Clearance, Metabolic Clearance, Metabolic Pathway/Pattern/Profile, Non-Compartment PK Analysis, Plasma Protein Binding, Partition Coefficient (P ; $\log_a P$ and $\text{clog}_a P$); Distribution Coefficient (D ; $\log_a D$), PBPK, and Pharmacodynamics. For specific definitions, see Refs. 231,232.

176. Plasma Protein Binding (PPB)

Typically, a readily reversible association between a xenobiotic and certain of the proteins present in plasma, particularly those that can be regarded as lipophilic in overall character, such as albumin, lipoprotein, glycoprotein and the α , β and γ globulins. In general, some of the association involves hydrophobic bonding, such that less polar xenobiotics tend to be more prone to this interaction than polar compounds. Alternatively, the slightly basic nature of albumin can allow for association with acidic functionality when present on a xenobiotic, as well as with neutral agents, while the acidic nature of certain of the glycoproteins can accommodate xenobiotics having basic centers. Because only unbound compounds can serve as substrates for enzymes or can undergo membrane permeation, significant PPB can impact the ADME profile of xenobiotics and their metabolites, as well as their efficacy profile.

Warfarin represents a classical example of a drug that undergoes highly significant PPB (more than 95 %). It also has a narrow therapeutic index, such that if another drug is administered that also binds strongly with the PPs so as to cause displacement of warfarin (drug-drug interaction), the latter's resulting higher unbound concentrations could lead to excessive bleeding or other toxicities not normally seen at this same dose.

Note 1: Historically, it was thought that PPB had a significant impact on ADME events only when a compound was bound at above 90 % or even above 95 %, because this typically rapid equilibrium's dissociation rate can otherwise readily supply unbound agent fast enough to satisfy the equilibrium requirements for significant interaction with various proteins associated with all of the potential ADME-related processes. This situation typically prevails in a practical manner when deploying an assay method to assess a drug's plasma concentration, i.e., such determinations typically account for both free and bound drug, even though the assay measurement itself invariably detects only unbound drug. This is because the sample preparation method generally pulls bound drug off of the PPs prior to executing the detection method.

Note 2: Xenobiotics bound to PPs at such extremely high levels can be thought to have entered another compartment that, in turn, serves to immediately decrease the levels of free xenobiotic in the plasma while serving as a reservoir to more gradually release the agent back into the system. This scenario probably has evolutionary ties, where mammals came to enhance the PPB proteins to assist in accommodating lipophilic natural products in their diets that could have concentration-dependent toxicity.

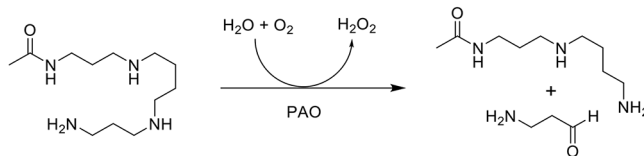
Note 3: More recently, it has become appreciated that the influence of PPB may be able to occur at less high levels of association with a xenobiotic. Given the dynamic nature of the flow-driven distribution for a xenobiotic, its initial unbound concentration, rather than a responsively increasable concentration, may be more important when the surface linked to a particular ADME event decreases in size, regardless of the specific ADME-related process's ability to capture unbound agent. Thus, a specific level where PPB generally becomes significant will not be cited as part of this term's definition. However, it should be clear that the more an agent undergoes PPB, the greater the chance for the latter to have an impact upon the agent's ADME profile: that impact would generally be to slow down the possible ADME processes that might occur on the agent.

See: Plasma and Serum; Plasma and Serum Concentration, Pharmacokinetics (PK), ADME, *AUC*, Clearance (*CL*), Compartment Model, Disposition, DMPK, Dose-Dependent Kinetics or Metabolism, Half-Life ($t_{1/2}$), Intrinsic Clearance, Metabolic Clearance, Metabolic Pathway/Pattern/

Profile, Non-Compartment PK Analysis, Partition Coefficient (P ; $\log_a P$ and $\text{clog}_a P$); Distribution Coefficient (D ; $\log_a D$), PBPK, Drug-Drug Interactions, and Pharmacodynamics.^{233,234}

177. Polyamine Oxidase (PAO)

This term is now known to encompass a group of related enzymes within the broader oxidoreductase family that cleave certain alkyl-amines to the des-alkyl amine and an aldehyde by using molecular oxygen with FAD and iron serving as cofactors. Specific members include: N^1 -acetylpolyamine oxidase EC 1.5.3.13; polyamine oxidase (propane-1,3-diamine-forming) EC 1.5.3.14; N^8 -acetylspemidineoxidase (propane-1,3-diamine-forming) EC 1.5.3.15; spermine oxidase EC 1.5.3.16; and non-specific polyamine oxidase EC 1.5.3.17. They are present in most mammalian tissues, where specific members play a role in the natural polyamines cycle, N^1 -acetylspermine and N^1 -acetylspemidine being particularly good substrates while spermidine itself is not. The PAO group works with MAO and DAO to generally deaminate xenobiotics where their structural requirements are similar to MAO, although as a group they can accommodate more complex and multiple amino-containing compounds. The example below depicts deamination of N^1 -acetylspermine to N^1 -acetylspemidine.



See: Catabolism, Deamination, Dealkylation, N -Dealkylation, Monoamine Oxidase (MAO), Diamine Oxidase (DAO), and Oxidation/Oxidase/Oxidoreductase.²³⁵

178. Polymorphism and Polymorphic Metabolism

Polymorphism reflects the existence of one or more alleles of a gene within a given population where the frequency of the rarer alleles is greater than can be explained by recurrent mutation alone and results in an observed phenotype with an incidence greater than 1 %. When the genes translate to metabolism-related machinery, the resulting polymorphic enzymes or other associated biomolecules can lead to differential metabolism or polymorphic metabolism among the individuals in this population. The

differences can be as large as distinct pathway changes, or as small as subtle alterations in the drug metabolism rates along the same biotransformation pathway.

See: Acetylation Phenotype, Aldehyde Dehydrogenase Polymorphism or Deficiency, Atypical Alcohol Dehydrogenase, Catechol-*O*-Methyl Transferase (COMT), Enzyme, Isoform or Isozyme (Isoenzyme), *N*-Acetyltransferases (NATs), Genotype/Genotyping, Pharmacogenetics and Pharmacogenomics, Phenotype/Phenotyping, Single Nucleotide Polymorphism (SNP), Extensive (or Rapid) Metabolizer and Poor (or Slow) Metabolizer.^{103,114-129}

179. Poor (or Slow) Metabolizer

A subpopulation within a given species that metabolizes a xenobiotic via a pathway under polymorphic influence at a significantly slower rate compared to the other subpopulations. The faster rate groups, in turn, can be classified as "extensive" or "rapid metabolizers."

Note 1: This profile can occur from a mutation or deletion in a drug metabolizing-related gene that results in lower expression of a fully functional metabolizing system; a similar expression of a less than fully functional metabolizing system; or a higher expression of a fully functional, even enhanced, catabolic system that degrades the metabolizing system.

See: Acetylation Phenotype, Aldehyde Dehydrogenase Polymorphism or Deficiency, Atypical Alcohol Dehydrogenase, Catechol-*O*-Methyl Transferase (COMT), Enzyme, Isoform or Isozyme (Isoenzyme), *N*-Acetyltransferases (NATs), Genotype/Genotyping, Pharmacogenetics and Pharmacogenomics, Phenotype/Phenotyping, Extensive (or Rapid) Metabolizer, and Polymorphism and Polymorphic Metabolism.^{103,114-129}

180. Pregnane X Receptor (PXR)

PXR is a nuclear receptor protein that serves as a transcription factor to regulate the expression of selected genes. In addition to being responsive to several endogenous ligands, such as many of the steroids, for which it is also sometimes referred to as a steroid sensing nuclear receptor (SXR), PXR is highly promiscuous in its formation of a complex with numerous xenobiotic partners. Exemplifying the latter is the complex formed with the

drug dexamethasone, which includes the formation of a heterodimer with the retinoid X receptor (RXR), which then binds to the response element of the CYP 3A4 promoter. Its interaction with the xenobiotic-responsive element (XRE) can likewise lead to upregulation of CYPs 3A4, 2C8, 2C9, and 2B6. Further underscoring its important role in the overall disposition of xenobiotics, the PXR complex can also upregulate certain of the drug conjugation enzymes, such as glutathione *S*-transferase, and even some of the efflux proteins, like P-glycoprotein (Pgp or MDR1).

See: Peroxisome Proliferator-Activated Receptors (PPARs), Aromatic Hydrocarbon Receptor (AHR), Glucocorticoid Responsive Element (GRE), Retinoid X Receptor (RXR), Constitutive Androstane Receptor (CAR) and CAR/RXR Heterodimers, and Xenobiotic Responsive Element (XRE).^{35,236}

181. Presystemic Elimination

Reduction in a xenobiotic's concentration prior to its distribution into the systemic circulation. Primarily used within the context of orally ingested agents that undergo first pass metabolism and excretion.

Note 1: In addition to elimination caused by the intestinal epithelium, hepatic tissues, and lungs, presystemic elimination can occur in the gut lumen due to degradation under the acidic conditions of the stomach, to breakdown by excreted peptidases, and from metabolism caused by the bacteria comprising the intestinal microflora.

See: Absorption, Bioavailability, Clearance, ADME, First Pass Metabolism, Hepatic Clearance, Hepatic Extraction, Metabolic Clearance, Presystemic Metabolism, and Enterohepatic Cycling.^{39,105,106}

182. Presystemic Metabolism

Reduction in a xenobiotic's concentration due to metabolism prior to its distribution into the systemic circulation. Primarily used within the context of orally ingested agents, which can undergo biotransformations caused by intestinal microflora, intestinal enterocytes, the liver, or the lungs prior to distribution within the general systemic circulation.

See: Absorption, Bioavailability, Clearance, ADME, First Pass Metabolism, Hepatic Clearance, Hepatic Extraction, Metabolic Clearance, Presystemic Clearance, and Enterohepatic Cycling.^{39,105,106}

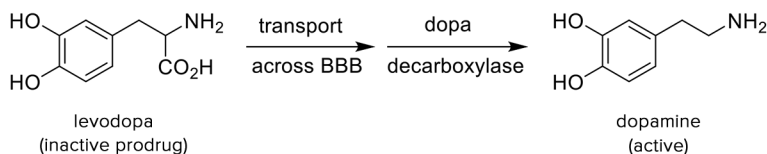
183. Primary Metabolite

A metabolite that is formed directly from the xenobiotic initially presented to the biological system (parent compound).

See: Metabolite versus Degradation and Decomposition Products, Metabolism, Drug Metabolism, Biotransformation, Secondary Metabolite, and Metabolic Pathway/Pattern/Profile.^{3,45,82,91}

184. Prodrug

As typically used: an inactive or weakly active pharmacological agent that undergoes conversion in the body to become an effective drug, the conversion generally involving a biotransformation or metabolic step. A more precise definition is: a compound that requires one or more structural changes to occur after its administration in order to elicit its desired pharmacological effects, such changes not including simple shifts in the equilibria of ionizable groups, nor the interchange of various salt or tautomeric forms that may be applicable to the compound within different environments. A classic example is shown below. While dopamine can be effective in treating Parkinsonism, it cannot cross the blood-brain barrier (BBB). Levodopa, itself inactive, is administered orally as a prodrug, because it can take advantage of an amino acid transporter system to gain entry into the body and the CNS. It then undergoes conversion to dopamine by brain dopa decarboxylase (although some is also prematurely converted by peripheral dopa decarboxylase, leading to the development of subsequent formulations also having a peripheral dopa decarboxylase inhibitor).



Note 1: The spelling and definition of this term have undergone an interesting evolution.¹⁶⁹ The term "pro-drug" was first introduced by Albert more than fifty years ago when he used it to describe compounds which require metabolic biotransformation in order to exhibit their pharmacological effects, such conversions being either an inherent property of the parent compound ("accidental" prodrug), or a property intentionally incorporated into an otherwise active drug by specific design.^{168a} Emphasizing the

latter, the term “drug latentiation” was also introduced at nearly the same time by Harper. This type of design strategy was defined as “chemical modification of a biologically active compound to form a new compound which upon *in vivo* enzymatic attack will liberate the parent compound.”^{169b} Kupchan et al. subsequently expanded the strategy to further include drug modifications that relied upon non-enzymatic processes to regenerate the parent compound within an *in vivo* setting, such as spontaneous hydrolysis of the modification as a function of pH.^{169c} Because “latentiation” implies a time-lag for the regeneration of the active substance and this type of event is not always required to be part of a prodrug’s most desirable pharmacokinetic profile, such terminology has not been sustained. Eventually, the term “prodrug” (without the former hyphen mark) became universally accepted.¹⁶⁹ Despite this consensus about what term to finally adopt, its intended meaning in a given usage has continued to vary, often being taken to include a much wider context of drug-related profiles and design strategies that, in some cases, are not appropriate. This situation has prompted the citation of a more precise definition in addition to the one that is typically encountered, as noted above.

Note 2: According to the more precise definition, prodrugs are inactive with regard to their desired effects within the range of their advantageous administration protocols until they undergo bioactivation by either a chemically driven conversion, an enzymatically driven biotransformation, or any multiple combination of these processes. However, this profile should not be taken to also encompass any undesired side-effects, which could be inherently present within the prodrug without the need for a preceding biotransformation. Also, according to this definition, efficaciously active agents whose metabolites are similarly active or contribute to the desired pharmacological profile by another mechanism should not be considered to be prodrugs. While such compounds have sometimes been referred to as “limited prodrugs,” they should retain their simple classification as “drugs,” no matter how short-lived the parent compound’s observable activity might be *in vivo*. That such drugs also happen to have active metabolites does not, then, alter their initial classification according to this term. Finally, although sometimes confused with prodrugs in the literature because they can also be used as a strategy to target selected biological compartments, “soft drugs” should not be regarded as prodrugs. The schematic relationships between a drug, prodrug, and soft drug can be found under the definition of a soft drug.

Note 3: Prodrug strategies can be attempted to enhance any of the ADMET properties associated with a drug candidate. A strategy that is particularly prominent today is to decrease toxicity by increasing selectivity for the desired, efficacious target, thus lowering the dose needed to interact with the latter and/or raising the dose needed to interact with the former, off-target site. To further appreciate the rich history of the prodrug field and its importance within today's drug design processes, readers are encouraged to consult the excellent review articles, monographs, and texts that have been dedicated specifically toward prodrugs by several distinguished experts across time.

Note 4: A classification system for different types of prodrugs has been proposed, but it has not been commonly adopted for general use. However, some system for the further subcategorization of prodrugs may be useful in the future due to the present rise in prodrug strategies that involve complicated molecular constructs to deliver therapeutic agents targeted toward specific tissues via connection to monoclonal antibodies, i.e., antibody-drug conjugates or ADCs. This possibility is further discussed in Appendix III, where interested readers can also consider the proposed classification system in an even further expanded manner suggested by the glossary's corresponding author.

See: DMPK, Biotransformation, Bioactivation, Blood-Brain Barrier (BBB), Transporters, Parent Compound, Pharmacokinetics (PK), ADME; ADMET and Soft Drugs.^{50-52,169,170,229}

185. Prosthetic Group

A non-protein substance whose tightly bound (sometimes covalent) association with an enzyme is required for the latter's function. It can be organic, such as a vitamin, or inorganic, such as a metal ion. An example particularly relevant for drug metabolism is the heme group, which has both an organic and inorganic component. Its association with the CYPs is requisite for their key role in the biotransformation of numerous xenobiotics. The heme group is composed of a protoporphyrin ring that utilizes its 4 pyrrole-ring nitrogen atoms to bind Fe^{+2} at its center.

See: Apoenzyme, Holoenzyme, and Cofactor.³⁴

186. Quantitative Structure-Metabolism Relationships (QSMR)

Multivariate data analyses that attempt to describe the relationship between quantifiable molecular parameters and the rates of a specific metabolic event for compounds within a series of substrates. Hansch is regarded as the “father” of conceptualizing quantitative structure-activity relationship (QSAR) studies applied to biological settings, including specific drug metabolism profiles, as well as toward observed efficacy.²³⁷ His tabulation of substituent constants for this use is landmark. Numerous molecular parameters have been additionally discerned for use during subsequent proliferation of such analyses and, for example, pertain to categories like: (i) size, volume, surface area, and molecular weight; (ii) aqueous solubility, polarity, and lipophilicity/hydrophobicity; (iii) point charge, charge distribution, and various electronic effects; (iv) hydrogen bonding; and (v) steric effects, particularly when assessed in close proximity to the site for the given metabolic event.

Note 1: The process is analogous to quantitative structure-activity relationship (QSAR) analyses that historically pertain to assessing the relationship between chemical structure and efficacy. Sometimes this phrase is retained for use within the context of drug metabolism, with “activity” then meant to additionally include metabolism as well as efficacy. Authors should then be careful to alert readers within the accompanying text that they are applying the phrase to mean either a given biotransformation event or a given efficacious event as may be appropriate for the situation undergoing analysis or discussion.

Note 2: In addition to defining meaningful and thus potentially useful molecular parameters to guide further structural modification, these types of analyses typically strive to predict the biological response that will be obtained for new compounds. To that end, reiterative improvements in the equation’s parameters are often derived by assessing several “test compounds” that have been selectively or randomly excluded from the initial analyses “training set” of compounds. With regard to both efficacy and drug metabolism analyses, predictive correlations have been most successful for large sets of compounds that are highly similar in overall structure and where the biological data has been derived from well-controlled *in vitro* assays, rather than from *in vivo* testing. Note that this is despite the fact that the original development of these types of analyses were from associations having *in vivo* data (see Random Walk).

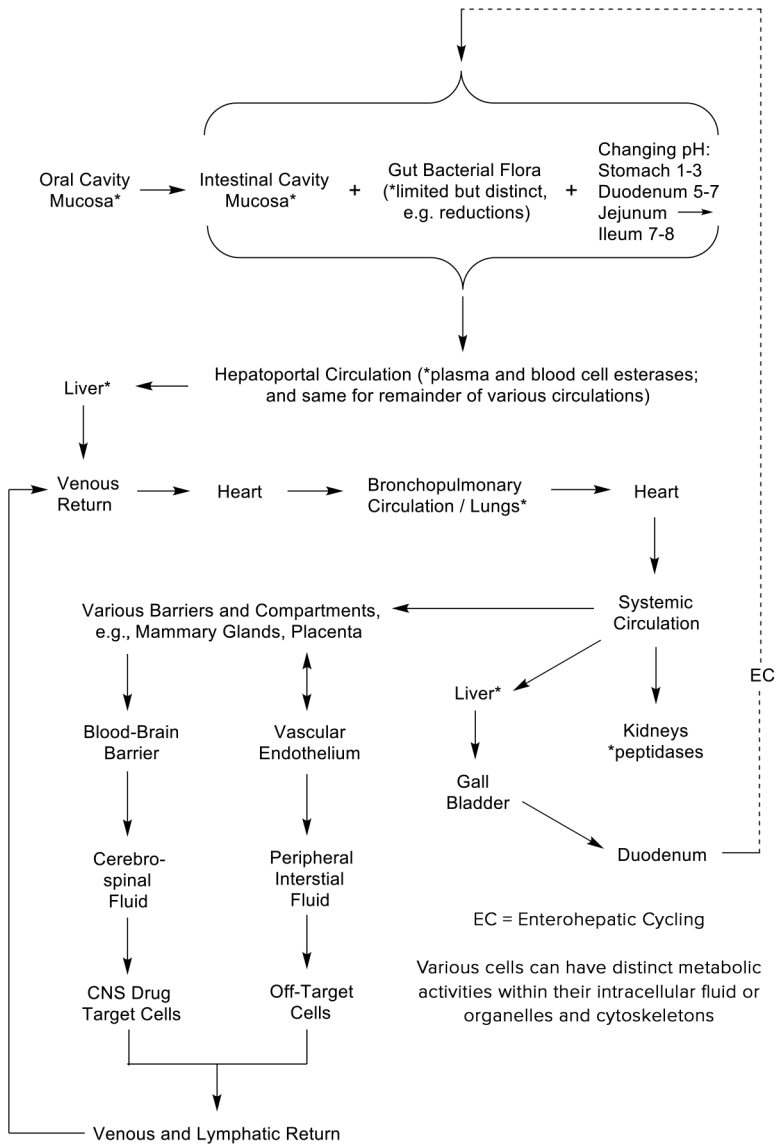
See: Metabophore, Metabophore Probe, Structure-Metabolism Relationship (SMR), Metabolic Prediction/Possibilities versus Probabilities, Metabolic Probe, Random Walk, and Rule of One. Landmark Refs.: 237, 238.

187. Random Walk

While this phrase originates within the principles of probability theory, Hansch was the first to apply it in the context of drug action, namely to emphasize the complexity of the path that a drug must traverse after its oral administration to an animal or human until the drug finally reaches its site of efficacious activity. For example, the path undertaken by a CNS drug after oral administration is depicted below. Sites regarded as being rich in drug metabolizing capability are denoted by an asterisk (*). More recently, this phrase has been used as part of the descriptive title for a quantitative structure-activity relationship (QSAR) method that attempts to predict drug-target interaction by random walk with restart on a heterogeneous network ("NRWRH").

Note 1: In probability theory, this is the stochastic process formed by the successive summation of independent, identically distributed random variables. Equations can be set up to appropriately accommodate the variables relative to sets of observed data. The situation for drugs, however, is not random, and their behavior derives from their chemical structures and the dictation by these of specific chemical properties, coupled to the largely identical distribution of the physiological variables that will be encountered within a given species. Hansch thus set up equations for this context that related the physicochemical properties of compounds to their observed biological properties, namely that of efficacious activity observed from an *in vivo* study. These were later termed Hansch equations and his assembly of them became the basis for considering him to be the "father of quantitative structure-activity relationship" (QSAR) studies.

Note 2: While ADME terminology had not yet become fashionable and the detailed study of each of their complexities was not yet feasible, these processes were at least appreciated in a gross manner, e.g., as more of a grey box. Thus, by analogy to probability theory, the ADME processes were essentially gathered and treated as a successive summation of identically distributed variables that then wrapped into the observable activity. Metabolism was not treated as a separate factor on the side of the equation



used to address the variable physicochemical properties. With time, however, metabolism has itself been treated as an observed activity, and for such analyses it is then placed on the other side of the equation, in place of observable efficacious activity.

See: ADME; ADMET, Absorption, Distribution, Metabolism, Biotransformation, Elimination, Excretion, Metabolic Pathway/Pattern/Profile, Blood-Brain Barrier (BBB), and Quantitative Structure-Metabolism Relationships. Landmark Refs.: 237, 238. For recent application of term, see Ref. 239.

188. Rapid (or Extensive) Metabolizer

See Extensive (or Rapid) Metabolizer.

189. Rate Constant (k or λ)

A constant which relates the rate of a linear kinetic process to the concentration or amount of the agent involved in the process.

See: Linear Kinetics, Pharmacokinetics (PK), Half-Life ($t_{1/2}$), Enzyme, Nonlinear Kinetics, and Michaelis-Menten Kinetics.^{39,40,104}

190. Reaction Phenotyping

In this context, the phrase typically refers to *in vitro* testing that attempts to determine which human metabolizing enzymes are involved in the biotransformation of a given xenobiotic.

Note 1: This is distinguishable from phenotyping an individual, which attempts to discern how that person will generally undertake biotransformations toward all types of xenobiotics.

See: Metabolic Pathway/Pattern/Profile, Metabolic Prediction/Possibilities versus Possibilities, Metabolic Profiling/Fingerprinting, Genotype/Genotyping, and Phenotype/Phenotyping.^{2,45,90,91,115,119,150-153,240}

191. Reactive Intermediates

Unstable (chemically reactive) species that have been postulated or observed in only a transient manner, which are rapidly formed in a catalyzed reaction and typically serve to decrease the energy barrier from reactant(s) to product(s), thereby increasing the overall reaction rate. For example, some of the biotransformations catalyzed by the CYPs produce reactive intermediates like a nitroso, carbene, ketene, quinone-imine, or quinone-methide while still on route to becoming metabolic products.

Note 1: Certain of the aforementioned examples can sometimes coordinate in a quasi-reversible fashion, with the iron present in the CYP's prosthetic heme leading to the transient formation of a catalytically inactive complex, or they can form a covalent bond with the CYP protein, thereby potentially deactivating the complex in a permanent manner. Should they escape the CYP catalytic site, they can quickly undergo reactions with neighboring macromolecules. Thus, reactive intermediates can be involved in mechanisms of toxicity, as well as accelerating the production of less active metabolites.

See: Non-Competitive Inhibition, Autoinhibition, Competitive Inhibition, Uncompetitive Inhibition, Mechanism-Based Product and Transition State Inhibitors, Covalent Binding, Suicide Inhibitor, Michaelis-Menten Kinetics, and Reactive Metabolites.^{42,62,146-148}

192. Reactive Metabolites

Unstable (chemically reactive) metabolic reaction products that are able to form covalent adducts with biomolecules. Depending on how aggressively promiscuous (reactive and nonselective) they are in pursuing a partner, their reactions can often extend beyond the CYPs and their immediately neighboring biomolecules. When electrophilic, they are instead candidates for bioinactivation via conjugation by the equally aggressive glutathione pathway. Likewise, the reactive aldehyde species produced by the CYPs during *N*-dealkylation, or by the oxidation of ethanol, are so commonplace that mammals have devised subsequent pathways to immediately deactivate them, e.g., by oxidation to their carboxylic acids or by reduction to their alcohols.

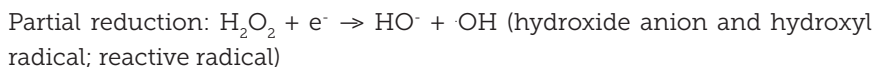
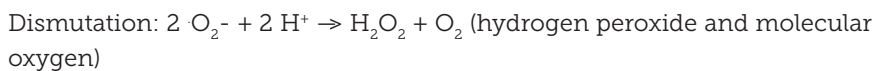
Note 1: CYP binding generally inhibits enzymatic activity; protein binding can lead to allergic responses, lipid binding to peroxidation or necrosis; and interactions with DNA can cause mutagenic, teratogenic, or carcinogenic effects; all depending upon the balance between toxic (reactive) metabolite formation versus the competing processes involving various detoxification mechanisms.

See: Non-Competitive Inhibition, Autoinhibition, Competitive Inhibition, Uncompetitive Inhibition, Mechanism-Based Inhibition, Covalent Binding, Suicide Inhibitor, Michaelis-Menten Kinetics, Reactive Intermediates, Bioactivation, Bioinactivation, Detoxification, *N*-Dealkylation, Dealkylation,

Alcohol Dehydrogenase (ADH), Aldehyde Dehydrogenase (ALDH), Aldehyde Oxygenase (AO), Glutathione (GSH), and Glutathione Conjugation.^{42,62,146-148}

193. Reactive Oxygen Species (ROS)

Endogenous ROS are components of oxygen metabolism that play important roles in cell signaling. However, environmental stress can increase their levels to a point where cell structures may be damaged, a syndrome known as "oxidative stress." Simple chemical species include: peroxide, superoxide, hydroxyl radical, singlet oxygen, and α -oxygen. The common pathway for reduction of molecular oxygen is depicted below, where several of these reactive species are shown. Dismutation is catalyzed by superoxide dismutase. Termination (full reduction) can be accomplished by both the glutathione and catalase pathways.



Note 1: Certain drug metabolism pathways can bioactivate a xenobiotic to produce related free radical species or xenobiotic metabolites that are themselves radicals, which may then undergo covalent interactions with enzymes and proteins or prompt lipid peroxidation. The formation of hydrogen peroxide can be particularly toxic, because its attenuated reactivity can allow it to distribute and interact with particularly sensitive biochemicals, such as DNA, within a cell's nucleus. The glutathione pathway protects cells from all of these events by either serving as a reducing agent (radical termination) or by undergoing a conjugation reaction with a reactive metabolite. Catalase protects cells from hydrogen peroxide. Toxicity results when oxidative stress overwhelms the local levels of these ubiquitous defensive pathways.

See: Active Metabolite, Bioactivation, Catalase, Covalent Binding, Glutathione (GSH), Glutathione Conjugation, Mercapturate/Mercapturic

Acid Conjugation, Oxidation/Oxidase/ Oxidoreductase, Oxidative Stress, Suicide Inhibitor, and Superoxide Dismutase.²⁴¹⁻²⁴³

194. Recombinant Enzyme

An enzyme expressed by a metabolically competent (host) cell line that has been transfected with a vector containing the enzyme's corresponding cDNA, so as to serve as a genetic insert: it is thus sometimes called a cDNA-expressed enzyme. It is common to simultaneously include a vector that can desensitize the host to an antibiotic, such that treatment of the cell culture with the latter allows only continued growth of the dually transfected cell line that will then produce the desired enzyme. The enzyme is either expressed in a functional form for direct use within the host cell, or, when separately isolated, can be re-activated by the addition of requisite cofactors or prosthetic groups. Examples relevant to drug metabolism include the expression of many of the CYPs, including their human (hCYPs) forms, flavin-containing monooxygenases, UDP-glucuronosyl transferases, and some glutathione *S*-transferases.

Note 1: These methods have become an important contributor to predicting what types of metabolic reactions (disposition) may occur for a new drug candidate once it becomes administered to humans (when it enters clinical studies).

Note 2: A wide range of isozymes produced in this manner are available from commercial sources, mainly from rodent and human genetic origins. It is also common for labs to be quite gracious in sharing vectors they have assembled for such purposes.

Note 3: The field has also moved toward the production of rodent animal models that have been transfected with numerous hCYPs and selected ADME-relevant transporters. A few of these cloned models are now commercially available as well.

See: Enzyme, Apoenzyme, Holoenzyme, Cofactor, Prosthetic Group, Metabolic Prediction/Possibilities versus Probabilities.^{244,245}

195. Reconstitution System

Medium required for catalytic activity by a given enzyme after its isolation or partial purification. In general, components are added so as to prompt the maximum achievable catalytic rate for a duration that is feasible according to the experiment of interest. For example, depending on the specific CYP, the following general conditions can typically promote a robust catalysis up to a ca. 15 to 30 min experimental timeframe: isolated CYP enzyme; NADPH-cytochrome *P*-450 reductase; cytochrome b5; a phospholipid mixture or isolated lipids; sodium cholate; divalent metal ions, such as Mg^{+2} ; reduced glutathione; and 50 to 100 mM pH 7.4 buffer at 37°C with exposure to air. An added NADPH-generating system is always also required. Many of these constituents are present in the standard microsomal fraction, where the CYPs can be regarded as being concentrated compared to the normal cellular milieu, if not partially purified.

See: Microsomal Fraction/Enzyme; Microsomes, Mixed-Function Oxidase, Cytochrome *P*-450 Enzymes (CYPs), Recombinant Enzyme, and Regenerating System.^{45,69-73,244}

196. Reductase

A broad term for enzymes that catalyze a reduction reaction, i.e., where the substrate receives one or more electrons or hydrogens from a donor molecule. One of the more common examples would be from the class of oxidoreductases, which typically require NAD(P)H as the hydrogen donor.

Note 1: The reductases are mainly located in the cytosol, especially from organs associated with biotransformations, such as the liver. They are also well-represented in the gut microflora and from there can often contribute significantly to the bioreduction of xenobiotics entering by the oral route.

See: Oxidation/Oxidase/Oxidoreductase, Gut Microflora, and Reduction.^{188,189,246}

197. Reduction

Biotransformation in which the substrate receives one or more electrons from a donor molecule, including via transfer of hydrogen, thus making a hydrogenation equivalent to a reduction reaction, but not always vice versa.

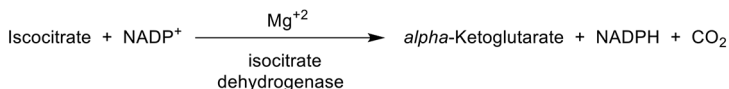
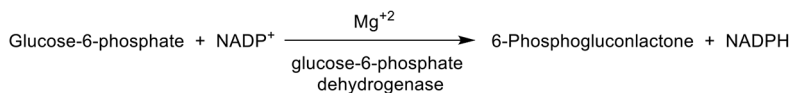
Note 1: Reductions are generally coupled to an oxidation where the electron-accepting substrate is reduced while the electron-donating partner is oxidized, characteristic of the oxidoreductase class of enzymes. Common partners during xenobiotic reductions are the reduced (H-containing) forms of glutathione, FAD, FMN, and NADP(H).

Note 2: Common substrate functionalities already present in xenobiotics or resulting from a xenobiotic's initial metabolism include: azo and nitro groups (as mainly catalyzed by the gut microflora and to a smaller extent by the liver); carbon-carbon double bonds; carbonyl groups, where the reduction of aldehydes (e.g., resulting from an initial *O*- or *N*-dealkylation biotransformation) to alcohols represents an important bioinactivation/detoxification pathway; certain disulfide linkages, which can lead to bioactivation; certain sulfoxide and *N*-oxide species; and certain carbon-halogen bonds, where reductive dehalogenation results in cleavage of the halide with replacement by a hydrogen atom.

See: Oxidation/Oxidase/Oxidoreductase, Reductase, Bioinactivation, Detoxification, Bioactivation, and Gut Microflora.^{188,189,246}

198. Regenerating System

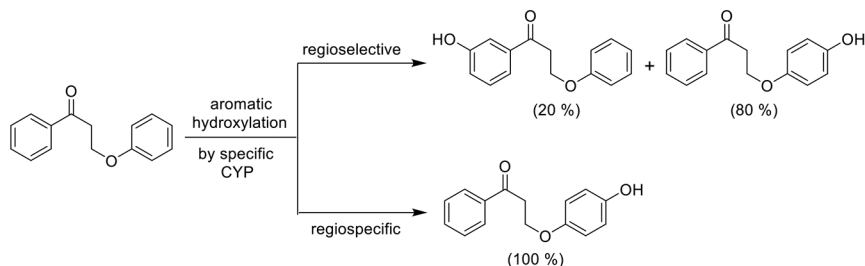
A method or set of conditions used to replenish a material that becomes depleted as part of an enzymatic reaction when conducted in an experimental setting where the normal process for the material's regeneration is no longer present. A common example relevant for xenobiotic metabolism would be the maintenance of NADPH while assessing CYP reactions utilizing a liver microsomal fraction. Two methods for the latter are shown below.



See: Microsomal Fraction/Enzyme; Microsomes, Mixed-Function Oxidase, Cytochrome *P*-450 Enzymes (CYPs), Recombinant Enzyme, and Reconstitution System.^{45,69-73,244}

199. Regioselective and Regiospecific Metabolism

Regioselective metabolism involves the differential formation of distinct metabolites that differ only in their regioisomeric locations of the same resultant modification by a single enzyme. When only one such possibility is observed, then the metabolic event can be considered to be regiospecific. A hypothetical example is shown below for the case of aromatic hydroxylation.



Note 1: Hydroxylation possibilities on the same aromatic ring, i.e., *ortho*- versus *meta*- versus *para*-, are almost always highly regioselective, if not completely regiospecific. Their selectivity/specificity follows the same electronic and steric factors contributing to the chemical reactivity observed for electrophilic aromatic substitution.

See: Aromatic Hydroxylation and Stereoselective Metabolism.^{247,248}

200. Retinoid X Receptor (RXR), Constitutive Androstane Receptor (CAR) and CAR/RXR Heterodimers

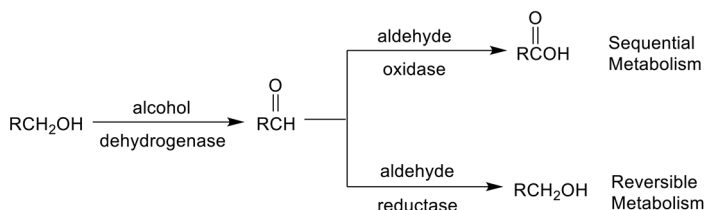
The retinoic acid receptors (RAR) and CAR are nuclear receptor proteins that serve as transcription factors to regulate the expression of selected genes. In addition to being responsive to endogenous ligands, where their signaling can regulate cellular differentiation, development, and basal-level endogenous metabolism (anabolism and catabolism), CAR can also interact with xenobiotic ligands, where its signaling can have an impact on drug metabolism and distribution. The interaction of endogenous ligands with the RXR- α , - β , and - γ members of the RAR family is not as well defined, such that the composite of their normal physiological roles remains unclear. However, like CAR, they can interact with xenobiotics and, in addition, can form heterodimers, such as RXR/PPAR and RXR/CAR, where subsequent signaling can impact pathways relevant to drug metabolism. The homodimers and RXR/CAR heterodimers can either promote or attenuate

signaling, depending on biological context. Examples include: RXR's induction of CYP 17A1, certain ABC-1 transporters associated with excretion, and down-regulation of certain CYP hydroxylases; CAR's activation or repression of genes coding for CYPs -2B, 2C, and 3A, sulfotransferases, and glutathione-S-transferases; and RXR/CAR's increased expression of CYP 2B6 and CYP 2B1 (aka "phenobarbital-responsive unit" or PBRU).

See: Aromatic Hydrocarbon Receptor (AHR), Glucocorticoid Responsive Element (GRE), Peroxisome Proliferator-Activated Receptors (PPARs), Pregnane X Receptor (PXR), and Xenobiotic Responsive Element (XRE).^{35,198,246,249-253} Drug Design Ref.: 254.

201. Reversible Metabolism

Occurs when the product from a given metabolic reaction is converted back to its initial structure, the return process generally mediated by another metabolic enzyme. An example of this within a closely proximal setting is shown below for the case of a simple alcohol. An example involving a distal arrangement is represented by the case where a phenol is converted to a glucuronide and then excreted via the bile, after which it undergoes hydrolysis by gut bacteria back to the original phenolic compound. This term is different from futile metabolism.



See: Futile Metabolism, Metabolic Pathway/Pattern/Profile, Enterohepatic Cycling, Gut Microflora, Glucuronic Acid Conjugation (Glucuronidation), Alcohol Dehydrogenase (ADH), Aldehyde Oxidase (AO), Aldehyde Dehydrogenase (ALDH), Oxidoreductases, Reduction, and Reductase.^{45,74,79-82,85,91,93}

202. Rule of Five

A molecule's ability to permeate membranes by passive processes will be reduced when: (1) its molecular mass is greater than 500 Da; (2) there are more than five hydrogen bond donor atoms; (3) there are more than

10 hydrogen bond acceptor atoms; and (4) its calculated octanol-water partition coefficient logarithmic value ($\epsilon\text{Log}_a P_c$) is greater than 5.²⁵⁵

Note 1: The “rules” derive from an assessment of about 2,000 drug molecules undertaken by Chris Lipinski,²⁵⁵ and they are often referred to as “Lipinski’s Rules.” While there are only four rules, their name stems from their common numerical theme that involves multiples of “5.”

Note 2: Large natural products and chemotherapeutic agents as a class were clearly stated to have been removed during the assessment. Likewise, the phrase “passive processes” intentionally emphasizes that when active transport systems or endocytosis are operative, they will supersede this type of determination. Finally, as a last proviso that draws from the context of the present, drug metabolism-related terminology, it should also be emphasized that any correlations to oral bioavailability are additionally subject to the “first pass effect.”

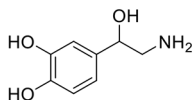
Note 3: Despite the provisos listed in Note 2, the rules are widely embraced and used as a guideline quite generally across a variety of chemical structures when undertaking drug design and decision-making, with the latter often also including considerations pertaining to oral bioavailability. This reliance tends to be directly proportional to the size of the compound library under investigation. Furthermore, during the early stages of a screening campaign, the molecular mass criteria is often lowered (e.g., to 400 Da), so that there will still be room to add substituents to any of the selected compounds during their subsequent structural optimization.

Note 4: Because the rules are particularly applicable when considering large numbers of compounds and because they can be used in a predictive manner for virtual compounds, $\epsilon\text{Log}_a P_c$ was set as the more useful parameter, rather than deriving experimental $\text{Log } P$ values.

Note 5: With regard to $\epsilon\text{Log}_a P_c$, while no lower limit was specified from the assessment, it is to be assumed that extremely polar molecules with values significantly less than 5 will likewise have difficulty. For example, a useful lower value would be $\epsilon\text{Log}_a P_c$ more negative than -1.0. Often not as apparent, however, is the reason for the cap at 5. For this, it is generally assumed that when lipophilicity rises above this value, the molecule will have trouble departing from the lipid bilayer into the aqueous layer on the

other side of the membrane, leading to low permeability into the cell despite ready uptake into the lipid membrane.

Note 6: Although Lipinski assigned a hydrogen bond donor ("HBD") count of 2 to an -NH_2 group in his original analysis, today's common practice of using the "rules" assigns a count of 1 to such a group,²⁵⁶ which is analogous to assigning a count of 1 for the hydrogen bond acceptor ("HBA") capability of an oxygen-containing group, even though the oxygen atom has two sets of lone-pair electrons. Thus, for counting purposes: common HBDs = $\text{OH} = \text{NH} = \text{NH}_2 = 1$ each; common HBAs = $\text{:N} = \text{:O} = 1$ each. An example is provided below by assessing norepinephrine.



Rule of 5 Assessment:

- (1) Molec Mass = $\text{C}_8\text{H}_{11}\text{NO}_3 = \text{ca. } 169 \text{ Da}$; $< 500 \text{ Da}$ so not a problem
- (2) $\text{HBD} = 4$; < 5 so not a problem
- (3) $\text{HBA} = 4$; < 10 so not a problem
- (4) $\text{Log}_p P = -1.2$ (from PubChem database); < 5 so seemingly not a problem but with the added corollary (this is more than -1.0 to the negative side) the extremely polar character could pose a problem; and likewise, when the basis amine is largely ionized at physiological pH, that particular rapid equilibrium form would not be expected to cross membranes in a passive manner.

See: Absorption, Bioavailability (Absolute and Relative), Partition Coefficient, Passive Transport, Permeation/Permeability.²⁵⁵⁻²⁵⁸

203. Rule of One (Metabolism's Rule of One)

Applicable to small-molecule xenobiotics in general: (1) if there is a simple ester present in the compound, it will typically get hydrolyzed as the predominant metabolic pathway unless it is sterically hindered; (2) all metabolic processes are exquisitely sensitive to steric features, where the rate for any given event is inversely proportional to the immediate steric environment at its biotransformation site; (3) decreasing the electron density at the site of a CYP *P*-450-mediated biotransformation will generally attenuate aromatic hydroxylation, but have little impact on *N*- and *O*-dealkylation processes; (4) replacing all of the hydrogens on the carbon *alpha* to a heteroatom by fluorines can completely block CYP-mediated *N*- or *O*-dealkylation at that location, while replacement of an aromatic hydrogen by fluorine may have little impact on hydroxylation at that location beyond that of electron withdrawal; and (5) as an often excepted parameter, increasing the xenobiotic's overall polarity will tend to decrease its metabolism, particularly with regard to oxidative pathways, and vice versa.

Note 1: The “rules” derive from a more than ten-year review of small-molecule drug metabolism information from a wide variety of public and private sector sources, including unpublished communications with numerous experts in the field. While there are five rules, their “singular” name stems from the overwhelmingly high predictive probability associated with the second rule compared to the other four, for which exceptions are not uncommon.

Note 2: As a predictive tool pertinent for how a new drug and potential analogs might behave during *in vivo* drug metabolism studies, and ultimately on administration to humans, no additional provisos are needed, as their application attempts to add probability assessments on top of the relevant metabolic possibilities.

Note 3: Although not previously iterated in this manner, it is clear that the fields associated with prodrugs and drug targeting have completely embraced the theme of the first rule as the most prominent strategy toward preferentially allowing for quick release of the active agent.

Note 4: In addition to specific drug design strategies, however, the rules are also applicable in ranking the merits of compounds within large libraries and virtual settings, where assessment of metabolic probabilities, rather than just metabolic possibilities, becomes beneficial.

Note 5: Finally, it is also apparent that investigators sometimes are quick to assume that replacement of an aryl-H by F will block hydroxylation at that site, and especially so when they have been successful experimentally. As iterated in rules 3 and 4, even those successes are likely a result of the net decrease in electron density prompted by such a substitution, rather than an actual blockade of this particular metabolic event (e.g., see NIH-Shift).

See: Esterases, Hydrolysis, Metabolic Pathway/Pattern/Profile, Metabolic Prediction/ Possibilities versus Probabilities, Metabolic Switching, Prodrug, Soft Drug.^{2,3,15,50-52,90,167-170,259}

204. S9-Mix

A mix of the S-9 fraction and an NADPH regenerating system, which can be used to study *in vitro* drug metabolism similar to the microsomal fraction, where both types are rich in the CYPs. The S-9 fraction is the supernatant

obtained from a tissue (usually liver) homogenate after centrifugation for 10 min at 9,000 G.

Note 1: Typically 1 mL of this mixture contains: 0.3 mL of the S-9 fraction (itself made from three volumes of 0.15 M KCl per gram of wet liver, so as to have a fairly uniform protein concentration among preparations, where 1 mL of S-9 typically contains microsomes from 250 mg of wet liver); 8 mM MgCl_2 ; 33 mM KCl; 5 mM glucose-6-phosphate; 4 mM NADP^+ ; and 100 mM sodium phosphate (pH 7.4).

Note 2: Since all of the cofactors are present, this mix is generally deployed immediately and, like microsomal fractions, will provide robust enzymatic activity for periods up to 15 to 30 min at 37°C.

See: Microsomal Fraction/Enzyme; Microsomes, Reconstitution System, Regenerating System, and Phenobarbital Induction and Sleeping Time.^{45,69-73,159,229,244}

205. Saturation Kinetics

Nonlinear kinetics observed when the local concentration or the plasma concentration of the analyte (drug or metabolite) does not change proportionately with the dose or the amount of the analyte placed or being generated in the analytical matrix or test system.

Note 1: This type of relationship may be associated with several pharmacokinetic processes, but a saturable transporter or metabolism system are the most common.

Note 2: The list of factors that can lead to dose nonlinearity with high drug concentrations includes both saturation and induction of metabolic enzymes, saturation of active intestinal absorption pathways, active renal tubular secretion and reabsorption, plasma protein binding, tissue binding, dissolution, and distribution.

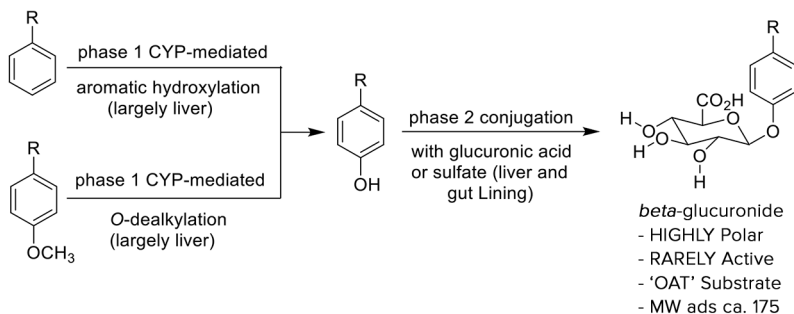
See: Clearance, Enzyme, Competitive Inhibition, Inhibition, Michaelis-Menten Equation/Kinetics, Half-Life ($t_{1/2}$), Linear Kinetics, and Plasma Protein Binding (PPB).^{38-40,104}

206. Secondary Metabolite

A sequential metabolite that is formed from a primary metabolite or any subsequent metabolites.

Note 1: There is a distinction between the various metabolites formed directly from the original xenobiotic or parent drug (all of which are "primary metabolites," regardless of their percentages and durations) versus metabolites subsequently formed from those or any other sequence of additional biotransformation products (all of which are "secondary metabolites," regardless of how many enzymatic steps may have occurred subsequent to the formation of the initial primary metabolite). There are no distinctions for tertiary or quaternary metabolites (all of these remain classified as "secondary metabolites"). Likewise, a secondary metabolite could be the major metabolite, as this classification is connected only with sequence and not with relative amounts.

Note 2: A common type of secondary metabolite is exemplified by a glucuronide when that conjugation occurs at an appropriate functional group that has either been generated or exposed by a preceding CYP-mediated aromatic hydroxylation or dealkylation of an aryl-ether. This is exemplified below.



See: Primary Metabolite, Metabolite versus Degradation and Decomposition Products, Metabolism, Drug Metabolism, Organic Anion Transporter (OAT), Biotransformation, and Metabolic Pathway/Pattern/Profile.^{3,45,82,91}

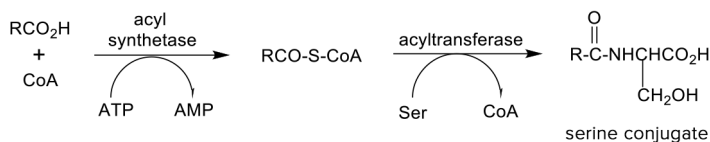
207. Second Pass Metabolism (Ψ First Pass Metabolism)

After a xenobiotic's absorption from the GI tract and "first pass metabolism" associated with the gut wall and dedicated hepatoportal circulation to the liver, the lungs can serve as a second metabolic compartment that is also well represented by the CYPs and analogously served by the dedicated bronchopulmonary circulation, all still occurring prior to returning to the heart's left ventricle for distribution into the systemic circulation. For administration routes that bypass the gut and liver, such as intravenous, intramuscular, subcutaneous, pulmonary, and topical, the lungs become a Ψ first pass metabolism pathway.

See: Absorption, ADME, Elimination, First Pass Effect/Metabolism (Pseudo-First Pass Effect), Extraction Ratio, Bioavailability (Absolute and Relative), and Oral Bioavailability.^{3,4,15,38-40,45,92,104-106}

208. Serine Conjugation

Conjugation of a xenobiotic's carboxylic acid group with endogenous serine according to the acylation pathway where the acid moiety is first activated by attachment to Coenzyme A.



Note 1: Glycine is one of the most commonly used amino acid partners; glutamine, ornithine, and taurine are also utilized.

Note 2: The conjugates resulting from the use of glycine are sometimes referred to as "hippuric acids" or "hippurates" after the historical characterization of this pathway with benzoic acid, the latter specifically called "hippuric acid." The formation of hippuric acid is still used today as a liver function test after administering benzoic acid.

See: Acylation, Amino Acid Conjugation, Acyltransferase, Glycine Conjugation, Hippuric Acid Conjugate (Hippurate), and Glutamine Conjugation.^{6,13}

209. Single Nucleotide Polymorphism (SNP)

A variation in a single nucleotide at a specific position within DNA that occurs within a genomic population to a significant degree, typically greater than 1 %. The two possible nucleotide variations are called “alleles” for this base position. SNPs can sometimes result in variations of the metabolism²⁶⁰ and/or pharmacokinetic profiles for certain xenobiotics observed in different individuals, e.g., extensive or poor metabolizers, and altered distribution or excretion patterns due to SNPs associated with the production of transporter proteins, such as Pgp.²⁶¹

Note 1: SNPs can occur within the coding sequence of genes, non-coding regions, or intergenic regions (sequences between genes). When they occur within the coding region, they can either be “synonymous,” in that they do not change the amino acid sequence of the resulting protein, or “nonsynonymous,” in that they change the resulting protein and alter the latter’s function. SNPs that are not in coding regions can still affect function, such as gene splicing, transcription factor binding, sequence of non-coding RNA, or messenger RNA degradation.

Note 2: Several maps (e.g., see Ref. 262) and databases are available that provide tracking information about the continuing identification of new SNPs and, most recently, at least one about their amino acid consequences. Examples for both include the National Center for Biotechnology Information’s *dbSNP*, and the *dbSNP* (e.g., see Ref. 263), both of which are intended for public use.

See: Acetylation Phenotype, Aldehyde Dehydrogenase Polymorphism or Deficiency, Atypical Alcohol Dehydrogenase, Extensive (or Rapid) Metabolizer, Genetic Polymorphism, Genotype/Genotyping, Phenotype/Phenotyping, Polymorphism, and Polymorphic Metabolism, Poor (or Slow) Metabolizer, and P-Glycoprotein (Pgp/MDR1/ABCB1).²⁶⁴⁻²⁶⁷

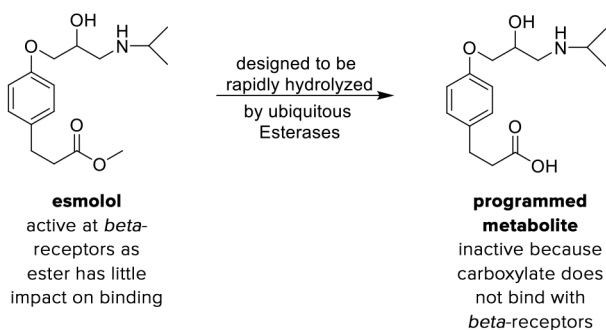
210. Slow (or Poor) Metabolizer

See: Poor (or Slow) Metabolizer.

211. Soft Drug

Pharmacologically active agent that has been designed to be inactivated to one or more nontoxic metabolites in a predictable and controlled manner

after achieving its therapeutic role. Note that a soft drug does not have to be a short-acting agent. Esmolol represents the prototypical soft drug, where a specific metabophore was appended to the classic pharmacophore for *beta*-adrenergic receptor blockade in order to program the resulting soft drug's metabolism according to a preselected metabolic pathway and biotransformation rate, into a metabolite that no longer would be acceptable to the *beta*-adrenergic receptor and thus inactive.^{268,269} In this case, the resulting PK profile is that of an ultra-short acting *beta*-blocker that provides therapeutic benefit by allowing for the moment-to-moment adjustment of adrenergic tone across the heart when it is administered by adjustable intravenous drip in either critical care or surgical settings. This technical definition should not be confused with the use of this same term in some countries as "slang" for certain narcotic agents, or in their legal systems' descriptions of the same (see Note 4 on the next page).



Note 1: While this definition stipulates a rational design of the soft drug, one can also state that a short-acting natural product is a "natural soft drug" when it is intentionally deployed in this type of therapeutic paradigm.

Note 2: Both prodrugs and soft drugs commonly incorporate esters to allow for hydrolytic bioactivation and bioinactivation, respectively, where the "rule of one" can then be used to effectively program reaction rates according to steric-driven structure-activity relationships.

Note 3: Bodor has published extensively in the soft drugs arena and has suggested a multi-layered classification system that further delineates several different types of soft drugs with separate tiers for tissue targeting and prodrug aspects.²⁷⁰ All of these classifications/sub-classifications, however, specify known metabolites as part of the initial design process

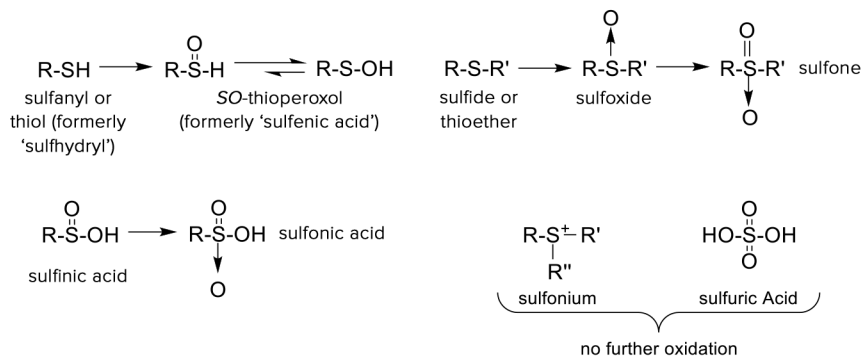
(i.e., Bodor's "retrometabolic drug design"), whereas the definition given above not only encompasses such demonstrable or actual literature-precedent features as rationale, but also further allows for *ab initio* design or unprecedented design strategies, the latter having proven to be useful by the clinically successful, marketed soft drug, esmolol. Furthermore, the principles elaborated for the esmolol strategy have been subsequently deployed in an *ab initio* design fashion by other investigators for several additional clinically successful soft drugs.

Note 4: This technical discussion should be distinguished from the use of this same term "on the street" as slang,²⁷¹ or in specific legal designations in certain European countries,²⁷² where in both situations "hard drugs" are associated with greater, and "soft drugs" with less, addicting properties. Those uses have nothing to do with the metabolic profiles of such compounds.

See: Prodrug, Bioactivation, Bioinactivation, Esterases, Metabophore, and Rule of One (Metabolism's Rule of One).^{50-52,167-170,229}

212. S-Oxidation

Any biotransformation where a sulfur atom is oxygenated. Somewhat analogous to the formation of *N*-oxides, although *S* has several more oxidation states to consider. Typically accomplished by the CYPs. Several examples pertaining to various *S* oxidation states are provided below.



Note 1: Sulfonium and sulfoxide forms can have asymmetric, tetrahedral-like coordinates. The sulfoxide stereoisomer consequences may need to be defined during the development of drugs undergoing such biotransformations.

Note 2: Carbon-sulfur systems can also undergo *S*-dealkylation reactions analogous to *O*-dealkylations so as to form sulfanyl groups, rather than hydroxy groups. The structure metabolism relationships for these pathways are similar.

Note 3: Carbon-sulfur double bonds can undergo an exchange of sulfur for oxygen.

See: *N*-Oxidation, *O*-Dealkylation, Oxidation/Oxidase/Oxidoreductase, and Stereoselective and Stereospecific Metabolism.²⁷³

213. Stereoselective and Stereospecific Metabolism

Stereoselective metabolism occurs when a biotransformation proceeds to a greater extent along preferred stereochemical pathways; stereospecific further indicates that the preference is exclusive for just one of the stereochemical options.

Note 1: When the choices involve only a pair of enantiomeric pathways, then the terms “enantioselective” and “enantiospecific metabolism” can also be used.

Note 2: The parent compound need not be chiral if it has one or more prochiral sites that can become chiral as a result of metabolism.

See: Enantioselective and Enantiospecific Metabolism, Substrate Specificity, and Regioselective Metabolism.^{247,248}

214. Structure-Metabolism Relationships (SMRs)

The correlation of one or more molecular parameters across a series of compounds versus the nature of the compounds' metabolic reactions. Molecular parameters can include net physicochemical properties and overall electronic surface potentials, as well as consideration of lipophilicity, electronic, or steric features in discrete regions of a molecule's three-dimensional structure.

Alternatively, the correlation of one or more chemical parameters for a given metabolic enzyme that relates to the possibility of compounds to serve as suitable substrates. These parameters can be the same as the above and most

often additionally include a characteristic functional group. For example, the SMR for a CYP capable of performing an aromatic hydroxylation might be defined within the context of preferentially doing so on a phenyl ring at the latter's most electron rich site and least sterically hindered position.

Note 1: This term is analogous to the more common phrase "structure-activity relationship" or "SAR." SAR is typically used relative to efficacious activity. However, it is also sometimes used to be inclusive of all types of biological responses, thus including SMRs as part of its overall meaning.

Note 2: In either case, SMRs can be summarized in terms of a "metabophore," which is analogous to the more common phrase "pharmacophore," where the latter is most often used with regard to efficacy but is sometimes used for other types of biological activities, including drug metabolism events.

See: Drug Metabolism, Biotransformation, Enzyme, Metabophore, Metabophore Probe, Rule of One, Substrate/Substrate Specificity, and Metabolic Prediction/Possibilities versus Probabilities.¹⁻³

215. Substrate/Substrate Specificity

In the present context, a substrate is an endogenous or xenobiotic compound that undergoes a given biotransformation reaction or active transport process because its structure contains the associated pharmacophore (metabophore or transportophore) required by the corresponding protein's active site. Specificity reflects the relative preference of an enzyme (or carrier protein) for specific substrates or a given substrate's preference for various enzymes (or carrier proteins). "Preference" translates functionally to a faster rate of metabolism (or transport) of the substrates.

Note 1: The affinity of a substrate for the protein is characterized by a binding constant (K_s or K_d) and the turnover efficiency by Michaelis-Menten constants $V_{\max}$ and K_m .

See: Enzyme, Apoenzyme, Holoenzyme, Cofactor, Prosthetic Group, Active Transport, Linear Kinetics, Nonlinear Kinetics, Michaelis-Menten Equation/Kinetics, Saturation Kinetics, Metabophore, and Structure-Metabolism Relationships (SMRs).^{1-3,38-40}

216. Substrate Inhibition/Product Inhibition

When an inhibitor of an enzyme is also a substrate/When the enzymatic products from a substrate's reaction cause inhibition.

Note 1: Since the presence of a substrate in the active site of an enzyme typically precludes another substrate from undergoing reaction at that same location at that same point in time, this is always taken as a transient, inhibitory situation. The same definition extends to the products from an enzymatic reaction. However, these terms are instead used to provide additional definition to the types of inhibitory pathways that any given compound might engage in.

Note 2: For example, an inhibitor can bind to an enzyme and either serve or not serve as a substrate. In either case, if binding is reversible, then these would be competitive inhibitors (one a substrate inhibitor and the other not), with their relative inhibitory efficiencies expressed as either IC_{50} or K_i values. Under normal operation, a substrate undergoes a reaction and the products are cleared from the active site. Thus, by definition a "substrate inhibitor" would always be a competitive inhibitor. If the products are not cleared as fast as would normally occur and themselves cause observable inhibition, then this situation would be called "product inhibition," where it would then be assumed that the initial compound must have served as a substrate in order to produce them.

Note 3: If binding is accompanied by the formation of a covalent bond such that it is no longer reversible, then this would be called non-competitive inhibition. Cases where the compound served as a substrate to produce products that bound covalently, or became bound itself during the reaction process, would be further classified as "suicide inhibitors." If the latter occurred for a species that resembled the transition state for the reaction, then these would be called "transition state inhibitors," with the same term used even when they do not form a covalent bond at that point.

See: Substrate/Substrate Specificity, Inhibition, Autoinhibition, Autoinduction, Competitive Inhibition, Non-Competitive Inhibition, Mechanism-Based, Product and Transition State Inhibitors, Covalent Binding, and Suicide Inhibitor. ^{42,62,146-148}

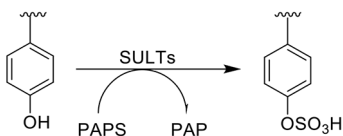
217. Suicide Inhibitor

A compound which undergoes a normal substrate's enzymatic reaction but instead produces a reactive intermediate or product that, in turn, forms a covalent bond in the active site and serves to inhibit the enzyme in a non-competitive manner.

See: Substrate Inhibitor/Product Inhibitor, Substrate/Substrate Specificity, Inhibition, Autoinhibition, Autoinduction, Competitive Inhibition, Non-Competitive Inhibition, Mechanism-Based, Product and Transition State Inhibitors, and Covalent Binding.^{42,62,146-148}

218. Sulfate Conjugation (Sulfation)

This biotransformation is important for a variety of endogenous molecules, as well as for xenobiotics. It involves the transfer of a sulfonate group to a hydroxy, and on occasion an amino group, when present within a substrate.



Note 1: The reaction is catalyzed by a family of sulfotransferases (EC 2.8.2), xenobiotics being particularly suitable for those members that are cytosolic and referred to as the "SULTs."²⁷⁴ The cofactor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) serves as a universal source of sulfate.²⁷⁵

Note 2: This pathway generally functions in parallel with the glucuronidation pathway, where each contributes according to the relative expression levels of their associated enzymes, the size of their cofactor pools at any given time, and their metabolophore matches with the substrate at hand. Among mammalian models, cats are distinct in their higher utilization of this pathway over that of glucuronic acid conjugation.

See: Cytochrome P-450 Sulfotransferases (Cytosolic Members or SULTs), Conjugation Reactions, Conjugate, Glucuronic Acid Conjugation (Glucuronidation), and Glucuronide.^{66,276,277}

219. Sulfotransferases (Cytosolic Members or SULTs)

A family of enzymes (EC 2.8.2) that transfer the sulfonate group from the cofactor 3'-phosphoadenosine-5'-phosphosulfate (PAPS), to hydroxyl or amino groups present on endogenous or exogenous substrates so as to form sulfate esters or sulfamates, respectively. Xenobiotics are particularly suited as substrates for the cytosolic family members or "SULTs."^{274,275}

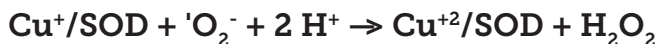
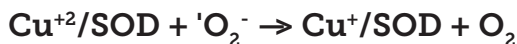
Note 1: The SULTs are cytosolic enzymes that function as homodimers having 32-35 kDa subunits. Thirteen distinct human SULTs have been identified and shown to be expressed differentially in several organs, including the liver, brain, lung, kidney, adrenal glands, and intestines.²⁷⁸

Note 2: Their nomenclature is similar to that delineated for the CYPs. It has been proposed that all SULTs constitute a gene superfamily. The 13 isoforms found in humans can be classified into four SULT families designated as SULT1, SULT2, SULT4, and SULT6. Within each SULT family, the constituent members are then further divided into subfamilies, such as SULT1A, B, C, and E.²⁷⁹

See: Sulfate Conjugation (Sulfation), Conjugation Reactions, Conjugate, Glucuronic Acid Conjugation (Glucuronidation), and Glucuronide.^{66,276,277}

220. Super Oxide Dismutases (SODs)

A family of metalloenzymes (e.g., EC 1.15.1.1) that catalyze the conversion of harmful superoxide radicals resulting from oxidative stress to less damaging species, namely molecular oxygen and hydrogen peroxide.



Note 1: The oscillating charge on the metal cofactor allows SOD to oxidize superoxide or, alternatively, to reduce it to hydrogen peroxide.

Note 2: This reaction represents the first step in detoxification within systems undergoing oxidative stress, catalase then further reducing the hydrogen peroxide to water.

See: Catalase, Oxidative Stress, and Reactive Oxygen Species (ROS).^{56,190-194,280}

221. Systemic/Systemic Circulation

The systemic circulation is that portion of the arterial flow that distributes blood to the entire body, i.e., both the peripheral tissues and central nervous system.

Note 1: The GI-tract, airways, skin surface, etc. are excluded from this definition, but would instead be encompassed by the phrase “whole body exposure.” Alternatively, these tissues, including their outer membranes, would still become accessed as part of the overall blood flow distributed by the systemic circulation and would be included in that context.

See: Whole Body Exposure, Hepatoportal Circulation, First Pass Effect/Metabolism, Second Pass Metabolism (ψ First Pass Metabolism), Enterohepatic Recycling, Blood-Brain Barrier (BBB), Presystemic Elimination, and Random Walk. General Medical Definition Refs.: 231, 232.

222. $T_{\max}$ or $t_{\max}$

The time after extra-vascular dosing when the drug concentration reaches a maximum within the fluid being measured, generally plasma, serum, or whole blood.

Note 1: The maximum concentration for intravascular dosing theoretically occurs immediately after administration, since there is no absorption process required. Hence, $T_{\max}$ is generally not reported for intravascular administration.

See: Pharmacokinetics (PK), ADME, AUC, Bioavailability (Absolute and Relative), DMPK, and PBPK.^{38-40,104}

223. Terminal Half-Life

The half-life for the slowest and final process of the parent drug's overall elimination.

See: Half-Life ($t_{1/2}$), Pharmacokinetics (PK), ADME, AUC, DMPK, PBPK, Elimination, Clearance, Excretion, and Phases of Drug Metabolism.^{38-40,104}

224. Therapeutic Window

The range of drug concentrations within a single subject or test group that can provide the desired therapeutic effect without prompting unacceptable side effects.

Note 1: While the most relevant concentrations are at the sites for the desired activity versus toxicities, a single value is most often obtained in plasma, serum, or whole blood due to practical limitations.

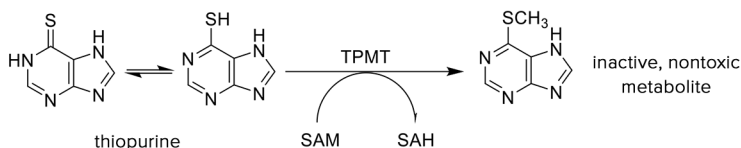
Note 2: The goal of drug therapy is to keep the active pharmaceutical agent concentration within the therapeutic window for as long as needed to alleviate the medical issue being treated.

Note 3: This term is related to the term “therapeutic index” (TI). The latter is typically the dose that prompts toxicity (TD_{50}) divided by the dose that is efficacious (ED_{50}), where each parameter has likely been obtained from two different models developed to optimize the assessment of each. The larger the TI, the “safer” the drug in terms of its potential side-effects.

See: Half-Life ($t_{1/2}$), Terminal Half-Life, Pharmacokinetics (PK), ADMET, AUC, DMPK, PBPK, Elimination, Clearance, Excretion, and Phases of Drug Metabolism.^{38-40,104}

225. Thiopurine S-Methyltransferase (TPMT)

TPMT (EC 2.1.1.67) catalyzes the methylation of aryl- and heteroaryl-sulfanyl groups using S-adenosyl-methionine (SAM) as the methyl donor. Detoxification of the anticancer drug 6-mercaptopurine (now “purine-6-thiol” or “thiopurine”) is exemplary:



Note 1: Genetic polymorphism of TPMT results in inter-individual variation in the sensitivity and toxicity toward thiopurine chemotherapeutic agents. Nearly 1/300 people are highly deficient for this enzyme. Measurement of TPMT status is recommended prior to administering thiopurine drugs to any population and prior to the use of cisplatin agents in children.

See: Conjugation Reactions, Conjugate, Transferase, Methylation, Methyltransferase, *N*-Methyltransferases, Catechol *O*-Methyltransferase (COMT), Genetic Polymorphism, Genotype/Genotyping, and Phenotype/Phenotyping.²⁸¹

226. Transferase

A general term for an enzyme that transfers an endogenous compound onto a polar functional group on another endogenous material (e.g., anabolism or control of function) or onto a xenobiotic compound (e.g., drug conjugation).

Note: The endogenous compound can either be polar, such as sugars, glucuronic acid, and amino acids, or non-polar, such as a methyl or acetyl.

See: Acetylation, *N*-Acetyltransferases, Acylation, Acyltransferases, Amino Acid Conjugation, Catechol-*O*-Methyl Transferase (COMT), Conjugate, Conjugation Reactions, Enzyme, *Gamma*-Glutamyltranspeptidase (γ -Glutamyltransferase; GGT), Glucuronic Acid Conjugation (Glucuronidation), Glucuronide, Glucuronosyltransferase, Glutamine Conjugation, Glutathione (GSH), Glutathione Conjugation, Glutathione-*S*-Transferase (GST), Glycine Conjugation, Mercapturate/Mercapturic Acid Conjugation, Methylation, Methyltransferase, *N*-Acetylation, *N*-Acetyltransferases (NATs), *N*-Methyltransferases, Serine Conjugation, Sulfate Conjugation (Sulfation), Sulfotransferases (Cystolic Members or SULTs), and Thiopurine-*S*-Methyltransferase (TPMT). General Dictionary Ref.: 282.

227. Transporters (Carrier Proteins)

Proteins which function to move ligands across membranes by processes that require energy (active transport).

Note 1: Analogous to enzymes, their ligands are referred to as “substrates,” even though no chemical change is made to the compounds that are transported. They can work against a concentration gradient and, while typically unidirectional, they can move substrates in either direction across the membrane, e.g., the Pgp transporter serves to pump compounds out of a cell.

Note 2: They can either be permanently embedded across the membrane (membrane spanning, e.g., Pgp) or move across the membrane while bound with one or more substrates (e.g., folate transporter).

Note 3: Several transporter molecules play critical roles during drug metabolism because they influence the uptake or export of xenobiotic compounds by cells possessing various drug metabolism enzymes. For example, within hepatocytes, the substrate carrier superfamily of proteins (SLC) are key basolateral (sinusoidal blood) uptake transporters that can serve to prolong the exposure of a given xenobiotic to the enriched array of metabolic possibilities present in the liver. Alternatively, the ATP-Binding Cassette superfamily of proteins (ABC), especially the Multidrug Resistance Protein (MDR or Pgp) and the Multiresistance Protein (MRP) subfamilies, represent key basolateral and canalicular (bile) export transporters that can serve to diminish the liver's metabolism of xenobiotics.

Note 4: Any possible metabolic event for a given xenobiotic at any locale within the body depends on: (i) the suitability of the xenobiotic's structure to serve as a substrate for the enzyme(s) associated with the event; (ii) the level(s) of the relevant enzyme(s) and competing enzymes present at that locale; and (iii) the concentration and residency time of the xenobiotic at that locale. Component (iii) is a function of the inherent physical properties of the substrate (e.g., $\log D$) and its suitability to serve as a substrate for transporters, where the latter's importance grows exponentially with the levels of the various transporters that may or may not be present and operative at that locale. While all three components represent a challenge for the prediction of a drug's metabolism *in vivo*, component (iii) arguably presents itself as the largest hurdle due to the presence of the transporters that additionally complicate the variables that need to be taken into account.

See: Absorption, ADME, Permeation/Permeability, Partition Coefficient (P ; $\log_a P$ and $\text{clog}_a P$), Distribution Coefficient (D ; $\log_a D$), Passive Transport, Rule of Five, Active Transport, Phases of Drug Metabolism, Random Walk, Blood-Brain Barrier (BBB), P-Glycoprotein (Pgp/MDR1/ABCB1), and Metabolic Prediction/ Possibilities versus Probabilities.²⁸³

228. Ultra-Rapid Metabolizer

An individual who has inherited multiple, functional copies of the same gene encoding a drug-metabolizing enzyme. This results in the over-

expression of the enzyme and a far greater than normal metabolism of its substrates.

See: Genetic Polymorphism, Genotype/Genotyping, and Phenotype/Phenotyping.^{103,114-129,230}

229. Ultra-Short-Acting Drug (USA Drug)

An ultra-short-acting (USA) drug is an agent whose duration of action (pharmacologic half-life) is significantly less than 15 minutes after its administration, to the extent that at least a pseudo-equilibrium (if not a true equilibrium) for an effective concentration has first been achieved within the relevant biocompartment. For comparative purposes (and not to be taken as a continuing strict definition), a short-acting drug might then reside in the range 15 to 30 min, a moderately long-acting drug in the range 0.5 to 5 h, a long-acting drug in the range 5 to 10 h, and an extremely long-acting drug in a range longer than 10 h. Thus, an agent's ultra-short duration is a consequence of quick bio-inactivation (e.g., drug metabolism) rather than being due to distribution out of the compartment associated with the drug's action. Alternatively, drugs exhibiting extremely long durations of action typically do exhibit a relationship with their distribution, regardless of their metabolism, namely that they may concentrate in and most certainly will leave only very slowly from the compartment associated with their biological activity (i.e., their observed duration is indirectly proportional to their rate of departure/furthered distribution). When the short duration for the agent has been engineered by drug design and synthesis, these agents are part of the broader family known as "soft drugs" (agents whose inactivating metabolism at any given rate, long or short, has been specifically programmed into the parent molecule, so as to achieve some desirable endpoint associated with the overall therapeutic efficacy-ADMET profile). Esmolol is the classic, prototypical soft drug (264, 265).

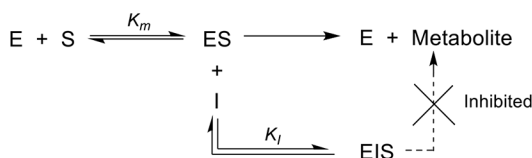
Note 1: Appreciating the distinctly different influences that metabolism and distribution play for these two extremes in a drug's duration of action and ADMET profile can allow for the two roles to be combined in a complimentary manner. For example, a delayed-release formulation placed within the target biocompartment that gradually releases a USA drug whose quick metabolism has been programmed to occur in the systemic circulation or liver, so as to produce innocuous metabolites, represents an ideal way to have a sustained (long, extremely-long duration) drug effect in

the desired compartment, while remaining free from off-target or systemic side-effect toxicity effects.

See: ADME, ADMET, Prodrug, and Soft Drug.^{50-52,167-170,229}

230. Uncompetitive Inhibition

A type of inhibition that occurs when an inhibitor binds reversibly only to the enzyme-substrate complex (ES complex) and does not bind to the enzyme alone. Both K_m and V_{max} decrease. The interactions between enzyme (E), substrate (S), and an uncompetitive inhibitor (I) are depicted below:



Note 1: Metabolism-independent inhibition occurs when a ligand inhibits an enzyme without undergoing biotransformation. Such direct inhibition has traditionally been divided into three categories: competitive, non-competitive, and uncompetitive.

See: Autoinhibition, Competitive Inhibition, Non-Competitive Inhibition, Mechanism-Based Inhibition, Covalent Binding, Suicide Inhibition, and Michaelis-Menten Kinetics.^{42,62,146-148}

231. Urinary Metabolic Ratio

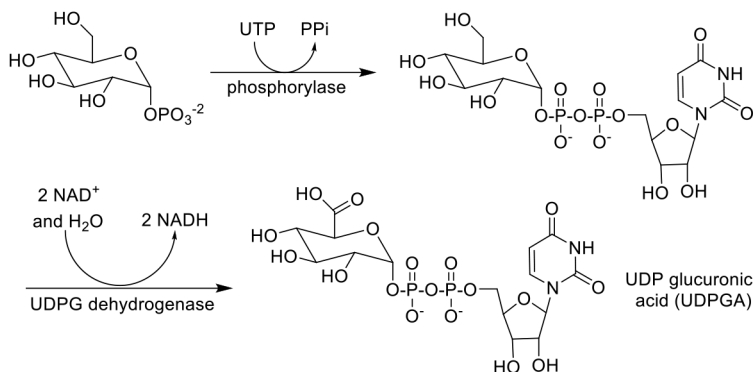
The amount of a particular metabolite found in the urine for a specified time period, as the numerator over one of the following in the urine for the same time period as the denominator: the amount of unchanged parent compound, the sum of the amounts of other metabolites, or the sum of unchanged parent and other metabolites.

See: Clearance (CL), Elimination, Excretion, Intrinsic Clearance, and Pharmacokinetics.^{38-40,104}

232. Uridine-diphospho-glucuronic Acid (UDPGA)

This cofactor for glucuronosyltransferase (GT; formerly UDP-glucuronosyltransferase or UGT) supplies the endogenous partner for the

glucuronidation pathway that is extremely common for xenobiotics. Its biosynthesis is shown below:



Note 1: Subsequent reaction with a drug is catalyzed by glucuronosyltransferase (GT) in an $\text{S}_{\text{N}}2$ manner to release UDP and a *beta*-glucuronide conjugate.

Note 2: The drug-conjugate has significantly increased polarity, generally little activity, and an increase in molecular mass of about 175 Da. Because of the added carboxylic acid group, the conjugate is also a candidate for excretion by organic anion transporters (OATs).

Note 3: In general, if the drug-conjugate's molecular mass is below 500 Da, it will be excreted via the urine; and if it is above 500 Da it will be excreted via the bile.

See: Glucuronosyltransferase (GT), Conjugation Reactions, Phases of Drug Metabolism, Aromatic Hydroxylation, Glucuronic Acid Conjugation (Glucuronidation), Glucuronide, Glucuronidase (β -Glucuronidase), and Sulfate Conjugation (Sulfation).^{45,63,66,130,131}

233. V_{max}

The maximum rate at which an enzyme can catalyze its reaction with a given substrate for a given set of experimental conditions. Increasing the concentration of substrate for a given amount of enzyme will eventually saturate the system at V_{max} ; no further increase in reaction rate will then occur.

See: Enzyme, Michaelis-Menten Equation/Kinetics, and Linear Kinetics.^{38-40,104}

234. V or V_d (Apparent Volume of Distribution)

Proportionality constant relating the drug concentration in the measured media, such as blood, plasma, or serum (C), to the amount of drug administered to the body (A), as expressed by the equation:

$$C = \frac{A}{V}$$

Note 1: The volume is termed “apparent” because it generally does not correspond to any true physiological volume in the body, except in rare special cases.

Note 2: V is only constant for pharmaceutical agents whose PK profile can be fit to a single compartment model. For agents requiring more than one compartment or PBPK models, V changes with time as the agent is distributed into other tissues.

See: Pharmacokinetics (PK), ADME; ADMET, Allometric Scaling, Allosteric Scaling, AUC , Bioavailability (Absolute and Relative), Clearance (CL), Compartment Model, Disposition, DMPK, Dose-Dependent Kinetics or Metabolism, Extraction Ratio, Half-Life ($t_{1/2}$), Intrinsic Clearance, IVIVE, Linear Kinetics, Metabolic Clearance, Metabolic Pathway/Pattern/Profile, Michaelis-Menten Equation/Kinetics, Non-compartment PK Analysis, Nonlinear Kinetics, Oral Bioavailability, Partition Coefficient (P ; $\log_a P$ and $\log_a P$); Distribution Coefficient (D ; $\log_a D$), PBPK, Pharmacodynamics, and Pharmacogenetics and Pharmacogenomics.^{38-40,104}

235. Whole Blood Concentration

Measurement used in PK analyses when significant amounts of the pharmaceutical agent can collect within red blood cells, but otherwise typically not as prevalent as using measurements of plasma or serum concentration.

Note 1: Collection requires mixing with an anticoagulant, such as heparin or EDTA, either during or immediately after drawing the whole blood sample.

See: Pharmacokinetics (PK), ADME; ADMET, Allometric Scaling, Allosteric Scaling, AUC , Bioavailability (Absolute and Relative), Clearance (CL), Compartment Model, Disposition, DMPK, Dose-Dependent Kinetics or

Metabolism, Extraction Ratio, Half-Life ($t_{1/2}$), Intrinsic Clearance, IVIVE, Linear Kinetics, Metabolic Clearance, Metabolic Pathway/Pattern/Profile, Michaelis-Menten Equation/Kinetics, Non-Compartment PK Analysis, Nonlinear Kinetics, Oral Bioavailability, Partition Coefficient (P ; $\log_a P$ and $\log_a P$); Distribution Coefficient (D ; $\log_a D$), PBPK, Pharmacodynamics, and Pharmacogenetics and Pharmacogenomics.^{38-40,104}

236. Whole Body Exposure

This phrase is used to indicate that all surfaces of the body, such as the GI-tract, airways, skin, eyes, etc., are included with the body's various compartments, as well as the venous and systemic circulation during the consideration of a xenobiotic's disposition after its exposure or administration to an individual.

See: Systemic/Systemic Circulation, Hepatoportal Circulation, First Pass Effect/Metabolism, Second Pass Metabolism (ψ First Pass Metabolism), Enterohepatic Recycling, Blood-Brain Barrier (BBB), Presystemic Elimination, and Random Walk.^{38-40,104}

237. Wild-Type Enzyme

The enzyme isolated from the natural gene product of the allele with highest frequency or highest enzyme activity.

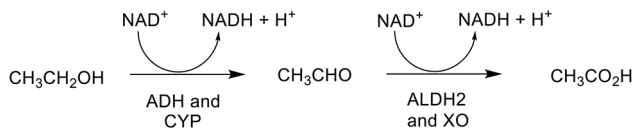
Note 1: Most commonly used in relation to an analogous form experimentally expressed and isolated using recombinant molecular biology methods and likely "humanized" while using a non-human host cell.

See: Genetic Polymorphism, Genotype/Genotyping, Phenotype/Phenotyping.^{103,114-129,230}

238. Xanthine Oxidase (XO)/Xanthine Dehydrogenase

This enzyme can interconvert between both activities (EC 1.17.3.2 and EC 1.17.1.4, respectively) depending on the protein's reversible sulfhydryl oxidation status, or will adopt only XO activity upon irreversible proteolytic modification. Important for the endogenous xanthine pathways, XO can also oxidize reactive aldehydes to their less toxic carboxylic acids, the latter role contributing to the second step in the clearance of ethanol. The metabolic disposition of ethanol is shown below, where alcohol

dehydrogenase (ADH) and certain CYPs perform the first oxidation and then aldehyde dehydrogenase (ALDH2) and XO become involved with the second oxidation.



See: Oxidation/Oxidase/Oxidoreductase, Aldehyde Dehydrogenase Polymorphism or Deficiency, Disulfiram-like Reaction, Alcohol Dehydrogenase, and Atypical Alcohol Dehydrogenase.²⁸⁴

239. Xenobiotic

A compound foreign to the living system under consideration. Thus, a natural product that is endogenous to a given plant becomes a xenobiotic when administered to humans. General Dictionary Ref.: 282.

240. Xenobiotic-Responsive Element (XRE)

The specific DNA sequence on the regulatory region of genes that can be bound by the aryl hydrocarbon receptor complex and usually causes an increased expression of that gene.

See: Pregnane X Receptor (PXR), Peroxisome Proliferator-Activated Receptors (PPARs), Aromatic Hydrocarbon Receptor (AHR), Glucocorticoid Responsive Element (GRE), and RXR and CAR/RXR.^{35,198,236,249-253}

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IUPAC standards for terminology technical reports. For the latter, final comments from Dr. Jürgen Stohner and Joshua Gannon proved particularly useful.

Appendix I: Defining the Term “Drug” in the Context of “Drug Discovery”

Given the numerous disciplines involved in drug discovery and the wide range of technical perspectives that exist among its participants, it is important to appreciate how using even a seemingly simple word like “drug” can become ambiguous among a group of interdisciplinary scientists. However, mandating a single, specific definition at the onset may not be the best way to enhance communication among drug discovery participants. An alternative approach is to provide a general or more fluid starting point that can allow the group of interacting scientists to devise a meaning suitable for their context that might be most beneficial to their particular discussion. In such cases, it will be the common knowledge base, potentially garnered from a tutorial about the general term, that will allow these practitioners to better understand each other’s perspectives and then devise their mutually beneficial working definition. This same situation arises when authoring a publication, where it may be necessary to provide some explanatory information for a term’s use/intended definition, depending on its specific context in the technical report and the differing perspectives of the intended audience. The following discourse about the term “drug” is taken from a textbook chapter pertaining to “Drug Discovery.”³ The text is intended for audiences consisting of both pharmacologists and medicinal chemists, primarily at the early stages of their careers, and so it became necessary during the writing of the chapter to devise a working definition applicable to the chapter’s educational thrust. However, the tutorial discourse leading to the final, rather succinctly stated singular definition, demonstrates how related knowledge can also allow a given term to remain dynamic and thus act as a bridge to other contexts across various disciplines and having individuals with varying technical perspectives.

The dictionary indicates that the word “drug” is derived from the Old English “dryge” which, in turn, was reconstructed from the Primitive Germanic “dreug” and hence the sound of its pronunciation even today. Because these older words meant to “dry,” it has been suggested that the word “drug” originally was used to convey how herbs were commonly processed so as to produce “*dried powders for administration as early medicinals*.” Even if historically correct, however, the field of medicine and the word “drug” have evolved well past this origin and a modern

usage must be sought. In today's broadest sense, the term "drug" is often taken to include any non-food substance that exhibits activity upon administration to any biological setting. The latter includes an *in vitro* test as well as an *in vivo* animal model or human subject. However, this definition also falls short of today's practice. It does not convey how the overall drug discovery process has so critically come to be a highly interdisciplinary effort all the way to its validated end-point within the clinic. Thus, the present chapter will specifically apply the term "drug" to include only single chemical substances that become marketed, albeit often then accompanied by added excipients, for use in human or veterinary medicine either by prescription ("ethical pharmaceuticals") or as "over-the-counter" products ("OTCs"). Inherent to this modern definition while still connecting to the past is the continuum of interdisciplinary activities needed to actually produce marketed substances that eventually become "*administered* as [today's] *medicinals*."

It can be noted that this definition does not exclude "recreational" or "abused substances." Because these chemicals involve non-conventional marketing strategies, to say the least, as well as non-therapeutic, if not harmful, applications, these "*drug shouldn't-be's*" will not be further considered within this chapter. Alternatively, the definition does exclude both herbals and dietary supplements or nutraceuticals even though some of these products may have a firmly established medicinal value. The latter are typically complex mixtures for which the complete range of components are often not well-understood and are sometimes not well-controlled in their lot-to-lot ratios. Thus, while dietary supplements and herbals can serve as excellent sources for new drugs, they are not themselves "drugs" by today's standards. Though they may sometimes represent very popular consumer products, they too will not receive further attention. Finally, in line with the working-definition and integral to fully appreciating how difficult it is to truly discover an actual drug substance, it will be important to keep in mind that all of the "compound libraries," "hit," "lead," "preclinical" and "clinical development compounds" that you will soon be reading about, should only be regarded as "*drug-wannabes*" since they are not yet marketed and thus all fall short of this strict definition.

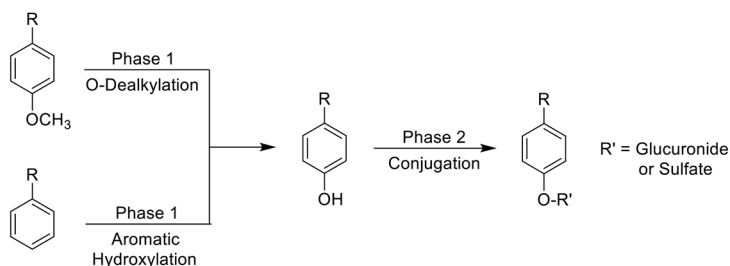
A similar view should be extended to the participants who work in the field of drug discovery. While investigators involved in studies at any given step in this process should be considered to be making very critical contributions, within the context of this definition the overall process of drug discovery entails numerous steps that need to be brought together as part of a much larger undertaking in order to eventually produce what we will be calling a true "drug." The huge challenge associated with this undertaking is illustrated in the next section of this chapter. Finally, in addition to the interdisciplinary emphasis afforded by this working-definition, the present chapter will primarily focus upon the process

of drug discovery as it specifically pertains to the pursuit of “small-molecule” agents. Although this will preclude in-depth consideration of biopolymers and vaccines, this division is commonly taken to separate these types of therapeutics from small-molecule drugs. The development and production of biopolymers are achieved in a distinctly different manner that relies heavily upon biotechnology-driven methods. Summarizing, for this chapter a drug is a single chemical substance marketed for use in human or veterinary medicine either by prescription or as an OTC.³

Appendix II: An Ongoing Terminology Controversy

The following discourse provides a historical backdrop of the differing views regarding the continued use of the terms “Phase 1” and “Phase 2” in the context of drug metabolism. **None of the definitions provided within this section should be taken as the final IUPAC recommendations for this glossary**, but rather as a basis for further consideration and comment related to how these particular terms might best be deployed in the future. Opinion on the future usage of these terms ranges from: “not to be used at all”; to “replaced by less sequential versions, such as Group 1 and Group 2 biotransformations”; to “as presently still being utilized by many”; to calls for “an even expanded version” that, although more complex, might become less controversial while potentially having some appeal for a much wider range of drug metabolism practitioners beyond those involved in drug discovery, e.g., toxicologists, nutritionists, and environmental scientists.

The terms “Phase 1” and “Phase 2” were introduced nearly 60 years ago by the late R. T. Williams,⁸¹ now recognized as one of the highly distinguished founders of the xenobiotic metabolism field. Dividing metabolic oxidation, reduction, and hydrolysis reactions (Phase 1) from “synthesis” reactions (Phase 2), which translate to what are now called “conjugation” reactions, his choice of such phraseology encompassed the general notion that this was also a common sequence of events, and further, that it typically yields inactive, more water-soluble metabolites. While the latter may often be applicable, there are many notable exceptions, such as *N*-acetylation (a Phase 2 or conjugation reaction), which would yield a less water-soluble metabolite than its amine precursor. Likewise, one of the major pathways that consistently behaves in this sequential manner is shown below. Today we further appreciate that even these types of xenobiotics would likely be simultaneously subject to several different types of biotransformation possibilities depending on the nature of their remaining structures (simplified to R for this example).



Nevertheless, these terms have been fully embraced over the years, often with what seems like an added emphasis on how important their sequential role is in the metabolic disposition of xenobiotics in general. Unfortunately, that a xenobiotic already having an aryl-hydroxyl-group (phenolic group) present is an immediate candidate for the indicated Phase 2 conjugation, without the need for a Phase 1 reaction, is sometimes not duly noted. Likewise, that a Phase 2 metabolite can still be subject to subsequent Phase 1 reactions may not be fully elaborated, e.g., the common case where an initial glutathione conjugate is further processed by a series of Phase 1 reactions to eventually produce a mercapturic acid. Finally, that an entire class of important conjugates are typically less polar, while others can retain activity, including in the latter certain glucuronides and sulfates, is often not noted either. In light of these exceptions and the resulting ambiguity, there are some practitioners who have advocated that the terms Phase 1 and Phase 2, despite their prestigious origin, should no longer be used, as they can be more confusing than helpful, especially for students new to the field.²²¹

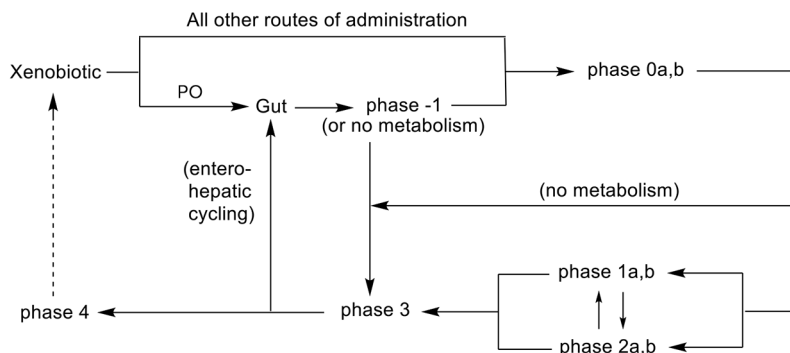
Amid such controversy, it appears that a majority of practitioners still feel that, in addition to their historical significance, there is merit to the retention of these terms when used today with appropriate tutorial material. For example, aside from the classic xenobiotic sequence noted above and from analogous pathways pertaining to key endogenous substrates (for example, see Ref. 222), there seems to be a compelling anatomical basis to consider these steps as having evolved to handle the indicated biotransformations in a tandem, enzymatic cascade fashion. The mutually high proportions of the enzymes associated with these pathways in the gut endothelium and liver suggest they work together toward biotransforming xenobiotics entering by the oral route. Especially noteworthy is the close physical proximity of many of the CYP enzymes and of uridine-diphospho-glucuronosyltransferase (UGT) along the endoplasmic reticulum lipid bilayer.^{223,224} The CYPs are

capable of catalyzing Phase 1 biotransformations that can generate an aryl-hydroxyl-group, and UGT is then capable of catalyzing the Phase 2 transfer of a glucuronic acid biochemical synthon to a hydroxyl-group. Even though this two-step pathway represents a small portion of the wide array of metabolic possibilities for a xenobiotic, the sheer number of natural products having aryl-methoxy-groups suggests that such chemical species had to be frequently dealt with as modern man evolved and ultimately developed a general diet and complimentary digestive system by sampling the edible materials available in Nature.

Likewise, from a systems biology perspective, there may be merit in an even further expanded use of the Phase 1 and 2 terminologies. For example, the term "Phase 3" has sometimes been deployed to designate the excretion of xenobiotics and their metabolites from the body, particularly emphasizing in this usage the importance of active transport pathways.²²⁰ More recently, the term "Phase 0" has seen some use to convey events like a xenobiotic's absorption and distribution prior to undergoing a biotransformation event.¹³⁰ Both terms have merit in that they draw attention to the importance of ADME parameters in conjunction with xenobiotic metabolism per se. This type of systems biology approach is, indeed, inherently fundamental to the overall process of xenobiotic metabolism.¹⁵⁸ Extending this paradigm in both directions, i.e., to include the role of gut microflora as part of the exposure process,²²⁵ and of metabolic events within the environment subsequent to excretion,²²⁶ actually forms a continuum or loop back into the human diet that can take on relevance across even more technical disciplines, while also prompting new ways to drive drug discovery and development.²²⁷

Taking the view that the Phase 1 and Phase 2 designations may be useful as process classifications, but not as strict sequential pathways, can allow for their continued usage. Furthermore, retaining their specific phraseology as a "phase number" pays tribute to the historical significance conveyed above, while underscoring that there are classical sequences of events which are often traversed by a substrate. Adopting an "a" designation to convey that an absorption or distribution process is occurring prior to any metabolic event, or that a biotransformation is occurring for the first time at a specific location on a parent xenobiotic or metabolite, can instead be used to track the actual sequence of events and molecular modifications. For the latter, it follows that a "b" then conveys that a metabolic event has already occurred anywhere on the compound in relation to tracking absorption or distribution

behavior, or at the same structural locale that is next undergoing another metabolic reaction at that location or on a Phase 2 appendage. Taking all of the above into consideration, a potential expansion of this nomenclature is illustrated below for the human case of exposure to a xenobiotic. Note that the term “phase,” as defined here in the context of drug metabolism, is no longer capitalized and uses Arabic numerals, so that these terms are more readily distinguished from the analogous terms used for the stages of drug clinical testing, which instead are capitalized and use Roman numerals, i.e., they are denoted as Phase I, Phase II, etc.



The depicted model and its designated nomenclature begin by distinguishing the potentially important role that gut microflora can play in the metabolic fate of a xenobiotic after its oral ingestion (PO) versus other routes of administration. Thus, **phase -1** (minus one) designates all of the possible biotransformations (some of which are quite unique to the microorganisms) that can be catalyzed by the gut microflora. As such, phase -1 also encompasses the role that these microorganisms may play during enterohepatic cycling, e.g., hydrolysis of a phenolic-glucuronide metabolite that has undergone biliary excretion back to the phenol, which can then be reabsorbed, rather than proceed toward fecal elimination.

Phase 0 designates all of the non-metabolic dispositional processes to which a xenobiotic or metabolite will traverse, such as absorption passively or by transport across membranes and association with biomolecules not involved in drug metabolism, while undergoing fluid-driven distribution through the living system. The important relationships of a drug's transport coupled to its metabolism, and a metabolite's transport coupled to its excretion, are thus recognized by this nomenclature. For this term,

the "a" designates that these phenomena occur prior to a xenobiotic's first mammalian metabolic event (as shown in the scheme), while the "b" designates that a biotransformation reaction has occurred and intends to further convey that these processes continue throughout the entire timeframe after a xenobiotic's arrival until it and all of its metabolites are cleared from the living system.

In line with the historical definition, **phase 1** includes all hydrolytic, oxidative, and reductive biotransformations. The "a" then provides a sub-classification for such events occurring at a given point on the substrate for the first time, while the "b" indicates that the reaction event occurs at a point on the substrate which has already participated in a preceding biotransformation. Typical phase 1a examples include ester hydrolysis, aromatic hydroxylation, arylether *O*-demethylation, and the first of possible *N*-dealkylations. Aside from the hydrolytic reactions, the most common phase 1a biotransformations are generally catalyzed by the CYP family. The phase 1b events utilize a broader range of enzymes to catalyze the same types of chemical reactions. Typical phase 1b examples include: the rapid reduction or further oxidation of the aldehyde metabolites resulting from CYP-mediated *N*- or *O*-dealkylations; further processing of an initial glutathione conjugate, so as to form a mercapturic acid metabolite; and successive β -oxidations of aliphatic carboxylic acid chains. It can be noted that phase 1b metabolism is often critical for avoiding metabolite toxicity (i.e., further processing of aldehydes) or for reducing a metabolite's molecular mass as a prelude to the latter's excretion (i.e., further processing of a glutathione conjugate reduces its initial molecular mass increase from 307 to only 162, which is then similar to the increase of 175 that results from formation of a common glucuronide conjugate).

Also in line with the historical definition, while additionally incorporating similar "a" and "b" subclassifications, **phase 2** metabolism includes all of the conjugation reactions that can take place, either as the first metabolic event at a given point on the substrate ("a"), or as a subsequent reaction at a point on the substrate having already undergone a biotransformation ("b"). Typical examples include: glucuronidation and sulfation of aromatic hydroxyl groups either present on the parent xenobiotic (phase 2a) or having been instilled there (phase 2b) by a preceding phase 1a, CYP-mediated aromatic hydroxylation or an aryl- *O*-demethylation biotransformation; glutathione entrapment of highly reactive electrophilic centers present in

the substrate; *N*-acetylation or *N*-, *O*-, and *S*-methylation reactions; and glycine conjugation of carboxylic acid moieties either present on the parent xenobiotic (phase 2a) or having resulted (phase 2b) from a preceding phase 1a, esterase-mediated hydrolysis reaction.

The term **phase 3** encompasses the various excretion-associated events that either the unmetabolized xenobiotic (note first line in the diagram immediately after the phase 0a,b processes) or its metabolites will eventually engage. Key examples include: physicochemical-driven partitioning behavior between biological milieu associated with excretion, such as the loop of Henle within the kidneys' nephrons; transport into the bile canaliculi at the hepatobiliary interface, especially for negatively charged metabolites by the organic anion transporters (OATs) present within the liver's hepatocytes; and transport across the GI epithelium into the gut lumen, especially for metabolites that are good substrates for P-glycoprotein (Pgp). Also accounted for in this expanded nomenclature's diagram is the possibility for enterohepatic cycling, a key example afforded by the steroids that, upon their glucuronide conjugation and excretion into the gut, can be hydrolyzed by the microflora and thus subject to reabsorption.

Finally, **phase 4** encompasses all of the processes that can continue to contribute to an excreted compound's environmental fate. Typical examples include: the possibility for both continued biodegradation (catabolism) or potential anabolism by a variety of microbial, plant, or animal species and "distribution" both by waterways or by edible plants and animals so as to enter the nutritional cycles of mammals, where reingestion by humans, as suggested by the dotted line in the nomenclature's accompanying scheme, then comes full circle along a systems biology theme.

After relating the differing views about the value of using the "phase 1" and "phase 2" terms, in the end the above diagram, along with a tutorial, has been placed in the list of definitions generated for this glossary. Far from providing a concise definition, it is certainly not intended as any type of mandate for usage. Instead, the goal for its inclusion is to provide a fertile ground for a wide variety of technical participants to consider and to discuss the theme of xenobiotic metabolism in the most broad scope possible while retaining all of its components connected within a rational framework that emphasizes a systems biology approach.

Appendix III: A Possible Classification System for Prodrugs

Today, a wide range of interdisciplinary technologies contribute toward prodrug strategies, such that the complexity for their design options has risen significantly.²⁸⁵ This encourages efforts to further classify prodrugs into subcategories that can be useful for clearly assigning their given type of technology and for potentially clarifying the associated regulatory processes needed for bringing prodrugs through preclinical development, clinical studies, and eventually, into the marketplace.²⁸⁵ While the definitions given above for prodrugs and soft drugs are in line with the most recent IUPAC nomenclature recommendations, no system of subcategories has yet been uniformly adopted by practicing technicians, nor have any been officially mandated by drug regulatory bodies and thereby prompting their recognition, let alone their formal adoption by IUPAC. Thus, the following discussion and suggested subcategorizations are, at this time, intended only for interested readers to consider, and perhaps to devise alternative systems that could be useful to the prodrug field of the future.

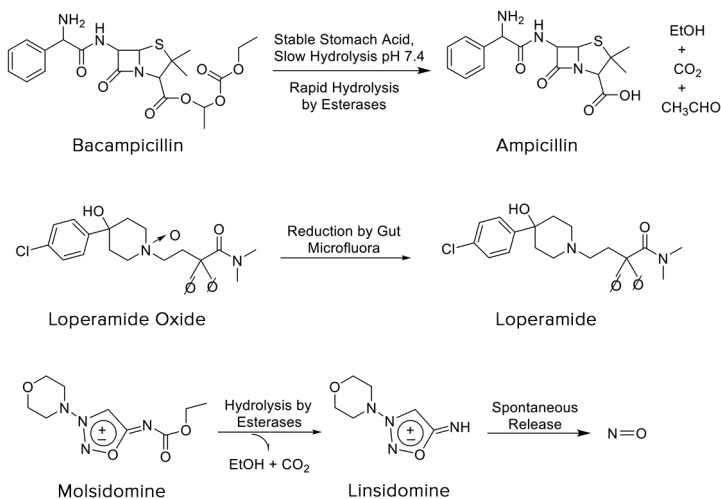
One potentially useful classification has proposed two distinct categories:^{286,287} Type I prodrugs, which undergo bioactivation within cells; and Type II, which undergo bioactivation within biological fluids outside of cells. The proposed system elaborated below also starts from this fundamental division. A few structural examples are provided in order to demonstrate how the various categories might be able to be adopted.

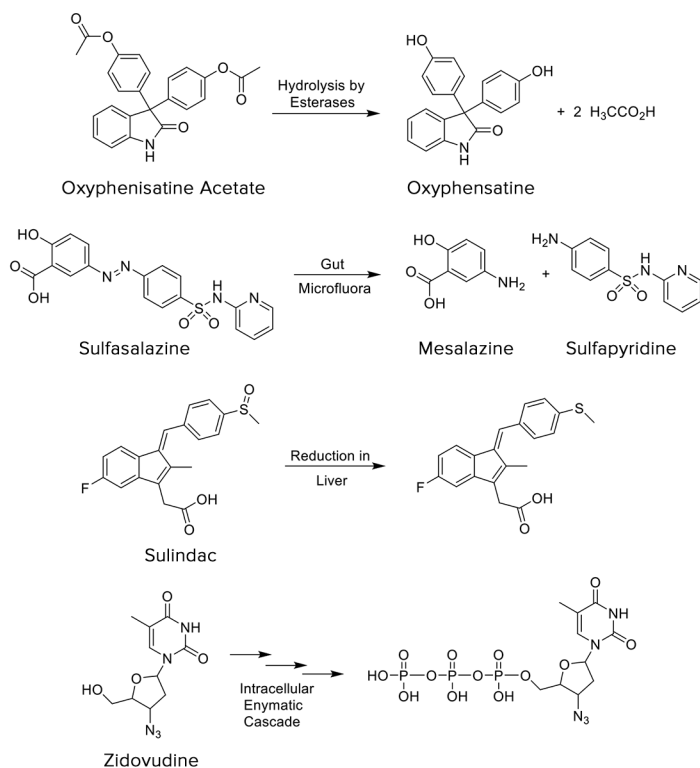
Expanding the system initially introduced by Wu et al.,^{286,287} the following table attempts to provide further definitions for several subtype categories, starting from this same classification system, but with minor alterations.²⁸⁵ Overall, this system is based on which region of a prodrug's "random walk"²³⁷⁻²³⁹ is primarily responsible for its bioactivation. This strikes a certain historical cord with medicinal chemists' early thoughts about the importance of drug distribution and how it can impact both therapeutic efficacy and offtarget toxicity. The table's title and descriptive phraseology have been modified from the original publication in order to further emphasize this theme. Some of the subcategories have also been modified to better accommodate the latest molecular biology-associated technologies, such as those like the antibody-drug conjugates (ADCs), which have recently become very popular as prodrug constructs.^{32,33} The accompanying figure then shows some of the structures and their key biotransformations for the examples listed on the table.

"Regional" Classification System for Prodrugs²⁸⁵ (a)

Type	Bioactivation Zip Code	Bioactivation Subtype	Street Address	Category Examples
I	Intracellular	IA	Therapeutic target tissue or cells	Acyclovir, L-DOPA, Zidovudine
		IB	Metabolic tissues (e.g., GI mucosa, liver, lungs)	Enalapril, Molsidomine, Sulindac
		IC	Molecular biology enhanced localization (mAb for select antigens; exogenous enzymes) (b)	ADCs, ADEPTs, GDEPTs, VDEPTs
II	Extracellular	II	GI fluids (includes gut microbiome) (c)	Loperamide Oxide, Oxyphenisatin Acetate, Sulfasalazine
		IIB	Systemic circulation and other extracellular fluid compartments	Bacampicillin, Bambuterol, Fosphenytoin
		IIC	Molecular biology enhanced localization (mAb for select antigens; exogenous enzymes) (b)	ADCs, ADEPTs, GDEPTs, VDEPTs

(a) This table has been adapted from an original version published by Wu and Farrelly.²⁸⁶ **(b)** ADCs whose mAbs have enhanced localization at the target cells, can be engineered to first enter the cells prior to releasing the active drug cargo (Type IC), or to first release the cargo in the immediate extracellular matrix so it can enter the cells without having the mAb still attached (Type IIC). Both approaches have distinct technical challenges. The same is true for mAb-delivered exogenous enzymes (ADEPTs). Similarly, once gene altered "exogenous-like" enzymes have been expressed (GDEPTs or VDEPTs), they can be either retained within the cell (Type IC) or shuttled to the extracellular matrix (Type IIC) in order to biotransform their subsequently administered prodrug partners that have been tailored to be ideal substrates. **(c)** While the microbiome's (i.e., gut bacteria or microflora) composite of metabolic activity is largely accomplished in their own "intracellular" region, this compartment is being regarded as "external" to that of all the human cells making up the body's tissues, organs etc.





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ESCAPE FROM PROTACS[®]: RECENT DEVELOPMENTS IN PROTEIN DEGRADATION WITH SMALL-MOLECULE GLUES

**Jennifer R. Riggs,* Deborah S. Mortensen, Thomas Clayton
and Christoph W. Zapf**

Bristol Myers Squibb, San Diego, California, U.S.A.

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1. INTRODUCTION

The regulation of cellular processes involves a delicate balance of critical protein interactions as well as protein expression and their removal from the cell. In a disease setting, these cellular processes are often dysregulated as a result of protein overexpression or functional upregulation. Returning the cell to a normal state can be achieved by functional inhibition of activated proteins or their removal through targeted protein degradation. Unfortunately, many biologically relevant proteins lack a typical ligand binding site that can be targeted by non-natural modulators and, consequently, have been termed “undruggable.” However, the cellular removal of some of these intractable targets (e.g., transcription factors) has been achieved and clinically validated utilizing targeted protein degradation.

Various mechanisms for proximity-driven targeted protein degradation have been uncovered.¹ These mechanisms rely on the induction of novel protein-protein interactions to facilitate the removal of therapeutically relevant proteins. Of particular interest are mechanisms that ubiquitinate

and degrade proteins by utilizing the cell’s natural ubiquitin proteasome system (UPS).^{2,3} Protein ubiquitination is a posttranslational modification requiring an enzyme cascade of an activating enzyme (E1), a conjugating enzyme (E2), and a ubiquitin ligase (E3). The E3 ligase is responsible for bringing the E2 and the target protein together, enabling the transfer of several ubiquitin proteins to the target protein, thus tagging the protein for degradation by the UPS (Figure 1).⁴ Although E3 ubiquitin ligases are critical for targeted protein degradation, they also regulate homeostasis, cell cycle processes, and DNA repair, making them potential tumor suppressors or promoters.⁵

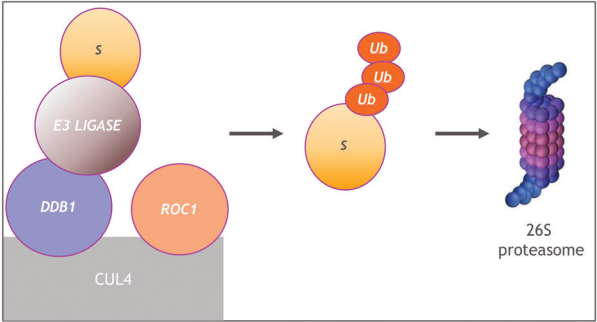


Figure 1 CRL4 ubiquitin ligase complex (CUL4, cullin 4; DDB1, DNA damage-binding protein 1; ROC1, regulator of cullins-1) facilitates transfer of multiple ubiquitin molecules (Ub) to a target protein (substrate, S) resulting in degradation of the substrate protein by the 26S proteasome.

Over 600 human E3 ligases exist, exemplifying the diversity and importance of this class of enzymes. The E3 ligase cereblon (CRBN) can be co-opted for targeted protein degradation by two distinct but related mechanisms: molecular glue and heterobifunctional degradation (Figure 2).

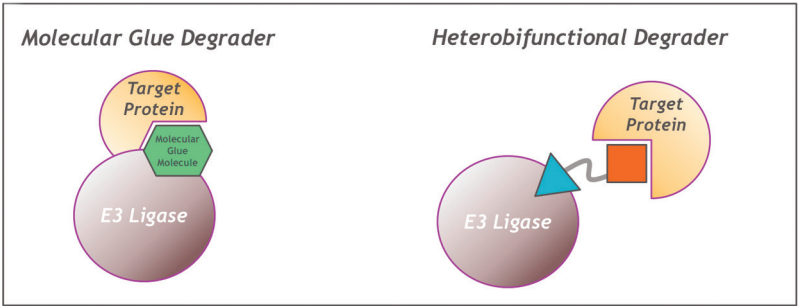


Figure 2 Conceptual depiction contrasting molecular glue degradation and heterobifunctional degradation utilizing the E3 ligase system. In heterobifunctional degradation, a ligase binder (blue triangle) is linked to a target protein binder (orange square) with an appropriate linker (gray line).

The term “molecular glue” was first used to describe the mechanism of action for the plant hormones auxin and jasmonate.^{6,7} These small molecules alter plant signaling by acting as “glues,” bringing together S-phase kinase-associated protein (Skp), Cullen, F-box containing complex E3s, and repressive transcription factors, resulting in the degradation of the transcription factors. Examples of small-molecule glues in the clinic include cereblon modulators that co-opt CRBN, altering the substrate specificity of the ubiquitin ligase complex (CUL4-RBX1-DDB1) and thus enabling the recruitment of specific target proteins. Cereblon modulating compounds have been shown to recruit a variety of otherwise unrelated proteins through a common recognition element, a short structural β -hairpin loop with a common glycine residue, also referred to as a structural degron.⁸ In contrast, heterobifunctional degraders, or proteolysis targeting chimeras (PROTACS®) require an E3 binding moiety and a binder to the protein of interest joined by a linker of optimal length and conformation bias.⁹ Heterobifunctional degraders do not require a degron in the target protein, as in the molecular glue mechanism, and thus can be utilized more widely. Although both protein degradation mechanisms have been applied to biological targets of interest, the field of molecular glue has been extensively clinically validated and is the subject of this review.¹⁰⁻¹⁵

2. TOOLS FOR MOLECULAR GLUE DRUG DISCOVERY

The mechanism of action for the first CRBN molecular glue compounds, Thalomid® (thalidomide, **1**) and Revlimid® (lenalidomide, **2**) was unknown at the time of their discovery (Figure 3). Rather, their identification and optimization was driven in a phenotypic manner. Elucidation of their mechanism of action has generated an ever-evolving toolbox of technologies for the discovery of molecular glue molecules, including cellular degradation screens, proteomic profiling, biochemical and biophysical assays for binary and ternary complex formation, and structural determination.

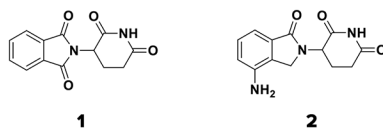


Figure 3 Chemical structures of thalidomide (**1**) and lenalidomide (**2**).

Cellular degradation screens look for the compound-dependent loss of protein via enzyme fragment complementation (EFC) assays, proteomic profiling, or fluorescent reporter systems. In enhanced ProLabel® (ePL) or HiBit EFC assays, loss of the tagged protein of interest in the presence of compound leads to loss of luminescence and can be utilized to measure degradation of endogenous or overexpressed proteins. Proteomic profiling, either via single-point pulse chase SILAC or isobaric chemical labeling, and multiplexing using tandem mass spec reagent (TMT) methods are used to evaluate proteome-wide changes with compound treatment,^{4,12,16,17} helping to define the degradome for particular compounds and assess compound selectivity. Cell-based discovery techniques in the presence of proteasome or NEDD8 inhibitors, and with CRISPR/Cas9 knockout of cereblon, have been used to establish that protein downregulation is indeed occurring via the ubiquitin proteasome system.^{4,9,10,12,16} Time course experiments using these assays allow for assessment of degradation kinetics and comparison of protein and mRNA levels, ensuring changes in protein levels are not transcriptionally regulated.

In vitro biochemical and biophysical assays such as time-resolved measurement of fluorescence with fluorescence resonance energy transfer (TR-FRET), surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), and *in vitro* ubiquitination are key to establishing a molecular glue mechanism. A hallmark feature of a molecular glue is the lack of an intrinsic interaction between the glue molecule and the non-native neo-substrate but rather a glue-mediated interaction between this neo-substrate and the E3 ligase.^{3,4,9,10,12} Natural substrates are recruited to their cognate E3s via a structural degron, the portion of the protein necessary and sufficient for proteasomal regulation. Additionally, *in vitro* assays are utilized in structural degron characterization, determining the neosubstrate domain of recruitment and the role of key amino acids at the ternary complex interface.^{3,4,9,10,12,16}

Binary and ternary complex structures have been instrumental in elucidation of the cereblon modulator molecular glue mechanism. Following the identification of CRBN as the molecular mediator for the activity of historical IMiDs®¹⁷ (immunomodulatory imide drugs), the structural elucidation of cereblon-DDB1 bound to thalidomide and lenalidomide^{16,18} led to the hypothesis that these molecules repurpose the surface of CRBN to recruit novel substrates (Figure 4a). Furthermore, ternary complex structures

of cereblon-DDB1 engaging the neosubstates G1 to S phase transition 1 (GSPT1) or casein kinase 1 α (CK1 α) in the presence of a cereblon modulating compound provided a structural rationale for how proteins of disparate sequence and structure could be recruited and degraded via the same class of molecules (Figure 4b).^{8,19} The cereblon modulating surface interacts with both GSPT1 and CK1 α through a structural degron. Interactions between the β -hairpin loop and cereblon are driven solely via CRBN backbone carbonyls and the glycine residue of the degron which stacks against the isoindolinone, taking on phi psi angles only accessible to glycine (Figure 4c). These structures confirmed that the cereblon modulator molecular glue mechanism of action occurs via a structural degron defined as the carbonyl-mediated interactions formed by the β -hairpin turn and the glycine residue immediately following them. Molecular modeling and subsequent structural studies established not only that the C2H2 zinc finger transcription factors IKZF1 (Ikaros) and IKZF3 (Aiolos) were similarly recruited via the same structural degron,^{8,19,20} but that the cereblon modulator degradome included a number of additional C2H2 zinc finger proteins. The recent publication detailing the degradation of zinc finger MYM-type protein 2 (ZMYM2) by avadomide **3** (Figure 5) establishes the presence of the structural degron in a different zinc finger fold and furthers the hypothesis that cereblon modulating molecular glue molecules can be used to recruit a plethora of structural degron containing targets.²¹⁻²⁴

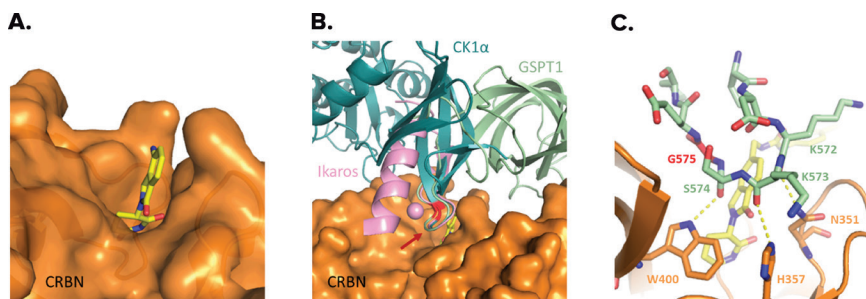


Figure 4 Mechanism of action of Cereblon Modulators. **A.** Structure of cereblon in complex with lenalidomide. **B.** Ternary complex structures overlay showing recruitment of GSPT1, CK1 α , and Ikaros via the structural degron. The structural degron is indicated with an arrow and the conserved glycine residue shown in red. **C.** Predicted polar interactions between the structural degron of GSPT1 shown in green and cereblon shown in orange.

Negative stain electron microscopy has been used as part of the molecular glue toolbox to establish ternary complex formation.⁸ Currently a resolution

revolution in cryogenic electron microscopy (cryo-EM) is underway, facilitating the structural interrogation of complexes recalcitrant to X-ray crystallography. As the opportunity to repurpose additional E3 ligases with molecular glues expands, cryo-EM and crystallography will be key to defining the mechanism of recruitment.

3. CRBN-BASED MOLECULAR GLUES

CRBN-based molecular glues are clinically effective in multiple hematological malignancies, leading to improved outcomes for patients. The first cereblon modulator to be approved for multiple myeloma (MM) was thalidomide (**1**, Figure 3). Although thalidomide was first approved for the treatment of morning sickness in the 1950s and withdrawn from the market due to severe birth defects, the mechanism of action of the drug was not elucidated until decades later.²⁵ It was found that thalidomide was indeed efficacious against leprosy and MM. Strict prescribing safeguards enabled appropriate patient subgroups to derive benefit from thalidomide treatment. The efficacy of thalidomide in MM patients led to a phenotypically driven drug discovery campaign resulting in lenalidomide (**2**, Figure 3) and Pomalyst® (pomalidomide, **4**, Figure 5). Years later, the degradation of the C2H2 zinc finger transcription factors Ikaros and Aiolos as neosubstrates of a CRBN-mediated protein degradation process was elucidated.²⁶⁻²⁹ Both lenalidomide and pomalidomide are approved for MM, consistent with the biological mechanism of targeting Ikaros and Aiolos in hematopoietic cell differentiation regulation.³⁰ Next-generation Aiolos and Ikaros degraders in Phase II clinical trials CC-122 (avadomide, **3**) and CC-220 (iberdomide, **5**) have proven efficacious in lenalidomide and pomalidomide refractory and relapsed (R/R) MM patients, and diffuse large B-cell lymphoma (DLBCL), R/R MM, and systematic lupus erythematosus (SLE), respectively (Figure 5).³¹⁻³⁴ This next generation of Aiolos and Ikaros degraders exhibited increased potency of CRBN binding and depth of substrate degradation.³²

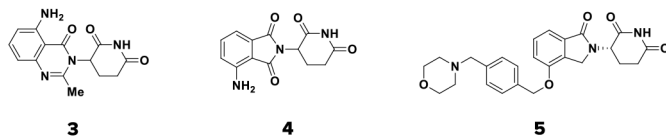


Figure 5 Chemical structures of avadomide (**3**), pomalidomide (**4**), and iberdomide (**5**).

A strong structural and biological understanding of CRBN facilitated the evolution from phenotypic drug discovery into a target-based approach, designing novel cereblon modulating compounds to degrade additional biologically relevant proteins. Although previous cereblon modulating agents had improved the MM response rate, relapsed and refractory patients were still in need of new therapies. After optimization, the exquisitely efficient and rapid Aiolos and Ikaros degrader, cereblon E3 ligase modulator (CELMoD®) CC-92480 (**6**, Figure 6), demonstrated improved antiproliferative activity over lenalidomide and pomalidomide in R/R MM cell lines.³⁵ CC-92480 is in Phase I clinical trials for MM. CELMoD CC-99282 (**7**, Figure 6) was optimized for rapid, deep, and sustained Aiolos and Ikaros degradation with a robust lymphoid tissue distribution profile. Preclinically, its superiority over lenalidomide has been demonstrated *in vitro* and *in vivo* in different non-Hodgkin lymphoma (NHL) tumor cell lines and disease models. This compound is currently being assessed either as monotherapy or in rationally chosen targeted combinations for patients with R/R NHL,³⁶⁻³⁷ as well as in combination with anti-CD20 treatment in a Phase I study for patients with R/R chronic lymphocytic leukemia (CLL).

While Aiolos and Ikaros have been the key substrates targeted clinically to date, the known cereblon modulating compounds have been shown to degrade additional substrates including kinases and translation termination factors. Lenalidomide not only degrades transcription factors Aiolos and Ikaros, but also CK1α through a CRBN-mediated molecular glue mechanism.³⁸ In addition, the translation termination factor GSPT1 is degraded by CC-885 (**8**, Figure 6), resulting in strong growth inhibition in multiple cell lines and patient-derived acute myeloid leukemia (AML) lines.⁸ The CELMoD agent CC-90009 (**9**, Figure 6), currently in Phase I clinical trials, was developed as a selective GSPT1 degrader for the treatment of AML.^{39,40} Although the discovery of CC-90009 was primarily driven phenotypically utilizing a panel of AML cell lines, the target substrate was quickly identified as GSPT1.

Multiple groups have reported the generation of IMiD®-based libraries and confirmed the identification of Aiolos and GSPT1 degrading compounds.⁴¹⁻⁴³ ZXH-1-161 was reported as an isoindolinone-containing selective GSPT1 degrader with antiproliferative activity in a MM cell line (**10**, Figure 6).⁴³ An additional potent and selective GSPT1 degrader, SJ6986, was recently disclosed from a focused thalidomide-based library screen with high oral

bioavailability (**11**, Figure 6).⁴² Taken together, these examples illustrate the utility and opportunity for selectivity of the CRBN-based molecular glue mechanism across multiple target classes.

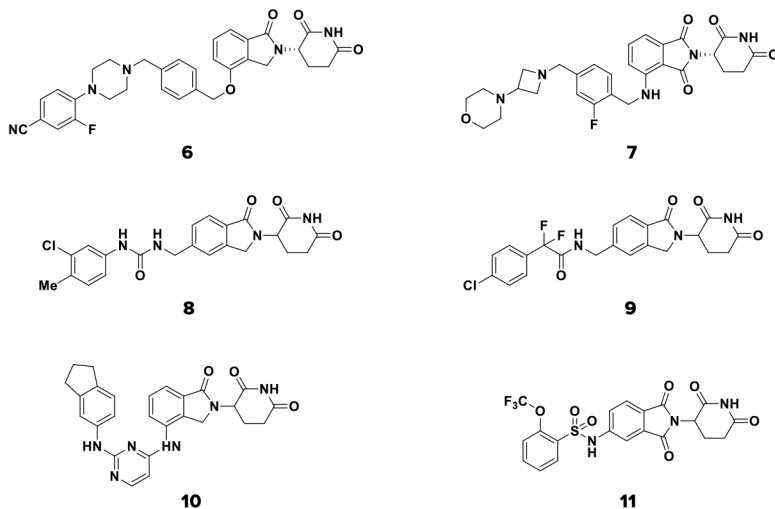


Figure 6 Chemical structures of CC-92480 (**6**), CC-99282 (**7**), CC-885 (**8**), CC-90009 (**9**), ZXH-1-161 (**10**), and SJ6986 (**11**)

Many in the pharmaceutical industry have recognized the utility of CRBN-based molecular glues. While the list of companies reportedly pursuing CRBN-based molecular glues is extensive, it is worth noting two advanced examples. Helios (IKZF2) degrader DKY709 (structure undisclosed) is in Phase I clinical trials for advanced solid tumors.^{36,44} In addition, the Aiolos and Ikaros degrader, CFT7455 (structure undisclosed), is reportedly entering Phase I in 2021 in R/R MM and NHL.⁴⁵ Continued vigorous activity from pharmaceutical and biotechnology companies in the molecular glue space highlights that exciting opportunities remain to target additional “undruggable” protein targets via this mechanism.

4. CONSIDERATIONS FOR THE DISCOVERY OF MOLECULAR GLUE CANDIDATES

While the CRBN-mediated removal of biologically relevant proteins has the potential to elicit therapeutic benefit, this approach must be weighed against the potential for unwanted side effects. The most infamous of

these unwanted side effects, the teratogenicity of thalidomide and related compounds, has now been linked to cereblon-mediated degradation of SALL4^{23,46} and PLZF,⁴⁷ as well as other suggested targets.⁴⁸

Selectivity in targeted protein degradation will be critical not only for the safety profile of potential therapeutics but also for target validation efforts utilizing such compounds. The profound cellular growth inhibition profile resulting from the degradation of GSPT1, for example, may significantly complicate on-target evaluation of molecular glue and heterobifunctional degrader efforts.⁴⁹ As with any drug discovery effort, the successful development of a therapeutic will come down to a careful balance of desired versus undesired effects.

When considering the identification and development of heterobifunctional degraders, the choice of ligase binding moiety should be carefully considered. The introduction of additional degradation targets by a molecular glue mechanism could be approached as a feature or a flaw. Heterobifunctional degraders utilizing a ligase binder targeting additional neosubstrates through a molecular glue approach could provide dual-mechanism targeted degraders. One example with improved preclinical activity was the IRAK4-targeting PROTAC KT-413 (structure undisclosed), which utilizes a CRBN binder that also acts as a molecular glue to recruit and degrade Ikaros and Aiolos. KT-413 was shown to be more effective in MYD88-mutant lymphoma lines as compared to either IRAK4 selective degrader KTX-545 or Ikaros/Aiolos degrader CC-220 alone.⁵⁰ Similarly, the identification of a BTK-PROTAC with molecular glue degradation of IMiD substrates for B-cell malignancies has also been reported.⁵¹

Species differences in the CRBN-mediated degradation of targets further complicates the pre-clinical efficacy and safety assessment of potential cereblon modulating therapeutics. Critical CRBN sequence differences in rodent versus primate have been linked to the lack of degradation of well-known substrates in rodent species. Mutational studies have shown that introduction of a single mutation in mouse CRBN (I391V) is sufficient to enable cereblon modulator-mediated degradation of CK1 α , IKZF1, and IKZF3 in mouse cells.^{19,38} Studies using human CRBN have similarly shown that E377V and V388I mutations, both known rodent polymorphisms, impair the ability to recruit and degrade GSPT1 and Ikaros, respectively.⁸

The evaluation of tumor-intrinsic activities using human tumor xenograft models expressing human CRBN is relatively straightforward. The use of rodent species for syngeneic disease models or toxicity evaluations, however, creates a challenge. Multiple research groups have developed CRBN-humanized murine models to aid in the assessment and development of CRBN based therapeutics.^{30,52} The use of CRBN-I391V mice has enabled *in vivo* evaluation of compounds such as thalidomide and its analogs, for both efficacy and toxicity. Thalidomide was shown to degrade Ikaros, Aiolos, Zfp91, and CK1 α *in vivo* in the humanized mouse model, and further, the CRBN-I391V was sufficient to confer thalidomide-induced fetal loss.³⁰ Lenalidomide has been shown to be protective against DSS-induced colitis in the CRBN-humanized and not wild-type mice.⁵³ Similarly, introduction of the single CRBN-I391V mutation was sufficient to render a syngeneic murine model of multiple myeloma sensitive to IMiD treatment by lenalidomide and pomalidomide.⁵²

The species homology-to-human CRBN has been found to be the most critical attribute for predicting species-specific degradation of a targeted substrate. In the case of the most well-known CRBN substrates studied to date, the homology of CRBN and not the degron of the protein of interest has been implicated in the species specificity of degradation. SALL4 represents a notable exception to this observation. Sequence differences in both CRBN and SALL4 prevent its degradation in mice and provide a rationale for the lack of IMiD-induced teratogenicity in this species.^{46,54} As more targets or alternate degrons are identified, the possibility of substrate-driven species selectivity remains.

CRBN-mediated protein degradation of Aiolos and Ikaros has been heavily studied in multiple myeloma patients and may provide insight into clinically relevant resistance mechanisms. In recent years, studies assessing the resistance mechanisms in these patients have been disclosed. Impairment of CRBN function by a variety of mechanisms including mutations, gene expression loss, and splice variants was discovered initially in a small patient population.⁵⁵⁻⁵⁸ A more extensive evaluation assessed 655 patients, including newly diagnosed, lenalidomide-refractory and pomalidomide-refractory cases.⁵⁹ The study showed that by the time patients progress to pomalidomide resistance, nearly one third of these patients will have developed genetic alterations in CRBN. Newly discovered CELMoD compounds, such as CC-92480 (**6**, Figure 6), with superior target

degradation potency and efficiency, have shown efficacy in pomalidomide-resistant MM cell lines,^{35,60} and an ability to degrade Ikaros and Aiolos in pomalidomide- and lenalidomide-resistant patients.⁶¹ While it remains to be seen if different resistant mechanisms arise in non-MM indications with molecular glue compounds targeting alternate substrates, the results of the MM resistance mechanism studies provide evidence that CRBN alterations should be considered in the development and evaluation of new cereblon modulating compounds.

5. EMERGING NON CRBN-BASED MOLECULAR GLUE

Structural biology insights enabled a fundamental understanding of the cereblon modulating mechanism of action.^{11,16,18} In an effort to expand the boundaries of this intriguing mode of action, extensive research has focused recently on the identification and rational design of molecular glues that target other ligase-substrate systems outside of CRBN. Arylsulfonamide indisulam (**12**, Figure 7) had been identified through phenotypic screening and advanced to clinical trials for solid tumors without an understanding of the compound's mechanism of action. Development of the compound was halted due to limited efficacy as opposed to a lack of tolerability. Subsequent mechanistic studies uncovered that indisulam resistance occurs in cells with mutations in the RNA Binding Motif Protein 39 (*RBM39*) gene. It was found that treatment of cells with indisulam and structurally related analogs such as E7820 (**13**, Figure 7) resulted in the proteasomal degradation of RBM39, a response that could be blocked by treatment with the 20S proteasome inhibitor bortezomib. Pull-down experiments revealed that indisulam recruits RBM39 to form a ternary complex with CUL4^{DCAF15} and thus acts as a novel molecular glue.^{62,63} Structurally related analogs with anti-cancer activity such as tasisulam (**14**, Figure 7) were hypothesized to also recruit RBM39 to DCAF15. Results from cellular experiments suggested that these sulfonamide compounds indeed share the same mechanism of action of DCAF15-mediated RBM39 degradation.⁶⁰ A cryo-EM structure (4.4 Å resolution), confirmed that E7820 acts as a molecular glue degrader by interacting with DCAF15, resulting in the selective binding and degradation of RBM39.⁶⁴

Dysregulation of the Wnt signaling pathway as a result of β -TrCP stabilization can result in cancer. Saha and coworkers disclosed an approach to prospectively identify molecular glue that enhances the interaction

between mutated β -catenin and its putative regulatory E3 ligase, SCF ^{β -TrCP}. Mutated β -catenin loses its affinity for β -TrCP if phosphorylation at Ser33 or Ser37 is abrogated, resulting in stabilization of this oncogenic transcription factor. Structure-based drug design was employed to optimize a series of small molecules capable of enhancing the interaction between a β -catenin mutant S33E/S37A (mimicking pSer33) peptide and β -TrCP. In the presence of 20 μ M NRX-252114 (**15**, Figure 7) the peptide's affinity to the ligase was restored to within 3-fold of the WT level. While these compounds were unable to degrade endogenous S37A mutant β -catenin in TOV-112D cells, *in vitro* substrate ubiquitination was clearly observed in a manner similar to WT β -catenin, thus validating the authors' discovery strategy to identify molecular glue for β -TrCP.⁶⁵

To identify drug molecules that act through E3 ligase-mediated protein degradation, the drug-sensitivity of over 4,000 preclinical and clinical compounds was assessed in cancer cell lines with respect to their impact on the mRNA levels of various E3 ligase components. This effort identified a correlation between the cytotoxic properties of CDK inhibitor (R)-CR8 (**16**, Figure 7) and the mRNA levels of the CUL4 adaptor protein DDB1 across several hundred cancer cell lines. A presumed mechanism involving protein degradation was supported by a proteomics experiment that revealed loss of cyclin K as a result of (R)-CR8 treatment. Control experiments involving inhibition of E1 ubiquitin activation, neddylation, and the proteasome suggest proteasomal degradation of cyclin K. *In vitro* experiments demonstrated that (R)-CR8 can instigate complex formation between CDK12-cyclin K and DDB1. This observation was corroborated by a crystal structure of CDK12(kinase domain)-cyclin K(1-267) bound to (R)-CR8 in complex with a truncated version of DDB1 (3.5 Å resolution) confirming that (R)-CR8 indeed acts as a molecular glue between CDK12 and DDB1 (BPC domain).⁶⁶

In an effort to identify molecular glue in a targeted manner, a hyponeeddylation strategy was devised by mutating UBE2M in KBM7 cells, thus creating a hypomorphic phenotype. This mutated cell line was used in a chemical screening campaign involving about 2,000 cytostatic and cytotoxic agents. The compound dCeMM1 (**17**, Figure 7) was identified as molecular glue between RBM39 and DCAF15, mechanistically similar to indisulam. In addition, the screening campaign also identified dCeMM2 (**18**, Figure 7) and other analogs that led to the degradation of cyclin K in

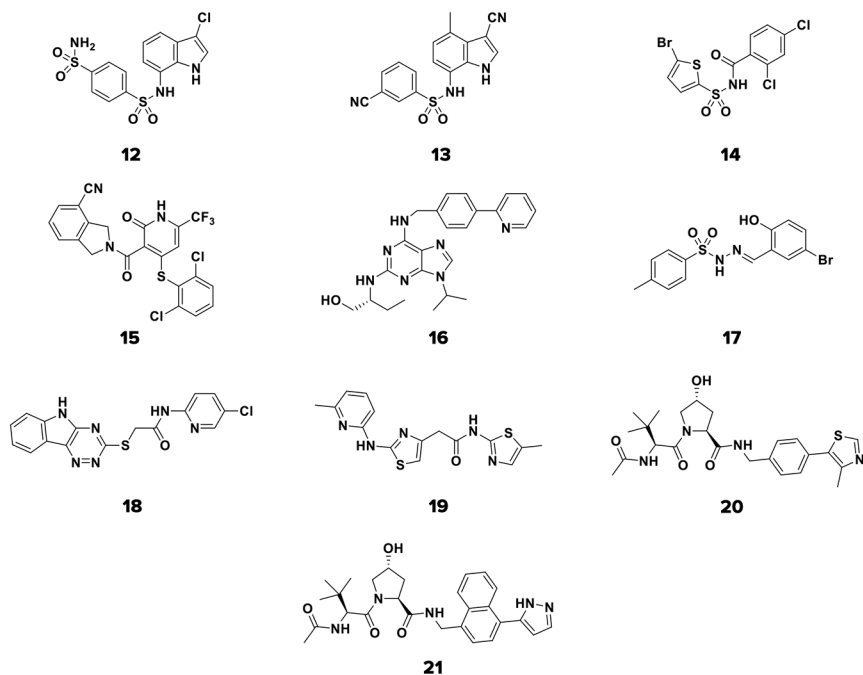


Figure 7 Chemical structures of disclosed small-molecule glues co-opting other E3 ligases: indisulam (**12**), E7820 (**13**), tasisulam (**14**), NRX-252114 (**15**), (R)-CR8 (**16**), and dCeMM1 (**17**), dCeMM2 (**18**) and HQ461 (**19**), Cpd4 (**20**), and Cpd5 (**21**).

KBM cells. As in the previous report, dCeMM2 serves as a molecular glue leading to a measurable drug-induced proximity enhancement between DDB1 and CDK12-cyclin K.⁶⁷

A third independent study published the same year identified a different chemotype capable of forming a ternary complex between DDB1 and CDK12. Following a high-throughput phenotypic screen, HQ461 (**19**, Figure 7) was characterized as a potent cytotoxic agent that binds to the ATP-binding pocket of the CDK12 kinase domain and, in turn, can recruit DDB1. This binary complex was also found to recruit cyclin K, which is ultimately degraded in a time- and concentration-dependent manner in A549 cells.⁶⁸

Recently, the well-known von Hippel-Landau (VHL) binder chemotype was disclosed as a molecular glue. A 2D-NMR study suggested that VHL protein can be recruited to cysteine dioxygenase 1 (CDO1) in the presence

of Cpd4 (**20**, Figure 7), a finding corroborated in a NanoBit assay measuring the proximity between the two proteins. Western blot data confirmed the degradation of CDO1 in the presence of Cpd4, which was suppressed by the addition of a neddylation or proteasome inhibitor. A ternary complex structure using structural analog Cpd5 (**21**) revealed that the chemotype is indeed capable of acting as a molecular glue between VHL and CDO1.⁶⁹

6. FUTURE DIRECTIONS AND CONCLUSIONS

IMiD-based molecular glue was serendipitously discovered as pharmaceutically relevant chemical matter in the mid-20th century. Its mode of action through engaging Cul4^{CRBN} was characterized in the early 21st century by the thoughtful use of chemical and structural biology. Subsequently, the discovery of novel and more efficient molecular glue compounds with vastly improved potency and selectivity profiles was enabled through phenotypic screening and targeted approaches. To date, several molecular glue drugs are on the market, and a substantial number of next-generation compounds are currently undergoing clinical evaluation in hematological and solid tumor cancer indications.

As we described, a growing number of ligases beyond cereblon are now enabled to drive protein degradation of known or novel drug targets by the molecular glue mechanism. Given the enormous diversity of human E3 ligases and the emerging strategies to identify molecular glue by *intent*, we are convinced that our industry is at the beginning of a new era of drug discovery allowing us to revisit undruggable proteins as drug targets, much to the benefit of patients.

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GLOSSARY AND TUTORIAL OF XENOBIOTIC METABOLISM TERMS USED DURING SMALL- MOLECULE DRUG DISCOVERY AND DEVELOPMENT

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Paul Erhardt*, **Kenneth Bachmann**, **Donald Birkett**, **Michael Boberg**, **Nicholas Bodor**, **Gordon Gibson**, **David Hawkins**, **Gabrielle Hawksworth**, **Jack Hinson**, **Daniel Koehler**, **Brian Kress**, **Amarjit Luniwal**, **Hiroshi Masumoto**, **Raymond Novak**, **Phillip Portoghese**, **Jeffrey Sarver**, **M. Teresa Serafini**, **Christopher Trabbic**, **Nico Vermeulen**, and **Steven Wrighton**

*Corresponding author: **Paul Erhardt**, Center for Drug Design and Development,
University of Toledo, Toledo, Ohio, U.S.A. (TGM and ST)

Kenneth Bachmann, Ceuticare, Inc., Sylvania, Ohio, U.S.A. (TGM and ST)

Donald Birkett, Department of Clinical Pharmacology, Flinders University, Adelaide, Australia
(now Emeritus) (TGM)

Michael Boberg, Metabolism and Isotope Chemistry, Bayer, AG, Germany
(now undetermined) (TGM)

Nicholas Bodor, Center for Drug Discovery, University of Florida, Belle Glade, Florida, U.S.A.
(now Emeritus Grad Res Prof/CEO Bodor Labs) (TGM)

Gordon Gibson, School of Biomedical and Life Sciences, University of Surrey, Surrey, U.K.
(now deceased) (TGM)

David Hawkins, Huntingdon Life Sciences, Huntingdon, U.K. (now retired) (TGM)

Gabrielle Hawksworth, Department of Medicine and Therapeutics, University Aberdeen,
Aberdeen, U.K. (now deceased) (TGM)

Jack Hinson, Division of Toxicology, University Arkansas for Medical Sciences, Little Rock,
Arkansas, U.S.A. (now Emeritus Dist Prof) (TGM)

Daniel Koehler, Department of Pharmacology, University of Toledo, Toledo, Ohio, U.S.A. (ST)

Brian Kress, Department of Medicinal and Biological Chemistry, University of Toledo,
Toledo, Ohio, U.S.A. (ST)

Amarjit Luniwal, NAMSA, Inc., Northwood, Ohio, U.S.A. (ST)

Hiroshi Masumoto, Drug Metabolism, Daiichi Pharm. Corp., Ltd., Chuo, Tokyo, Japan (now
retired) (TGM)

Raymond Novak, Institute of Environmental Health Science, Wayne State University,
Detroit, Michigan, U.S.A. (now undetermined) (TGM)

Phillip Portoghese, Department of Medicinal Chemistry, University of Minnesota,
Minneapolis, Minnesota, U.S.A. (TGM)

Jeffrey Sarver, Department of Pharmacology, University of Toledo, Toledo, Ohio, U.S.A. (ST)

M. Teresa Serafini, Department of Pharmacokinetics and Drug Metabolism,
Laboratories Dr. Esteve, S.A., Barcelona, Spain (now Head Early ADME) (TGM)

Christopher Trabbic, MPI Research, Inc., Mattawan, Michigan, U.S.A. (ST)

Nico Vermeulen, Department of Pharmacochimistry, Vrije University, Amsterdam, Netherlands
(now Emeritus Section Molecular Toxicology) (TGM)

Steven Wrighton, Eli Lilly, Inc., Indianapolis, Indiana, U.S.A. (now retired) (TGM)

TGM: Task Group Member ST: Submission Team

Sponsoring bodies: IUPAC Chemistry and Human Health Division (VII) and Subcommittee on
Drug Discovery and Development. Specific information is provided on page 639.

Our final submission is dedicated to Gordon Gibson and Gabrielle Hawksorth, who were
collegial participants on this project's Founding Working Party. The field of drug metabolism
misses them.

Abstract

This project originated more than 15 years ago with the intent to produce a glossary of drug metabolism terms having definitions especially applicable for use by practicing medicinal chemists. A first-draft version underwent extensive *beta*-testing that, fortuitously, engaged international audiences in a wide range of disciplines involved in drug discovery and development. It became clear that the inclusion of information to enhance discussions among this mix of participants would be even more valuable. The present version retains a chemical structure theme while expanding tutorial comments that aim to bridge the various perspectives that may arise during interdisciplinary communications about a given term. This glossary is intended to be educational for early-stage researchers, as well as useful for investigators at various levels who participate on today's highly multidisciplinary, collaborative small-molecule drug discovery teams.

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ALPHABETICAL LIST OF TERMS

Several of the terms included in this project also appear in other IUPAC glossaries, or "Color Books," such as the Red Book (2005), Blue Book (2013), and Gold Book (2014 PDF plus online version updates), wherein alternative definitions may sometimes be found due to differences in specific context or strictly intended focus. None of the present definitions are meant to displace former IUPAC recommendations, particularly when used within the context of their originating technical disciplines. Instead, the present terms and related tutorial materials are meant to enhance the discourse between technical disciplines. An alphabetical list of terms is provided below. The actual definitions follow in alphabetical order. Readers are encouraged to start with the Introduction section, so as to appreciate the early history and evolution of this list, and to fully relate to the final intent of the definitions and their use within the overall context of today's highly interdisciplinary process for small-molecule drug discovery and development.

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Project Origin

Aside from being a notable calendar event, passage into the new millennium was a significant turning-point for many practitioners involved in drug discovery, because it marked the beginning of major operational changes that soon swept across the entire global pharmaceutical enterprise. Prompted by rapid advances in biotechnology, some of the disciplines that had served as a long-standing foundation for drug discovery were scrambling to relate and to remain relevant as their roles began to change.¹ The IUPAC Medicinal Chemistry Subcommittee's response was highly proactive, and it became one of the leaders in the generation of informational materials pertaining to the potential adoption of new technologies in ways that might improve the overall process of drug discovery. For example, the IUPAC text, *Drug Metabolism: Databases and High-Throughput Testing During Drug Design and Development*,² helped to usher in medicinal chemistry's serious consideration of ADMET parameters, as well as efficacy, during the very early stages of drug design. As part of the effort associated with this text, it was determined that a useful follow-up project would be a glossary of *Drug Metabolism Terms* specifically directed toward providing standardized definitions for use by medicinal chemists.

Historical Development and Reorientation

Getting off to a rapid start, by 2005 the project's working party consisted of 15 highly regarded experts in drug metabolism recruited from around the world (denoted as "Task Group Member" or TGM in the list of authors). They initially identified more than 600 terms that could be included in a comprehensive coverage of this topic, while also providing a healthy overlap with closely allied technical fields. The need to pare this list down to just the most relevant terms for drug discovery was recognized, and a list of about 200 was finally selected by ballot, wherein any candidate term, phrase, or acronym receiving 10 or more votes was retained. With the list divided nearly equally among the team members, preliminary definitions for these terms began to be filled in.

For the next several years the project took advantage of an opportunity to gain "first-hand" input from a variety of drug discovery practitioners, eventually leading to a reorientation of the glossary's intent. This resulted from delivering invited presentations and a short course about "drug metabolism considerations during drug design and development" while on a global lecture circuit that included numerous small, medium, and large pharma organizations, as well as several academic institutions and audiences at international technical meetings. Thus, this period served as a valuable *beta*-test for the still-evolving glossary while deploying its terms among a wide variety of audiences who were quick to provide useful feedback. Two key lessons became clear. The first was that the terms and short definitions would be more useful if they were accompanied by both chemical structure examples and tutorial information applicable to structure-metabolism relationships (SMR) relevant to drug design. The second was that nearly all of the multi-disciplinary technical staff, including non-medicinal chemists, from small pharma would typically attend the short-course and, similarly, more than 25 % of the typical 200-plus attendees during a presentation to big pharma were scientists from disciplines other than medicinal chemistry. Importantly, these interdisciplinary scientists' jobs often involved discussing drug metabolism-related issues with medicinal chemists during collaborative drug discovery and development team meetings. Thus, their attendance reflected a desire to enhance this dialogue by better understanding the context in which their medicinal chemistry counterparts tended to operate.

Returning to the project, the original definitions were adjusted so as to provide structural examples that would be of practical interest to medicinal chemists and informative to non-chemists in a manner useful for interdisciplinary discussions. Additionally, tutorial comments were crafted for many of the terms to enhance cross-communication among the different types, and professional levels, of individuals engaged in the various technical aspects and stages of drug discovery.

Impact of Reorientation

This change represents a significant departure from the long-standing theme that the IUPAC has traditionally adopted for its chemistry-related technical glossaries. Historically, definitions primarily have been devised to clarify and harmonize the use of a given term among different nations while providing what should be considered as a "standard definition" to be adhered to while being deployed across different languages. This clearly remains the preferred orientation for many chemical terms, such as those associated with distinct physical properties, such as a solid compound's melting point. Conversely, this approach may not be optimal for more interdisciplinary networks, where the words used in a given definition can have varying meanings among different technical fields, and where the terms' use may be trying to capture and emphasize differing components or aspects while working within an overarching, collaborative system. Drug metabolism is exactly such a topic, and its participants are represented by a highly interdisciplinary, wide range of investigators who, for this glossary's overarching context, have come together to pursue drug discovery.

As a common example to illustrate this key distinction, even the simple word "drug" within the phrase "drug discovery" can itself become ambiguous. Advocating a "single definition intended to fit all" may not be the best way to enhance communication, as opposed to allowing a group of interdisciplinary scientists to devise a meaning which is suitable for the context that might be needed in their particular setting. In such cases, it will be the common knowledge base potentially garnered from a tutorial about the term that will allow these practitioners to better understand each other's perspectives and then devise a mutually beneficial working definition. This same situation becomes applicable when authoring a publication wherein it may be necessary to provide some explanatory information for a term's definition depending upon its specific context within the technical report

and depending upon the differing perspectives of the envisioned readers. The appended discourse on the seemingly simple term “drug” (see Appendix I) is taken directly from a textbook chapter about “Drug Discovery.”³ This venue was intended for audiences consisting of both pharmacologists and medicinal chemists primarily at the early stages of their careers, and so it became necessary to devise a working definition within that particular context that was also applicable to the chapter’s educational thrust. However, the tutorial discourse leading to the final, rather succinctly stated singular definition, demonstrates how related knowledge can also allow a given term to remain dynamic and thus provide a bridge to whatever other context might be better served across various disciplines having individuals with differing technical perspectives. Finally, this simple example also demonstrates how quickly tutorial material can expand when multiple perspectives are considered for even a very basic term. Although worthwhile for the context of that application within a basic educational text’s chapter entitled “Drug Discovery,” this same level of information would be unwieldy for a list of terms having more than 200 entries. Thus, in the end, revision of this project’s definitions became a careful and judicious exercise of delicately balancing useful tutorial comments with the concise definitions initially laid out by the Founding Working Party for each term. Structural examples were retained or added when relevant.

Submission and Present Intent

Since several years elapsed during *beta*-testing, it became necessary to form a second team to accomplish the last steps leading to the final release of this project’s hybridized product to the public. A new team (labeled “Submission Team” or ST in the authors list) was assembled to polish the revisions and to provide a final review of the definitions while assessing a term’s contributions toward enhancing knowledge in general and improving specific communications among practitioners having various levels of cross-technical savvy. Thus, a mix of early-career, mid-career, and seasoned investigators were recruited within several different disciplines and employment sectors. After completing the refinement of the glossary’s terms, its name was also adjusted to reflect how the final product is now being delivered.

For the reasons discussed above, the product has been slanted toward bridging medicinal chemistry perspectives with those of various other disciplines, while focusing on their common bond as practitioners of

collaborative drug discovery. During this final period, the value of this particular slant has been further underscored by advances in public domain scientific resources, e.g., databases and knowledge bases that are readily accessible via user-oriented interfaces. Typically, these sources provide well-referenced, high-quality information, such as historical notes and concise definitions for terms and acronyms. Likewise, they often recognize the importance of either alerting readers to cases where a given entry may be used differently by different disciplines or actually listing these multiple definitions. However, even when noting such distinctions, these sources may not be as useful for bridging the perspectives different experts bring to the overarching arena or enterprise. This has become critical for interdisciplinary drug discovery discussions, allowing them to be most effective, and ultimately for drug discovery practice to be most fruitful.

In summary, the definitions provided here are not intended to mandate how a given term should always be used by a given discipline, but rather are suggestions for how they might be deployed as a starting point to enhance interdisciplinary communications within the context of drug discovery. Similarly, our use of expressions and symbols, such as percent (%) are not intended as a mandate for how they must be correctly conveyed across different languages. Notably, we have adopted the % format prescribed by the International System of Units (ISO 31-0 standard) and IUPAC where there is a space between the number and the % sign, while the style in English typically deploys this expression without a space. As a closing example, practitioners should be able to use the definition provided here for "Glucuronic Acid Conjugation (Glucuronidation)" as a starting point for a joint discussion. Then, from the accompanying tutorial and the related, cross-referenced definitions/tutorials, all parties should gain a general background/base knowledge level that will allow them to better appreciate the following viewpoints: (1) The *medicinal chemist's* emphasis on the key role of the requisite "nucleophilic functional group" present on the substrate's chemical structure and how it might be masked by incorporating steric features so as to attenuate this particular biotransformation; (2) The *bioanalytical/drug metabolism chemist/biologist's* suggestion about the feasibility for devising an assay to assess such a series of sterically hindered analogs by using LC-MS/MS to detect and sort the substrates and potential glucuronide metabolites from the biological matrix while relying on the glucuronide's distinctive "increases in molecular mass of 175"; and, (3) The *biochemist's/pharmacologist's* comments about the prediction accuracy ("probability") from various *in vitro* and *in vivo* studies, accompanied by a

warning that, while many mammals might be utilized as a model for the latter studies, there would be “one notable exception in that the deployment of cats to monitor glucuronidation would not be recommended when trying to extrapolate the data to human behavior.” The corresponding author looks forward to additional critique of these terms from readers engaged in any aspect of the overall process of drug discovery, and in this same regard, remains prepared to further clarify or evolve their utility as may be helpful for all such disciplines. The author is especially receptive to comments that pertain to the two, related terms noted in the following section and as thoroughly discussed in Appendix II, because a controversy often exists regarding the continued use of these particular terms, even among practitioners within the same discipline.

An Ongoing Terminology Controversy

The discourse covered in Appendix II provides historical background and the differing views on the continued use of the terms “Phase 1” and “Phase 2” in the context of drug metabolism. **None of the definitions provided within the appendix should be taken as the final IUPAC recommendations for this glossary**, but rather as the basis for further consideration and comment related to how these particular terms might best be deployed in the future. Views on this matter range from: (i) “not to be used at all”; (ii) and “to be replaced by less sequential versions, such as Group 1 and Group 2 biotransformations”; to (iii) “Use, as presently still being utilized by many”; and (iv) “Use an even more expanded version” that, although more complex, might become less controversial while potentially having broader appeal for a much wider range of drug metabolism practitioners beyond those involved in drug discovery, e.g., environmentalists and system biology investigators in general.

DEFINITIONS WITH STRUCTURAL EXAMPLES AND TUTORIALS

1. Absorption

Passage of a xenobiotic through physiological membranes, leading to its uptake into the blood circulation.

Note 1: Oral (PO) and intraperitoneal (IP) administrations of pharmaceutical agents initially deliver drugs to the hepatoportal circulation that flows

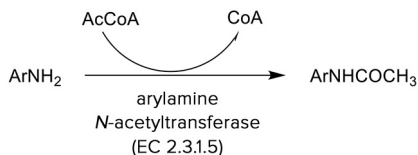
to the liver. Inhaled administration initially delivers drugs to the bronchopulmonary circulation's venous return to the heart. Sublingual (buccal), rectal (intrathecal), topical, subcutaneous (SC), and intramuscular (IM) administrations initially deliver drugs to the peripheral circulation's venous return. Intracerebral administration delivers drugs to the central circulation's venous return. Intravenous (IV) and intra-arterial (IA) administrations do not require an absorption step while directly delivering drugs to the venous return and arterial blood supply, respectively. Administration via the lymph system may allow for localized permeation of tissues with subsequent absorption following one of the above patterns and eventually adding to the venous blood flow.

Note 2: Pharmacokinetic analyses (PK studies) typically obtain samples from the peripheral circulation for the quantification of a drug in order to assess absorption and bioavailability according to various PK models that can accommodate different routes of administration.

See: Permeation/Permeability, Passive Transport, Active Transport, ADME, First-Pass Effect, Extravascular Dosing, Random walk, Pharmacokinetics, and Bioavailability.⁴

2. Acetylation

Conjugation of a xenobiotic's amino, hydroxy, or sulfanyl group with an acetyl moiety donated by acetylcoenzyme A and mediated by an acyltransferase (EC 2.3.1). Primary aromatic amines are the principal substrates (as exemplified below), with sulfonamides being both historically significant⁵ and still important as a major drug class today. The process is also applicable to various other nitrogen compounds, including endogenous amino acids, which become important for the regulation of proteins, and to drugs having hydrazine functional groups. It is a common xenobiotic biotransformation across numerous species, although dogs represent an exception.



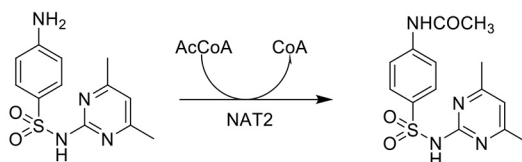
Note 1: Unlike in chemical terminology, in the context of xenobiotic metabolism, the terms "acetylation" and "acylation" characterize two

distinctly different types of biotransformations. Acetylation is closest to the chemical classification system, in that the substrate's (starting material's) nucleophilic center is acylated.

See: Acetylation Phenotype, *N*-Acetylation, *N*-Acetyltransferases and Acylation.⁶

3. Acetylation Phenotype

Different levels of *N*-acetyltransferase (NAT1 and NAT2) activities that lead to individual variation in acetylation rates. Common drug therapies that can be impacted include the half-life for procainamide⁷ and for the sulfonamide antibiotics, wherein the acetylation rate of sulfadimidine (shown below) is often deployed as a standard during phenotyping studies.⁸



Note 1: Variations in NAT2 were one of the first known drug metabolism-related pharmacogenetic traits, namely polymorphic “fast” and “slow” acetylators. About 30 to 45 % of Caucasian, 80 to 90 % of East Asian, and 100 % of Inuit, Eskimo, and Aleut populations are “fast” acetylators.

Note 2: The “fast” NAT polymorphism has been linked with an increased risk for bladder, colorectal, and other cancers associated with certain aromatic amines, which can be bioactivated into carcinogens.

See: Acetylation, *N*-Acetylation, *N*-Acetyltransferases; Extensive (or Rapid) Metabolizer, Poor (or Slow) Metabolizer, Slow (or Poor) Metabolizer, and Phenotype/Phenotyping.⁶

4. Active Metabolite

A metabolic reaction product that exhibits biological activity locally or at more distal sites upon subsequent distribution within an *in vivo* setting.

Note 1: The profile of activity may be the same as, or different from, the parent molecule, including especially the possibility for producing toxicity

at off-target sites. "Whether a metabolite is more or less pharmacologically active than its parent drug depends on the specific compound being studied, the biological properties measured, and the sensitivity of individual tissues or organs."⁹

Note 2: Prodrugs typically rely on bioactivation to produce an active metabolite in order to obtain the desired biological effect. In this case, it is also acceptable to refer to the release or generation of the active form of the drug (aka parent drug) from its inactive precursor or masked prodrug form.

See: Bioactivation, Reactive Metabolite, and Prodrug.

5. Active Transport

The passage of a substrate across a biological membrane by a process that requires energy.

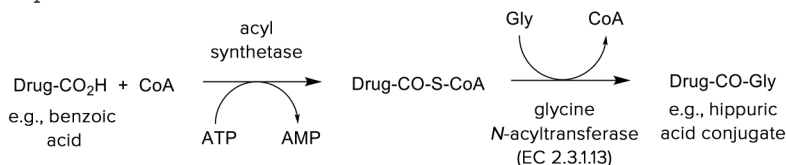
Note 1: Substrates can be moved from sites having a low concentration to sites having higher concentrations, but such gradients are not required and may or may not be important for the distribution and/or metabolism of a given xenobiotic.

Note 2: Several transporter molecules play critical roles during drug metabolism because they influence the uptake or export of xenobiotic compounds by cells possessing various drug metabolism enzymes. For example, within hepatocytes, the Substrate Carrier superfamily of proteins (SLC) are key basolateral (sinusoidal blood) uptake transporters that can serve to prolong the exposure of a given xenobiotic to the enriched array of metabolic possibilities present in the liver. Alternatively, the ATP-Binding Cassette superfamily of proteins (ABC), especially the Multidrug Resistance Protein or *P*-glycoprotein (MDR or Pgp), and the Multiresistance Protein (MRP) subfamilies, represent key basolateral and canalicular (bile) export transporters that can serve to diminish the liver's metabolism of xenobiotics.

See: ADME, Passive Transport, Membrane Permeability, Phases of Drug Metabolism, Transporters, *P*-Glycoprotein (Pgp), Organic Anion Transporters (OATs), Organic Cation Transporters (OCTs), and Random Walk.¹⁰

6. Acylation

Activation of a xenobiotic carboxylic acid's acyl group by attachment to coenzyme A followed by an acyltransferase-mediated (EC 2.3.1) conjugation with an endogenous amine to form an amide. Glycine (Gly) is the most commonly used amino acid. The resulting Gly conjugates are called "hippurates,"¹¹ borrowing historically from this pathway's initial characterization involving the metabolism of benzoic acid in 1842, notably credited as the first biotransformation reaction to be discovered.¹² It is exemplified below:



Note 1: Although the carbonyl moiety undergoes an initial activation step analogous to a chemical synthetic manipulation during a typical acylation procedure, this biotransformation process is notably different from the chemical classification of such reactions because the substrate (starting material) instead undergoes what would be termed an "amidation reaction," while it is the endogenous partner (reagent) that actually becomes acylated. Alternatively, by definition, biochemical acetylation reactions acylate xenobiotic substrates that have a nucleophilic heteroatom moiety.

Note 2: Glutamine is also commonly used as the endogenous amino acid and is the more predominant partner for xenobiotic substrates having an aryl-acetic acid moiety. Taurine and ornithine can also serve as partners, but are less frequently used.

See: Acyltransferase, Acetylation, Amino Acid Conjugation, Glutamine Conjugation, Glycine Conjugation, and Hippurate.¹³

7. Acyltransferase

The broad family of enzymes (EC 2.3.1) that transfer various acyl moieties, other than amino-acyl functionality, from acylated coenzyme A to substrates having receptive heteroatoms, such as O, S, and especially N, including for the latter the endogenous amino acid partners incorporated during the conjugation of xenobiotic acids.

Note 1: For drug metabolism, the most significant members include glycine *N*-acyltransferase (EC 2.3.1.13), depicted above, and the two arylamine *N*-acetyltransferase (EC 2.3.1.5) genetic polymorphs, NAT1 and NAT2.

See: Acylation, Acetylation, Acetylation Phenotype, *N*-Acetylation, and *N*-Acetyltransferases (NATs).^{6,13}

8. ADME; ADMET

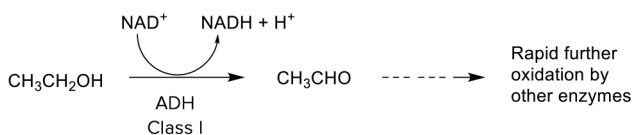
Acronym for the series of absorption, distribution, metabolism, and excretion processes that can occur when a living system is exposed to a xenobiotic, the most common context being the delivery of a drug to humans and *in vivo* animal models. The extended acronym ADMET is also often used when toxicity is included as an additional parameter within the overall profile conveyed by this term.

Note 1: Because they often become hurdles to overcome during drug development, ADME and ADMET processes are typically characterized and potentially optimized among efficacious analogs during the early stages of drug discovery by deploying *in vitro* models and *in vivo* PK studies that attempt to predict such behaviors within the clinic (e.g., see reference 14).

See: Absorption, Distribution, Metabolism, Excretion, Membrane Permeation, Phases of Drug Metabolism, Random Walk, DMPK, Pharmacokinetics (PK), and Toxicity.^{3,15}

9. Alcohol Dehydrogenase (ADH)

A family of cytosolic enzymes (EC 1.1.1.1) existing in multiple dimer forms having five distinct Classes (I-V). Each Class oxidizes various aliphatic alcohols, typically with a preference for primary over secondary and with specific selectivities for differing chain lengths. ADH is important in the processing of the retinoids, while Class III readily oxidizes formaldehyde to formic acid, so as to avoid toxicity.



Note 1: The Class I forms are among the most relevant for drug metabolism. They are expressed at high levels in the liver and stomach wall as three subunits (α , β and γ), which can form multiple dimers. They prefer shorter-chain alcohols, as shown above for a commonly ingested xenobiotic.

Note 2: Overall these enzymes are zinc-containing dimeric proteins having two 40 kDa subunits that utilize the oxidized form of nicotinamide adenine dinucleotide (NAD^+) as a cofactor to capture a hydride ion. In humans, the subunits are encoded by seven gene loci for which some transcribe to multiple allelic variants. All subunits can combine as homodimers and a smaller set can form heterodimers. They are divided into five major classes: Class I, as described above, is encoded by ADH1, 2, and 3; Class II is encoded by ADH4 and prefers longer aliphatic alcohols and aralkyl alcohols as substrates; Class III, encoded by ADH5, has been shown to be identical to the ubiquitous formaldehyde dehydrogenase (EC 1.2.1.46) that readily oxidizes formaldehyde, as well as longer-chain (aliphatic and aralkyl alcohols, particularly those with five carbons or more; Class IV, encoded by ADH7; and Class V, which is encoded by ADH6 and has representation in the upper GI tract for oxidizing medium-chain alcohols, with a particularly high capacity to oxidize retinol (vitamin A_1).

See: Atypical Alcohol Dehydrogenase.¹⁶

10. Aldehyde Dehydrogenase (NAD) (ALDH)

A family of cytosolic and membrane-bound enzymes (EC 1.2.1.3) existing in multiple dimer and tetramer forms and having three distinct Classes (I-III). A wide range of aldehydes are substrates for these enzymes, which also play a role in retinoid processing.



Note 1: The Class 1 and 2 forms are generally the more important for xenobiotic metabolism. They are found in many tissues of the body and have high concentrations in the liver. They can oxidize aldehydes, having a range of chain lengths and, as exemplified above, are important in detoxifying the acetaldehyde produced after the consumption of alcohol. As noted above, formaldehyde dehydrogenase does not belong to the ALDH family, but is instead a Class III ADH enzyme.

Note 2: In general, these enzymes' active sites utilize distinct amino acid residues to bind substrates, with magnesium sometimes also playing a role during the catalytic process. Oxidized nicotinamide adenine dinucleotide (NAD⁺) functions as a cofactor to capture a hydride ion. All three Classes have constitutive and inducible forms: Class 1 with low K_m is cytosolic, Class 2 with low K_m is mitochondrial, while Class 3 can have high K_m when expressed in the stomach, the cornea, and in certain tumors. The first two Classes, ALDH1 and ALDH2, are tetrameric enzymes having 54 kDa subunits.

See: Oxidation/Oxidase/Oxidoreductase, Aldehyde Dehydrogenase Polymorphism or Deficiency, Disulfiram Reaction, Alcohol Dehydrogenase, and Atypical Alcohol Dehydrogenase.¹⁷

11. Aldehyde Dehydrogenase Polymorphism or Deficiency

A genetic polymorphism that leads to an inactive allelic variant of ALDH termed ALDH2*2. This phenotype is present in ca. 30 to 50 % of various Asian populations around the Pacific Rim and affects their ability to metabolize alcohol.

Note 1: When coupled with the ALDH2*2 phenotype, the initial production of acetaldehyde from ethanol is no longer subsequently oxidized to acetic acid at a pace to prevent accumulation of this toxic electrophile notorious for its chemical reactions with amines to form Schiff bases. Its bioaccumulation leads to the classic "flushing" syndrome and hangover effects that can then occur at even lower amounts of consumption for this population when drinking alcohol.

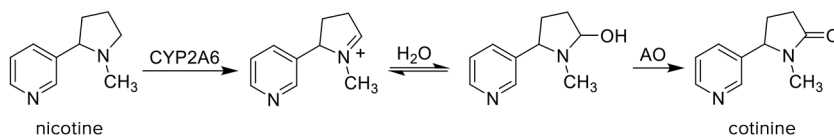
Note 2: Small-molecule inhibitors of ALDH2 can cause this same effect and are deployed to treat alcoholism because they result in an unpleasant drinking experience. Disulfiram was the first of such agents, and this drug-induced metabolic effect is thus called a "disulfiram-like reaction."

See: Aldehyde Dehydrogenase, Alcohol Dehydrogenase, Atypical Alcohol Dehydrogenase, and Disulfiram Reaction.¹⁸

12. Aldehyde Oxidase (AO)

One of two molybdenum-containing enzymes, aldehyde oxidase (AO; EC 1.2.3.1) plays an important role in xenobiotic metabolism, namely the oxidation of carbon atoms within nitrogen-containing heterocyclic

systems.^{19,20} As its name implies, AO can also oxidize certain aldehydes to carboxylic acids. The example below shows AO performing the second step in the oxidation of the common xenobiotic present in cigarettes.²¹ The iminium species is thought to be the substrate for AO.



Note 1: AO is expressed primarily in the liver, where it can play a role in ethanol metabolism and have an influence upon alcohol-induced liver damage. However, xenobiotic aromatic aldehydes are markedly preferred over aliphatic aldehydes. Endogenously, a number of important substrates include homovanillyl-aldehyde and 5-hydroxyindol-3-ylethanal, derived from cytochrome *P*-450-mediated catabolism of dopamine and serotonin, respectively, and catabolism of retinal as the activated form of retinol (vitamin A_1).

Note 2: Working in concert with the CYP enzymes, which generally prefer to oxidize electron-rich carbon atoms, AO prefers carbon atoms with lowered electron density. For example, naphthalene is a good substrate for CYPs and a poor substrate for AO, quinazoline is a substrate for both, and pteridine is a good substrate for AO while a poor substrate for the CYP enzymes.

Note 3: Further demonstrating the importance of AO's role in oxidizing xenobiotic heterocycles, some medicinal chemistry efforts have been specifically directed toward avoiding this type of biotransformation (e.g., see reference 22).

See: Alcohol Dehydrogenase (ADH), Aldehyde Dehydrogenase (ALDH), and Xanthine Dehydrogenase/Xanthine Oxidase (XD/XO).²³

13. Allometric Scaling

Extrapolation of xenobiotic activities (including drug metabolism events and PK profiles) between different species as a function of body weight or surface area. Assumes that physiologic variables, such as metabolic processing and clearance, are related to body weight or surface area according to the following general allometric equation:

$$y = aW^b$$

wherein y represents the physiological parameter, W represents the weight or surface area, and a and b represent allometric constants expressed as a coefficient and as an exponent, respectively. The logarithmic transformation of this relationship is linear.

See: PBPK (Physiologically Based Pharmacokinetic Model) and Pharmacokinetics (PK).²⁴⁻²⁶

14. Allosteric Regulation

A biochemical process wherein the function of a protein's receptor or enzyme active site can be influenced by interaction with another compound (ligand) whose association occurs at a location different from the receptor or active site. The influence can be either positive, so as to enhance functional activity, or inhibitory. Today, it is recognized that an ensemble of proteins can often interact in this manner, expanding the context for the word "ligand" and for the overall process, so as to include cell signaling systems. In general, this type of regulation has not previously appeared to be significant for the biotransformation of xenobiotics. However, studies using the wider dimensions of today's definition for this type of regulation remain ongoing for the area of drug metabolism and are key to understanding signal transduction.

Note 1: Cofactors and prosthetic groups needed to form a functional holoenzyme are not regarded as components operating by a process of allosteric regulation.

Note 2: In some systems, a receptor's ligand or an enzyme's substrate is also the ligand that can interact with a distal, allosteric site.

See: Enzyme, Apoenzyme, Cofactor, Holoenzyme, Ligand, Nonlinear Kinetics, Prosthetic Group, Substrate/Substrate Specificity, Substrate Inhibition/Product Inhibition, and Uncompetitive Inhibition.²⁷

15. Amino Acid Conjugation

Biotransformation wherein a xenobiotic's carboxylic acid moiety forms an amide bond with the amino group of an endogenous amino acid, usually glycine or glutamine, and sometimes taurine, via an acylation reaction accomplished by an acyltransferase enzyme.

Note 1: In this distinctive mechanism, the xenobiotic's carboxylic acid group is first activated by coupling with coenzyme A (CoA), so as to produce an acyl-CoA thioester that then reacts with the amino group of the endogenous amino acid.

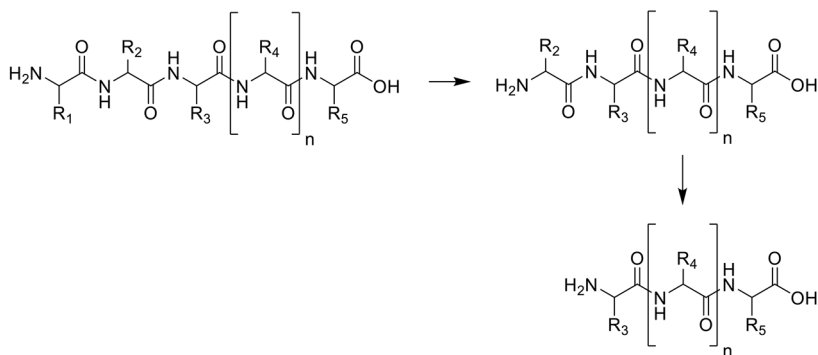
Note 2: Xenobiotics or initial metabolites having an aromatic hydroxylamine are also considered to be undergoing "amino acid conjugation," although their activation engages aminoacyl-tRNA synthetase and typically uses serine or proline for the amino acid partner.

Note 3: Alternatively, the conjugation of xenobiotics having electrophilic centers by the tripeptide glutathione is classified as a separate pathway for metabolism, because its chemistry and associated enzymes are distinctly different.

See: Acylation, Acyltransferase, Hippurate, Glutathione Conjugation, and Glycine Conjugation.¹³

16. Aminopeptidases

An important class within the protease family (EC 3.4), many of which are Zinc metalloenzymes, aminopeptidases readily catalyze amide bond hydrolysis at the amino-terminus of a protein or peptide, sometimes in rapid succession along the peptide's backbone. The latter is depicted generally below. Because they play key roles in the processing and catabolism of many endogenous proteins, avoiding such hydrolyses becomes a critical ADME hurdle for short peptide and peptidomimetic drug candidates.



Note 1: There are several types of aminopeptidases and various biochemical formats for their assembly, the majority having widespread distributions

within the *in vivo* setting. Cell-based assays can display considerable activity as well.

Note 2: *N*-substitution, inversion of stereochemistry, or other peptidomimetic alterations made near the amino-terminus can remedy this type of ADME-related bioinactivation hurdle. Alternatively, simple prodrug approaches that attempt to use the amino-terminus are generally complicated in this case, because the rapid rate of deactivation after bioactivation to an active amine can still remain problematic.

Note 3: When an initial peptide lead compound is converted to a peptidomimetic structure and finally to a small-molecule drug scaffold (with little peptide character remaining), the overall metabolic fate of the series of analogs can generally be expected to follow the pattern shown below.

Metabolism by	Peptide Drug →	Peptidomimetic Drug →	Small-Molecule Drug
Peptidases:	100%	Mix of Both	0%
Phase1 and/or 2 Processes:	0%	Mix of Both	100%

See: Carboxypeptidases.²⁸⁻³⁰

17. Anabolism

Composite of biochemical pathways that construct or liberate endogenous molecules intended for distinct functions.

Note 1: This term is not used within the context of xenobiotic metabolism, even though many enzymes important for this process also have specific roles in various anabolic pathways. Likewise, the metabolic conjugation of a xenobiotic or the liberation of an active metabolite from a xenobiotic after a hydrolytic biotransformation are not regarded as anabolic processes.

See: Catabolism.³¹

18. Antibody-Drug Conjugate (ADC)

The chemical connection (conjugation) of a monoclonal antibody (mAb) with a small-molecule drug, wherein: the mAb serves to selectively deliver the conjugate to a target cell via its specific recognition of a protein expressed on the target cell's surface; the chemical connection uses a prodrug strategy

to release the drug from the mAb, typically also having chemical linkages to prevent the prodrug's metabolically labile connection from becoming sterically hindered by the mAb; and, upon its release, the small molecule exhibits a therapeutic action. At least one such connection for a single mAb biomolecule is present and, in general, several can occur, so that several molecules of the drug may be liberated. The ADCs are becoming very useful in the treatment of cancers, where the latter often over-express distinct cell surface proteins and where the highly toxic nature of small-molecule, natural product toxins can then remain masked until their selective release at the cancer cells.

See: Prodrugs.^{32,33}

19. Apoenzyme

The protein portion of an enzyme complex without any cofactors or prosthetic groups that may be required for activity. Many of the phase 1 and all of the phase 2 drug metabolizing enzymes require such complexes in order to catalyze their biotransformations.

Note 1: Although they may be able to associate with substrates, apoenzymes are inactive until they combine with their cofactors and prosthetic groups to form an intact complex called a holoenzyme.

Note 2: Cofactors are small organic or inorganic molecules that are typically easy to remove by laboratory manipulations. Prosthetic groups are tightly bound partners that are often difficult to remove, such as the heme molecule present in a CYP complex.

See: Cofactor, Prosthetic Group, and Holoenzyme.³⁴

20. Aromatic Hydrocarbon Receptor (AHR)

Cytosolic transcription factor component having high affinity for polycyclic aryl hydrocarbons that activate the signaling pathway to eventually upregulate genes coding for several cytochrome *P*-450 drug metabolizing enzymes.

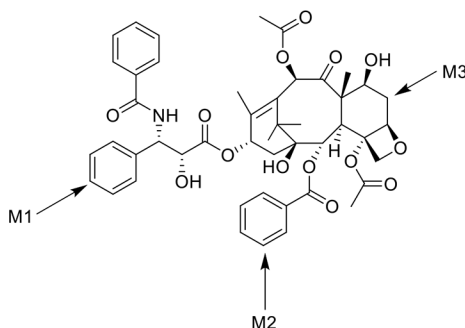
Note 1: Upon binding with polycyclic aryl hydrocarbons, AHR loses its association with heat shock protein (hsp) 90 and forms a heterodimer

complex with a nuclear translocator protein (ARNT). Serving as a transcription factor, this complex moves to nuclear DNA and binds with the xenobiotic response element (XRE) sequences that upregulate genes encoding for CYP 1A1, 1A2 and 1B1.

See: Peroxisome Proliferator Activator Receptor (PPAR), Pregnane X Receptor (PXR), Glucocorticoid Responsive Element (GRE), and Xenobiotic Responsive Element (XRE).³⁵

21. Aromatic Hydroxylation

Frequent biotransformation mediated by various members of the CYP family (see specific nomenclature separate from the EC system) wherein a hydroxy group replaces a hydrogen atom (see **Note 2** below), typically at the most electron-rich site within an aromatic system, while also accounting for steric accessibility.



Note 1: The example above depicts some of the CYP-mediated metabolites observed for paclitaxel in rodents and humans.³⁶ In rodents, aromatic hydroxylation is predominately at M1, followed by M2, with no observation of an aliphatic oxidation at M3. In humans, aliphatic hydroxylation by CYP 2C8 at M3 becomes most prevalent, followed by CYP 3A4 aromatic hydroxylation at M1. The four esters are remarkably stable due to the large steric bulk of the central scaffold.

Note 2: The resulting aryl-hydroxy groups often undergo subsequent biotransformation to form either the glucuronide or sulfate conjugates, which are good candidates for some of the excretory pathways. This sequence follows the classical phase 1, phase 2 pattern of biotransformation (see discussion in the Introduction section).

Note 3: After CYP-mediated insertion of an oxygen atom, re-aromatization and formation of the hydroxy group is thought to occur via an intermediate stabilized in complexation with the CYP's prosthetic heme group, or as a bound epoxide, either of which can then undergo a hydride shift called the "NIH Shift." Thus, mechanistically, in addition to replacing a hydrogen atom, halide atoms and other groups capable of undergoing such a shift can also serve as substrates for an aromatic hydroxylation reaction. Although their electronegative character will diminish the overall reactivity of the aryl-system for these types of biotransformations in general, this effect has been observed experimentally, initially by the U.S. National Institutes of Health (NIH) laboratories, which gives the effect its name.

Note 4: Reactive epoxides failing to collapse to the re-aromatized hydroxyl-containing metabolites can become a source of localized toxicity, such cases then representing one example of an enzyme "suicide inhibitor." Moderately stable epoxides can sometimes bypass re-aromatization and dissociate from the enzyme complex, so as to become active metabolites that undergo further distribution and eventually cause distal toxicity. Epoxide hydrolase and glutathione-S-transferase (GST) serve to intercept these types of active metabolites and to detoxify them by forming non-aromatic diols and glutathione conjugates, respectively. Benzo(a)pyrene is a classic example of an exogenous material, found in soot and charcoal, that forms a moderately reactive epoxide metabolite able to evade epoxide hydrolase and GST to ultimately become a carcinogen by reacting with RNA via covalent bond formation at the C-2-amino group of guanosine. Highly stable epoxides can dissociate from the enzyme complex and potentially be eliminated without further metabolic processing or incidents of toxicity.

See: NIH Shift, Phases of Drug Metabolism, Glucuronide Conjugation, Sulfate Conjugation, Reactive Metabolite, Active Metabolite, Epoxide Hydrolase, and Glutathione Conjugation.³⁷

22. Atypical Alcohol Dehydrogenase

A member of the ADH2 isozymes within Class I of the alcohol dehydrogenase family (EC 1.1.1.1) for which the human phenotype results in the rapid oxidative conversion of ethanol to acetaldehyde.

Note 1: This phenotype is present in ca. 90 % of the Pacific Rim Asian population, whereas it is either not present or is much less prevalent among

different Caucasian populations (below 5 % in Americans of Caucasian descent and ca. 10 to 20 % among various European populations).

Note 2: When this population also has an aldehyde dehydrogenase genetic polymorphism (ALDH2*2), such that subsequent oxidation to acetic acid is slowed, individuals are highly susceptible to the flushing syndrome and hangover effects associated with the consumption of alcohol.

See: Alcohol Dehydrogenase (ADH), Aldehyde Dehydrogenase (ALDH), and Aldehyde Dehydrogenase Polymorphism.¹⁶⁻¹⁸

23. AUC (Area Under the Curve)

Common acronym for the area under the curve, *A*. Applicable to xenobiotic (drug) and metabolite pharmacokinetic (PK) studies, it is defined to be the integral of the given analyte's (most often drug's) concentration measurement in a given biological media (typically plasma, serum, whole blood or urine) with respect to time. This area can be derived from the formula:

$$A = \int_0^{\infty} C \, dt$$

Note 1: Analyte concentration *C* is typically measured and quantified by either HPLC (UV/Visible wavelength) or tandem mass spectrometry (LC-MS/MS).

Note 2: AUC is commonly used in the measurement of bioavailability and to determine if a drug's presence follows linear kinetics in a given media or compartment with time.

See: Plasma Concentration, Serum Concentration, Whole Blood Concentration, Pharmacokinetics, Linear Kinetics, Nonlinear Kinetics, Bioavailability, Absolute Bioavailability, and Relative Bioavailability.³⁸⁻⁴⁰

24. Autoinduction

Substrate mediated induction (up-regulation) of an enzyme that is involved with the metabolism of that substrate. In its most common usage, this

definition is taken to mean that more of the enzyme is produced due to enhanced genetic signaling for its biosynthesis (anabolism). In a broader sense, however, the same physiological observation can occur if less of the enzyme is degraded due to diminished genetic signaling for its catabolic enzyme or enhanced genetic signaling of an enzyme that degrades the catabolic enzyme. These latter possibilities would need to be specified while using this term if they were suspected of being operative. Even though the endpoints are essentially the same, these alternate possibilities represent distinctly different drug design targets for conducting structural modification of their associated ligands.

Note 1: When it occurs for xenobiotics or their metabolites undergoing subsequent biotransformations, it is generally observed after repeated exposure to the xenobiotic. While it can be applicable to a given metabolite, it most typically pertains to the parent xenobiotic compound and leads to a nonlinear PK profile, characterized by its lower-than-expected concentration *in vivo*. For certain cases, it may be able to be detected at the cell-culture level when studies have been designed to assess this possibility.⁴¹

See: Induction, Autoinhibition.

25. Autoinhibition

Both historically and today, substrate-mediated inhibition of an enzyme that is involved with the metabolism of that substrate. Unlike receptors, which can undergo gene-signaled down-regulation upon over-stimulation by an agonist ligand, enzymes are generally not subject to such negative feedback control upon exposure to their substrate ligands and, instead, may undergo up-regulation (autoinduction). Also see **Note 2** below for another, more recent usage of this term.

Note 1: When autoinhibition occurs, it is most often due to product inhibition from the substrate's biotransformation, such that it can be observed upon the first, as well as on repeated, exposures of the xenobiotic. While it can be applicable to a given metabolite, it most typically pertains to the parent xenobiotic compound and leads to a nonlinear PK profile characterized by a higher-than-expected concentration *in vivo*.

Note 2: A related usage of this term is now being applied to enzyme systems that can equilibrate between functional conformations and non-functional

("autoinhibited") conformations, either as a single protein species acting independently of a substrate's presence, or in response to a substrate, such as by an allosteric interaction or as protein complexes of dimers, trimers, etc., wherein one or more of the members is non-functional and, in turn, modulates the activity of the overall complex (again either independently or in an interactive manner with a potential substrate). The multiple protein complex has become important for understanding the many systems wherein the genome produces one or more additional, but inactive, versions of a functional enzyme, sometimes by utilizing duplicate paralog genes that read to analogous proteins having altered enzymatic activity.⁴²

See: Inhibition, Autoinduction.

26. Bioactivation

The biotransformation of a xenobiotic to a metabolite that is more biologically active or is active in a manner different than that of the parent compound.

Note 1: Activity may be efficacious or toxic. For drugs, bioactivation is generally unwanted, since the metabolites typically interact with "off-target" sites that can lead to undesired side-effects and toxicity. Prodrugs are an exception, because they have been designed to rely upon a metabolic step in order to unmask a desired activity from the inactive parent molecule.

Note 2: Biotransformation to an active metabolite that is equivalent to that of the parent compound does not constitute bioactivation.

See: Active Metabolites and Prodrugs.³

27. Bioavailability (Absolute and Relative)

For xenobiotics in general (not having formal PK studies) and for dietary supplements, herbs, and other nutrients, bioavailability is defined as the fraction that is absorbed, i.e., the per cent of the mass unaccounted for at the site after an exposure. More specifically for drug administration, this is the fraction of the dose that reaches its site of action or that reaches a biological fluid from which the drug is presumed to gain access to its site of action. Generally depicted by the term "F," which ranges from 0 to 1, where unity represents 100 % bioavailability.

Note 1: Typically, the biological fluid from which drugs are presumed to gain access to their sites of action is the blood of the general circulation. For a drug taken orally (PO), examples of factors that can limit bioavailability include formulation issues, such as slow dissolution rate; drug stability, such as degradation under the acidic conditions of the stomach; metabolic transformation by the gut microflora, intestinal epithelial cells, or liver; efflux by transporters within epithelial cells, such as P-glycoprotein (Pgp); or direct biliary excretion.

Note 2: For determination of absolute bioavailability, F is measured experimentally during PK studies as the ratio of the AUC from plasma samples taken for a dose given by a non-intravenous route (e.g., PO), divided by those taken for a dose given intravenously, with the comparison normalized to account for different doses or varying weights of the subjects.

Note 3: Relative bioavailability compares the AUC between different formulations of a drug using the same route of administration, or between different routes of administration for the same drug formulation when neither route is an intravenous injection.

See: Absorption, Gut Microflora, P-Glycoprotein (Pgp), First Pass Effect, Pharmacokinetics, AUC, and Bioequivalence.³⁹

28. Bioequivalence

Different formulations or generic products for a given active agent are regarded as bioequivalent when the active agent is absorbed, metabolized, and excreted in approximately the same amount over approximately the same time period as the active agent in the original product.

Note 1: Studies directed toward demonstrating bioequivalence typically include *in vitro* dissolution testing and relative bioavailability after administration to humans, emphasizing blood concentration, how fast it is achieved, and how long it is sustained.

Note 2: For example, in the U.S., regulatory approval requires a generic to demonstrate a 90 % confidence interval for the ratio of the mean responses of its product to that of the original drug while remaining within limits of 80 % to 125 % for maximum achieved concentration ($C_{\max}$), the time it takes to reach $C_{\max}$ ($T_{\max}$), and the overall duration of a measurable concentration

(AUC profile). According to the Hatch-Waxman Act, when these conditions are met, a generic brand-name product may be able to take advantage of an abbreviated new drug application (ANDA) to obtain approval for marketing.

See: Absorption, Bioavailability, Pharmacokinetics, and AUC.^{43,44}

29. Bioinactivation

Biotransformation of a xenobiotic or an active metabolite to a metabolite that is significantly less active.

Note 1: Virtually all tissues possess at least some degree of inactivating enzymes, but the greatest xenobiotic metabolizing activities are present in the liver, gastrointestinal tract, kidneys, and lungs. Alternatively, metabolic activity that can afford protection from xenobiotics is not significantly enhanced in the blood-brain barrier (BBB), mammary glands, or placenta, although the latter does have some added capabilities.

Note 2: In general, bioinactivation is not a pre-programmed feature of a drug, but typically something to be avoided or attenuated during ADME optimization, so as to prolong the efficacious half-life of clinical candidate compounds. However, there is one strategy for drug design, namely that of soft drugs, that intentionally deploys bioinactivation mechanisms as the central theme of their design process.

See: Detoxification, Bioactivation, Active Metabolites, and Soft Drugs.^{3,45}

30. Biotransformation

Enzyme-mediated alteration of a xenobiotic or its metabolites by living systems or isolated enzyme preparations.

Note 1: By strict definition, the hydrolysis of an ester due to the basic nature of physiological pH (i.e., 7.4), or the acidic nature of the stomach or the lysosome within a cell, are not examples of a biotransformation. Instead, these are chemical events afforded by the specific environments present within various compartments of a living system.

See: Bioinactivation, Bioactivation, Active Metabolites, Prodrugs, and Soft Drugs.⁴⁶

31. Blood-Brain Barrier (BBB)

A highly selective barrier between the circulating blood and the brain's interstitial (extracellular) fluid.

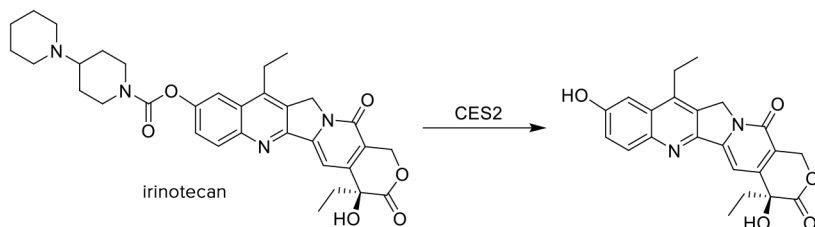
Note 1: Physically, the BBB is formed by tight junctions of the brain's endothelial cells arranged in numerous, partially overlapping zones contained in a highly anisotropic lipid bilayer that is additionally supported biochemically by astrocytes (star-shaped brain glial cells). The tight junctions are key for precluding the paracellular diffusion that is normally operative across other membrane structures. Likewise, brain endothelial cells have comparatively few pinocytotic vesicles, diminishing that mode of crossing membranes. The BBB allows the passage of water, oxygen and some other gasses, as well as lipid-soluble small molecules, by passive diffusion. Essential nutrients that are polar, such as glucose and amino acids, are afforded passage by specific, active transport systems. Similar to the gut epithelium, brain endothelial cells are enriched with certain metabolizing capabilities and express high levels of the efflux transporter *P*-Glycoprotein (Pgp), which is well suited for excluding lipophilic molecules across a wide range of structures. Thus, both polar and non-polar xenobiotics will have diminished distribution into the Central Nervous System (CNS). Large-molecule therapeutics are not able to traverse the BBB, and estimates suggest that less than 5 % of all small-molecule drugs may be able to do so.

Note 2: Drugs or prodrugs targeting the CNS after oral or peripheral administration are typically designed to have: (i) a structural scaffold modeled after a prototypical agent already known to traverse the BBB; (ii) Log*P* values optimized to be near 2.0 to 2.5, so as take advantage of potential lipoidal entry, while minimizing Pgp's affinity for the same physicochemical profile and also considering that Pgp inhibitors might be additionally deployed as adjunct therapeutic agents; (iii) an overall pharmacophore that can prompt efficacy while also being suitable for active transport; or (iv) further attachment via a stable or prodrug-type linker to a separate structural moiety that is a known substrate for an active transport system. Alternatively, drugs can be delivered directly to the brain in such a way that traversal of the BBB is not necessary. Given the invasive nature of the latter method, less frequent administration becomes imperative and prolonged dosage forms or surgical implants, such as drug-containing wafers, become important ADME-related elements during drug design.

See: Absorption, Passive Transport, Active Transport, Bioinactivation, P-Glycoprotein (Pgp), Bioactivation, and Prodrugs.⁴⁷⁻⁴⁹

32. Carboxyl Esterases (CESs)

An important class (EC 3.1.1) within the esterase family that can hydrolyze a range of xenobiotic ester and ester-like moieties. These enzymes are often exploited as a bioactivating process during the design of prodrugs. CESs are divided into five major groups (CES1-5) according to sequence homology to human CES1A1. Most members belong to either CES1 or CES2, with the former being further classified into eight subfamilies. CES1 prefers esters having small alcohol groups, while CES2 is more tolerant in that regard. An example for these preferences is shown below for the bioactivation of the prodrug irinotecan.



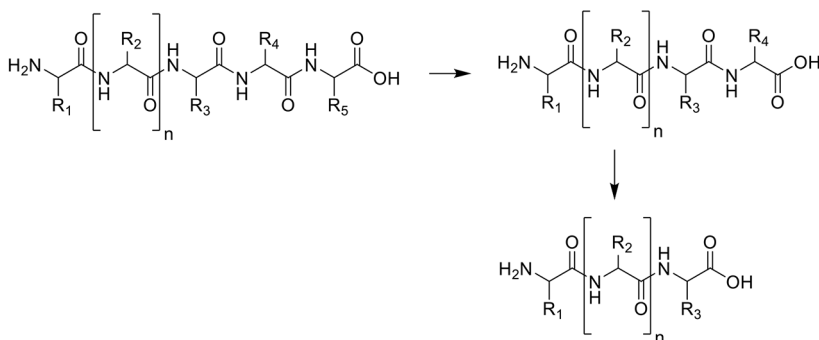
Note 1: The more relevant human CESs are about 60 kDa glycoproteins. While CES1 can exist in monomer, dimer, trimer, and hexamer arrangements, CES2 is typically only assembled as the monomer. CES1 and 2 are found in a wide variety of tissues, including serum, with high levels being present within the liver, where they are mostly in association with the endoplasmic reticulum, followed by lower amounts in lysosomes and the cytosol. As members of the broader class of hydrolases, however, the overall composite for this type of biotransformation can be considered as being ubiquitous throughout the body. Likewise, together the hydrolases constitute a very aggressive metabolic capability toward a wide range of ester-related groups when present in xenobiotics, unless the latter are sterically sheltered from such a barrage.

Note 2: As is the case for esterases in general, the catalytic triad consists of a Ser, Glu, and His imbedded at the bottom of a cleft that has a pocket on one side for the alcohol portion and one on the other side for the acyl moiety present in an ester or ester-like linkage.

See: Hydrolases, Prodrugs, and Bioactivation.^{50,51}

33. Carboxypeptidases

An important class (EC 3.4.16-3.4.18) within the protease family that readily catalyzes amide bond hydrolysis at the carboxy-terminus of a protein or peptide, sometimes in rapid succession along the peptide's backbone. The latter instance is depicted in a general way below. Because they play key roles in the processing and catabolism of many endogenous proteins, avoiding such hydrolyses becomes a critical ADME hurdle for short peptide and peptidomimetic drug candidates.



Note 1: There are several types of carboxypeptidases. The majority have widespread distributions within the *in vivo* setting, and even cell-based assays can display considerable activity. In addition to the standard peptidase nomenclature based upon reaction mechanism, they can be classified by their substrate preferences. Most important for xenobiotics are carboxypeptidase A and B, the former preferring terminal amino acids having neutral aromatic or aliphatic sidechains and the latter preferring basic amino acids, like Arg and Lys, that tend to be charged at physiological pH.

Note 2: Inversion of stereochemistry or other peptidomimetic alterations made near the carboxy-terminus often remedy this type of ADME-related bioinactivation hurdle. Alternatively, simple prodrug approaches are generally complicated in this case because the rapid rate of deactivation after bioactivation to an active acid can still remain problematic.

See: Peptidases/Proteases, Aminopeptidases, Peptidomimetic Drug Metabolism, and Prodrugs.^{51,52}

34. Catabolism

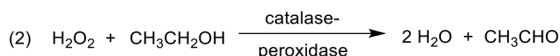
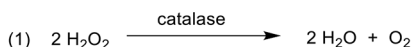
Composite of biochemical pathways that break down endogenous and specific xenobiotic molecules, such as food and vitamin components, to either release energy that can be captured for functional purposes or to provide building blocks for anabolism.

Note 1: This term can be applicable within the context of xenobiotic metabolism when the latter pertains to nutritional compounds, but is otherwise generally not used to describe xenobiotic biotransformations, even though many enzymes important for the latter also have specific roles in various catabolic pathways.

See: Anabolism.^{31,53}

35. Catalase and Catalase-Peroxidase

Catalase is a specific enzyme (EC 1.11.1.6) that converts hydrogen peroxide to water and molecular oxygen, for which its stoichiometry is shown in reaction (1). A closely related enzyme, catalase-peroxidase (EC1.11.1.21), further participates in the oxidation of certain xenobiotics and their metabolites, such as for ethanol, as shown in reaction (2).



Note 1: Regarded as a detoxifying enzyme, catalase prevents cell damage associated with reactive oxygen species (ROS) and oxidative stress in general. These enzymes are found in all living systems exposed to oxygen.^{54,55} In humans, it is present in all tissues, with particularly high levels being present in the liver. Its subcellular location is largely confined to organelles called peroxisomes.

Note 2: Catalase is a tetramer having four polypeptide chains of over 500 amino acids, each accompanied by a prosthetic heme-porphyrin moiety that plays a role during reaction with hydrogen peroxide.

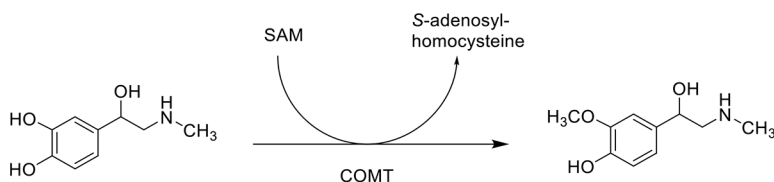
Note 3: Several genetic variants have been identified, some of which can give rise to catalase deficiency, a syndrome called "acatalasia" (homozygotes) or

"hypocatalasia" (heterozygotes). Although there may be some association with an increased likelihood of developing type 2 diabetes, these deficiencies do not otherwise appear to lead to any significant ill effects, perhaps owing to the major role that the peroxiredoxins play in also scavenging hydrogen peroxide in mammalian cells.

See: Alcohol Dehydrogenase (ADH), Aldehyde Dehydrogenase (ALDH) and Aldehyde Oxidase (AO), Reactive Oxygen Species.⁵⁶

36. Catechol-O-Methyl Transferase (COMT)

An enzyme (EC 2.1.1.6) that conjugates a methyl group to one of the hydroxyl moieties present in a catechol arrangement. *S*-adenosylmethionine (SAM) serves as a cofactor. While applicable xenobiotics are ready substrates, COMT plays a major role in the bioinactivation of the endogenous catecholamine neurotransmitters, wherein it demonstrates exquisite regiospecificity for the meta-hydroxy group of the parent compounds and their deaminated metabolites (shown below for epinephrine).⁵⁷



Note 1: Although low concentrations of extracellular enzyme have been noted, COMT is essentially located in the cytoplasm of all tissues, with the highest concentrations being found in the liver, followed by the kidney. As for other methyltransferases, COMT's active holoenzyme takes advantage of a complex with SAM imbedded deep within the active site to supply the methyl group.

Note 2: The human COMT gene possesses alleles coding for high and low activity forms (COMT^H and COMT^L), with the latter reflecting a valine to methionine mutation at position 158, (Val¹⁵⁸Met)rs4680, that reduces catabolic rate by greater than 4-fold. There is an even distribution of these allelic variants in the Caucasian population (25 % homozygous for high, 25 % for low, and 50 % heterozygous becoming intermediate metabolizers), while the African-American population has a higher frequency of the

COMT^H allele. High COMT can be associated with more difficult therapeutic management of Parkinson's disease (see Note 3 below).

Note 3: Levodopa is a catechol-containing prodrug used to treat Parkinson's disease by virtue of its passage into the CNS and subsequent bioactivation by dopa decarboxylase to dopamine, which cannot itself traverse the BBB. Because both levodopa can be metabolized and dopamine can be bioinactivated by COMT, an inhibitor of this enzyme (e.g., entacapone) is used as an adjunct agent during levodopa therapy, generally with an inhibitor of peripheral dopa decarboxylase (e.g., carbidopa) to prevent premature conversion to dopamine outside of the CNS, and sometimes with an inhibitor of monoamine oxidase B (e.g., selegiline) to additionally attenuate the bioinactivation of dopamine by deamination.⁵⁸

See: Methylation, Methyltransferases, Bioinactivation, Monoamine Oxidase (MAO), Deamination, Absorption, Blood-Brain Barrier (BBB), Prodrugs, and Bioactivation.

37. Clearance (CL)

The rate of elimination of a xenobiotic, normalized to its concentration in a given biological fluid, usually blood, serum, or plasma. CL has units of volume per time because it represents the volume of biological fluid, per unit of time, from which the xenobiotic would have to be completely removed in order to account for the observed elimination rate. The total systemic clearance (CL_{tot}) is the sum of the clearances of each organ serving to remove the xenobiotic from the biological fluid, e.g., blood. The equation below expresses this relationship for a drug that is cleared from the body by both the liver and kidneys.

$$CL_{tot} = CL_{hepatic} + CL_{renal}$$

Note 1: At steady-state concentrations, CL_{tot} will equal the dose rate (DR), such that it can be calculated as follows, where C_{ss} is the steady-state concentration of the drug:

$$CL_{tot} = DR / C_{ss}$$

See: Elimination, Excretion, Intrinsic Clearance, and Pharmacokinetics.³⁸⁻⁴⁰

38. Cocktail Study

Study of two or more simultaneously administered xenobiotics, either to assess their own metabolism, metabolism-related interactions or PK or ADMET properties.

Note 1: Multiple enzyme activities can be simultaneously evaluated *in vivo* through the combined administration of several selective enzyme probes, i.e., a metabolic or phenotypic probe cocktail study. Such studies can characterize the subject's metabolic phenotype.

Note 2: The metabolism of multiple drug candidates (drug cocktail) can be simultaneously assessed within a single animal, providing that cross-over metabolic induction or inhibition (drug-drug interactions) do not occur and that there are no cross-over interferences of the assay method. It is not uncommon for more than five "hit compounds" to be tested in this simultaneous manner when supporting large compound screening campaigns.

See: N-in-One Dosing, Drug-Drug Interactions, Induction, Inhibition, Competitive Inhibition, and Non-competitive Inhibition.⁵⁹⁻⁶⁰

39. Cofactor

A non-protein substance whose loosely bound association with an enzyme is required for the latter's function.

Note 1: Cofactors may be ions, such as those derived from various oxidation states of copper, iron, or zinc, or they may be small organic molecules, such as vitamins, NADH, or NADPH.

Note 2: Cofactors may initially facilitate or enhance an enzyme's ability to bind with its substrates, and may subsequently be required for the specific enzymatic reaction to occur.

See: Apoenzyme, Holoenzyme, and Prosthetic Group.^{34,61}

40. Compartment Model

Pharmacokinetic (PK) model where the body is represented by one or more inter-accessible compartments that a xenobiotic may enter. Absorption,

distribution, and excretion are defined by rate terms for the movement of the xenobiotic into, out of, or between different compartments, while metabolism is defined by a rate term for the biotransformation of the agent. When all of these terms follow linear kinetics, the compartment model for plasma, serum, or whole blood concentration (C) versus time predictions (t) can be expressed as a sum of exponential terms having the form shown below, where the A terms are coefficient constants and the k terms are linear kinetic rate constants.

$$C = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + \dots + A_n e^{-k_n t}$$

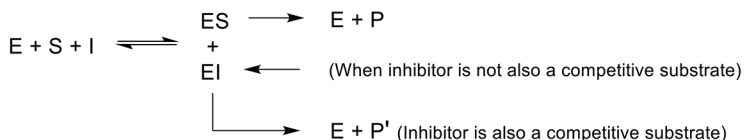
Note 1: Each compartment is taken to be internally homogeneous and well-mixed, thus representing a collection of fluids and tissues that have similar concentration versus time profiles. While inter-accessible, a compartment's components need not be directly interconnected, e.g., a two-compartment model categorized as all extracellular aqueous media versus all fat tissue would separately encompass all of the appropriate components for each of these two categories, regardless of their locations and lack of direct physical connections.

Note 2: The number of exponential terms for a linear kinetics model without absorption (intravascular administration) is equal to the number of compartments, while that requiring an absorption phase (extravascular administration) is equal to one more than the number of compartments.

See: Absorption, ADME, Clearance, Pharmacokinetics, PBPK Model, and Non-compartment Model.³⁸⁻⁴⁰

41. Competitive Inhibition

Reversible inhibition of an enzyme or transporter by a xenobiotic (inhibitor) that competes with the natural substrate, or with another intended substrate, for the enzyme's binding domain in a non-covalent manner. The interaction between a non-reversible enzyme (E), its intended substrate (S) leading to product (P), and an inhibitor (I), which itself may or may not serve as a secondary substrate leading to an alternative product (P'), can be depicted as follows:



Note 1: Depending upon conditions, many enzymes can also operate in reverse, i.e., $E + P \leftrightarrow EP \leftrightarrow E + S$. Likewise, in some cases the product can serve as an inhibitor whether it is a substrate or not. These processes will lead to non-Michaelis-Menten kinetics.

Note 2: Competitive inhibition is a mass-action phenomenon that can be overcome with a sufficiently high concentration of substrate. For enzymes that metabolize substrates in accordance with Michaelis-Menten kinetics, competitive inhibition will raise the enzyme's K_m , but will not alter its V_{max} .

See: Inhibition, Non-Competitive Inhibition, Uncompetitive Inhibition, Michaelis-Menten Kinetics.⁶²

42. Conjugate

Within the context of xenobiotic metabolism,⁶³ the metabolite resulting from a biotransformation that forms a covalent linkage with an endogenous reactant and a functional group present on a xenobiotic or its metabolite.

Note 1: This is different from the term "drug conjugate," or "DC," which derives from the practice of drug design and involves connecting an efficacious drug molecule to another molecule that can home in on (or preferentially distribute/concentrate in) a desired biological target (physiologic compartment). For example, one strategy is to link an anticancer agent with an antibody that can recognize proteins being over-expressed by a particular cancer cell, the resulting complex then called an "antibody-drug conjugate," or "ADC."⁶⁴ The DC/ADC strategies are currently so popular that several technical journals, emphasizing such subject matter, presently bear some aspect of this phraseology as part of their name.

Note 2: Though conjugates frequently possess diminished biological activity and are generally more polar and subject to rapid excretion (e.g., the very common formation of a glucuronide on a phenolic hydroxyl-group), in some cases conjugation leads to less polar metabolites (e.g., acetylation of an amino-group or methylation of a sulfhydryl-group), which can extend the duration of activity, including potential toxicity.

Note 3: Certain conjugates are subject to further metabolic processing (e.g., reaction of glutathione with xenobiotics having an electrophilic group followed by a specific sequence of biotransformations that lead to formation

of mercapturic acids), and in some cases they can be converted back to their starting material (e.g., hydrolysis of glucuronides by the gut microflora).

See: Conjugation Reactions and Phases of Drug Metabolism.⁶³

43. Conjugation Reactions

Biotransformations that form a covalent bond between an endogenous molecule that is programmed biochemically to serve as a partner during metabolic processing and a functional group present on a xenobiotic or its metabolite. A special path occurs for a xenobiotic carboxylic acid moiety, because in this singular case the xenobiotic itself becomes biochemically programmed to undergo a conjugation reaction with an endogenous amino acid partner.

Note 1: Conjugation reactions are often referred to as “Phase 2” biotransformations because they so frequently follow introduction of a functional group by a preceding metabolic event that, in turn, is referred to as a “Phase 1” biotransformation. However, this is not a required sequence for using this terminology and, whenever a parent xenobiotic already has a suitable functional group, Phase 2 metabolism can occur directly and without the need for a preceding Phase 1 metabolic event. See thorough discussion of this topic in the introduction.

Note 2: Examples include: (1) glucuronidation reactions that combine glucuronic acid quite commonly with phenols, sometimes with alcohols, occasionally with amines, and more rarely with carboxylic acids; (2) sulfation reactions that combine sulfate with phenols and, to a lesser extent, with alcohols, including hydroxylamines; (3) acetylation reactions that combine an acetyl moiety with amines and sometimes with hydroxy and sulfanyl groups; (4) rapid reactions that combine glutathione with xenobiotics or their metabolites when they happen to contain an electrophile that might lead to toxicity via their reaction with other endogenous nucleophiles; (5) methylation reactions commonly involving sulfhydryl-groups and catechols, and to a lesser extent other amine and alcohol moieties; and (6) amino acid reactions typically utilizing glycine or taurine that combine with carboxylic acid groups.

Note 3: Among the six conjugation reactions listed above, their relative contributions during drug metabolism are approximated in the following

pie chart, adapted from reference 65. This summary reflects the types of structures that have become deployed as small-molecule drugs (namely the specific suitability of their functional groups), as well as the distribution accessibility, compartment abundance, and specific reaction rates for each of the processing systems associated with a given type of conjugation pathway. Note that category (6) encompasses all remaining conjugation reactions, as well as amide formation with specific endogenous amino acids.

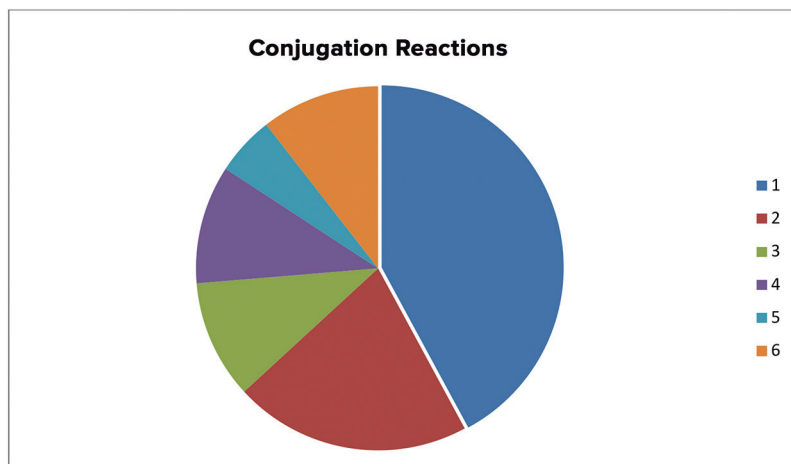


Figure of relative contributions for the various types of conjugation reactions toward overall human metabolism of small-molecule xenobiotics. Types: (1) Glucuronidation; (2) Sulfation; (3) Acetylation; (4) Reaction with glutathione; (5) Methylation; and (6) Reaction with an endogenous amino acid such as glycine or taurine.

See: Acetylation, Acylation, Acyltransferase, *N*-Acetyltransferases, Catechol-*O*-Methyl Transferase (COMT), Conjugate, Covalent Binding, Glucuronidation, Glucuronosyltransferases, Glutathione Conjugation, Glutathione-*S*-Transferase, Methylations, Phases of Drug Metabolism, Sulfation, and Sulfotransferases.⁶⁶

44. Covalent Binding

A biochemical process where a xenobiotic or metabolite forms a chemical bond with an endogenous molecule by sharing a pair of electrons between the two bonding atoms.

Note 1: Relative to various aspects of drug metabolism, covalent binding can occur as a requisite feature of conjugation or phase 2 biotransformations;

within enzyme active sites as an inherent but reversible mechanistic step (as described below) or by either intentional (drug design) or inadvertent (from production of a reactive species) reaction that alters the enzyme's function; or, for reactive metabolites, spuriously, with off-target protein sites that can lead to unwanted side effects and toxicity.

Note 2: In general, when the endogenous molecule's bonding atom resides on the functional surface of a protein, such as within an active site of an enzyme, this type of interaction can lead to permanent inhibition of that function, i.e., non-competitive, non-Michaelis-Menten type of kinetics for which the inhibition cannot be overcome by increasing the concentration of the normal substrate. Alternatively, some enzymes, such as certain members of the Hydrolases and Proteases, utilize a covalent bond-forming reaction as the first step toward hydrolysis of their ester or amide containing substrates. In these specific cases, other functional groups are also present in the active site to assist in re-establishing the enzyme's non-covalent bound "ground state." Lacking specific biochemical machinery for recovery, covalently inhibited systems must rely upon protein turnover to re-establish function.

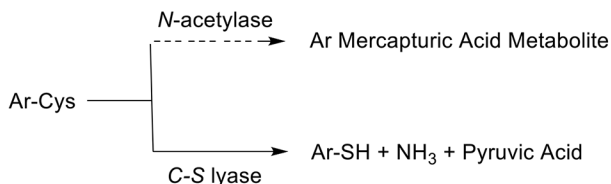
Note 3: For drug molecules, this term is most frequently associated with undesirable interactions, i.e., inhibition of metabolizing enzymes leading to drug-drug interaction issues, or interaction with off-target proteins whose functions are not meant to be inhibited, such that toxic side-effects may occur. Thus, in the selection of "drug-like" molecules among compound libraries, structures having reactive functional groups capable of forming covalent bonds, such as an *alpha-beta* unsaturated ketone, are often removed from further consideration.

See: Competitive Inhibition, Non-Competitive Inhibition, Michaelis-Menten Kinetics, Conjugation Reactions, Hydrolases, Esterases, Proteases, Drug-Drug Interactions, Drug-Like Properties/Profile.⁶²

45. Cysteine-S-Conjugate β -Lyase (C-S Lyase)

A pyridoxal phosphate-dependent β -lyase enzyme (EC 4.4.1.13) that can hydrolyze a cysteine conjugated xenobiotic intermediate otherwise on route to becoming a mercapturic acid metabolite. This process is shown below for a general aryl-containing xenobiotics (Ar) metabolic intermediate

(Ar-Cys) in a manner that depicts this key departure from the more typical route toward becoming a mercapturate (dotted line).



Note 1: While the parent xenobiotic, Ar, may have started as a highly reactive and potentially toxic electrophile, instead of progressing to the innocuous mercapturate, that center has now been masked with a less reactive (but still reactive) sulfanyl group. As a result, the latter is often quickly further metabolized by a methylation reaction, so as to produce the much less reactive thioether metabolite.

See: Glutathione and Mercapturic Acid.⁶⁷

46. Cytochrome b_5

A protein which can donate the second of two electrons required by the CYPs during their catalytic cycle associated with the metabolism of xenobiotics. In combination with the flavoprotein cytochrome b_5 reductase (EC 1.6.2.2), this pair normally functions as a non-phosphorylating electron-transport chain associated with the endoplasmic reticulum, where it plays a key role in the desaturation of endogenous fatty acids.

Note 1: In addition to increasing the catalytic rate, cytochrome b_5 increases the apparent affinity with which certain of the CYPs appear to bind their substrates. Thus, it may be increasing $V_{\max}$ and/or decreasing the apparent K_m , since it is the ratio $V_{\max}/K_m$ that relates to catalytic efficiency and intrinsic clearance.

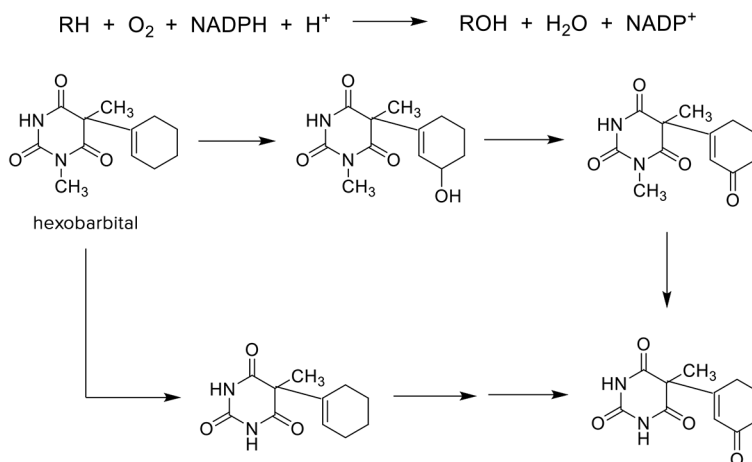
Note 2: While there are numerous isoforms of the various CYPs within liver microsomes, there appears to be only one form of NADPH-cytochrome P-450 oxidoreductase (accepted name: NADPH-hemoprotein reductase; EC 1.6.2.4) and only one form of cytochrome b_5 that serve as the principal partners for the CYPs.

Note 3: Since the reductase is important for reducing heme iron from Fe^{+3} to the oxygen-carrying Fe^{+2} state, disruption of this system can lead to methemoglobinemia.⁶⁸

See: Cytochrome *P*-450 Enzymes (CYPs), NADH-Cytochrome b_5 Reductase, Microsomes, Reconstitution System, Regenerating System, and NADPH-Cytochrome *c* Reductase/NADPH-Cytochrome *P*-450 Oxidoreductase/Cytochrome *P*-450 Reductase (CYPR).

47. Cytochrome *P*-450 Enzymes (CYPs)

The CYPs belong to the superfamily of heme proteins that have a heme (Fe^{+3}) cofactor, the P450 phrase in this broad term actually deriving from these enzymes' diagnostic spectrophotometric peak shift to 450 nm when the reduced heme iron (Fe^{+2}) is complexed with carbon monoxide and enzymatic activity is poisoned. In addition to having numerous roles in mammalian biochemical pathways involving the processing of endogenous materials, the CYPs are a major contributor toward a variety of xenobiotic oxidative metabolic events. The overall process is shown below for a general xenobiotic (R). Due to rearrangements, however, products are not limited to just aliphatic alcohols and phenols. A specific case that exemplifies several of hexobarbital's CYP-mediated sequential biotransformations is also shown below. In certain cases, most often prompted by anaerobic conditions, the CYPs can also perform reductive biotransformations. The CYPs have a distinct nomenclature based upon their protein homology, rather than substrate preferences. These designations are elaborated in Note 1. Note 3 provides additional mechanistic details.

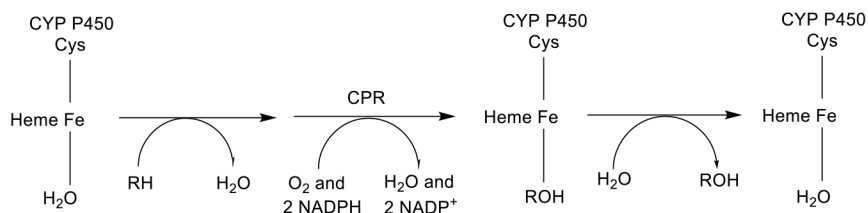


Note 1: CYP Family: a subset of the cytochrome *P*-450 superfamily of enzymes that shares at least 40 % amino acid sequence homology. A specific family is denoted by an Arabic numeral following the uppercase letters "CYP," e.g., CYP 2. **CYP Subfamily:** a subset of the CYP 450 superfamily that

shares at least 55 % amino acid sequence homology. A specific subfamily is denoted by adding an Arabic uppercase letter after the numerical family designation, e.g., CYP 2D. **CYP Isoform:** an individual form (distinct amino acid sequence) of the CYP 450 superfamily. A specific isoform is denoted by adding an Arabic numeral after the designation of its family and subfamily, e.g., CYP 2D6.

Note 2: More than 20,000 CYP isoforms have been identified in nature. However, for human drug metabolism, only three major families, about one-half-dozen subfamilies, and less than a dozen specific isoforms seem to be the most important. Just six isoforms account for 90 to 95 % of the metabolism of marketed drugs. The top four of these can be ranked: CYP 3A4 > CYP 2D6 > CYP 2C9 > CYP 1A2, with several others contributing considerably less (and nearly equally to each other) to human drug metabolism. Common examples of xenobiotic biotransformations mediated by the CYPs include: aromatic hydroxylation; *N*- and *O*-Dealkylations, especially demethylations; and aliphatic and alicyclic oxidations and ring scissions.

Note 3: The most prevalent mechanism utilized by various CYPs during metabolism of xenobiotics is a monooxygenase, hence they are often referred to as "mixed-function oxidases" in relation to drug metabolism discussions. NADPH is a requisite cofactor from which electrons are transferred by a required coenzyme, generally NADPH cytochrome *P*-450 oxidoreductase (CPR), so as to balance the reduction of molecular oxygen, while the CYP simultaneously utilizes the latter as its source to introduce an oxygen atom into a xenobiotic substrate (RH). This overall process is summarized below. It should be noted that the depiction of the various enzyme-bound oxygen species has been simplified to just a single oxygen atom, and the changes in the iron's oxidation state are not shown where $\text{Fe}^{+3} \rightarrow \text{Fe}^{+2} \rightarrow \text{Fe}^{+3}$ so as to maintain balance of the net charge throughout the biotransformation.



Note 4: While the CYPs can be found in nearly every tissue, their expression in the liver, the lungs, and the gut wall is especially noteworthy for drug metabolism and distribution in general, as is their acceptance toward the biotransformation of a wide range of xenobiotic structural types. Largely localized within the endoplasmic reticulum (ER) of cells and to a lesser extent within the mitochondria, their membrane-bound nature within that locale allows for their functional isolation by disruption of the cells and centrifugation to form lipid sacs called “microsomes” or the “microsomal fraction.” Microsomes are highly enriched with the CYPs and are used to conduct *in vitro* drug metabolism studies from various mammalian donors, particularly rodents. Expression and isolation of the key humanized isoforms has also allowed for biochemical studies with the distinct human enzymes.

Note 5: CYP-mediated biotransformations are often referred to in a general manner as “Phase 1” metabolic processes because they frequently instill or unmask a functional group that subsequently lends itself to one of the metabolic conjugation reactions, which, in turn, are referred to as “Phase 2” biotransformations. However, this is not a required sequence for using this terminology and, even after a conjugation reaction has occurred, there are numerous cases where additional CYP-mediated “Phase 1” biotransformations are observed. A thorough discussion of this topic can be found in the Introduction and the Appendix.

Note 6: Genetic polymorphism in CYP expression can lead to considerable individual variation. There are two general phenotypes based on how quickly individuals metabolize debrisoquine. These are commonly referred to as: (1) debrisoquine-related extensive metabolizers (DR EMs); and (2) debrisoquine-related poor metabolizers (DR PMs). The variations caused by these phenotypes extend to well over 20 other types of marketed drugs. DR PM reflects an inherited defect in CYP 2D6 expression that affects 5 to 10 % of the Caucasian, 2 % of the East Asian, and 1 % of the Arabic populations. Due to the potential for this variation, developers of new drugs often try to avoid relying on the CYP 2D6 pathway for the metabolic clearance of their candidate compounds. Another phenotype is associated with phenytoin-related metabolism and likewise extends to numerous other types of drugs. Phenytoin-related poor metabolizers (PR PMs) have an inherited defect in CYP 2C18, with an occurrence of 15 to 20 % in East Asian and 2 to 6 % in Caucasian populations. For this reason, the CYP 2C18 pathway is often also avoided during the development of new drug candidates.

See: Aromatic Hydroxylation, Dealkylation, *N*-Dealkylation, *O*-Dealkylation, Oxidation, Mixed-Function Oxidase, Monooxygenase, Phases of Drug Metabolism, NADPH-Cytochrome c Reductase/NADPH-Cytochrome *P*-450 Oxidoreductase/Cytochrome *P*-450 Reductase (CYP_R), Cytochrome *b*₅, NADH-Cytochrome *b*₅ Reductase (C_{BR})/Methemoglobin Reductase, Microsomal Fraction/Enzyme; Microsomes, Regenerating System, Hexobarbital Sleeping Time, Induction, and Drug-Like Properties/Profile.^{45,69-73}

48. Deacetylation

Typically used within the context of xenobiotic metabolism to specifically refer to the hydrolysis of *N*-acetyl groups.



Note 1: This special designation likely derives from the commonality of this particular functionality in drug molecules coupled with the resulting frequency for this type of deacetylation reaction. Reactions that remove an acetyl group, as well as other acyl groups, from other heteroatoms, such as oxygen are, instead, generally referred to as “hydrolyses” or “hydrolytic reactions.”

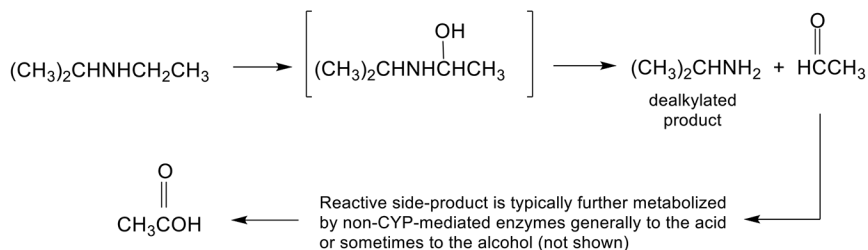
Note 2: Within the context of DNA packaging, histone acetylation versus deacetylation plays a key role in the extent of chromatin organization. Inhibitors of histone deacetylase (HDAC inhibitors) prevent cleavage of some of the acylated amines on the histone protein complex, which in turn decreases their organization with DNA, because the latter’s negatively charged phosphate groups cannot associate with as many protonated ammonium partners. Because some HDAC inhibitors have been found to be effective anticancer agents, this endogenous pathway involving key acetylation/deacetylation processes has become a popular area for usage of this term.

See: Hydrolysis and Hydrolases.⁵¹

49. Dealkylation

Common biotransformation wherein an alkyl group is removed from a heteroatom. Most often mediated by the CYPs, the alkyl group first becomes oxidized to an alcohol *alpha* to the heteroatom and then collapses to leave as

an aldehyde or ketone. Since the aldehyde side-products can be toxic, they are generally quickly processed by further, non-CYP-mediated oxidation to the carboxylic acid or, sometimes, by reduction to an alcohol. These pathways are illustrated below.



Note 1: For CYP-mediated dealkylations, substrates must possess at least one *alpha*-hydrogen, which becomes extracted during the early stages of the biotransformation. While *N*-dealkylations are quite general, *O*- and *S*-examples tend not to be nearly as prominent. In these latter dealkylations, only demethylation remains important, and especially so when an arylmethoxy-group is present.

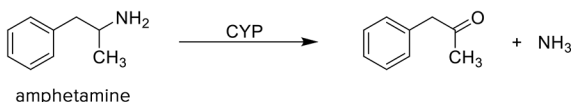
Note 2: The overall process is readily subject to steric hindrance: tertiary amines are less subject to dealkylation than secondary amines, which are similar to primary amines; isopropyl groups undergo less reaction than propyl groups or other non-branched longer chains; and as indicated above, simple methyl groups are typically the quickest substrates.

Note 3: Sterically hindered tertiary amines typically become prone to *N*-oxidation, rather than serving as substrates for dealkylations.

See: Demethylation, Deamination, Monoamine Oxidase (MAO), Diamine Oxidase (DAO), Polyamine Oxidase, *N*-Dealkylation, *O*-Dealkylation, *N*-Oxidation, Aldehyde Dehydrogenase, and Aldehyde Reductase.⁷⁴

50. Deamination/Oxidative Deamination

For xenobiotics, this represents a special case of *N*-dealkylation, mostly applicable to primary amines that can be mediated by CYPs, monoamine oxidase, diamine oxidase, or polyamine oxidase. Analogous mechanistically, after formation of the *alpha*-hydroxy intermediate, further collapse liberates ammonia and either an aldehyde or ketone moiety. The biotransformation of amphetamine is representative:



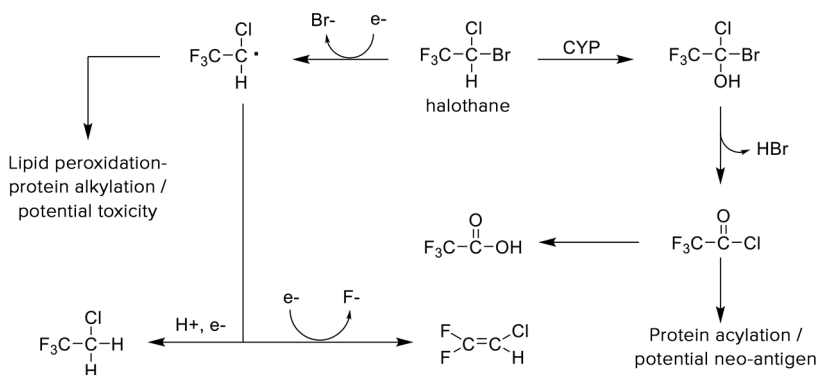
Note 1: This example is mediated by a CYP because the other applicable oxidase enzymes are far less tolerant of substrates whose *alpha*-carbon bears two substituents, which results in a steric impediment.

Note 2: For endogenous biochemistry, this term is used similarly in several other contexts. Important examples include: catabolism of amino acids, such as the conversion of glutamic acid to α -ketoglutaric acid (now 2-oxopentanedioic acid), wherein the liberated ammonia joins the urea cycle and the keto-acid participates in subsequent transamination reactions, so as to also deaminate other amino acids; similar catabolism of excess proteins within the body; and analogous reactions involving nucleosides for which some spontaneous (hydrolytic driven) deaminations can lead to mutations that prompt repair mechanisms.

See: Dealkylation, *N*-Dealkylation, Monoamine Oxidase (MAO), Diamine Oxidase (DAO), and Polyamine Oxidase.⁷⁴

51. Dehalogenation

Removal of one or more aliphatic halogens from a xenobiotic. This can be accomplished by any combination of three mechanisms (see Note below), all of which traverse reactive intermediates that can lead to potential toxicity. The metabolism of halothane is representative:



Note 1: The three most prominent mechanisms for this type of biotransformation are: reductive dehalogenation, in which a halide is replaced by a hydrogen; oxidative dehalogenation, in which a halide and a hydrogen on the same carbon are replaced by an oxygen; and removal of two halides or of one halide and one hydrogen (also called "dehydrohalogenation") on adjacent carbon atoms, so as to produce a double bond. Each of these pathways is evident in the metabolic composite that is undertaken by halothane.

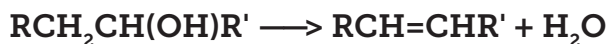
Note 2: All three types of dehalogenation are catalyzed by the CYPs. Formation of the double bond can also be catalyzed by glutathione-*S*-transferase.

Note 3: The halothane example also illustrates the different sources of potential toxicity during these mechanisms. In general, when a carbon atom contains two halides and a hydrogen, the dihalohydrin intermediate that is formed during oxidative dehalogenation collapses to an acylhalide, which, unless it is quickly hydrolyzed to a carboxylic acid, can acylate proteins that then become neo-antigens (antigens not present in the normal genome, generally resulting from an aberrant metabolic step) and ultimately lead to immune hepatitis, as is sometimes seen after repeated exposure of humans to halothane and other structurally related volatile anesthetics.

See: Bioactivation and Bioinactivation.⁷⁵

52. Dehydration

For xenobiotics, dehydration most frequently pertains to the formation of unsaturated metabolites by the elimination of water from hydroxy-containing compounds, typically those having an acidic hydrogen at an adjacent carbon atom.



Note 1: In addition to being enzymatically catalyzed,⁷⁶ the dehydration of certain alcohols can occur non-enzymatically as a one-step acid-catalyzed reaction or as a two-step process involving the metabolic activation of the hydroxyl group, e.g., by sulfation or glucuronidation and the subsequent elimination of sulfuric acid or glucuronic acid, respectively. Dehydration of aldoximes to nitriles can be catalyzed by the CYPs in their reduced state.

See: Dehydrogenation.

53. Dehydrogenase

General term for a large family of enzymes that oxidize a substrate by removing one or more hydrides and transferring them to an endogenous acceptor, such as $\text{NAD}^+/\text{NADP}^+$ or FAD/FMN , which in turn becomes reduced. They are further designated by the type of substrate involved. Common examples include alcohol dehydrogenase (ADH, EC 1.1.1.1) and aldehyde dehydrogenase (ALDH, EC 1.2.1.3).

See: Alcohol Dehydrogenase, Aldehyde Dehydrogenase, and Dehydrogenation.⁷⁷

54. Dehydrogenation

This term is used to designate biotransformations that convert single bonds to double bonds without undergoing an equivalent loss of water and especially for the specific production of unsaturated aliphatic compounds from saturated compounds. These conversions are typically catalyzed by the dehydrogenases and occasionally by the CYPs (see Notes).

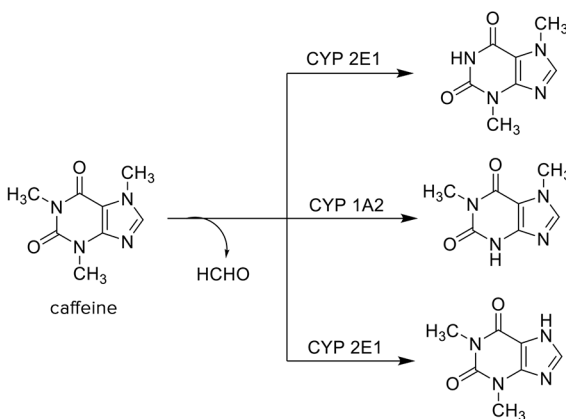
Note 1: While ADH and ALDH perform this type of reaction, they are more typically thought of as causing oxidations.

Note 2: The CYPs occasionally catalyze the de-saturation of hydrocarbons by a mechanism that does not proceed via hydroxylated intermediates. The oxidation of dihydropyridines to pyridines is likewise CYP-mediated, as is the formation of quinones from hydroquinones and the oxidation of secondary alcohols to ketones.

See: Dehydrogenase, Cytochrome P-450 Enzymes (CYPs), Alcohol Dehydrogenase, and Aldehyde Dehydrogenase.⁷⁸

55. Demethylation

This is a very common biotransformation, wherein a methyl-group is removed from arrangements associated with an amine, ether (especially phenolic methoxy ethers), or a thioether when they are present on a xenobiotic or its metabolite. Typically mediated by the CYPs, the process begins by oxidation of the methyl group. The metabolism of caffeine represents an interesting case of various *N*-demethylation possibilities, including the regio-promiscuity that can be demonstrated by the same CYP isoform:



Note 1: This is a special case of the more general dealkylation reactions typically catalyzed by the CYPs which involves an oxidative cleavage mechanism. CYP 2E1 is EC 1.14.13.178 (methylxanthine N^1 -demethylase), and CYP 1A2 is EC 1.14.13.179 (methylxanthine N^3 -demethylase).

Note 2: The formaldehyde side product is quickly metabolized further by other enzymes (e.g., ALDH), so as to avoid its potential toxicity.

See: Dealkylation, *N*-Dealkylation, *O*-Dealkylation, and Cytochrome *P*-450 Enzymes (CYPs).^{79,80}

56. Detoxification

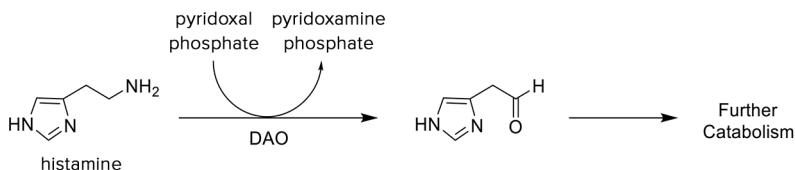
A biotransformation that eliminates a toxicant or prevents formation of a toxicant.

Note 1: Includes all metabolic pathways of the toxic substance that lead to non-toxic metabolites. In principle, these processes compete with those forming toxic metabolites and reactive intermediates.

See: Bioinactivation, Bioactivation, Active Metabolites, Reactive Intermediates, Soft Drugs, Glutathione, and Hydrolases.^{81,82}

57. Diamine Oxidases (DAO)

A pair of copper-containing amine oxidases that catalyze the oxidative deamination of short chain diamines: primary amine oxidase (EC 1.4.3.21) and diamine oxidase (EC 1.4.3.22). The catabolism of histamine is exemplary:



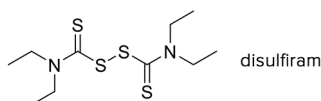
Note 1: In terms of xenobiotic structure-metabolism relationships (SMRs), any compound having a basic center with more than two but less than seven carbon atoms' distance away from a primary amine or secondary methylamine center is a candidate for deamination of the latter by DAO.

Note 2: DAO catabolizes short chain diamines, such as putrescine (4 C spacer) and cadaverine (5 C spacer), while longer, more complicated polyamines, such as spermine, are catabolized by spermine oxidase or by monoamine oxidase (MAO).

See: Catabolism, Deamination, Dealkylation, *N*-Dealkylation, and Monoamine Oxidase (MAO).⁸³

58. Disulfiram Reaction

Disulfiram inhibits aldehyde dehydrogenase (ALDH), causing a buildup of its aldehyde substrates, which in turn leads to toxicity and what is referred to as the "disulfiram (physiological) reaction."



Note 1: Disulfiram is used to treat alcoholism because the buildup of acetaldehyde after the ingestion of ethanol leads to an acute sensitivity, much like the unpleasant toxicity associated with a "hangover."⁸⁴

See: Alcohol Dehydrogenase (ADH), Aldehyde Dehydrogenase (ALDH), Aldehyde Dehydrogenase Polymorphism or Deficiency, Aldehyde Oxidase (AO), and Atypical Alcohol Dehydrogenase.

59. Disposition

All processes that occur to a xenobiotic (and its metabolites) within the living recipient after it has been absorbed, especially as they relate to the pharmacokinetic profile.

Note 1: While distribution (to and from tissues/compartments) and elimination (by metabolism and excretion) represent the major dispositional processes, additional examples include binding to proteins, wherein the latter may prompt desirable biological activity (e.g., efficacy at the desired target site of a drug), as well as toxicity (e.g., off-target drug interactions) or a passive association (e.g., plasma protein binding).

Note 2: While the term is generally meant to be all-encompassing, in some uses one or more of the ADME processes may be further distinguished, such as is the case for the journal entitled *Drug Metabolism and Disposition*.

Note 3: After the excretion of a xenobiotic and/or its metabolites, these compounds enter the environment, where their further dispositions are repeated by continued exposures to other living systems. Each of the latter can be separately tracked using this same terminology and a composite chart can be composed to represent the overall environmental fate for all of the eventual components. A discussion about the phases of drug metabolism is provided in the Introduction.

See: Absorption, First Pass Effect, Distribution, Plasma Protein Binding, Metabolism, Random Walk, Excretion, ADME/ADMET, Pharmacokinetics, and Phases of Drug Metabolism.³⁸⁻⁴⁰

60. Distribution

Generally taken as the pharmacokinetic-related parameter for the movement of a xenobiotic and its metabolites between the blood circulation and other tissues or fluids in the body. The term can also be used to designate more localized parameters, such as an uneven distribution of a xenobiotic or metabolite among the organelles within a given cell where, for example, drug metabolizing enzyme concentrations are known to be significantly different.

Note 1: After excretion of a xenobiotic and/or its metabolites, these compounds enter the environment, where their environmental distribution is impacted by physical factors and their further dispositions can be repeated by continued exposures to other living systems. Each of the latter can be separately tracked using this same terminology and a composite chart can be composed to represent the overall environmental fate for all of the eventual components. See also the discussion about the phases of drug metabolism provided in the Introduction.

See: Absorption, Disposition, First Pass Effect, Plasma Protein Binding, Metabolism, Random Walk, Excretion, ADME/ADMET, Pharmacokinetics, and Phases of Drug Metabolism.³⁸⁻⁴⁰

61. DMPK

Acronym for drug metabolism and pharmacokinetic processes, typically used in conjunction with their study, with their related profile for a given molecule, or to designate a lab or group of investigators involved with such studies.

Note 1: The same acronym is used for dystrophin myotonic protein kinase, the latter being important within the context of protein coding genes for a family of associated diseases.

See: ADME, Drug Metabolism, and Pharmacokinetics.⁴⁵

62. Dose

Amount of xenobiotic delivered to the site of an intentional administration or an unintentional exposure.

See: Absorption.⁸⁵

63. Dose-Dependent Kinetics or Metabolism

In this context, dose-dependency is the alteration of pharmacokinetic parameters that occurs with changing dose levels, where the result leads to nonlinear relationships.

Note 1: A system is linear when all pharmacokinetic parameters are independent of a xenobiotic's concentration. Linearity is demonstrated by superimposition of the data when normalized for dose. A system is nonlinear when one or more pharmacokinetic parameters changes with the xenobiotic concentration or dose. Lack of linearity is demonstrated by the absence of superimposable data when normalized for dose.

Note 2: Dose-dependent kinetics can be due to saturable processes encountered during any of the ADME parameters, e.g., transport across membranes, protein binding, metabolic enzyme activity, or active excretion pathways. While autoinhibition can likewise cause this effect, autoinduction

is generally observed only with repeated administration or exposure to the xenobiotic.

See: Pharmacokinetics, ADME/ADMET, Autoinduction, and Autoinhibition.³⁸⁻⁴⁰

64. Drug Delivery Formulations

Historically, these have been created by the inclusion of non-active excipients that have little impact on drug metabolism and by encapsulations that enhance the release of the active agents for absorption by specified segments of the gut across specified (slow release) timeframes. The field has now moved on to biocompatible polymeric assemblies, which can provide for delayed release from selected sites of (often implanted) administration and into micro-particle and nanotechnology-derived carriers, which can target specific sites for release of the active agents.⁸⁶ These new types of formulations can have profound effects on drug pharmacokinetics and biotransformations, sometimes even relying on the latter as part of their end-point design strategies, much like prodrugs and soft drugs rely on programmed drug metabolism to control the pharmacokinetic profiles of their active forms.

See: Prodrugs and Soft Drugs.

65. Drug-Drug Interactions

These interactions occur when one drug alters the efficacy, toxicity, or the pharmacokinetic profile observed for another drug, either upon their overlapping administration or from previous administrations of the drug that can cause the alteration.

Note 1: In general, these interactions occur at the pharmacokinetic level, particularly with regard to drug metabolism, and can, in turn, often cause undesirable variations in efficacy and/or toxicity. Because of this, the reclinical testing of candidate compounds typically includes co-administration with other established drugs that are likely to also be present in the intended patient population to *in vitro* models of metabolism, as well as to appropriate *in vivo* models. Criteria are generally then set so that there are no (or only minimal) interactions. Alternatively, these interactions may be intentionally utilized: the use of disulfiram represents such an example of an intentional drug-drug interaction.

Note 2: If an inhibitory mechanism is responsible for the interaction, then the effect can be observed immediately, whereas if a common ADME process is enhanced due to induction, then a prior and often repeated exposure of the altering drug is required.

Note 3: The phrase is sometimes used in a general manner to include cases where a xenobiotic or food component demonstrates similar interactions with a given drug or, more specifically, a food-drug interaction.⁸⁷ A classic example of the latter is the ability of the furanocoumarins present in grapefruit juice to inhibit CYP 3A4, such that the PK half-life may become prolonged for a drug which engages this metabolic pathway, e.g., atorvastatin.⁸⁸

See: ADME/ADMET, Aromatic Hydrocarbon Receptor (AHR), Autoinduction, Autoinhibition, Cocktail Study, Competitive Inhibition, Covalent Binding, Disulfiram Reaction, Dose-Dependent Kinetics or Metabolism, Induction, Inhibition, Phenotype/Phenotyping, Pregnane X Receptor (PXR), and Suicide Inhibitor.^{89,90}

66. Drug-Like Properties or Profile

The range of physicochemical properties and functional groups typically displayed by small-molecule compounds that are being used in the clinic. The profile can be selectively refined depending on the route of administration and the clinical indication, e.g., CNS drugs are generally different from systemic drugs,^{47,48} and oral agents are nearly always different from injectable drugs.⁴ Notably, the concept is often deployed in the reverse manner, since it also delineates chemical groups that should be excluded from compounds if they are to be regarded as being “drug-like.” Groups that are known to cause toxicity are especially likely to be excluded. An example pertinent to drug metabolism would be the exclusion of a 1*H*-imidazole group, which is known to chelate with the iron of the CYPs, causing them to be poisoned and potentially leading to hepatotoxicity.

Note 1: Differing versions of the range of desirable properties and the list of groups to be avoided are often used as criteria that new drug candidates must meet during the early stages of the drug discovery process. Different participants will emphasize different degrees of adherence at different stages of drug development, additionally refining the ranges based on the anticipated progression of molecular changes that may be needed for any given program.

Note 2: The “rule of five” represents a historical landmark for adopting such considerations during drug design and development.

See: Rule of Five.^{1,3,15}

67. Drug Metabolism

The general term for all aspects of the biotransformation of xenobiotics upon interaction with biological systems.

Note 1: Biological systems include isolated enzymes, cellular fractions, intact cells, and tissues and organs, as well as intact organisms and various species, including humans. Aspects include: the structural modifications that xenobiotics and their metabolites undergo; the anabolism, function, homeostasis, induction, inhibition, and catabolism of the enzymes and their prosthetic groups and cofactors involved in metabolic reactions; and, in turn, the effects that xenobiotics and their metabolites exert on the systems.

See: Metabolism, Enzyme, Prosthetic Groups, Cofactors, and Phases of Drug Metabolism.^{2,45,79,80,91-93}

68. EC Number (Enzyme Commission Number)

A distinct identity number assigned to a given enzyme, where the letters “EC” are followed by four sets of digits, separated by periods, that are used to convey classification-related information. The acronym is often taken to mean “enzyme classification number.” Truncated versions of the EC numbers are also sometimes used to convey groups of enzymes according to the same classification system.

Note 1: The first digit conveys a general reaction type from among seven classification groups: 1 Oxidoreductases; 2 Transferases; 3 Hydrolases; 4 Lyases; 5 Isomerases; 6 Ligases; and 7 Translocases. Subsequent digits convey sequentially more specific information about the types of substrates that the enzyme will transform.

Note 2: Presently, newly discovered enzymes are submitted to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB), which meets with the Joint Commission on Biochemical Nomenclature (JCBN) for classification. EC numbers are then assigned by the IUBMB.

See: Enzyme.^{94,95}

69. Elimination

The processes that cause reductions in the concentration of a xenobiotic and its metabolites.

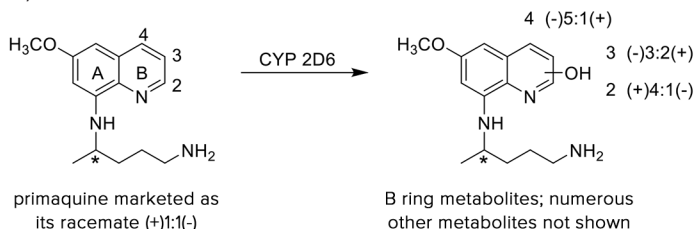
Note 1: Processes include biotransformation reactions, spontaneous degradation, and excretion.

Note 2: This term should not be used for the “E” portion of the “ADME” acronym, wherein “E” is meant to signify only excretion.

See: ADME, Clearance, Excretion, Extraction Ratio, and Phases of Drug Metabolism.^{45,92}

70. Enantioselective and Enantiospecific Metabolism

This biochemical process occurs when a biotransformation proceeds to a greater extent along a preferred stereochemical pathway. “Enantiospecific” further indicates that the preference is exclusive to just one of the enantiomers. The example shown below demonstrates this effect for several of primaquine’s aromatic hydroxylation reactions on its “B ring,” which can occur with varying regiochemistry and multiplicities (di- and tri-hydroxylations are not depicted, nor are the oxidative biotransformations that occur on the “A ring” or *N*-dealkylation followed by reduction of the aldehyde).⁹⁶



Note 1: The inherent stereochemical bias present in all living systems is often manifest during xenobiotic metabolism, and this term is meant to encompass all diastereomeric consequences that can result when a biochemical surface interacts with a xenobiotic having one or more chiral or prochiral centers. Within the context of drug metabolism, this becomes most interesting when enantiomeric species exhibit preferential

biotransformation even when the enzymes are quite promiscuous relative to their array of substrates and to their display of regiochemistry variation for a given substrate (as demonstrated by the example shown above).

Note 2: Based on chiral enzymic discrimination between enantiomeric substrates or between two sites of enantiotopic groups, substrate, and product selectivity are distinguished. An example of substrate selectivity involves the formation of different amounts of single metabolites from two enantiomers of a racemic xenobiotic. An example of product selectivity involves the metabolism of an achiral substrate at a prochiral center where the two types of enantiomeric metabolites are formed in different amounts.

Note 3: Both substrate and product selectivity are common across a wide range of biotransformation reactions. They vary considerably between different species and within humans can impact both a drug's efficacious and toxicological profiles.

See: Substrate Specificity, Regioselective Metabolism, and Stereoselective Metabolism.

71. Endoplasmic Reticulum (ER)

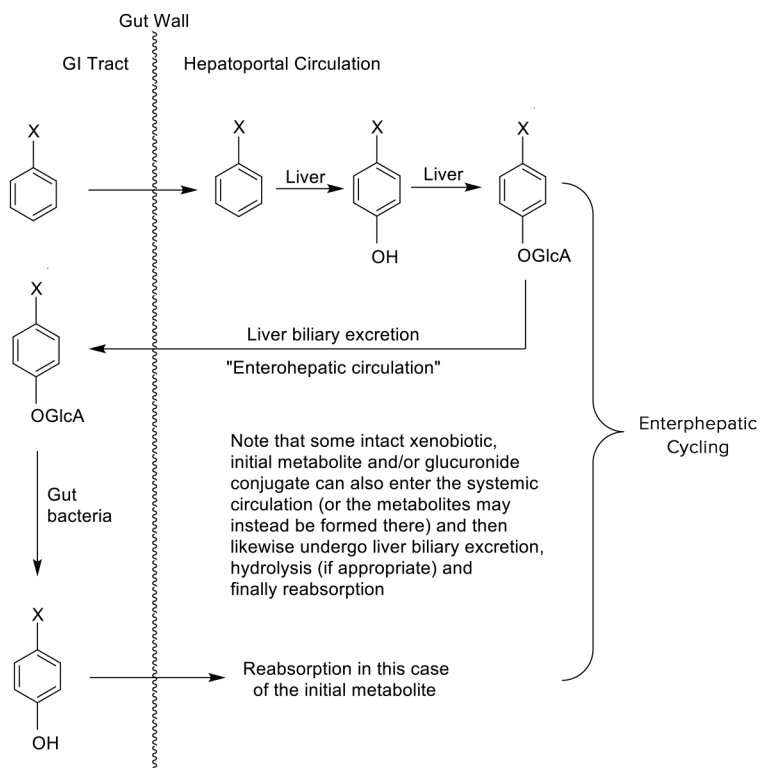
A network of tube-like structures in nearly all eukaryotic cells, called cisternae, that extend throughout the cytosol from the outer nuclear membrane. Ribosomes are attached to certain portions of the ER in clusters, giving them a studded appearance and their further classification as "rough ER," compared to the non-ribosome portions, which are called "smooth ER." The CYP enzymes are intimately associated, anatomically and functionally, with the ER lipid bilayer, while certain of the enzymes responsible for conjugation reactions, such as glucuronide formation, are present within the ER lumen. Since only the smooth ER is noticeably responsive to induction of the CYPs by the barbiturates, it is thought to be more important than the rough ER for xenobiotic metabolism in general.

Note 1: The ER membranes, along with the CYPs and their other associated enzymes, are captured within the microsomal fractions isolated to perform drug metabolism studies in an *in vitro* setting.

See: Cytochrome P450 Enzymes (CYPs), Glucuronic Acid Conjugation, Phases of Drug Metabolism, Microsomes, Microsomal Enzyme, and Hexobarbital Sleeping Time.⁹⁷

72. Enterohepatic Cycling

This physiological process occurs when a xenobiotic is absorbed from the gastrointestinal tract (GI), excreted into the bile, and then reabsorbed from the GI. It can also be applied to the metabolites from a xenobiotic, either directly as such or upon their reversion to either the original xenobiotic or to any of its metabolites after their excretion into the GI via the bile. An example of the latter is portrayed below for a xenobiotic material "X" undergoing: aromatic hydroxylation; conjugation with glucuronic acid (GlcA); biliary excretion; hydrolysis by β -glucuronidase enzymes present in the GI bacterial flora; and finally, reabsorption of the initial metabolite. Note that the involvement of gut bacteria is not a requisite step for enterohepatic cycling, but in this case is needed to remove the glucuronic acid conjugate, since these types of compounds typically have very low absorption and bioavailability.



Note 1: The enterohepatic cycling of drugs can have a major impact on the distribution and PK profile, either directly or indirectly through one or more metabolites. For example, it can increase the systemic exposure to a drug or metabolite even when the hepatic extraction of the drug or metabolite is high. Likewise, if passage through the systemic circulation is a step that precedes liver biliary excretion, then a pulsating increase and decrease in systemic exposure may result after just a single dose of compound. Repeated exposures and prolonged elevated blood levels due to this process are thought to contribute to the toxicity observed for arsenic.⁹⁸

Note 2: Some of the endogenous steroids take advantage of this pathway both in order to influence systemic concentration with time and as a catabolic salvage pathway to recoup larger building blocks for steroid anabolism. Likewise, the biliary acids themselves utilize this cycling pathway while assisting in both the digestion/absorption of nutrients and in the excretion of xenobiotics and the latter's metabolites.

See: Hepatoportal Circulation, First Pass Metabolism, and Extraction Ratio.^{74,79-82,85,91,93}

73. Enzyme

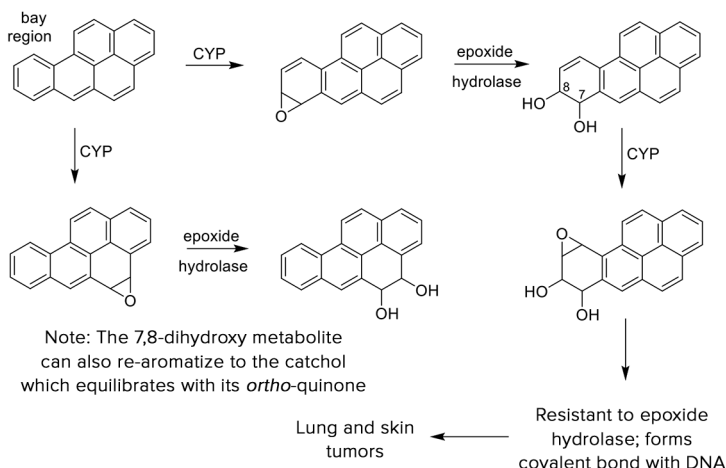
A macromolecule (typically protein) that functions as a biocatalyst (often complexed with cofactors and prosthetic groups) by increasing the reaction rate. In general, a given enzyme catalyzes only one reaction type (reaction selectivity) and operates on only one type of substrate (substrate selectivity), which are transformed at the same site (regioselectivity), where only one chiral substrate or racemate is preferentially transformed (stereoselectivity or enantioselectivity, respectively). However, see note below.

Note 1: Within the context of xenobiotic metabolism, many of the participating enzymes are very promiscuous in terms of their ability to act on a wide range of substrate types and sometimes in terms of their differing action upon a given substrate. An example of the latter is the differing *N*-dealkylations that CYPs can perform on caffeine.

See: Apoenzyme, Haloenzyme, Cofactors, Prosthetic Groups, Enantioselective and Enantiospecific Metabolism, Substrate Specificity, Regioselectivity, Dealkylation, and Demethylation (caffeine *N*-dealkylations).^{62,99}

74. Epoxidation

Oxidation of an aromatic or olefin group within a xenobiotic to form an epoxide (oxirane) metabolite. Epoxidation is typically accomplished by members of the CYP family. These metabolites are often toxic, but can also be non-toxic or even serve to prevent toxicity,¹⁰⁰ depending on the reactivity of the product. The metabolism of benzo[a]pyrene is shown below. It represents a classic example of how the epoxidation of certain aryl systems can lead to toxicity.



Note 1: Within the context of drug metabolism, these biotransformations are typically done by the CYPs. An oxygenated biradical intermediate is thought to form first and rearrange to the epoxide as one of several possible products.

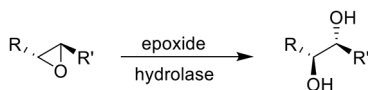
Note 2: For aryl systems, aromaticity is lost: the arene oxide products generally are unstable electrophiles that can undergo ring opening and rearrangement to phenols. In addition, and as shown in the example, epoxide hydrolase can usually convert the reactive epoxides to diols that are less toxic. Conjugation with glutathione likewise serves to detoxify these types of metabolites. The polycyclic hydrocarbons (PAHs) are known for their insidious carcinogenicity because they form epoxides having transient stability while retaining their reactivity, so as to form covalent bonds with distinct biological systems, such as DNA. As shown in the example, epoxides formed in the "bay region" of the PAHs are resistant to detoxification by epoxide hydrolase.

Note 3: Epoxides formed from olefins are usually less reactive and more stable. However, they can also undergo similar intramolecular rearrangement reactions to form ketones or become hydrolyzed or trapped by nucleophiles.

See: Aromatic Hydroxylation, Active Metabolite, Bioactivation, Covalent Binding, Epoxide Hydrolase, and Glutathione Conjugation.

75. Epoxide Hydrolases

A pair of enzymes that add water to three-membered cyclic ethers, forming products that are usually 1,2-diols: microsomal epoxide hydrolase (EC 3.3.2.9) and soluble epoxide hydrolase (EC 3.3.2.10). Examples for arene oxides are shown under the term "Epoxidation." A generalized example for an alkene-derived epoxide is provided below with the specific stereoselectivity also designated.



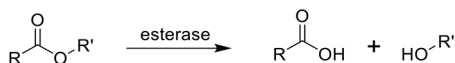
Note 1: These are key participants in the common xenobiotic metabolism process where lipophilic materials are converted to more polar metabolites.

Note 2: Most epoxides demonstrate moderate reactivity, but some are highly reactive, the latter being responsible for the electrophilic insults associated with many known carcinogens, mutagens, and toxins. Thus, along with glutathione, the epoxide hydrolases play an important role in the detoxification of such species.

See: Epoxidation, Active Metabolite, Bioinactivation, and Glutathione Conjugation.¹⁰¹

76. Esterases

A ubiquitous family of enzymes (EC 3.1) that catalyze the hydrolysis of ester-containing xenobiotics into their respective free acids and alcohol components. A generalized example is depicted below.



Note 1: Numerous biomolecules are also processed by these enzymes into active forms or are catabolized into inactive components, the hydrolysis of the neurotransmitter acetylcholine by acetylcholine esterase representing a common example for the latter.

Note 2: The esterases are further subdivided according to their principal types of substrates: carboxyl esterases (CESS; EC 3.1.1) as specifically shown above; thiol esterases (EC 3.1.2); the various phosphatases (ECs 3.1.3, 3.1.4, 3.1.5, 3.1.7 and 3.1.8); sulfatases (EC 3.1.6); the various exonucleases (ECs 3.1.11 to 3.1.16); and the various endonucleases (EC 3.1.21 to 3.1.31), for which this list continues to be refined. However, many of these enzymes exhibit broad and overlapping substrate specificity, such that their distinct classification can be difficult.

Note 3: Amid such overlapping participation during the metabolism of xenobiotics and their different expression levels in various compartments, the carboxyl esterases appear to play the most important roles during drug metabolism. This subdivision is specifically described as a distinct term.

Note 4: Given their ubiquitous and often high levels of distribution as an overall enzymatic class, coupled with their promiscuous and aggressive activity toward a wide array of substrates, it can be generally assumed that if a xenobiotic contains an ester group that is not sterically encumbered, then it will likely become rapidly metabolized at that site at an initial probability that is highest among all of the other biotransformation possibilities.

See: Carboxyl Esterases, Prodrugs, Soft Drugs Rule of One (Metabolism's Rule of One), and Metabolic Probabilities versus Possibilities.^{45,50,51,79,80,93,102}

77. Excretion

Removal of xenobiotics or their metabolites from the systemic circulation by transfer into bile, saliva/nasal fluid, perspiration, or body wastes, such as urine, feces, or exhaled gases. This is the "E" in "ADME."

Note 1: Biliary excretion generally prefers substrates having molecular masses greater than 500 Da, while renal excretion generally prefers those under 500 Da.

See: ADME and Elimination.^{38-40,91}

78. Extensive (or Rapid) Metabolizer

A subpopulation within a given species that metabolizes a xenobiotic via a pathway under polymorphic influence at a significantly higher rate compared to other subpopulations. The slower rate groups, in turn, can be classified as “poor metabolizers.” The terms “intermediate” and “ultra-extensive” metabolizers are also sometimes used to provide further delineations.

Note 1: As an example, individual variations in *N*-acetyltransferase (NAT) activity that result in phenotypically “fast” and “slow acetylators” were one of the first pharmacogenetic traits recognized in humans. Similarly, 7 % of the Caucasian population can be regarded as poor metabolizers for drugs that primarily rely upon CYP 2D6.¹⁰³ Due to the potential to cause individual variation in the response to drugs, companies often try to have their lead compound candidates either avoid this pathway altogether, or they try to also engage several alternative pathways for the compound’s clearance.

Note 2: Genetic testing has now been firmly adopted as part of the drug selection and development process, as well as part of the process for determining clinical study populations. The growing list of genetic tests already includes CYP 2D6, 2C9, 2C19, and 1A2.

See: Acetylation, Acetylation Phenotype, Aldehyde Dehydrogenase Polymorphism or Deficiency, Atypical Alcohol Dehydrogenase, Genetic Polymorphism, Genotype/Genotyping, *N*-Acetylation, *N*-Acetyltransferases, and Poor Metabolizer.

79. Extraction Ratio

This ratio represents the fraction of a xenobiotic or given metabolite’s blood concentration that enters an organ of elimination and is extracted or metabolized by that organ during a single pass. Symbolized as “*E*,” the pharmacokinetic (PK) representation for this is shown below:

$$E = (C_{\text{in}} - C_{\text{out}}) / C_{\text{in}}$$

where C_{in} is the concentration in the blood entering on the arterial side and C_{out} is the concentration leaving on the venous side. By definition, *E* is always greater or equal to 0 and less than or equal to 1.

Note 1: It follows that “clearance” by the organ, CL , is proportional to E and the amount of blood flow, Q , according to $CL = Q \times E$. Thus, the clearance of drugs with a high extraction ratio is flow limited, $CL \propto Q$.

$$Q = (C_{in}) \rightarrow \text{Elimination Organ} \rightarrow C_{out}$$

See: Pharmacokinetics (PK), ADME, Elimination and Excretion, First Pass Effect/Metabolism, and Second Pass Metabolism (ψ First Pass Metabolism).^{38-40,104}

80. Extravascular Dosing

Drug administration to any body site other than the systemic blood circulation.

See: Absorption and Intravascular Dosing.^{38-40,85,91,104}

81. First Pass Effect/Metabolism (Pseudo-First Pass Effect)

Strictly intended as the metabolism of a xenobiotic that occurs after its administration or exposure, before it reaches the general systemic circulation and becomes distributed to all tissues. Most frequently taken as the metabolism of a drug that occurs after oral delivery until it passes the liver on route to the heart, thus including the metabolic capability of the gut microflora and emphasizing capabilities in the gut lining, hepatoportal circulation, and liver, while typically excluding the metabolic capabilities present in the bronchopulmonary circulation and lungs (see **Note 2**).

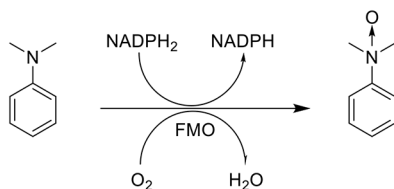
Note 1: For drugs that can serve as good substrates for metabolism, the “first pass effect” can significantly limit oral bioavailability, because the hepatoportal circulation delivers all of its blood flow (100 % Q) to the liver, which is the most aggressive drug “elimination organ” in terms of overall xenobiotic metabolizing capability.

Note 2: Drugs administered intravenously bypass the ‘first pass effect’ as the phrase is most commonly used. However, such drugs initially flow to the heart and through the bronchopulmonary circulation prior to being distributed to the general circulation. Because the lungs are also an aggressive drug metabolizing organ, these cases can then be thought of as undergoing a “pseudo-first pass effect.”

See: Absorption, ADME, Elimination, Extraction Ratio, Bioavailability, and Oral Bioavailability.^{105,106}

82. Flavin Monooxygenase (FMO)

A flavoprotein enzyme (EC 1.14.13.8) that can act, for example, on xenobiotic dialkylarylamines to form *N*-oxides.



Note 1: FMO (*N,N*-dimethylaniline, NADPH₂:oxygen oxidoreductase) is part of the oxidoreductase family (EC 1.14). Its holoenzyme composite serves as a paired donor for the incorporation and reduction of molecular oxygen.

Note 2: Alterations in the gene which transcribes to FMO3, an important liver version that metabolizes trimethylamine to the *N*-oxide, is suspected to be the cause of several types of trimethylaminuria, which is a metabolic disorder where individuals excrete a fishy body odor in their urine, sweat, breath, and other body excretions.

See: Holoenzyme, and *N*-Oxidation.^{107,108}

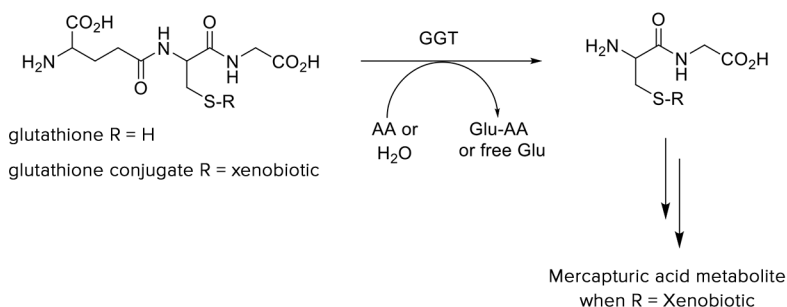
83. Futile Metabolism

Initial metabolic events that are ineffective because they rapidly revert back to the parent compound by non-enzymatic, chemical stability-driven mechanisms. Although difficult to detect, one example is the first, one-electron reduction step for nitro-imidazoles that generates an extremely reactive radical anion which, under well-oxygenated conditions, is rapidly oxidized back to the parent compound, leaving no intermediate for the second step of the overall pathway.¹⁰⁹ These events are different from "reversible metabolism."

See: Reversible Metabolism.

84. Gamma-Glutamyl Transpeptidase (γ -Glutamyltransferase; GGT)

Distinct from the acyltransferases (EC 2.3.1), this family of transpeptidases (EC 2.3.2) transfers specific aminoacyl functionality from a peptide to another peptide, amino acid, or water, the latter liberating the aminoacyl group as its free amino acid. The family member most significant to drug metabolism is GGT (EC 2.3.2.2), which transfers a glutam-5-yl moiety that is important for: (i) the "gamma-glutamyl cycle" and its role in the synthesis and degradation of glutathione; and, (ii) the initial step in the secondary processing of xenobiotic-glutathione-conjugates eventually leading to 'mercapturic acid' metabolites. GGT's degradative step involving glutathione is shown below for the parent compound (R = H) as part of the gamma-glutamyl cycle, and for a xenobiotic conjugate (R = X).



Note 1: The family is ubiquitous in humans, with GGT having especially high levels in the renal tubules, liver, and pancreas.

Note 2: The level of these enzymes can be elevated by a variety of diseases and especially so for liver disease and hepatic biliary obstructions. GGT is particularly sensitive to alcohol. Levels can be elevated from moderate intake as well as chronic alcoholism, even without clinical evidence of hepatic injury.

See: Acylation, Acyltransferases, Amino Acid Conjugation, Glutathione Conjugation, and Mercapturic Acid Metabolites.¹¹⁰⁻¹¹³

85. Genetic Polymorphism

A trait determined by a single gene that results in at least two phenotypes within a normal population, neither of which is rare, i.e., they are observed

for larger than 1 %. It is an important contributor to the large inter-individual variations that exist in the biotransformation of xenobiotics by humans, where different subpopulations metabolize the same compound with significantly different rates because of genetic polymorphism among at least one of the enzymes contributing to the associated metabolic pathway.

Note 1: Individuals can be classified as either “extensive” (rapid) or “poor” (slow) “metabolizers” on the basis of phenotypic differences, assessed by the amount of drug or metabolic standard compound that is excreted through any given metabolic pathway that is subject to genetic polymorphism.

Note 2: In addition to classifying the populations recruited for clinical studies, it is important to study species during preclinical and even early-stage drug development that are most representative for the anticipated metabolic pathways, including the latter’s potential variation. Given such complexity during development and the variation that can still result when approved drugs eventually become available to the general public, it is a common strategy not to advance candidate compounds through development that are suspected to become subject to metabolic pathways known to have genetic polymorphism that can be difficult to characterize for an individual within the population at large. CYP 2D6 is one example of such a metabolic pathway to try to avoid.

Note 3: Historically, the first genetic polymorphism described was for the *N*-acetylation of isoniazide. In this case, the slow acetylator phenotype is homozygous for the mutant allele. Early genetic polymorphisms pertaining to the common oxidative pathways were uncovered by the phenotypic differences observed for debrisoquine and mephenytoin metabolism.

See: Genotype/Genotyping, Phenotype/Phenotyping, Acetylation, Acetylation Phenotype, Alcohol Dehydrogenase (ADH), Atypical Alcohol Dehydrogenase, Aldehyde Dehydrogenase (ALDH), Aldehyde Dehydrogenase Polymorphism or Deficiency, Cytochrome P-450 Enzymes (CYPs), Drug-Like Properties or Profile, Extensive (or Rapid) Metabolizer, Poor (or Slow) Metabolizer, Cocktail Study, Single Nucleotide Polymorphism (SNP), and ADME. Specific Enzyme Refs.: 103,114-129.

86. Genotype/Genotyping

The term “genotype” can take on various meanings in different contexts, applicable to different aspects of xenobiotic metabolism considerations. For example, starting from the more global level and proceeding to a more localized context: (i) the type species of a genus; (ii) the entire genetic constitution of an individual; and (iii) the alleles present at one or more distinct gene loci. Genotyping, within the specific context of human disease and drug therapy, is the process of reading selected components of the sequence in selected strands of DNA in order to understand how mutations influence the onset, progression, and treatment of some diseases, including an individual’s drug metabolism profile.

Note 1: Because the genotypes responsible for the observed phenotypes associated with certain xenobiotic metabolism events have become very well established, it is possible to do some metabolic “genotyping” in an indirect manner for an individual by assessing the phenotypic response to administered standard probe compounds.

See: Genetic Polymorphism, Phenotype/Phenotyping, Cocktail Study, and ADME. Specific Enzyme Refs.: 103,114-129.

87. Glucocorticoid Responsive Element (GRE)

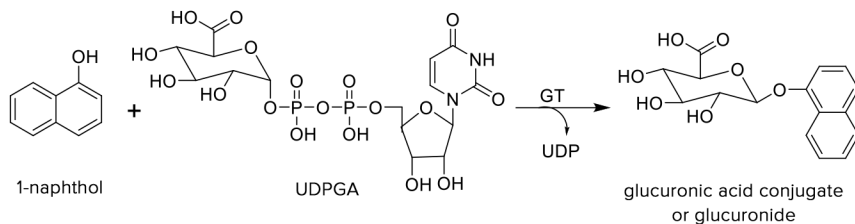
Short DNA sequence within the regulatory regions of genes that are recognized by the activated glucocorticoid receptor and provide specificity to induction of gene transcription by glucocorticoids. This control mechanism, in turn, impacts the status/phenotype for various components of the overall biotransformation machinery associated with the metabolism of xenobiotics, as well as several endogenous processes.

See: Aromatic Hydrocarbon Receptor (AHR), Peroxisome Proliferator Activator Receptor (PPAR), Pregnane X Receptor (PXR), and Xenobiotic Responsive Element (XRE).³⁵

88. Glucuronic Acid Conjugation (Glucuronidation)

A biotransformation where a substrate having a nucleophilic group is coupled to endogenous glucuronic acid. The latter is donated by uridine-5'-diphospho- α -D-glucuronic acid (UDPGA) serving as a cofactor, while the S_N2 -like coupling is enzymatically catalyzed via glucuronosyltransferase

(GT; EC 2.4.1.17). In addition to endogenous compounds, such as estrogen, xenobiotics having hydroxyl, sulfanyl, carboxylic acid, or primary amino groups can serve as substrates, with phenolic hydroxyl groups being particularly prone to this metabolic pathway. The latter is exemplified below for 1-naphthol.



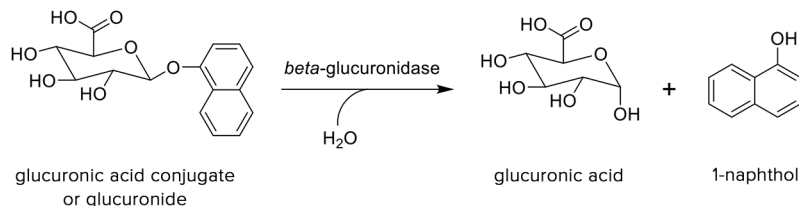
Note 1: This is a major pathway for the biotransformation of phenolic compounds. Because GT is abundantly expressed in both the gut wall and liver, as well as other locations, xenobiotics having such functionality are typically subject to a high clearance rate during first pass metabolism and thus have very poor bioavailability after oral administration or ingestion. GT's predominant intracellular location is within the lumen of the endoplasmic reticulum, where it resides in close proximity to the CYPs (see discussion in the Introduction section pertaining to the phases of drug metabolism).

Note 2: Both enzyme polymorphism and differing levels of the cellular UDPGA pools can lead to individual variation, but the human population on average remains well-equipped to accomplish this biotransformation and no significantly varying phenotypes have risen to merit further classification. Alternatively, in any individual case, the sustained exposure of this pathway to a substrate can lead to localized depletion of UDPGA and result in a shift to another, closely related conjugation pathway, also present in a significant functional capacity, namely that of sulfate conjugation or sulfation. The latter occurs on the same types of nucleophilic groups and is actually a competitive pathway. Although glucuronidation typically predominates, in some cases sulfation is the preferred pathway. Among mammalian models, the cat is distinct in its higher utilization of the sulfation pathway compared to glucuronidation.

See: Uridine-diphospho-glucuronic Acid (UDPGA), Glucuronosyl Transferase (GT), Conjugation Reactions, Phases of Drug Metabolism, Aromatic Hydroxylation, Glucuronide, Glucuronidase (β -Glucuronidase), and Sulfate Conjugation (Sulfation).^{45,130,131}

89. Glucuronidase (β -Glucuronidase)

This enzyme (EC 3.2.1.31) can catalyze the hydrolysis of glucuronic acid conjugates of xenobiotics, as well as endogenous materials, such as the mucopolysaccharides. A xenobiotic example is depicted below for the glucuronide of 1-naphthol.



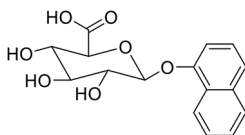
Note 1: Found in lysosomes, this enzyme is important for the catabolism of several endogenous materials. An inherited deficiency can lead to toxic accumulation of mucopolysaccharides, known as "Sly syndrome."

Note 2: Its presence in the gut wall and bacterial flora of the human GI tract can lead to enterohepatic cycling of glucuronide metabolites by reverting them back to their aglycone precursors, which can be reabsorbed. Repetition of this cycle can significantly prolong the residence time (half-life) of the aglycone in the body. This pathway may also be an energy conserving pathway that serves to recycle certain endogenous steroid hormones, rather than to rely only on their re-syntheses.

See: Glucuronic Acid Conjugation (Glucuronidation), Glucuronide, and Enterohepatic Cycling. Chemistry Ref.: 132.

90. Glucuronide

A common name for the metabolite derived from the conjugation of glucuronic acid to a xenobiotic. The term *beta-* or β -glucuronide is synonymous and is also frequently used. The β -glucuronide of 1-naphthol is depicted below.



Note 1: The original α -configuration of UDPGA, which serves as the donor for glucuronic acid, is inverted to a β -configuration for the latter during its S_N2 conjugation reaction with the xenobiotic.

Note 2: Glucuronides have an increase in molecular mass of about 175 Da, which, depending on the mass of the initial substrate, can result in relatively more biliary excretion compared to renal excretion, since the latter tends to prefer substrates having molecular masses below 500 Da, whereas the liver route continues to process compounds having masses above 500 Da.

Note 3: Glucuronides generally have a significant increase in polarity due to the additional hydroxyl groups, with the free carboxylic acid moiety, especially, being largely ionized at physiological pH. The acid moiety can also serve to enhance the suitability for excretion via the organic anion transporter (OAT) family. The OATs are particularly active in the liver, where they can transport such substrates into the bile.

Note 4: Glucuronides generally are inactive or have significantly reduced activity, although certain morphine conjugates represent an important exception. In addition, they are sometimes subject to hydrolysis by β -glucuronidase, which, in turn, may restore activity to the non-conjugated xenobiotic.

See: Glucuronide Conjugation (Glucuronidation), Uridine-diphosphoglucuronic Acid (UDPGA), Uridine-diphosphoglucuronosyl Transferase (UGT), Conjugation Reactions, Phases of Drug Metabolism, Excretion, Glucuronidase (β -Glucuronidase), and Enterohepatic Cycling.^{45,130,131}

91. Glucuronosyltransferase (GT) (Previously Uridinediphosphoglucuronosyl-transferase and UGT or UDPGT)

A family of enzymes (EC 2.4.1.17) that catalyze the conjugation of the glucuronic acid component of uridinediphosphoglucuronic acid to an appropriate nucleophilic functional group on small-molecule substrates.

Note 1: The GTs are 50 to 60 kDa integral membrane proteins where ca. 15 to 20 of the C-terminal amino acids form a transmembrane span of the endoplasmic reticulum (ER) lipid bilayer, with the remainder of the enzyme, including its catalytic domain, located within the lumen of the ER.

Note 2: These specific transferase enzymes, and their requisite glucuronic acid pools, are anatomically situated close to the many CYPs also residing along the ER, such that they seemingly can join in sequential metabolic reactions as part of a two-step enzymatic cascade involving a phase 1 and phase 2 biotransformation.

Note 3: About 25 human GT genes have been identified and divided into two families, based on at least 50 % homology of their amino acid sequences: TG 1 and TG 2. The GT 1A and 2B subfamilies appear to be the most important for drug metabolism, with several of their isoforms being highly expressed in the liver: GT 1A1, 1A3, 1A4, 1A6, 1A9 and UGT 2B4, 2B7, 2B10, 2B11 and 2B15.

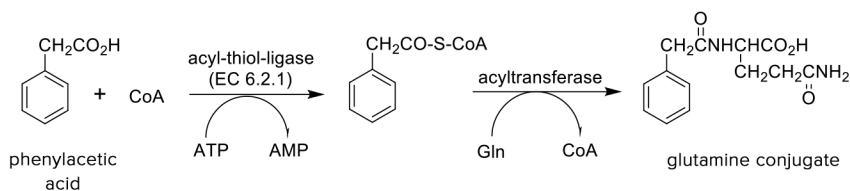
Note 4: Maturation of the GT pathways can lag during early life: neonates with underdeveloped GTs exhibit decreased clearance of bilirubin, a fairly common syndrome that can be alleviated by exposure to fluorescent light until enzymatic maturation normalizes, generally within just a few days.

Note 5: Several other inter-individual variations in GT expression levels have also been identified. At least six polymorphisms are known: GT 1A1, 1A6, 1A7, 2B4, 2B7 and 2B15. In addition, certain cancer cells can enhance their expression of the UGTs, thus promoting a phenotype with greater resistance to treatment by certain chemotherapeutic agents.

See: Uridinediphosphoglucuronic Acid (UDPGA), Conjugation Reactions, Phases of Drug Metabolism, Aromatic Hydroxylation, Glucuronic Acid Conjugation (Glucuronidation), Glucuronide, Glucuronidase (β -Glucuronidase), Sulfate Conjugation (Sulfation), Genetic Polymorphism, Genotype/Genotyping, and Phenotype/Phenotyping.^{45,63,66,130,131}

92. Glutamine Conjugation

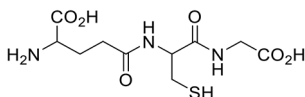
Conjugation of a xenobiotic's carboxylic acid group with endogenous glutamine according to the acylation pathway, where the acid moiety is first activated by attachment to coenzyme A. It is most applicable to xenobiotics having an aryl-acetic acid group (shown below), while glycine more generally serves as the endogenous partner across a much wider range of acid-containing compounds.



See: Acylation, Acyltransferase, Acetylation, Amino Acid Conjugation, Glycine Conjugation, Hippuric Acid Conjugate (Hippurate), and Serine Conjugation.¹³

93. Glutathione (GSH)

An endogenous tripeptide consisting of γ -Glu, Cys and Gly that serves as the major antioxidant produced by cells. It is also an important partner in biotransformation reactions catalyzed by glutathione transferase (EC 2.5.1.18) that can conjugate xenobiotics and metabolites, which contain potentially toxic electrophilic centers. Because of the latter, it is often referred to as “the body’s defensive nucleophile.” The structure of GSH is shown below in its reduced form.

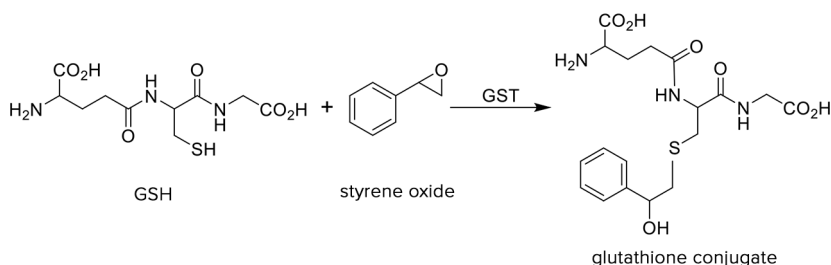


Note 1: GSH exists largely in the reduced form in cells (ca. 90 %), but is in equilibrium with its oxidized form (ca. 10 %), which is the disulfide linked dimer or “GSSG.” Higher levels of GSSG are indicative of oxidative stress.

See: Glutathione Conjugation, Glutathione Transferase, Cysteine Conjugate β -Lyase (C-S Lyase), Mercapturate/Mercapturic Acid Conjugation, and Phases of Drug Metabolism.^{45,133-138}

94. Glutathione Conjugation

A biotransformation where a xenobiotic substrate having a reactive electrophilic center is coupled to endogenous glutathione (γ -Glu-Cys-Gly or GSH). The coupling is catalyzed by a family of predominantly liver cytosolic enzymes called the glutathione transferases (GSTs; EC 2.5.1.18). The example below depicts the reactive xenobiotic styrene oxide undergoing bioinactivation due to conjugation by GSH.



Note 1: Serving as a “defensive” pathway to inactivate xenobiotics having electrophilic centers that might otherwise prove toxic, this biotransformation is applicable to a broad range of substrates, including: alkyl-, alkenyl-, aryl- and aralkyl-halides; carbons having sulfate or nitro leaving groups; small ring systems such as epoxides and β -lactones; and activated β -carbons of α,β -unsaturated carbonyl compounds (Michael acceptors).

Note 2: The initial GSH adduct has an increase in molecular mass of 307 Da, which, in turn, often enhances biliary excretion, since the latter tends to prefer substrates having molecular masses above 500 Da, whereas the renal route prefers molecular masses below 500 Da. However, these initial adducts frequently become subject to further metabolic processing by a specific pathway that sequentially removes the Glu and Gly and then acetylates the remaining Cys amino-group. The conjugate resulting from this catabolic process is called a “mercapturic acid” metabolite, and its molecular mass is only 162 Da. Not too dissimilar from the mass gained when a xenobiotic forms a conjugate with glucuronic acid (i.e., 170), this smaller increase leaves either the renal or the biliary route as viable options for excretion, depending on the mass of the initial xenobiotic compound.

See: Glutathione (GSH), Glutathione Transferase (GST), Bioinactivation, Catabolism, Mercapturate/Mercapturic Acid Conjugation, Cysteine Conjugate β -Lyase (C-S Lyase), ADME; ADMET, and Excretion.^{45,133-138}

95. Glutathione Transferase (GST)

A family of enzymes (EC 2.5.1.18) that catalyze the conjugation of endogenous glutathione (GSH) with xenobiotics having reactive electrophilic centers that could otherwise cause toxicity.

Note 1: Most GSTs are cytosolic homo- or heterodimers composed of ca. 25 kDa subunits from one of four major protein structural classes called *Alpha*,

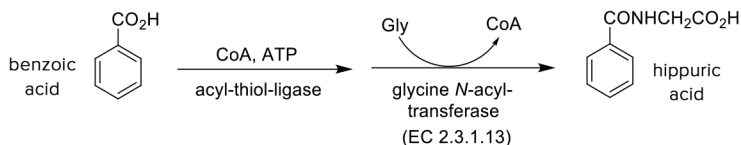
Mu, *Pi*, or *Theta*. There are multiple isoforms and allelic variants. Their nomenclature has adopted some of this structural description, e.g., GST A1, GST M1, etc.

Note 2: GST A1 is found in the kidney, intestine, lung, and liver. GST M1 is found mainly in the liver. GST P1 is widely distributed, except for in the liver, and is the most abundant in many types of tumor cells. A null GST M1 genotype has been identified, and these individuals may be more susceptible to lung cancer.

See: Glutathione (GSH), Glutathione Conjugation, Bioinactivation, and Mercapturic Acid.^{45,133-138}

96. Glycine Conjugation

Conjugation of a xenobiotic's carboxylic acid group with endogenous glycine according to the acylation pathway where the acid moiety is first activated by attachment to coenzyme A. This biotransformation is used to test human liver function by administering benzoic acid, which is converted to hippuric acid (exemplified below).



See: Acylation, Amino Acid Conjugation, Acyltransferase, Hippuric Acid Conjugate (Hippurate), Glutamine Conjugation, and Serine Conjugation.¹³

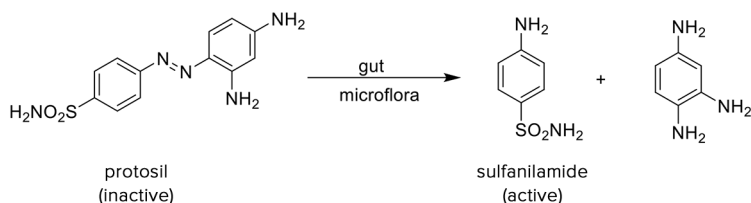
97. Glycosylation (or Glycosidation)

Enzymatic conjugation of an endogenous sugar with a substrate having an OH, NH, or SH group. While glucose is the most common partner for endogenous substrates, glucuronic acid is by far the most important partner for xenobiotic substrates. The latter is typically referred to by the more specific term "glucuronidation."

See: AGlucuronide and Glucuronic Acid Conjugation (Glucuronidation). Therapeutic Ref.: 139.

98. Gut Microflora

The community of mutualistic microorganisms living within the digestive tract of animals. They can impact drug metabolism in humans after oral administration, particularly by performing anaerobic biotransformations that are less prevalent among the various human pathways. An example would be the reduction of various types of double bonds. The sulfonamide antibiotics were discovered as a result of this type of microbial biotransformation (shown below) after oral administration of the azo dye protosil, which became recognized as an effective antibiotic prodrug.



Note 1: In some cases, the gut microflora can hydrolyze glucuronide conjugates excreted via the bile. When the parent compound is liberated, this can lead to enterohepatic cycling.

Note 2: Oral administration of antibiotics leads to an alteration of the various individual populations of bacteria within the overall gut microflora, which, in turn, can lead to altered metabolic profiles and clearance of other orally administered drugs and ingested xenobiotics.

See: Drug Metabolism, First Pass Metabolism, Bioactivation, Prodrug, Glucuronide, Glucuronic Acid Conjugation (Glucuronidation), and Enterohepatic Cycling. Therapeutic Ref.: 140.

99. Half-Life ($t_{1/2}$)

Used in xenobiotics in general, the time required to decrease the concentration of the solute of interest by one-half from a specified matrix. Most typically used in relation to the elimination of a pharmaceutical agent, in which case it is called the "elimination half-life" and refers to the time it takes for 50 % of the agent to be cleared from the body by metabolism and/or excretion. Half-life values are only constant (always the same and independent of the concentration and dose) for linear kinetic processes.

The half-life for any linear kinetic process can be related to a corresponding rate constant (k) by the natural logarithm ($\ln$) relationship:

$$t_{1/2} = \ln(2) / k \approx 0.693 / k$$

Note 1: Matrices can range in complexity from a whole animal, as indicated above for the elimination half-life, to various compartments within or taken from a whole animal, as well as to specified cells or *in vitro* systems, including simple aqueous media, where decreasing concentrations of the solute can still result from spontaneous decomposition or precipitation.

Note 2: For pharmacokinetic studies (PK analyses), the term can be applied to any such PK processes, e.g., in addition to the elimination half-life, absorption and distribution half-lives can also be defined. When compartmental or non-compartmental PK models have several exponential terms, the half-life value that is generally of the most interest and most frequently reported is called the “terminal half-life” or “terminal elimination half-life,” which can be derived from the exponential term with the smallest rate constant and hence the longest half-life. As such, it then constitutes the slowest and final removal process.

Note 3: It is also possible to cite an “effect” or “pharmacodynamic half-life” where no measurement of the xenobiotic’s concentration has been obtained.

See: Disposition, Distribution, Clearance, Pharmacokinetics, Compartment Model, Non-Compartment Model, and Pharmacodynamics.^{38-40,104}

100. Hepatic Clearance/Extraction

The ability of the liver to remove substances from the blood. Clearance is dependent on hepatic blood flow in terms of percentage (100 % directed by the hepatoportal circulation; 25 % of the cardiac output/systemic circulation) and rate, hepatic uptake of the substance by passive and active transport versus efflux by active transport, excretion of the substance into the bile, and the liver’s rich complement of metabolizing processes.

See: Hepatoportal Circulation, Bioavailability, Clearance (CL), Excretion, Drug Metabolism, and Extraction Ratio.^{38-40,104}

101. Hepatocytes

The predominant cell type in the liver, taking up about 90 % of this organ's volume. Their primary function is associated with the intermediary metabolism of food-related nutrients and endogenous materials and in the biotransformation and clearance of xenobiotic substances.

Note 1: These cells, and thus the liver, are the richest source of the overall CYP family members expressed in humans. They also have high levels of the metabolic components associated with glucuronidation.

Note 2: Hepatocytes from various mammalian species are often harvested, in a form called the microsomal fraction or microsomes, for use in conducting *in vitro* drug metabolism studies.

See: Hepatoportal Circulation, Hepatic Clearance/Extraction, Bioavailability, Drug Metabolism, First Pass Metabolism, Bioinactivation, Bioactivation, Cytochrome *P*-450 Enzymes (CYPs), Conjugation Reactions, Glucuronic Acid Conjugation (Glucuronidation), and Microsomes.¹⁴¹⁻¹⁴³

102. Hepatoportal Circulation

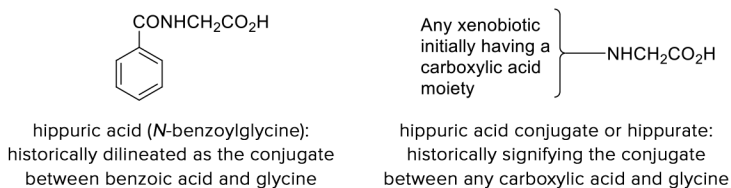
The vasculature system that provides venous drainage from the capillaries in the spleen, stomach, small and large intestines, pancreas, and gallbladder and delivers all of its flow to the liver. This localized uptake and delivery forces all materials absorbed after oral ingestion (food and xenobiotics) to enter liver hepatocytes, where they have the potential to be metabolized, before flowing to the heart/lungs and into the general circulation.

See: Absorption, Hepatic Clearance/Extraction, Bioavailability, Hepatocytes, Drug Metabolism, First Pass Metabolism, Bioinactivation, Bioactivation, and Enterohepatic Cycling.¹⁴⁴

103. Hippuric Acid Conjugate (Hippurate)

Historically, this phrase has been used to designate the metabolite obtained from the conjugation of a xenobiotic having a carboxylic acid moiety, with a glycine residue via an acylation type of biotransformation. This process was first described for benzoic acid, which forms actual "hippuric acid,"¹² and the phrase was then extended to include any glycine conjugate with a

carboxylic acid group that is present on a xenobiotic. Today, this phrase is instead falling in usage, and it is no longer officially recognized by IUPAC. The pathway is itself part of the even broader classification noted as amino acid conjugation. Regarded as the first biotransformation discovered, this "first metabolite" is depicted below, along with how the term has historically been used more broadly in practice.



Note 1: The molecular mass of the initial carboxyl-containing xenobiotic will be increased by 57 Da, while the glycine residue adds a new carboxylic acid group, thus retaining the potential to be excreted by the organic anion transporter (OAT), which is rich in the liver and is directed toward bile formation.

See: Conjugation Reactions, Acylation, Amino Acid Conjugations, Glycine Conjugation, and Serine Conjugation.¹³

104. Holoenzyme

The complete and generally functional complex of an enzyme plus all of its requisite prosthetic groups and cofactors, assuming a source of energy is likewise available.

Note 1: By definition, this complex will be active in its native environment. However, since it is additionally necessary to adopt a functional conformation within an exogenous setting, the complex may not be active in an *in vitro* matrix. Membrane-bound enzymes and transporters are particularly subject to this situation.

Note 2: Alternatively, it is also possible to achieve "functional" activity, even in an *in vitro* setting, using specifically modified and truncated protein forms of the enzyme, as long as the key residues of the active site are preserved, appropriate conformational arrangements remain accessible, and all of its requisite prosthetic groups, cofactors, and an energy supply

are provided. This strategy is often deployed to render the enzymes more amenable toward crystallization followed by X-ray diffraction studies.

See: Enzyme, Apoenzyme, Cofactor, and Prosthetic Group.³⁴

105. Hydrolases

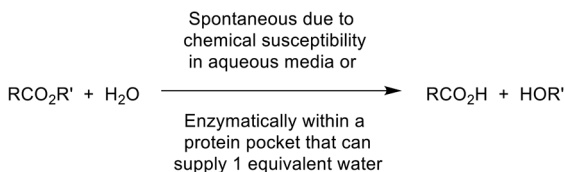
Within the context of drug metabolism, this term generally refers to hydrolysis enzymes that specifically catalyze the *trans*-addition of water to alkene epoxides and arene oxides, e.g., the microsomal and soluble epoxide hydrolases (EC 3.3.2.9 and EC 3.3.2.10). Epoxides and arene oxides are sometimes formed during CYP-mediated oxidation of aliphatic alkenes and aromatic hydrocarbons, respectively. Because they can be reactive, the hydrolases thus serve as important detoxification enzymes.

Note 1: In mammals, two major forms of epoxide hydrolase (EH) handle the majority of xenobiotic-related biotransformations, one being membrane-bound (microsomal), mEH, and the other present in the soluble fraction, sEH.

See: Hydrolysis, Epoxidation, Epoxide Hydrolases, and Detoxification.¹⁰¹

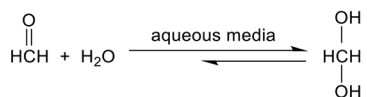
106. Hydrolysis

A reaction involving the addition of water to a functional group so as to permanently form one or more new species prompted by either spontaneous chemical behavior or an enzymatically catalyzed biotransformation. The case of a simple ester hydrolysis is shown below in a general manner where two new compounds are produced.



Note 1: Within the context of xenobiotic metabolism (but not necessarily that of chemical terminology), this definition becomes distinguishable from "hydration," which designates the formation of new species in rapid chemical equilibrium with water, e.g., hydrogen-bonded alcohols and

amines in an aqueous media, and likewise for even the gem-diol forms of hydrated ketones or aldehydes, as shown below for formaldehyde, whose equilibrium favors the "water-added" species.¹⁴⁵



Note 2: Common metabolizing enzymes that catalyze the hydrolysis of both endogenous and xenobiotic compounds include: peptidases; carboxylesterases, such as the cholinesterases and pseudocholinesterases, organophosphatases; and epoxide hydrolases.

See: Hydrolases, Epoxide Hydrolase, Esterases, Carboxyl Esterases, Carboxpeptidases, and Biotransformation.⁵¹

107. Hydroxylation

A biotransformation where a hydrogen atom on a xenobiotic is replaced by a hydroxyl-group. The most common type occurs on an aryl system, namely "aromatic hydroxylation."

See: Aromatic Hydroxylation.³⁷

108. Inducing Agent

A substance that causes enzyme induction and an enhancement of metabolizing activity. For xenobiotics, this is most often observed following repeated exposure to such a substance.

See: Induction and Autoinduction.¹⁴⁶⁻¹⁴⁸

109. Induction

An increase in enzyme activity above its normal, homeostatic level. Induction can result from increased expression of the enzyme or proenzyme, increased conversion of a proenzyme, increased liberation from a storage complex, decreased inactivation by negative control systems, such as the kinases, or decreased catabolism of the enzyme. In cases where levels of a cofactor or prosthetic group become the rate limiting factor for a holoenzyme's activity, their induction by any of these same mechanisms can likewise result in enzymatic induction. The most common mechanism

associated with the metabolism of xenobiotics is increased expression of the active enzyme.

Note 1: Mutations that enhance activity can occur, and these are then typically also referred to as having been “induced” when prompted by a xenobiotic. While prominent in microorganisms for both induction and inhibition, this type of induction is not a common occurrence within the mammalian drug metabolizing enzymes.

See: Inducing Agent, Autoinduction, Enzyme, Apoenzyme, Holoenzyme, Cofactor, Prosthetic Group, Ah Receptor, Xenobiotic Response Element (XRE), Pregnane X Receptor (PXR), Anabolism, Catabolism, Drug-Drug Interactions, and Phenobarbital Sleeping Time.¹⁴⁶⁻¹⁴⁸

110. Inhibition

A reduction in enzyme activity below its normal, homeostatic level relative to a given substrate that results from the interaction of the enzyme with an inhibiting substance. For xenobiotics, this can be observed upon the first exposure to substances capable of inhibiting the system.

Note 1: In some cases, the substance may be the substrate itself or a substrate’s enzymatic product; these events are also referred to as “autoinhibition” or “product inhibition,” respectively.

Note 2: The inhibiting substance may interact with the enzymes’ active (functional) site in either a competitive (reversible binding and also itself serving as a substrate) or non-competitive (typically covalent binding and itself not serving as a substrate) manner. Alternatively, the inhibiting substance may interact with a regulatory site on the enzyme, in which case the event is also referred to as “allosteric inhibition.”

Note 3: Exogenous inhibitors can be present in dietary materials, as well as drugs. The furanocoumarins present in grapefruit juice represent a classical example for inhibition of CYP 3A4.¹⁴⁹

Note 4: In addition to these drug metabolism related comments, it can be mentioned in a very general manner in relation to drug discovery aimed at enzymatic targets or specific receptor systems that it is typically far easier to

inhibit a given enzyme or receptor than it is to design a superior substrate or agonist for that system.

See: Autoinhibition, Competitive Inhibition, Noncompetitive Inhibition, Substrate Inhibitor, Mechanism-Based inhibitor, and Suicide Inhibitor. Drug Metabolism Related Refs.: 146-148.

111. Intraperitoneal Dosing

Administration of a pharmaceutical agent into the body cavity (peritoneum) surrounding the lower stomach/upper GI tract, such that its absorption is dependent on the hepatoportal circulation but is not dependent on "oral absorption" across the lining of the stomach and intestines. By definition, this venous return circulation proceeds to the liver, thus still retaining this particular component of the "first pass effect."

Note 1: While intraperitoneal ("IP") dosing is not a common route in humans, it is frequently deployed when testing substances during *in vivo* studies in small animals to eliminate some of the variables associated with oral bioavailability.

See: Absorption, Hepatic Clearance/Extraction, Bioavailability, Drug Metabolism, First Pass Metabolism, Bioinactivation, Bioactivation, and Intravascular Dosing.¹⁴⁴

112. Intravascular Dosing

Administration of a pharmaceutical agent directly into the systemic blood circulation, intravenously (commonly abbreviated as "IV") utilizing the venous return to the heart or intra-arterially utilizing arteries delivering blood to the body. By definition, there is no "absorption" step when a drug is given in this manner.

Note 1: Intravenous administration is a common route for agents where: (i) an immediate drug effect is needed, such as in emergency or critical care situations; (ii) the drug has poor or erratic oral bioavailability, and particularly so when it does not need to be frequently taken in a repetitive manner for prolonged periods; or (iii) the drug can be highly toxic to normal cells, as is the case for certain chemotherapeutic agents, particularly several of the anticancer drugs.

Note 2: For the purposes of PK studies, the timeframe for absorption can either be dropped or taken as instantaneous when devising mathematical models.

Note 3: While this route eliminates the entire “first pass effect” by bypassing the GI tract, its membrane linings, and the liver, the IV route will still pass through the bronchopulmonary circulation prior to arterial delivery to the entire systemic blood circulation. Since the lungs represent a significant source of drug metabolizing capability and provide a “second pass effect” after oral absorption of xenobiotics, this type of passage after IV administration can be thought of as a “pseudo first pass effect” where the pharmaceutical agents are instead first exposed to the lungs.

See: Absorption, Extravascular Dosing, Bioavailability, First Pass Effect, and Pharmacokinetics (PK).⁹¹

113. Intrinsic Clearance

The relationship between a xenobiotic's rate of metabolism and its concentration at the enzyme site.

Note 1: In biochemical terms, it is derived from the ratio of the Michaelis-Menten parameters for maximum rate of metabolism ($V_{\max}$) and the drug interaction constant (K_m).

Note 2: Conceptually important, in that no matter how fast a xenobiotic may be determined to be metabolized during an *in vitro* study involving its incubation with selected enzymes, it may undergo little metabolism and essentially no intrinsic clearance by those same enzymes unless it also becomes exposed to them in adequate concentration and time periods *in vivo*. Alternatively, if the *in vitro* study demonstrates essentially no intrinsic clearance of the xenobiotic by a given enzyme, then it becomes unlikely that this particular biotransformation will take place no matter how high and long the exposure of the xenobiotic to that same enzyme *in vivo* (assuming no other enzymes are able to cause the identical biotransformation).

See: Clearance, Michaelis-Menten Kinetics, and ADME.^{38-40,45,104}

114. Isoform or Isozyme (Isoenzyme)

A member of an enzyme family that shares comparable catalytic sites, prosthetic groups, cofactors, and reaction mechanism but where the isoforms exhibit different substrate specificities or reaction rates due to genetically determined differences in the primary structure of their protein components. Although certainly relevant historically, there has been a recent trend to abandon these distinctions and simply call them “enzymes,” accompanied by whatever distinct informational content may then be needed.

Note 1: Individual variation in drug metabolizing capabilities can result from expression of different isoforms and from differential expression levels of the same isoform, i.e., individuals having the same genetic makeup in terms of drug metabolizing enzymes can still differ in their drug metabolizing capabilities due to differences in their expression levels.

See: Acetylation Phenotype, Aldehyde Dehydrogenase Polymorphism or Deficiency, Atypical Alcohol Dehydrogenase, Cytochrome P450 Enzymes (CYPs), Extensive (or Rapid) Metabolizers, and Genetic Polymorphism.^{103,114-129}

115. IVIVE (Sometimes IVIV)

Acronym designating the use of *in vitro* to *in vivo* extrapolation to predict pharmacokinetics and metabolic drug-drug interactions for the *in vivo* case based upon results obtained from *in vitro* studies.

See: ADME; ADMET, Pharmacokinetics, and Metabolic Prediction/Possibilities versus Probabilities.^{150,151}

116. Ligand

Within the context of xenobiotic metabolism, this term can be used to describe two different physical settings. The first refers to the metal ions that often play a role as requisite cofactors in many of the holoenzyme complexes, the latter usually having a distinct chelation arrangement with preference for a specific metal ligand species. The second refers quite generally to any compound that can bind to an enzyme's active or allosteric regulatory sites in any capacity, whether or not they behave as substrates or regulators.

See: Allosteric Regulation, Cofactors, Enzymes, Holoenzymes, and Prosthetic Groups.¹⁵²

117. Linear Kinetics

Any kinetic process for which the rate (R) of a xenobiotic's or metabolite's appearance (e.g., absorption or production) or disappearance (e.g., clearance) is directly proportional to the local concentration (C) or amount of the xenobiotic or parent agent (A). This relationship can be expressed mathematically as

$$R = kC \text{ or } R = kA$$

where, in both cases, " k " is a constant called the "rate constant." By convention, the rate of appearance of the substance is taken to be positive, while the rate of disappearance is taken to be negative.

Note 1: Nonlinear kinetic processes that follow Michaelis-Menten kinetics (e.g., saturable enzymes or transporters) are often approximated by linear kinetics, as long as the local concentration is much less than the Michaelis-Menten constant, K_m . This relationship can be expressed as

$$R = V_{\max} C / (K_m + C) \approx (V_{\max} / K_m) C$$

when $C \ll K_m$, because $V_{\max}/K_m$ is a constant, such that the rate equation approximates the linear kinetic equation.

See: Half-Life ($t_{1/2}$), Enzyme, Nonlinear Kinetics, and Michaelis-Menten Kinetics.^{38-40,104}

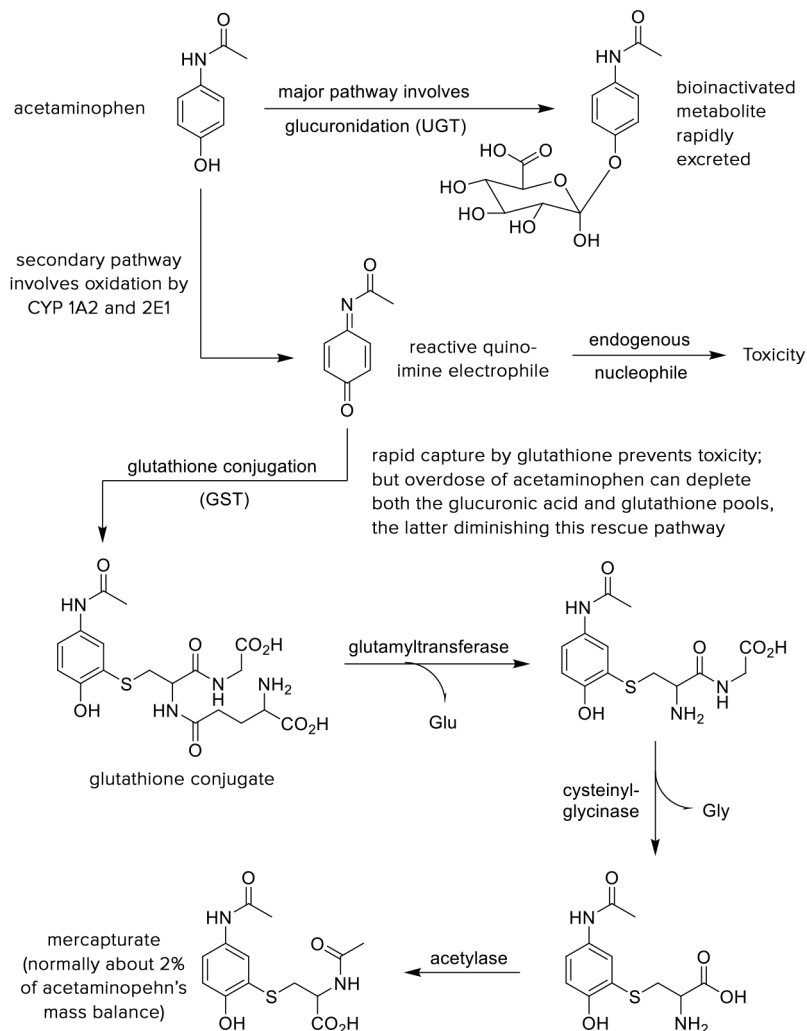
118. Mechanism-Based, Product and Transition State Inhibitors

Mechanism-based inhibition of an enzyme results from the interaction of an intermediate or product (aka "product inhibition") formed during the metabolic reaction. Typically occurring within the enzyme's active site, the interaction can become either competitive or noncompetitive. When the intermediate is a transient species representative of the reaction's transition state, such compounds are also referred to as "transition state inhibitors." The latter term additionally encompasses agents designed to directly mimic the transition state without the need for any mechanism-based conversion to occur.

See: Autoinhibition, Competitive Inhibition, Noncompetitive Inhibition, Substrate Inhibitor, and Suicide Inhibitor.⁶²

119. Mercapturate/Mercapturic Acid Conjugation

The *N*-acetylcysteine metabolite of a xenobiotic after the latter has first undergone a conjugation reaction with glutathione and then been further metabolized by a three-step process that modifies the initial glutathione adduct. The overall process of glutathione conjugation followed by the sequence of three metabolic events is referred to as "mercapturic acid conjugation." The example below depicts a reactive quino-imine electrophile being captured and detoxified by this overall pathway.



Note 1: The initial glutathione adduct of a xenobiotic parent or the latter's metabolite has an increase in molecular mass of 307 Da, which, in turn, often enhances relative biliary excretion, since it tends to handle substrates having molecular masses above 500 Da, compared to the renal route, which prefers molecular masses below 500 Da. After further metabolic processing by this specific catabolic pathway that sequentially removes the Glu and Gly and then acetylates the remaining Cys amino-group, the resulting mercapturate's molecular mass is only increased by 162 Da compared to the parent xenobiotic. This smaller increase, which is not too dissimilar from the mass gained when a xenobiotic forms a conjugate with glucuronic acid (i.e., 175), leaves either the renal or the biliary route as viable options for excretion, depending on the mass of the initial xenobiotic compound or its metabolite.

See: Glutathione (GSH), Glutathione Conjugation, Glutathione-S-Transferase (GST), Bioinactivation, Bioactivation, Catabolism, Cysteine Conjugate β -Lyase (C-S Lyase), ADME; ADMET, and Excretion.^{45,133-138}

120. Metabolic Capacity

This term finds wide use in the context of nutrition and exercise, where it includes all of the body's anabolic and catabolic processes across all endogenous and exogenous substances. It takes on two, much narrower, definitions when used in the context of drug metabolism, where it becomes either the maximum rate at which a given compound can be metabolized by the sum of the participating enzymes, whether identified or unknown, or, alternatively, the maximum catalytic rate that a specified (and thus known) enzyme can achieve under optimal conditions for an ideal substrate.

Note 1: Excretion is excluded from this biotransformation-dependent definition, just as it is also not encompassed by the phrase "metabolic clearance." Alternatively, the phrase "overall clearance" encompasses both metabolism and excretion considerations.

See: Drug Metabolism, Anabolism, Catabolism, Clearance, and Metabolic Clearance.^{38-40,104}

121. Metabolic Clearance

The volume of blood cleared of a specified compound by the organism's metabolism per unit of time.

See: Metabolic Capacity, Drug Metabolism, Anabolism, Catabolism, and Clearance.^{38-40,104}

122. Metabolic Fate

Typically, the delineation of all metabolic events that contribute to, and only to, a parent xenobiotic's complete clearance. In a less common but broader sense, the delineation of all metabolic events and pathways that occur on a xenobiotic plus all of its metabolites until the levels for all of these materials are no longer detectable anywhere in the body.

See: Drug Metabolism, Metabolic Clearance, Clearance, and Metabolic Pathway/Pattern/Profile.^{38-40,45,91,104}

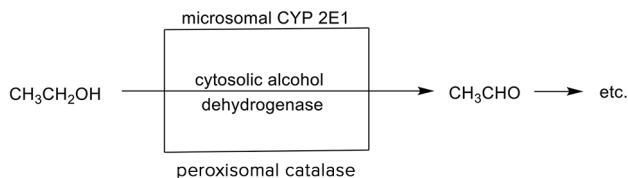
123. Metabolic Pathway/Pattern/Profile

Starting from a parent, xenobiotic compound, a metabolic pathway is either a single, end-point metabolic event or, more frequently, a series of metabolic events that proceed sequentially in a step-wise manner. The latter is also referred to as "sequential metabolism." The parent compound is most often subject to multiple metabolic pathways (also referred to as "competing metabolism"), which can each, in turn, also divide into multiple pathways, some of which can be designated as "complementary" or "overlapping metabolism." An accounting of the relative formation of metabolites along all of the pathways engaged by a given xenobiotic constitutes the latter's metabolic pattern or profile. Examples are provided below in a general manner for a xenobiotic "X" and its various metabolites M#. One example specifically uses ethanol.

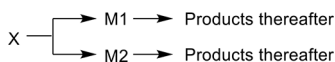
Single step pathway: $X \rightarrow M1 \rightarrow \text{Excretion}$.

Sequential pathway/metabolism: $X \rightarrow M1 \rightarrow M2 \rightarrow \text{etc.} \rightarrow \text{Excretion}$.

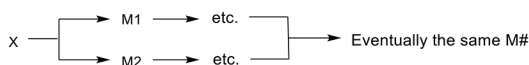
Complementary pathways/metabolism:



Competing pathways/metabolism:



Overlapping pathways/metabolism:



Note 1: A common theme in drug metabolism involves the sequential pathway wherein a xenobiotic compound is first “functionalized” in an appropriate manner by an oxidative (“Phase I”) metabolic step so as to subsequently become a good substrate for a conjugation (“Phase 2”) biotransformation. An example of this would be aromatic hydroxylation to insert a phenolic hydroxyl-group that then undergoes rapid glucuronidation, which, in turn, would likely be followed by excretion. A thorough discussion of this topic can be found in the introduction.

See: Drug Metabolism, Aromatic Hydroxylation, Conjugation, Glucuronic Acid Conjugation (Glucuronidation), Phases of Drug Metabolism, Alcohol Dehydrogenase (ADH), Catalase, and Metabolic Ratio.^{45,82,91}

124. Metabolic Prediction/Possibilities versus Probabilities

A prediction of possible metabolites and metabolic pathways after the administration of a clinical candidate compound to humans can be obtained by software programs associated with either expert systems or user-friendly databases. These programs typically convey all of the metabolic possibilities that could happen to a given query molecule across an “averaged mammalian” (and thus hypothetical) species. As such, they are generally extremely exhaustive and are unlikely to miss something that is eventually observed experimentally. However, their ability to consider relative rate data between the potentially competing possibilities, and thus provide a ranked list of only the metabolic probabilities that are most likely to actually occur, has yet to be successful.

Note 1: Even the software packages having expert systems which assign a probability ranking to a suggested metabolic possibility typically designate a number between four and six for a given possibility, while using a ranking scale that runs from 1 to 10, and thus lack adequate discriminatory power.

See: Drug Metabolism, Biotransformation, ADME, Metabolic Fate, Metabolic Pathway/Pattern/Profile, and Metabolic Ratio.^{2,90,115,153}

125. Metabolic Probe

A substance whose rate of metabolism or metabolic clearance can be used as a marker for the activity of a specific enzyme or for a distinct enzyme system associated with a specific metabolic pattern. Similarly, a selective inhibitor or inducer of a specific enzyme that can be used to alter the role that the enzyme might be playing in another compound's metabolism.

Note 1: Tables are available of substances that have at least one metabolite known to be reproducibly formed after interaction with a specific enzyme, which allows monitoring of that enzyme by measuring the probe's metabolite. Likewise, tables are available of substances known to be selectively cleared by a single metabolic pathway, and these can be used to directly monitor the status of that pathway. Such data can be used to profile an individual's genotype/phenotype and/or help characterize the metabolic pathways undertaken by a new drug candidate. Tables of selective inhibitors and inducers of specific enzymes are available, and these can also be deployed to study their effect on the metabolic fate of new drug candidates, so as to implicate pathways likely to be operative for the new compound.

See: Drug Metabolism, Pharmacogenetics and Pharmacogenomics, Metabolic Fate, and Metabolic Pathway/Pattern/Profile.^{115,119,126,128,150,151}

126. Metabolic Profiling/Fingerprinting

The process of experimentally assessing the metabolic fate or metabolic pattern/profile for a given xenobiotic, while assigning as much structural detail as possible among the metabolites, along with their metabolic ratios. When structural information cannot be ascertained for a given metabolite or for several metabolites, their presence can be said to be "fingerprinted" in a qualitative manner if they are detectable in a reproducible fashion from one experimental run to another. Quantitation for the fingerprint of a group

of unidentified metabolites may still be possible when a radiolabel is being deployed as part of the experiment.

Note 1: These types of experiments are often undertaken by deploying liquid chromatography-dual mass spectrometry (LC-MS/MS) so that both the molecular weight and distinct fragmentation pattern for a given metabolite can be ascertained after its separation by chromatography. Software is available to predict metabolites based on this type of data relative to the known structure of the parent compound.

See: Drug Metabolism, Pharmacogenetics and Pharmacogenomics, Metabolic Fate, Metabolic Pathway/Pattern/Profile and Metabolic Ratio.^{115,119,126,128,150,151}

127. Metabolic Ratio

The mass ratio of a given metabolite to the parent compound or to another metabolite, most often accounted for by tracking an initial radiolabel-to-mass relationship present in the parent compound. When only a mass balance is pursued, it is necessary to account for increases in molecular weights due to the formation of conjugates, assuming that such structural information can be discerned. Alternatively, when structural detail allows, this ratio is sometimes accounted for by mole percent, which inherently adjusts for changes in molecular weights in either direction from the parent compound.

See: Metabolic Pathway/Pattern/Profile.^{38-40,91,104}

128. Metabolic Switching

In relation to drug discovery, today's common use of this phrase pertains to when a derivative of an initial xenobiotic undergoes a different biotransformation pathway after a specific metabolic event becomes avoided or diminished due to a synthetic modification of the initial xenobiotic's structure. Differences in metabolic profile due to the same xenobiotic's administration by different routes does not constitute metabolic switching.

Note 1: It is a standard strategy during drug development to alter a candidate compound's metabophore if it is responsible for too short a duration of action, so as to avoid the implicated metabolic pathway and potentially

prolong the duration. This becomes a challenge, however, when the modified drug candidate then switches to another metabolic pathway that is still rapid enough to cause the new analog to retain too short a duration of action.

Note 2: Historical use of this phrase has pertained to such switching relative to isotope exchange effects during biochemistry studies¹⁵⁴ and to various enzyme modifications during molecular pharmacology studies,¹⁵⁵ as well as to the dynamics of a living system's gross metabolic/catabolic state relative to changes in diet, etc.¹⁵⁶

See: Drug Metabolism, Biotransformation, Metabolic Pathway/Pattern/Profile, and Metabophore.¹⁵⁷

129. Metabolism

A general term that encompasses all of the physical and chemical processes involved in the maintenance and reproduction of life in which nutrients and endogenous materials are broken down (catabolism) to liberate energy that can be captured and used by the organism (heterotrophic organisms), as well as to generate simpler molecules that may be used in turn as building blocks to form more complex molecules (anabolism) that can perform specific functions at the molecular level. To convey a more specific context relative to xenobiotics or drugs, the phrase "xenobiotic or drug metabolism" should be used.

See: Drug Metabolism, Biotransformation, Anabolism, Catabolism, and Metabolomics.^{53,79,80,82,158}

130. Metabolite versus Decomposition and Degradation Products

Metabolites are any intermediate or product resulting from an enzymatically catalyzed event. Materials resulting from autoxidation and spontaneous chemical reactions are not regarded as metabolites, even when prompted within select anatomical compartments or under specific physiological conditions, such as pH 7.4 and 37°C. Such materials would be called decomposition products. The interrelated term "degradation products" can encompass both metabolic degradation and spontaneous decomposition.

Note 1: Autoxidation can complicate the study of a xenobiotic's metabolism. In some cases, this can be remedied by the use of antioxidants, including examination of the roles of the CYP-450s, despite the latter's inherent requirement for oxygen.¹⁵⁹⁻¹⁶¹

See: Drug Metabolism and Biotransformation.^{2,45,46,79,80,91-93}

131. Metabolomics/Metabonomics

The study of all endogenous biomolecules, metabolites, and degradation materials within a cell or organism under both normal and other conditions, including exposure to xenobiotics, as well as various disease states.

Note 1: The focus is on qualitative, and when possible quantitative, analyses of the endogenous materials, rather than on the xenobiotic and its metabolites, so as to ascertain the endogenous materials' functions, interconnections, and regulation, while adopting a systems biology approach. In relation to xenobiotic exposures, this can include alterations that perturb normal ratios and concentrations, or binding and fluxes, of endogenous biomolecules, either by the xenobiotic's direct chemical reaction or by its binding to regulatory proteins or nucleic acids that control metabolism.

See: Metabolism, Drug Metabolism, and Pharmacogenetics and Pharmacogenomics. Refs. associated with xenobiotics: 162-166.

132. Metabophore

The specific spatial arrangement of the key structural features within an overall molecule that prompt the compound to be a substrate for a given metabolizing enzyme.

Note 1: The features can also be thought of in terms of their distinct electronic surface potentials, which match corresponding regions for recognition, binding, and catalysis by the enzyme, either simultaneously or in a dynamic fashion involving mutual molding across a sequence of associations.

Note 2: Generally taken to encompass the specific set of molecular features on a xenobiotic that can lead to a distinct metabolic reaction, such as the specific structure-metabolism relationships applicable to *N*-dealkylation by CYP 2D6.

Note 3: The term is analogous to the more commonly used terms “pharmacophore” and “toxiphore,” which apply to pharmacological efficacy and toxicology, respectively.

See: Drug Metabolism, Biotransformation, Enzyme, Metabophore Probe, and Structure-Metabolism Relationship (SMR).^{1-3,15,62,79,80,82,85,90,91,153} Refs. associated with prodrugs and soft drugs: 167-170.

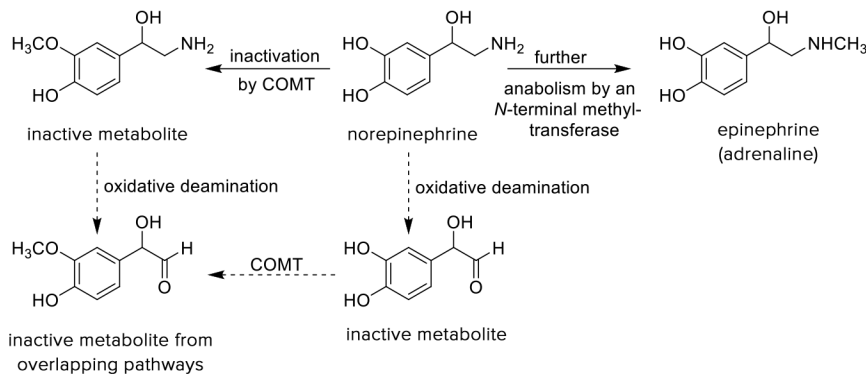
133. Metabophore Probe

A molecule with defined chemical features that is used to assess the relevance of those features for recognition, binding, and/or metabolism by a distinct enzyme or by a select enzyme system associated with a given biotransformation. Deployment of a series of such probes (metabolism-related directed-library) can be used to assemble a database of structure-metabolism relationships relevant for the enzyme/system under investigation.

See: Metabophore and Structure-Metabolism Relationship (SMR).^{1-3,15,62,79,80,82,85,90,91,153} Refs. associated with prodrugs and soft drugs: 167-170.

134. Methylation

Biotransformation of a xenobiotic involving conjugation of catechols, *N*-heterocyclic systems, and some sulfanyl- and phenolic hydroxy-groups, with a methyl-group donated in nearly all cases by *S*-adenosyl-L-methionine. These metabolites are less water soluble than their parent molecules, but generally are less active/toxic, particularly for a catechol group. Numerous endogenous materials also undergo this reaction as part of anabolism or as a regulatory step that can cause either activation or inhibition. The example below shows how methylation of norepinephrine can serve as both an anabolic-related event and a bioinactivation event.



Note 1: Several methyltransferase enzymes (EC 2.1.1) form an overall family that can accommodate a wide range of endogenous and exogenous structural types. Class I members have a protein fold that specifically binds *S*-adenosyl-*L*-methionine as a cofactor, and these are the most prominent for methyl conjugation of xenobiotics. Common examples include catechol *O*-methyltransferase (COMT), as shown above, and thiol methyltransferase, which adds a methyl-group to captopril.

See: Conjugation Reactions, Conjugate, Deamination/Oxidative Deamination; Methyltransferase, *N*-Methyltransferases, and Catechol *O*-Methyltransferase (COMT). General Ref: 171.

135. Methyltransferase

A heterogeneous family of enzymes (EC 2.1.1) that catalyze the transfer of a methyl-group to several endogenous materials and to xenobiotic compounds having a catechol, *N*-heterocyclic system, or a sulfhydryl- or phenolic hydroxyl-group. *S*-adenosyl-*L*-methionine (SAM) serves as the source of the methyl-group for the biotransformations that are most relevant for drug metabolism in humans.

Note 1: A practical categorization for this broad family's members can be arranged according to general substrate types: protein methyltransferases, which are often important for functional regulation of the proteins; DNA methyltransferases; xenobiotic methyltransferases; and non-SAM-dependent methyltransferases.

See: Conjugation Reactions, Conjugate, Methylation, *N*-Methyltransferases, and Catechol *O*-Methyltransferase (COMT). Refs. associated with xenobiotics: 172.

136. Michaelis-Menten Equation/Kinetics

A mathematical description of the relationship between a substrate's reaction rate and its responsible enzyme. It is written as

$$V = V_{\max} [S] / (K_m + [S]),$$

where *V* is the reaction rate, [*S*] is the concentration of the substrate, and, for a fixed amount of enzyme, *V*_{max} is the maximum rate at infinite substrate

concentration, while the rate constant K_m becomes equivalent to the substrate concentration at one-half the maximum velocity.

Note 1: The equation is representative for most enzymes, including those where an intermediate enzyme-substrate complex is formed. It describes the typical situation, where rate is dependent on the concentration of both enzyme and substrate.

Note 2: When product formation exhibits a hyperbolic relationship to substrate concentration, the reaction is said to follow Michaelis-Menten kinetics. There are a number of linear and nonlinear regression methods, as well as classical graphical analyses, that can be used to derive V_{max} and K_m values from experimental data obtained by measuring the biotransformation of a substrate to its product.

See: Enzyme, Half-Life ($t_{1/2}$), and Linear Kinetics.^{38-40,104}

137. Microdosing; Phase 0 Clinical Study

The common definition for microdosing is administration of a drug, generally to humans, at significantly lower dose than what is normally used to prompt an efficacious response (e.g., 100 times less), so that the lower dose circumvents toxicity while potentially demonstrating some other beneficial effect that may be achievable at this level. This approach is also undertaken with drug candidates to study their disposition and pharmacokinetic profile as a first step in clinical testing, where it is then also referred to as a "Phase 0 clinical study."

Note 1: Phase 0 clinical studies usually deploy a radio-labeled compound with exposure levels low enough to be considered non-radioactive by regulatory agencies. Analytical sensitivity is typically achieved by using accelerator mass spectrometry (AMS) methods originally developed to accomplish radiocarbon dating of precious samples within the field of archaeology.

See: ADME, ADMET, Disposition, Distribution, Pharmacokinetics (PK), Random Walk, Phases of Drug Clinical Testing, and Phases of Drug Metabolism.¹⁷³

138. Microsomal Fraction/Enzyme; Microsomes

A fraction of the cellular homogenate that contains the endoplasmic reticulum (ER) and thus many drug-metabolizing enzymes. It is typically reformed as lipoidal spheres called "microsomes."

Note 1: This fraction is generally obtained while operating at 2 to 3°C throughout by: (i) homogenizing cells, such as from extracted liver tissue; (ii) centrifugation of the homogenate at 9,000 G to pellet mitochondria and other heavy cellular components; (iii) ultracentrifugation of the supernatant at 100,000 G for ca. 1 hour; and (iv) reconstituting the pellet in physiological saline, where they spontaneously form "microsomes" that contain the ribosomes and are filled with numerous drug-metabolizing enzymes. Microsomes can be stored for several days at low temperature and are typically used to study drug metabolism *in vitro* upon the addition of the required enzymatic cofactors and warming to 37°C, after which enzymatic functional activity is then typically robust for periods up to 15-30 minutes.

Note 2: Associated enzymes include the entire family of CYPs, Flavin Monooxygenase, UDP Glucuronosyltransferase, and Epoxide Hydrolase.

Note 3: When isolating liver tissue from rodents, it is common practice to first treat the animals with a standard, enzyme-inducing agent, such as phenobarbital, so as to provide increased functional capacity for the various biotransformation reactions residing in the eventual microsomes.

See: Metabolism, Biotransformation, Enzyme, Apoenzyme, Holoenzyme, Cofactor, Prosthetic Group, Regenerating System, Cytochrome *P*-450s (CYPs), Glucuronic Acid Conjugation (Glucuronidation), Epoxide Hydrolase, Flavin Monooxygenase (FMO), Induction, and Phenobarbital Sleeping Time.⁶⁹

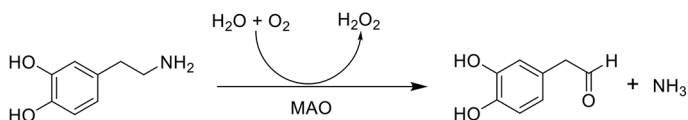
139. Mixed-Function Oxidase

Older terminology that is still widely used today in a general manner for enzyme systems that oxidize xenobiotics. It was initially derived from the oxygenating enzymes' requirements for both molecular oxygen and reducing agent cofactors. The isolation and purification of distinct forms of the CYP and FMO enzymes has progressed considerably, while accompanied by the elaboration of more specific and descriptive nomenclatures, all of which can be used in a more specific manner.

See: Cytochrome P450 Enzymes (CYPs) and Flavin Monooxygenase (FMO).⁶⁹

140. Monoamine Oxidase (MAO)

A family (EC 1.4.3.4) of flavin-containing amine oxidases that catalyze the oxidative deamination of monoamine substrates and diamines when the latter's second basic center is located more than six atoms away from the primary amine (shorter separations instead being catalyzed by diamine oxidase). The catabolism of dopamine is exemplary and demonstrates the importance of this pathway with regard to endogenous neurotransmitters:



Note 1: The mechanism involves the initial formation of an imine intermediate, which is hydrolyzed to the aldehyde and ammonia. The reactive and potentially toxic aldehyde undergoes rapid bioinactivation by other enzymes to either a carboxylic acid or an alcohol, the former being the more typical path for the endogenous neurotransmitters.

Note 2: In humans, MAO occurs as two forms, called MAO-A and MAO-B, which are separate gene products having about 70 % sequence homology. Both forms are present throughout the brain and in neurons, where they reside on the outer surface of mitochondrial membranes. MAO-A also has high levels in the liver, lung, kidney, intestine, and placenta, while MAO-B is found in blood platelets.

Note 3: MAO function has been implicated as playing a role in certain CNS disorders. For example, inhibitors (MAOIs) have been deployed clinically to treat depression. However, their use has largely become limited to secondary therapy because of side effects and the development of alternative agents that can serve as front-line therapy.

See: Catabolism, Deamination, Dealkylation, *N*-Dealkylation, and Diamine Oxidase (DAO).¹⁷⁴

141. Monooxygenase/Monooxygenation

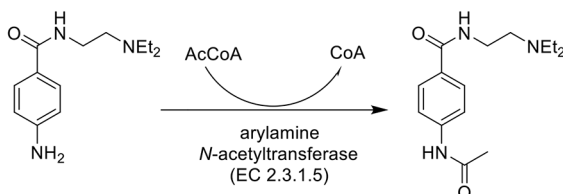
A specific term for the general class of oxygenase enzymes (EC 1.13 and EC 1.14) that occurs when one oxygen atom of molecular oxygen is added to a substrate while the other atom becomes reduced by NADPH to form water. Common examples of monooxygenases important to the metabolism of xenobiotics include the CYPs and FMOs.

Note 1: Alternatively, the term dioxygenation specifies a biotransformation where both atoms of molecular oxygen are incorporated together into one substrate or sometimes separately into two.

See: Cytochrome P-450 enzymes (CYPs), Flavin Monooxygenase (FMO), and Oxidoreductase.¹⁷⁵

142. *N*-Acetylation

Conjugation of a xenobiotic's amino group with an acetyl moiety donated by acetylcoenzyme A and catalyzed by an *N*-acetyltransferase (NAT). Aryl primary amines and hydrazines are particularly susceptible. Bioactivation of the local anesthetic procaine to the antiarrhythmic agent procainamide serves as an example:



Note 1: Primary aromatic amines are the principal substrates, followed by hydrazines, although several other nucleophiles can sometimes undergo this reaction, such as primary aliphatic amines (including amino acids), sulfonamides, and hydroxylamines at the chemically reactive oxygen atom.

Note 2: Two isoforms are especially important for drug metabolism in humans, namely the arylamine-*N*-acetyltransferases NAT1 and NAT2. Their polymorphisms lead to distinct metabolic phenotypes.

See: Acetylation, Acetylation Phenotype, *N*-Acetyltransferases (NATs), Acylation (which is notably a different metabolic pathway), and Bioactivation.⁶

143. *N*-Acetyltransferases (NATs)

The family of enzymes which catalyze the conjugation of amino groups with an acetyl moiety donated by acetylcoenzyme A. Two arylamine-*N*-acetyltransferase (EC 2.3.1.5) isoforms (NAT1 and NAT2) play an important role in humans to acetylate primary arylamines.

Note 1: Both NAT1 and NAT2 are 33 kDa cytosolic proteins having a high degree of polymorphism, which leads to two distinct phenotypes, namely "fast" and "slow acetylators." NAT1 is expressed in numerous tissues, while NAT2 is primarily expressed in the liver and gut wall. The two isoforms have considerable overlap in their acceptance for substrates, although certain trends in the preference for selected materials are also apparent.

See: Acetylation, Acetylation Phenotype, *N*-Acetylation, and Acylation (which is notably a different metabolic pathway).^{6,176} Specific forms Refs.: 177-179.

144. NADH-Cytochrome b_5 Reductase (CBR)/ Methemoglobin Reductase

This enzyme (EC 1.6.2.2) mediates an electron transfer from NADH to cytochrome b_5 using FAD as a cofactor. Cytochrome b_5 , in turn, serves as a ubiquitous electron carrier that participates in numerous biochemical pathways. For xenobiotic metabolism, a membrane-bound form of CBR located on the cytosolic side of the endoplasmic reticulum is particularly important because it participates in cytochrome *P*-450 (CYP) related biotransformations.

Note 1: A soluble form of CBR is present in erythrocytes, where it participates in the conversion of methemoglobin (Fe^{+3}) to hemoglobin (Fe^{+2}). Because of this, CBR is also referred to as "methemoglobin reductase." Deficiencies of this enzyme can lead to methemoglobinemia, where the Fe^{+3} form does not bind and carry oxygen as well as the Fe^{+2} form.

See: Cytochrome b_5 , Cytochrome *P*-450 Enzymes (CYPs), NADPH-Cytochrome *c* Reductase/NADPH-Cytochrome *P*-450 Oxidoreductase/Cytochrome *P*-450 Reductase (CYPR), Microsomal Fraction/Enzyme, Microsomes, and Regenerating System.^{43,64-69}

145. NADPH-Cytochrome *P*-450 Reductase (preferably)/ NADPH-Cytochrome *c* Reductase/NADPH-Cytochrome *P*-450 Oxidoreductase/Cytochrome *P*-450 Reductase (CYPR)

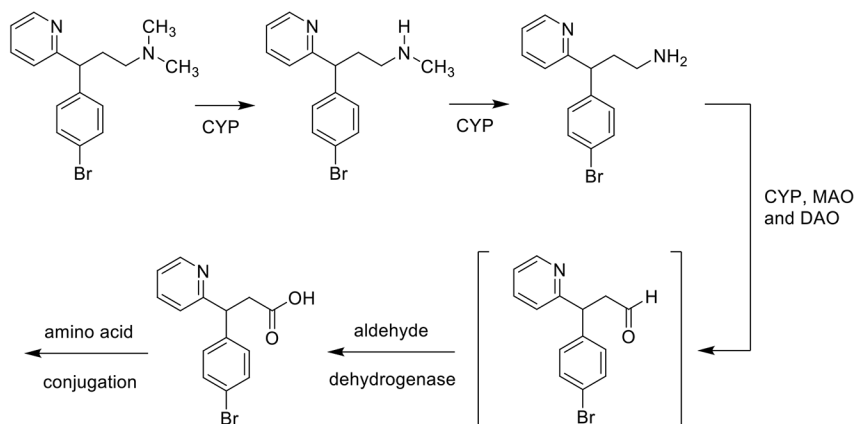
This enzyme (EC 1.6.99.1) mediates an electron transfer from NADPH to cytochrome *c* using FAD and FMN as cofactors. After the preferred term, the sequence of still acceptable names follows historically along the progression of its more detailed functional characterization. CYPR, along with other microsomal enzymes, depending upon their oxidation status, readily interacts with NADPH. It can also interact with NADH, but has less affinity for this interaction and also partners with CBR in that regard. In this context, CYPR is critical for certain of the cytochrome *P*-450 (CYP) catalyzed biotransformations of xenobiotics.

Note 1: The CYPs require two electrons to perform their typical monooxygenation reactions. The first is mainly delivered by CYPR to the substrate-cytochrome *P*-450⁺³ complex, while the second electron, which subsequently reduces the substrate-cytochrome *P*-450⁺²-O₂ complex, can be supplied by either CYPR or cytochrome *b*₅ via CBR.

See: Cytochrome *b*₅, NADH-Cytochrome *b*₅ Reductase (CBR)/Methemoglobin Reductase, Cytochrome *P*-450 Enzymes (CYPs), Microsomal Fraction/Enzyme, Microsomes, and Regenerating System. General Refs.: 45,68-73.

146. *N*-Dealkylation

A biotransformation catalyzed by the CYPs, where a nitrogen-alkyl-carbon bond (most typically in a xenobiotic's secondary or tertiary *N*-alkyl amine or amide) is oxidatively cleaved, resulting in an aldehyde derived from the alkyl group (and for the typical substrates, in the corresponding primary or secondary amine or amide, respectively). *N*-demethylation of alkylamines is a frequently observed pathway owing, in part, to how common this structural motif is within drug molecules. In addition to alkyl amines and amides, a diverse range of nitrogen compounds can be substrates, e.g., carbamates, ureas, and sulfonamides. One of the metabolic pathways for brompheniramine is illustrative, where its first two steps are classified as *N*-dealkylations, while the third is classified as a deamination:



Note 1: In this example, the deamination step can involve catalysis by monoamine oxidase (MAO) and diamine oxidase (DAO), as well as by a cytochrome *P*-450 (CYP). Analogous to an *N*-dealkylation, all of these enzymes will produce the aldehyde from cleavage of the *N*-alkyl group, which then becomes quickly oxidized to the carboxylic acid that, in this particular case, is both excreted into the urine and further conjugated to an amide using glycine as an endogenous amino acid partner.

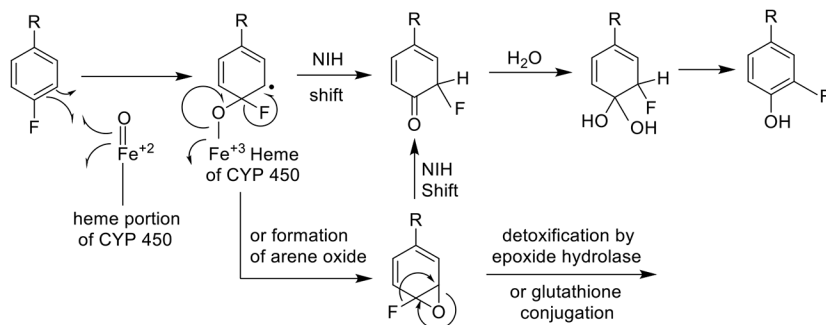
Note 2: CYP-mediated *N*-dealkylation of aliphatic amines occurs by a stepwise, electron transfer process that is initiated by a rate-limiting, one-electron *N*-oxidation reaction to form an aminium radical cation. Abstraction of a hydrogen atom from the α -carbon of the alkyl ligand is then rapid and forms an iminium intermediate that undergoes hydrolysis via a carbinolamine. Alternatively, the process for alkyl-amides is initiated by abstraction of an α -carbon hydrogen atom without a preceding electron abstraction.

See: Dealkylation, Demethylation, Deamination, Monoamine Oxidase (MAO), Diamine Oxidase (DAO), Polyamine Oxidase, and Cytochrome *P*-450 Enzymes.^{74,79,80,83,174}

147. NIH Shift

An intramolecular rearrangement of the transient intermediates produced during CYP-mediated aromatic hydroxylation. The rearrangement involves a radical or hydride shift and is also applicable to the migration of

other groups capable of undergoing these types of 1,2-transfers. The two mechanistic pathways are depicted below for the case of a fluorine atom as the radical (top line) or as the fluoride anion (lower line):



Note 1: This reaction was named after the U.S. National Institutes of Health (NIH), where it was first observed and characterized. It is more prominent when R = OMe, NMe₂, SO₂Ph, Ph, CN, NO₂, CONH₂, and halides; and less so when R = OH, NH₂, NHAc, NHCOPh, NHSO₂Ph, and NHCHO.

Note 2: The common strategy of adding substituents, such as one or more fluorine atoms, to block or attenuate metabolic degradation at a given locale on a drug candidate's structure can be impacted by the NIH shift when dealing with these specific molecular arrangements and their related metabolic pathway. It may work, because halide atoms are electron withdrawing, such that their addition to the phenyl-ring will decrease the latter's overall electron density or "richness." That, in turn, will decrease the overall susceptibility of the phenyl-ring to undergoing an aromatic hydroxylation biotransformation at any location. The strategy may not work, however, because halide atoms cannot be relied on to block a given site for an aromatic hydroxylation because of the possibility for an NIH shift.

See: Aromatic Hydroxylation and Epoxidation.¹⁸⁰

148. N-in-One Dosing

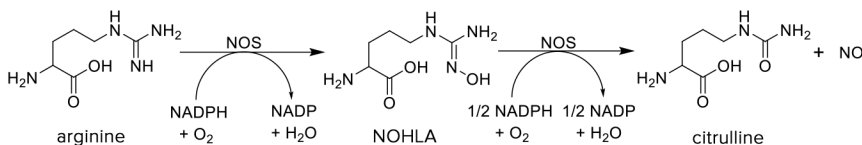
Study of two or more simultaneously administered xenobiotics, either to assess their own metabolism or to assess metabolism-related interactions, PK, or ADMET properties.

Note 1: Generally used more often during *in vivo* studies in order to increase throughput for the assessment of interesting compounds while reducing the number of animals that might be needed. Thus, within the context of drug metabolism studies, this is distinguishable from the practice of high-throughput screening for efficacy and toxicity at the *in vitro* level, where it is common to test mixtures having large numbers of compound candidates followed by various methods to de-convolute the resulting dataset.

See: Cocktail Study.^{59,60}

149. Nitric Oxide Synthases (NOSs)/Nitrogen Monoxide/Nitrogen (II) Oxide/Oxidonitrogen

A family of CYP-related enzymes (EC 1.14.13.39) that generate nitric oxide (NO) and citrulline from arginine, oxygen, and NADPH. Because of their high specificity, they do not appear to play a role in the metabolism of xenobiotics having a guanidine moiety. Alternatively, they can be mechanistic targets for drug design.



Note 1: There are three major isoforms: neuronal (nNOS), endothelial (eNOS), and inducible (iNOS).

See: Cytochrome *P*-450 Enzymes (CYPs).¹⁸¹ Inhib. Ref.: 182.

150. *N*-Methyltransferases

Members within the family of methyltransferase enzymes (EC 2.1.1) that can mediate the conjugation of amino groups with a reactive methyl group provided by *S*-adenosyl-L-methionine (SAM)

Note 1: Histamine-*N*-methyltransferase and indolethylamine-*N*-methyltransferase are commonly known for their roles with endogenous partners. However, the latter has a broader range for substrates, including the potential to catalyze reactions with xenobiotics.

See: Methylation and Methyltransferase.^{171,172}

151. Non-Compartment PK Analysis

Pharmacokinetic (PK) model where the body is considered as an unknown "black box," rather than a series of interconnected compartments, as in a compartment or PBPK model. Measured drug concentrations (C), generally in plasma, serum, or blood, are fitted to a series of exponential terms of the form shown below. The form of this equation and the definition for its terms' symbols are the same as those provided for the equation pertaining to *Compartment Model*.

$$C = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + \dots + A_n e^{-k_n t}$$

Without requiring any type of assumed model structure, the terms of this fitted empirical equation can then be used to estimate useful PK parameters numerically, e.g., AUC, bioavailability, clearance, terminal half-life, and the mean residence time of a drug in the body.

See: Absorption, ADME, Clearance, Pharmacokinetics (PK), Compartment Model, and PBPK Model.^{38-40,104}

152. Non-Competitive Inhibition

Can occur by two different mechanisms: (i) the inhibitor binds irreversibly within an enzyme or transporter's active site for a substrate, e.g., by forming a covalent bond with one of the protein's amino acids; or (ii) the inhibitor binds either reversibly or irreversibly to another site on the enzyme or transporter that is not subject to substrate binding, but when bound by the inhibitor has a negative influence on the substrate's binding within the active site.

Note 1: Unlike competitive inhibition, non-competitive inhibition cannot be overcome by raising the concentration of substrate. For enzymes that metabolize substrates in accordance with Michaelis-Menten kinetics, non-competitive inhibition will lower the enzyme's $V_{\max}$ but will not alter its K_m .

See: Autoinhibition, Competitive Inhibition, Uncompetitive Inhibition, Mechanism-Based Inhibition, Covalent Binding, and Michaelis-Menten Kinetics.^{62,146-148}

153. Nonlinear Kinetics

Any kinetic process for which the rate of the analyte's appearance or disappearance is not directly proportional to the local concentration or amount of the agent.

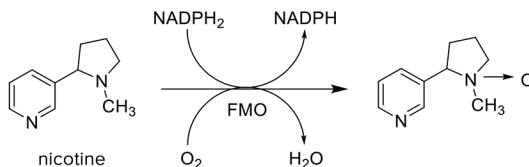
Note 1: Nonlinear kinetics are typically encountered for enzymes or transporters that follow Michaelis-Menten kinetics or some other type of saturable or rate-limited process.

Note 2: PK models that attempt to address nonlinear kinetic processes are more difficult to solve than those with only linear kinetic parameters and generally require the use of computer-assisted numerical methods rather than direct algebraic solution.

See: Linear Kinetics, Michaelis-Menten Kinetics, and Pharmacokinetics (PK).^{38-40,104}

154. N-Oxidation

In the broadest sense, this phrase can be used for any biotransformation of an organic nitrogen-containing xenobiotic where the oxidation state of the nitrogen atom is increased. Most commonly, however, it is taken to refer to the formation of an *N*-oxide, particularly with regard to tertiary amines (see Notes). The more common usage is exemplified below.



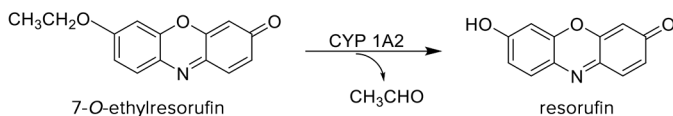
Note 1: The process is catalyzed by either an FMO or a CYP and involves electron transfer from nitrogen to the enzyme. Most primary amines can potentially undergo three successive two-electron oxidations that can give rise to hydroxylamines (or imines) (-2), nitroso groups (or oximes, cyanos) (-4), or nitro groups (-6). In all cases the indicated valencies represent the combined change in oxidation state for nitrogen and its *alpha*-carbon atoms. Secondary amines can undergo a maximum of two, two-electron oxidations that give rise to hydroxylamines (or imines) (-2) or nitrones (-4). Tertiary amines can only undergo a single, two-electron oxidation that gives rise to their *N*-oxides.

Note 2: Initial one-electron *N*-oxidation of amines affords highly unstable aminium cation radicals (-1), which can give rise to a variety of end products. Tertiary amines have a much greater propensity to form stable *N*-oxide metabolites than either secondary or primary amines. As a result, tertiary amines, which are sterically hindered from the common *N*-dealkylation pathways, are often metabolized to observable *N*-oxides.

See: Oxidation, Dealkylation, *N*-Dealkylation, Demethylation, Deamination, Cytochrome *P*-450 Enzymes (CYPs), Flavin Monooxygenase (FMO), Monoamine Oxidase (MAO), Diamine Oxidase (DAO), and Polyamine Oxidase.¹⁸³

155. O-Dealkylation

A biotransformation catalyzed by the CYPs where an oxygen-alkyl-carbon bond in a xenobiotic's ether linkage is oxidatively cleaved, resulting in the corresponding alcohol and an aldehyde derived from the alkyl group. Most commonly observed for aralkylethers, as exemplified by the fluorescent CYP functional probe shown below.



Note 1: *O*-dealkylation is not as prominent as *N*-dealkylation. It is readily subject to steric hindrance (branching) and is also attenuated by increasing the length of the alkyl group. *O*-demethylation, particularly of an arylmethoxy group, however, presents itself as a highly probable metabolic possibility.

Note 2: Because of their reactivity and potential toxicity, other metabolic pathways are in place to rapidly biotransform the initial aldehyde metabolites into less reactive species, mainly aldehyde dehydrogenase (ALDH). This is a well-established sequential pathway/pattern/profile observed in drug metabolism.

Note 3: Alternatively, when an arylhydroxy group becomes exposed in the metabolite, it is often a good candidate for a subsequent conjugation reaction, mainly glucuronidation. This is a common sequential pathway/pattern/profile observed in drug metabolism.

See: Oxidation, Dealkylation, *N*-Dealkylation, Cytochrome *P*-450 Enzymes (CYPs), Metabolic Possibilities versus Probabilities, Metabolic Pathway/Pattern/Profile, Aldehyde Dehydrogenase (ALDH), and Glucuronidation.^{74,184}

156. Oral Bioavailability

The ratio of the AUC from plasma samples taken for a dose of drug given orally (PO) divided by those for a dose of the drug given intravenously, with the comparison normalized for different doses or varying weights of the subjects.

Note 1: In this context, the blood of the general circulation (or its plasma component as a practical matrix for analytical measurements) is always utilized, whereas for the term “bioavailability” the compartment under consideration may vary and so must be specified by the user, e.g., “bioavailability within the cerebrospinal fluid” for drugs intended to have effects in the CNS.

Note 2: For a drug taken PO, examples of factors that can limit oral bioavailability include: formulation issues, such as slow dissolution rate; drug stability, such as degradation under the acidic conditions of the stomach; metabolic transformation by the gut microflora, intestinal epithelial cells, or liver; efflux by transporters within epithelial cells, such as P-glycoprotein (Pgp); or direct biliary excretion. Many of these factors work together to limit the access of xenobiotics into the circulation, a process called the “first pass effect or metabolism.”

See: Absorption, Bioavailability (Absolute and Relative), Gut Microflora, P-Glycoprotein (Pgp), First Pass Effect/Metabolism, Pharmacokinetics, AUC, and Bioequivalence.³⁹

157. Organic Anion Transporters (OATs)

A family of transmembrane proteins that actively transport substrates having organic anion groups, such as a carboxylic acid moiety, when ionized at physiologic pH. More than a dozen members have been identified at significant expression levels in various locations throughout the body.

Note 1: In addition to their influence on the distribution profile of endogenous and xenobiotic compounds, of particular relevance for metabolism is the

OATs' facilitation of drug and metabolite excretion. For example, OAT1, OAT3, OATP4C1, MDR1, MRP2, MRP4, and URAT1 are expressed in cells forming the S_2 segment of the proximal tubules of the kidneys, where the first three are thought to transport from the plasma into the cells and the latter four from the cells into the lumen of the proximal tubules, such that their substrates are then excreted into the urine. OAT2, with only low levels in the kidneys, is highly expressed in the liver, where it and other family members ultimately deliver substrates to the bile in an analogously tandem manner.

Note 2: Notably, both the common glucuronide and glutathione/mercapturate metabolites add a carboxylic acid group to their substrates as part of their conjugation molecules.

Note 3: Various of the OATs can also be classified according to their systematic names, associated with either the solute carrier (SLC) or ATP-binding cassette (ABC) transporter super families. For example, OAT1 is also SLC₂₂A6, while MDR1 is also ABCB1.

See: Active Transport, Organic Cation Transporters (OCTs), Distribution, Excretion, Glucuronic Acid Conjugation (Glucuronidation), Glutathione Conjugation, Mercapturate/Mercapturic Acid Conjugation, Hippuric Acid Conjugate (Hippurate). Renal-related Refs.: 185,186.

158. Organic Cation Transporters (OCTs)

A family of transmembrane proteins that actively transport substrates having positively charged groups, such as basic amines, which become highly ionized at physiologic pH. However, some of their family members can also transport non-charged molecules, such as paclitaxel, and even certain negatively charged compounds. Several members have been identified at high expression levels in various locations throughout the body.

Note 1: In addition to their influence on the distribution profile of endogenous and xenobiotic compounds, of particular relevance for metabolism is the OCTs' facilitation of drug and metabolite excretion. For example, their expression at specific subcellular sites in renal proximal tubular cells and in hepatocytes becomes sequentially engaged like that of the OATs, so as to promote excretion into the urine and into the bile, respectively. Over-expression of the P-glycoprotein member, PgP, within cancer cells can result in multidrug resistance.

Note 2: Various of the OCTs can also be classified according to their systematic names, associated with the solute carrier (SLC) or ATP-binding cassette (ABC) transporter super families.

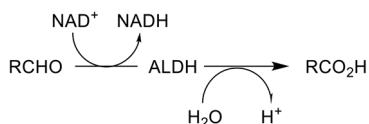
See: Active Transport, Organic Anion Transporters (OATs), Distribution, Excretion, P-Glycoprotein (Pgp)/MDR1/ABCB1.¹⁸⁷ Renal Ref.: 185

159. Oxidation/Oxidase/Oxidoreductase

Oxidation is an encompassing term used within the context of drug metabolism to generally categorize biotransformations that ultimately increase the oxidation state of the substrate, often incorporating one or more oxygen atoms donated from either molecular oxygen or from a water molecule. They can be catalyzed by numerous types of enzymes (e.g., ALD, ALDH, AO, MAO, DAO, FMO, and CYPs) that remove one or more electrons or hydrides and/or directly add one or more oxygens to a substrate or its metabolized fragments (e.g., further conversion of aldehyde products from metabolic dealkylation reactions into carboxylic acids). Similarly, the general terms used for the variety of enzymes/enzyme systems that can perform oxidation reactions can be either oxidases or oxidoreductases, depending on how much of the biochemical system's functional components are being incorporated into the intended description for a given process. The broadness of the term "oxidoreductase" is reflected by its EC number, namely EC 1, which is then further delineated into more than 20 subclasses.

Note 1: Oxidation, as opposed to reduction, reactions constitute the vast majority of xenobiotic biotransformations within mammals. Conversely, after oral ingestion the gut microflora can contribute to a xenobiotic's metabolism by performing a considerable array of reductive biotransformations. Metabolic enzymes that normally catalyze oxidations may sometimes perform a reduction, e.g., certain of the CYPs have this capability under anaerobic conditions.

Note 2: The following example intends to clarify the variety of terminology that can be used in relation to the oxidation of a xenobiotic by an oxidoreductase pathway as typically catalyzed by an enzyme with its cofactors:



Catalyzed by aldehyde dehydrogenase (ALDH), which serves as an "oxidoreductase," the aldehyde-containing xenobiotic RCHO becomes an "electron donor" or "reductant" when it transfers a "hydride ion" to cofactor NAD⁺, which, in turn, becomes an "electron acceptor" or "oxidant." At this point bound to a cysteine within the ALDH active site as its thioester intermediate, the xenobiotic can already be considered to have been "oxidized," while the NAD⁺ cofactor has been "reduced" to NADH. Continuing its role as an "oxidase," ALDH then catalyzes hydrolysis of the thioester, so as to produce the now formally "oxygenated," carboxylic acid-containing "oxidized" product that results from this particular "oxidation" reaction, wherein a water molecule has provided its "oxygen atom" in the form of a hydroxy group after being polarized and positioned for the transfer by hydrogen bonding as a donor to a glutamate within the ALDH active site.

See: Aldehyde Dehydrogenase (ALDH) and Cytochrome *P*-450 Enzymes (CYPs), among many other examples. Thorough Ref.: 188; Chem. Mechanisms Ref.: 189.

160. Oxidative Stress

In general, an imbalance between the level of reactive oxygen species (ROS), such as superoxide and hydroxyl radicals (O₂⁻ and ·OH) or hydrogen peroxide (H₂O₂), compared to a biological system's ability to detoxify the ROS or repair their damage. There are several interrelationships with the metabolism of xenobiotics. For example: (i) many of these biotransformations engage oxidoreductase enzymes, which are dependent on or sensitive to localized oxygen tension (balance); (ii) some reactive xenobiotics or metabolites also draw from (and can thus contribute toward the depletion of) the pool of systems in place to detoxify normal levels of reactive oxygen species (e.g., glutathione conjugation of reactive electrophiles derived from exogenous materials or catalase, which converts hydrogen peroxide to water and molecular oxygen); and (iii) alternatively, oxidative stress can deplete glutathione stores and thus alter the pathways available for xenobiotic metabolism.

See: Oxidation/Oxidase/Oxidoreductase, Mixed-Function Oxidase, Catalase, Glutathion (GSH), Glutathione Conjugation, Cytochrome *P*-450 Enzymes (CYPs), Flavin Monooxygenase (FMO), and Reactive Oxygen Species (ROS).¹⁹⁰⁻¹⁹² Research Topic Refs.: 193,194.

161. Parent Compound

Within the context of xenobiotic metabolism, the molecule (drug) initially presented to the biological system, which may then undergo biotransformation.

Note 1: In drug design, this phrase typically refers to an initial molecule that serves as a structural template for the production of new analogs or scaffold-hopping strategies. This alternative usage is particularly prevalent during the design of prodrugs and soft drugs, and this can lead to confusion. Even though both are correct in the following example, the context is crucial for appreciating their meaning: A prodrug designed/modified from a parent drug becomes the parent compound upon its administration to the biological system in terms of assessing and discussing its ADME behavior.

See: Metabolite versus Degradation and Decomposition Products, Metabolism, Drug Metabolism, and Biotransformation.³

162. Partition Coefficient (P ; $\log_a P$ and $\epsilon \log_a P$); Distribution Coefficient (D ; $\log_a D$)

For drug metabolism, a partition coefficient (P) is a property of a xenobiotic that reflects the ratio of this solute's concentration as a neutral (uncharged) species in a non-polar phase (generally defined to be octan-1-ol), compared to that in an aqueous phase, after the two phases are equilibrated by mixing and allowed to separate. This property is generally expressed as a $\log_a$ value ($\log_a P$, where a signifies the basis of the logarithm), as calculated below, where α reflects the degree of dissociation for compounds capable of ionizing in an aqueous environment according to their ionization constants and thus accounts for their enhanced solubility in that phase. In case of base 10 (decadic logarithm), the expression reads

$$\text{Log}_{10} P = \log_{10} ([S]_o / [S]_w) - (1 - \alpha)$$

where $[S]$ is the concentration of the solute in octanol (subscript o) and water (subscript w).

Note 1: Neutral or weakly ionizing compounds ($\alpha \approx 1$) generally fit this equation well, but those that significantly ionize in water, such as xenobiotics having basic amine or acidic carboxylic acid groups, can be

problematic. Thus, another convention for these compounds is to drop the $(1 - \alpha)$ term and allow the enhanced aqueous solubility to be maintained in the calculation while stipulating that this is now a distribution coefficient (D) having $\log_a D$ values. Because the contribution from ionization will be pH-dependent, it then becomes important in $\log_a D$ determinations to specify what specific pH was utilized during the partition experiment, e.g., pH 7.4. Similarly, temperature changes can also affect the degree of ionization and so these are also reported, e.g., 37°C.

Note 2: Compounds having greater solubility in water have P values less than 1, while those having greater solubility in octan-1-ol have values greater than 1. Studies have demonstrated that there is a bell-shaped-curve relationship between the $\log_a P$ values for various compounds and their rate of passive transport across biological membranes. Relevant for oral bioavailability, the optimal $\log_a P$ for many drug-like molecules tends to be less than 5, because too much lipophilicity can cause them to remain/concentrate within the membrane. Conversely, compounds having significantly lower values tend to stay in the aqueous phase, rather than entering the membrane as a prelude to passive transport. A $\log_a P$ near 2 is thought to be relevant for penetration into the brain.

Note 3: Since P derives from the summation of the properties for each of a xenobiotic's molecular components/substituents as displayed across its overall scaffold, various computational methods have been devised to calculate this net property for a given molecule, such results then being designated as $\log P_c$ values, as opposed to experimentally determined values. Comparisons between $\log_a P$ and $\log_a P_c$ values for the same compounds are generally acceptable, but become most reliable when a given computational method has been refined with the aid of experimental data to best reflect specific classes of chemical structures.

Note 4: Historically, $\log_a P_c$ has been symbolized as simply $\text{clog } P$, and while it has remained customary for the latter to represent $\log_a P_c$, no mathematical symbol clog exists; the calculated property is the partition coefficient P_c . Specification of the basis is now mandatory wherein other symbols exist, for example $\lg$ for $\log_{10}$, $\ln$ for $\log_e$, or ld for $\log_2$.

See: Permeation/Permeability, Passive Transport, Rule-of-Five, Absorption, Oral Bioavailability, ADME, Distribution, Pharmacokinetics (PK), Active Transport, and Blood-Brain Barrier (BBB).^{195,196}

163. Passive Transport

The passage of a solute across a biological membrane by a process that does not require the expenditure of energy.

Note 1: This process is driven by the solute's concentration gradient. Thus, solutes cannot move from sites or compartments having lower concentrations into sites or compartments having higher concentrations.

See: Absorption, Membrane Permeation, Active Transport, ADME, First-pass Effect, Extravascular Dosing, Random Walk, Pharmacokinetics, and Bioavailability.^{4,10,195,196}

164. PBPK (Physiologically Based Pharmacokinetic Model)

Acronym for physiologically based pharmacokinetic model, in which compartments represent specific tissues, and the rates into and out of a compartment represent transport by blood flow, permeation across a physiological barrier, or metabolism within a given tissue.

Note 1: Many of the numerical values for the parameters in PBPK models can be taken directly from physiological tables, such as those for species specific tissue volumes and blood flow rates. Metabolism data from *in vitro* studies is also sometimes incorporated into these models.

Note 2: PBPK models generally provide a more reliable means of PK-related extrapolations between species than allometric scaling, because they can be modified to represent the actual physiological differences between species, rather than an empirical scaling to body weight or surface area.

See: Pharmacokinetics (PK), Allometric Scaling, Blood Brain Barrier (BBB), Compartment Model, ADME; ADMET, Clearance (CL), Distribution, DMPK, Disposition, Elimination, Excretion, Extraction Ratio, Gut Microflora, Half-Life ($t_{1/2}$), Hepatoportal Circulation, Hepatic Clearance/Extraction, IVIVE, Linear Kinetics, Metabolic Pathway/Pattern/Profile, Michaelis-Menten Equation/Kinetics, Non-Compartment PK Analysis, and Nonlinear Kinetics.^{38-40,104}

165. Permeation/Permeability

Within the context of drug metabolism, the movement/capability of a xenobiotic to enter a given compartment. The latter most often refers to lipid membranes, where this term includes passage and exit to the opposite side, such as for the gastro-intestinal tract epithelial lining. This property is important for a xenobiotic's absorption and oral bioavailability or for the blood-brain barrier (BBB), where it is important for penetration of a drug into the central nervous system (CNS).

Note 1: Under static conditions, a xenobiotic's permeability is simply reflected by its physicochemical properties, e.g., its solubility in a given compartment, such as the lipid/hydrophobic nature of a membrane *versus* its aqueous/hydrophilic solubility. This makes aqueous-lipophilic solvent (generally octan-1-ol) partitioning experiments valuable to determine partition coefficient values for a given compound or series of compounds, where this data can then be directly proportional to passive transport and distribution profiles.

Note 2: However, within the dynamic conditions of a living system, other factors can additionally influence a xenobiotic's permeability, such as a drug's ability to serve as a substrate for active (influx or efflux) transporters. Since the transporters are, in turn, subject to up- and down-regulation, substrate xenobiotics that can also interact with the transporters' associated translation and signaling pathways may exhibit a differing permeability profile upon their continued exposure to the system.

See: Partition Coefficient, Passive Transport, Rule-of-Five, Absorption, Oral Bioavailability, ADME, Distribution, Pharmacokinetics (PK), Active Transport, and Blood-Brain Barrier (BBB).^{4,10,195-197}

166. Peroxisome Proliferator-Activated Receptors (PPARs)

The PPARs are a family of nuclear receptor proteins that serve as transcription factors to regulate the expression of selected genes. In addition to their regulation of cellular differentiation, development, and basal-level endogenous metabolism (anabolism and catabolism), some of these family members can interact with xenobiotic metabolism. This interaction generally results in gene overexpression and induction of the biotransformation pathway. For example, the binary complex of PPAR and

retinoid X receptor (RXR) acts as a transcription factor that increases the expression of CYP 4A genes by binding peroxisome proliferator responsive element.

Note 1: The family is divided into three subfamilies: (i) PPAR-alpha; (ii) -beta or -delta; and (iii) -gamma, with this last group being further divided into $\gamma 1$, $\gamma 2$, or $\gamma 3$ as a result of alternative splicing.

Note 2: This type of overlapping interaction (between gene regulation factors associated with basal-level endogenous signaling pathways and certain xenobiotic metabolism pathways) is also observed for the hormone nuclear receptors. For example, regulation of CYP 3A genes can occur through a receptor-mediated mechanism where the complex of ligand and steroid receptor binds to the glucocorticoid responsive element (GRE). The PPAR and steroid receptor pathways are thus similar to each other while being significantly different from the aromatic hydrocarbon receptor (AHR) pathway that can upregulate certain of the CYP 1A and 1B subfamily members by relying on the xenobiotic response element (XRE) without playing a fundamental role in any other endogenous processes. In this regard, the pregnane X receptor (PXR) sits between these two classification types, because it behaves more like a promiscuous nuclear receptor protein that interacts with both a variety of endogenous and xenobiotic compounds, ultimately being capable of upregulating some of the CYP 2B, 2C, and 3A members, as well as components for the glutathione drug-conjugation pathway and P-glycoprotein (Pgp) drug-efflux transporter system.

See: Aromatic Hydrocarbon Receptor (AHR), Pregnane X Receptor (PXR), Glucocorticoid Responsive Element (GRE), Retinoid X Receptor (RXR), Constitutive Androstane Receptor (CAR) and CAR/RXR Heterodimers, and Xenobiotic Responsive Element (XRE).³⁵ Res. Topic Ref.: 198.

167. P-Glycoprotein (Pgp/MDR1/ABCB1)

Pgp is an ATP-dependent efflux transporter protein that spans the cell membrane and serves to pump a broad range of compounds out of cells, including, especially, xenobiotics that present themselves as suitable substrates. The importance of the relationship that transporters have with drug metabolism events, as well as with pharmacokinetic profiles in general, cannot be emphasized enough (see Note 4).

Note 1: Borrowing from enzyme-related terminology, a transporter's cargo is acceptably referred to as a "substrate," even though no chemical change is imparted to the structure.

Note 2: Pgp was first identified from cancer cells that had become resistant to chemotherapeutic agents during prolonged treatment by its overexpression, as well as by several other possible mechanisms. Since the observed resistance included a broad range of drug structural types, the term first adopted for Pgp was "multidrug resistance protein (MDR1)," which is still in use today. It was later determined that there is a family of these types of transporters (some being uptake as well as additional efflux members), where Pgp falls into the category of an ATP-binding cassette sub-family B group and is designated as member number 1. Thus, the acronym "ABCB1" is also utilized to designate Pgp. Among this family, Pgp remains one of the foremost and most significant of the transporters.

Note 3: Especially high levels of Pgp are normally expressed in: the intestinal epithelium, where it can serve to pump xenobiotics back into the lumen and thus limit the latter's oral bioavailability; liver cells, where it pumps its substrates (e.g., parent drugs and their metabolites) into the bile duct and thus serves to excrete them from the body by the biliary route; the proximal tubule cells of the kidney, where it pumps its substrates into the urine-forming ducts as a prelude to their excretion by the renal route; and the capillary endothelial cells associated with the blood-brain barrier (BBB), where it serves to limit the access of many endogenous compounds and xenobiotics into the brain.¹⁹⁹⁻²⁰¹

Note 4: Any possible metabolic event for a given xenobiotic at any locale within the body depends on: (i) the suitability of the xenobiotic's structure to serve as a substrate for the enzyme(s) associated with the event; (ii) the level(s) of the relevant enzyme(s) and competing enzymes present at that locale; and (iii) the concentration and residency time of the xenobiotic at that locale. Component (iii) is a function of the inherent physical properties of the substrate (e.g., $\log_a D$) and its suitability to serve as a substrate for transporters, where the latter's importance grows exponentially with the levels of the various transporters that may or may not be present and operative at that locale. While all three components represent a challenge for prediction of a drug's metabolism *in vivo*, component (iii) arguably presents itself as the largest hurdle due to the presence of the transporters that additionally complicate the variables that need to be taken into account.

See: Absorption, Permeation/Permeability, Partition Coefficient (P ; $\log_a P$ and $\text{clog}_a P$); Distribution Coefficient (D ; $\log_a D$), Passive Transport, Active Transport, Blood-Brain Barrier (BBB), and Metabolic Prediction/Possibilities versus Probabilities. Structure/Mechanism Refs.: 202-205.

168. Pharmacodynamics

In one usage, this is the time course for a drug's effects (therapeutic or undesired), such that it is possible to cite a "pharmacodynamic onset" and, importantly, a "pharmacodynamic half-life" subsequent to a given dose (various routes of administration are possible, but should be indicated in a specific manner) without ever determining the drug's concentration in any particular tissue/compartment. In another usage, this is the relationship between drug concentration and the magnitude of its effects. While the concentration at the site of action is the most appropriate for this latter relationship, most often a more readily sampled tissue or fluid is used, such as plasma, serum, or blood.

See: Pharmacokinetics (PK) and Half-life ($t_{1/2}$).^{3,38-40,104}

169. Pharmacogenetics and Pharmacogenomics

Pharmacogenetics is the study of inherited genetic differences that impact directly on an individual's responses to drugs in terms of efficacy and ADMET profile. Pharmacogenomics encompasses pharmacogenetics and further includes an individual's acquired genetic differences. Both fields track germline single-nucleotide polymorphisms (SNPs), with pharmacogenomics also studying somatic mutations, e.g., within diseased cells, such as cancer. Finally, pharmacogenomics also considers an individual's ongoing exposure to xenobiotics and other circumstances that can alter the expression of one's inherited and acquired genetic code (that composite leading to the observed phenotype).

Note 1: The characterization of rapid versus slow metabolizers and various other phenotypes associated with xenobiotic metabolism preceded the "omics" field and can be considered to have established a solid base of interest to pursue individual variation toward drug responses well before the genetic code and the study of its variation were unraveled.

See: Acetylation Phenotype, Aldehyde Dehydrogenase Polymorphism or Deficiency, Atypical Alcohol Dehydrogenase, Drug-Drug Interactions, Extensive (or Rapid) Metabolizer, Genetic Polymorphism, Genotype/Genotyping, Inducing Agent, Induction, Inhibition, Isoform or Isozyme (Isoenzyme), Metabolic Prediction/Possibilities versus Probabilities, Metabolic Probe, Metabolic Profiling/Metabolic Fingerprinting, Metabolomics/Metabonomics, Phenotype, Polymorphism, Single Nucleotide Polymorphisms (SNPs), and Ultra-Rapid Metabolizer. Representative Refs. (across this topic's history as it connects drug metabolism to clinical practice): 206-215.

170. Pharmacokinetics (PK)

Either the study of, or the resulting data/profile reflecting, the time course for a pharmaceutical agent's absorption, distribution, metabolism, and excretion (ADME) from the body, assessed in a quantitative manner by tracking the agent's concentration with time. These assessments most commonly focus on plasma, serum, or whole blood concentration versus time after administration of the test agent. However, any of the various tissues (physiological compartments) may be additionally assayed, such as the use of cerebrospinal fluid so as to further assess penetration into the brain for agents designed to be potential CNS drugs.

Note 1: Historically, radiolabeling was typically relied on as a sensitive method to detect and track the test agent. While still useful today, most of these studies are now able to deploy liquid chromatography-mass spectrometry (LC-MS) to sufficiently generate a PK profile. In addition, LC-MS/MS can now be used to also gain a preliminary, if not final, determination of what metabolites may have been produced from the parent compound within the given test subjects/species.

See: ADME, ADMET, Allometric Scaling, Allosteric Scaling, AUC, Bioavailability (Absolute and Relative), Clearance (CL), Compartment Model, Disposition, DMPK, Dose-Dependent Kinetics or Metabolism, Extraction Ratio, Half-Life ($t_{1/2}$), Intrinsic Clearance, IVIVE, Linear Kinetics, Metabolic Clearance, Metabolic Pathway/Pattern/Profile, Michaelis-Menten Equation/Kinetics, Non-Compartment PK Analysis, Nonlinear Kinetics, Oral Bioavailability, Partition Coefficient (P ; $\log_a P$ and $\text{clog}_a P$); Distribution Coefficient (D ; $\log_a D$), PBPK, Pharmacodynamics, and Pharmacogenetics and Pharmacogenomics. ^{3,38-40,104}

171. Phases of (Drug) Clinical Testing

Investigations pertaining to the first time that a new drug is administered to humans are undergoing global harmonization. The following terms have been taken from the system presently used by the U.S. Federal Drug Administration (FDA) and are generally representative. Phase 0, also often called "human microdosing" (HMD), is not a mandatory first step, but can be used to gain a preliminary assessment about a new agent's anticipated PK profile after administering doses that are too small to prompt efficacy and, importantly, toxicity. A Phase I trial involves testing in healthy human volunteers to establish the agent's short-term safety/tolerability; PK profile, including likely metabolites, and a dosing paradigm that may be able to be used in subsequent efficacy studies (whenever possible, assays for relevant biomarkers of toxicity and physiological mechanistic parameters leading to anticipated efficacy are now also being added, where the latter is often considered to be a practical/investment-worthy validation of the therapeutic concept). Phase II testing/trials utilize volunteer patients to see if the drug can treat the intended disease in a beneficial manner, thus firmly establishing efficacy while further assessing possible side-effects and continuing the refinement of administration protocols toward an eventual recommended dosing paradigm. Phase III trials recruit large numbers of volunteer patients, so as to establish a broader knowledge base about the efficacy, side-effect profile, and general safety of the agent relative to a recommended dosing paradigm that can include considerably longer exposure cycles. Finally, Phase IV involves post-marketing surveillance to watch the new drug's reports from longer-term use across the general public where patients have sought treatment from a physician.

Note 1: Drug metabolism is a critical component of the ADMET profile in humans that often has direct relationships with the desired efficacious activity and any undesired off-target or side-effect toxicity. Its thorough characterization, including the possibility of metabolism-related drug-drug interactions as appropriate for anticipated future usage, is mandated for a new drug candidate to successfully proceed to the marketplace. Numerous types of preclinical studies at the *in vitro* level and several *in vivo* studies in various animal models, as well as computer program databases and expert systems, attempt to discern what is likely to occur upon the administration of a drug to humans, but the above series of clinical trials are ultimately intended to provide the final answer before a new drug can be released for sale to the general public.

Note 2: In the U.S., the composite of chemical production information; analytical quality control measures for the drug substance and its measurement in various biological matrices; and preclinical efficacy, PK, and ADMET testing studies, with a special emphasis on the evaluation of toxicity across several animal species for durations longer than those to be deployed in humans; is all assembled into an Investigational New Drug (IND) document that is an FDA-mandated application needed to enter into human testing. A similar New Drug Application (NDA) must be submitted before entering the marketplace—it additionally includes all of the information gathered during the Phase I to III clinical trials.

Note 3: The terms used to designate clinical trials and those used for the phases of drug metabolism are very similar. They should be distinguished by the use of a capital “P” and by using a Roman numeral for each of the clinical phase testing/trial designations, whereas the phases in drug metabolism are not capitalized and are denoted by Arabic numerals.

See: Microdosing, Phase 0 Clinical Study, Drug Metabolism, Biotransformation, ADME, Metabolic Fate, Metabolic Pathway/Pattern/Profile, Metabolic Ratio, Metabolic Prediction/Possibilities versus Probabilities, and Phases of Drug Metabolism.^{173,216–219}

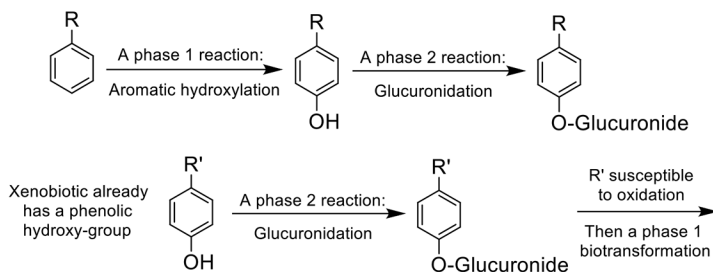
172. Phases of Drug Metabolism

Historically, the now controversial terms “phase 1” and “phase 2” metabolism have been used for many years to designate xenobiotic biotransformations involving hydrolytic, oxidative, and reductive processes (“phase 1”) versus conjugation reactions (“phase 2”).⁸¹ Subsequently, the term “phase 3” has sometimes been used to designate the excretion of xenobiotics and their metabolites from the body.²²⁰ More recently, the term “phase 0” has seen some use in order to convey events, such as a xenobiotic’s absorption and distribution, generally in the context where these take place prior to a biotransformation event.¹³⁰ Some experts want to move away from using any of these types of terms (for the reasons indicated in Note 2 below),²²¹ while others suggest an even broader expansion of the terminology to incorporate systems biology and cross-disciplinary concerns relative to a xenobiotic’s metabolic fate within both the individual that it is initially administered to, plus its subsequent appearance in the environment (Note 3). **Readers are encouraged to see the sections within the glossary’s Introduction and detailed Appendix II that discuss the “pros and cons” of preserving the**

use of these historical terms and their potential expansion, as well as considering the notes provided below.

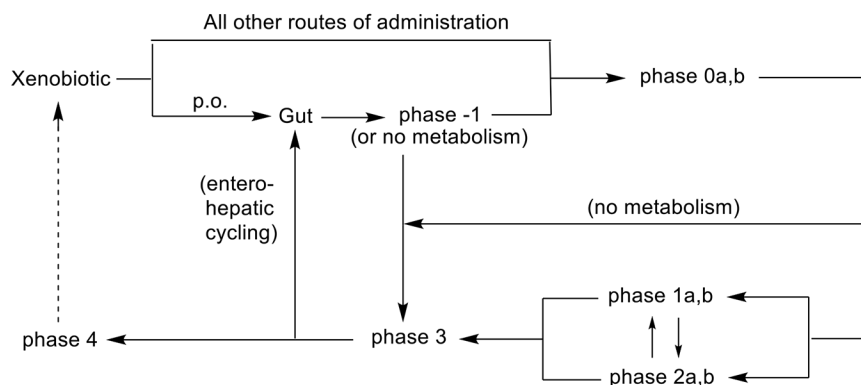
Note 1: The clinical testing phases use very similar nomenclature. However, they can be distinguished by the use of a capital "P" and a Roman numeral for each of their sequential steps, whereas the phases in drug metabolism are not capitalized and are denoted by Arabic numerals.

Note 2: The early identification of a very common metabolic pathway, where a xenobiotic having a susceptible aryl-group undergoes aromatic hydroxylation followed by glucuronide conjugation to the instilled hydroxyl-group, grew into the general theme where lipophilic compounds are first metabolized (especially by an oxidative process) so as to gain a functional-group handle that allows the body to couple (conjugate) one of its own, highly polar adducts so as to dramatically increase the initial xenobiotic's water solubility and enhance its excretion from the body. This theme, in turn, became the basis for the phase 1 and 2 designations. But when a xenobiotic already has an appropriate functional group present, then conjugation can occur without the need for a preceding phase 1 event. Likewise, in some cases further oxidative metabolic events will follow a conjugation reaction. This has led to the confusion implied by this terminology, which has led some experts to suggest moving away from its usage altogether. The historical and confusing aspects about this nomenclature are illustrated below by the upper and lower pathways.



Note 3: Appreciating that these designations are more like process classifications, rather than strictly sequential pathways, allows for their continued usage. Alternatively, retaining their specific phraseology as a "phase" recognizes the historical note conveyed above, while underscoring that there are specific sequences of events often required by the substrate to

traverse an overall metabolic pathway. In this context, a potential expansion of this nomenclature is illustrated below for the human case of exposure to a xenobiotic. This is not intended to be a recommended “final” definition, but rather the basis for further discussion. It encompasses a systems biology approach that could provide a useful context for investigators interested in the “phase -1” and “phase 4” regions of xenobiotic metabolism, areas that reflect distinctive, cutting-edge directions for the field in general.



This nomenclature begins by illustrating the potentially important difference in the metabolic fate of a xenobiotic contributed by the gut microflora after oral ingestion (PO) versus other routes of administration. Thus, phase -1 (minus one) designates all of the possible biotransformations (some being quite unique to the microorganisms) that can be catalyzed by the gut microflora. As such, phase -1 also encompasses the role that these microorganisms can sometimes play during enterohepatic cycling, e.g., hydrolysis of a phenolic-glucuronide metabolite that has undergone biliary excretion back to the phenol, which can then be reabsorbed rather than proceed toward fecal elimination. Phase 0 designates all of the non-metabolic dispositional processes to which a xenobiotic or metabolite will traverse, such as absorption passively or by transport across membranes and association with biomolecules not involved in drug metabolism, while undergoing fluid-driven distribution through the living system. The important relationships of a drug's transport coupled to its metabolism, and a metabolite's transport coupled to its excretion, are thus recognized by this nomenclature. For this term, the “a” designates that these phenomena occur prior to a xenobiotic's first mammalian metabolic event (as shown in the scheme), while the “b” intends to further

convey that these processes continue throughout the entire timeframe after a xenobiotic's arrival until it and all of its metabolites are cleared from the living system. In line with the historical definition, phase 1 includes all hydrolytic, oxidative, and reductive biotransformations. The "a" then provides a sub-classification for such events occurring at a given point on the substrate for the first time, while the "b" indicates that the reaction event occurs at a point on the substrate which has already participated in a preceding biotransformation. Typical phase 1a examples include ester hydrolysis, aromatic hydroxylation, arylether *O*-demethylation, and the first of possible *N*-dealkylations. Aside from the hydrolytic reactions, the most common phase 1a biotransformations are generally catalyzed by the CYP family. The phase 1b events utilize a broader range of enzymes to catalyze the same types of chemical reactions. Typical phase 1b examples include: the rapid reduction or further oxidation of the aldehyde metabolites resulting from CYP-mediated *N*- or *O*-dealkylations, further processing of an initial glutathione conjugate so as to form a mercapturic acid metabolite, and successive β -oxidations of aliphatic carboxylic acid chains. It can be noted that phase 1b metabolism is often critical for avoiding metabolite toxicity (i.e., further processing of aldehydes) or for reducing a metabolite's molecular mass as a prelude to the latter's excretion (i.e., further processing of a glutathione conjugate reduces its initial molecular mass increase of 307 Da to only 162 Da, which is then similar to the increase of 175 Da that results from the formation of a common glucuronide conjugate). Also in line with the historical definition, while additionally incorporating similar sub-classifications, phase 2 metabolism includes all of the conjugation reactions that can take place, either as the first metabolic event at a given point on the substrate ("a") or as a subsequent reaction at a point on the substrate having already undergone a biotransformation. Typical examples include: glucuronidation and sulfation of aromatic hydroxyl groups either present on the parent xenobiotic (phase 2a) or having been instilled there (phase 2b) by a preceding phase 1a, CYP-mediated aromatic hydroxylation or an aryl-*O*-demethylation biotransformation; glutathione entrapment of highly reactive electrophilic centers present in the substrate; *N*-acetylation or *N*-, *O*-, and *S*-methylation reactions; and glycine conjugation of carboxylic acid moieties either present on the parent xenobiotic (phase 2a) or having resulted (phase 2b) from a preceding phase 1a, esterase-mediated hydrolysis reaction. The term phase 3 encompasses the various excretion-associated events that either the un-metabolized xenobiotic (note first line immediately after the phase 0a,b processes) or its metabolites will eventually engage. Key

examples include: physicochemical-driven partitioning behavior between biological milieu associated with excretion, such as the loop of Henle within the kidneys' nephrons; transport into the bile canaliculi at the hepatobiliary interface, especially for negatively charged metabolites by the organic anion transporters (OATs) present within the liver's hepatocytes; and transport across the GI epithelium into the gut lumen, especially for metabolites which are good substrates for P-glycoprotein (Pgp). Also accounted for in this expanded nomenclature's diagram is the possibility for enterohepatic cycling, a key example afforded by the steroids that, upon glucuronide conjugation and excretion into the gut, can be hydrolyzed by the microflora and thus subject to reabsorption. Finally, phase 4 encompasses all of the processes that can continue to contribute toward an excreted compound's environmental fate. Typical examples include: the possibility for both continued biodegradation (catabolism) or potential anabolism by a variety of microbial, plant or animal species; as well as "distribution" by waterways or by edible plants and animals so as to enter the nutritional cycles of mammals, where ingestion by humans, as suggested by the dotted line in the nomenclature's accompanying scheme, then comes full circle along a systems biology theme.

See: Phases of (Drug) Clinical Testing; Drug Metabolism, Biotransformation, ADME, Metabolic Fate, Metabolic Pathway/Pattern/Profile, Metabolic Ratio, Cytochrome P450s Enzymes (CYPs), Aldehyde Dehydrogenase (ALDH), Aldehyde Oxidase (AO), Aromatic Hydroxylation, Conjugation Reactions, Conjugate, Glucuronidation, Glutathione Conjugation, Mercapturate/Mercapturic Acid Conjugation, Enterohepatic Cycling, and Phenobarbital Induction and Sleeping Time.^{81,130,158,220-227}

173. Phenobarbital Induction and Sleeping Time

In addition to its clinical indications, phenobarbital is often deployed to induce the over-expression of liver enzymes within rodents destined to serve as donors for the preparation of liver fractions (e.g., microsomal) that are to be used during *in vitro* drug metabolism studies. It induces many members of the CYP enzyme family, with CYP 2B6 being particularly responsive via the CAR/RXR nuclear receptor signaling pathway.

Note 1: A typical protocol involves intraperitoneal injection of the donor group daily for three days. Starting with a dose that can cause all animals to sleep for ca. an hour, even the second dose will have an observably weakened

pharmacodynamic effect due to the agent's more rapid metabolism, e.g., perhaps 50 % will sleep and then only for 0.5 hours. By day three, that same dose is likely to cause less than 25 % of the study group to sleep, and those for only a few minutes. A detectable increase in body weight can occur, the latter resulting from a very observable and pronounced increase in liver weight when compared to untreated control animals.

Note 2: Phenobarbital itself largely undergoes the classical pattern of CYP-mediated aromatic hydroxylation followed by glucuronide conjugation; this metabolite is then primarily excreted by the renal route; this is historically called the phase 1 and phase 2 metabolism sequence, or, according to certain proposed newer nomenclature, a phase 1a, phase 2b, phase 3 and phase 4 sequence, with the latter's details remaining unspecified.

See: Induction, Inducing Agent, Microsomal Fraction/Enzymes; Microsomes, Phenotype, RXR and CAR/RXR, Pharmacodynamics, and Phases of Drug Metabolism.²²⁸ Applied Research Refs.: 159,229.

174. Phenotype/Phenotyping

Within the context of drug metabolism, a phenotype is the observed manner in which an individual handles the disposition of an administered agent. It begins with the individual's inherited genotype, which can then become influenced by environmental factors through interactions at the genetic level (e.g., induction or inhibition of expression) or at the immediate functional level for any of the processes associated with each of the ADME parameters (e.g., agonist or antagonist effects in play at any given process). A common environmental factor would be prior and/or simultaneous exposure to one or more other drugs (e.g., drug-drug interactions) or to xenobiotics present in one's diet. Equally common is when continued exposure to the same drug causes an alteration of one or more of the ADME processes with time, thus causing an alteration in the agent's observed disposition (i.e., the person's phenotype). Compounds with well-defined disposition patterns across normal and/or various genotype-defined individuals can be deployed for both genotyping and phenotyping of an individual.

See: Genotype/Genotyping, Genetic Polymorphism, Phenobarbital Induction and Sleeping Time, ADME, Drug-Drug Interactions, and Cocktail Study.^{116,119,125,127,230}

175. Plasma and Serum; Plasma and Serum Concentration

Pertaining to drug metabolism, plasma is the fluid portion of whole blood. It can be separated from the latter by adding an anticoagulant (e.g., heparin or EDTA) and centrifuging to remove cellular components. If no anticoagulant is added and clotting is allowed to occur, then the centrifugation's separated fluid is called serum (plasma that also lacks the majority of the clotting factors, such as the fibrinogens).

Note 1: Plasma/serum represents more than 50 % of the total blood volume. They are more than 90 % water and, in addition to a plethora of small inorganic and organic molecules, contain numerous proteins, including various enzymes, the most important of which for drug metabolism probably belong to the Esterase family. A family of highly lipophilic plasma proteins unique to plasma/serum (hence their name) can sometimes have a significant impact on the disposition of a xenobiotic, even when there is no susceptible ester group present. This is especially the case when non-polar xenobiotics bind tightly to such proteins, which can then serve as a reservoir while decreasing the immediate plasma/serum concentration of unbound agent that would otherwise be free to undergo metabolism or to potentially cause toxicity.

Note 2: Because blood is the primary means of transporting xenobiotics throughout the body and provides for readily accessible sampling, it and/or its subsequently separated plasma or serum is commonly used during PK studies to track the distribution and disposition of parent compounds and their metabolites, such as, for example, their plasma concentrations.

Note 3: Total blood volume can be ascertained by the following steps: 1) injection of a standard dye (e.g., Evans blue); 2) allowing for its distribution throughout the body; 3) removal of a blood sample, which is treated with an anticoagulant and centrifuged; and 4) measurement of the dye's plasma concentration. The concentration of a test drug can be determined similarly, while also following a protocol that instead specifies several measurements to be taken across a continuing timeline (typically for several hours after a single oral dose) so as to deduce a half-life within this compartment that, in turn, is generally taken as an estimate of the terminal half-life for the agent relative to the entire body.

Note 4: Serum measurements should not be used when there is a suspicion that significant amounts of the test agent may bind to blood clotting factors or that the agent may be susceptible to entrapment during the blood clotting process. Similarly, plasma protein binding should be assessed for either fluid during analytical method development so as to account for how it might impact the agent's recovery from the matrix and its ultimate response in the assay. Finally, although plasma can be relied upon as a preliminary assessment of an agent's susceptibility to Esterases, the use of whole blood in follow-up studies is additionally recommended, because the surface and interior of red blood cells also contain considerable Esterase activity.

See: Pharmacokinetics (PK), ADME, *AUC*, Clearance (*CL*), Compartment Model, Disposition, DMPK, Dose-Dependent Kinetics or Metabolism, Half-Life ($t_{1/2}$), Intrinsic Clearance, Metabolic Clearance, Metabolic Pathway/Pattern/Profile, Non-Compartment PK Analysis, Plasma Protein Binding, Partition Coefficient (P ; $\log_a P$ and $\text{clog}_a P$); Distribution Coefficient (D ; $\log_a D$), PBPK, and Pharmacodynamics. For specific definitions, see Refs. 231,232.

176. Plasma Protein Binding (PPB)

Typically, a readily reversible association between a xenobiotic and certain of the proteins present in plasma, particularly those that can be regarded as lipophilic in overall character, such as albumin, lipoprotein, glycoprotein and the α , β and γ globulins. In general, some of the association involves hydrophobic bonding, such that less polar xenobiotics tend to be more prone to this interaction than polar compounds. Alternatively, the slightly basic nature of albumin can allow for association with acidic functionality when present on a xenobiotic, as well as with neutral agents, while the acidic nature of certain of the glycoproteins can accommodate xenobiotics having basic centers. Because only unbound compounds can serve as substrates for enzymes or can undergo membrane permeation, significant PPB can impact the ADME profile of xenobiotics and their metabolites, as well as their efficacy profile.

Warfarin represents a classical example of a drug that undergoes highly significant PPB (more than 95 %). It also has a narrow therapeutic index, such that if another drug is administered that also binds strongly with the PPs so as to cause displacement of warfarin (drug-drug interaction), the latter's resulting higher unbound concentrations could lead to excessive bleeding or other toxicities not normally seen at this same dose.

Note 1: Historically, it was thought that PPB had a significant impact on ADME events only when a compound was bound at above 90 % or even above 95 %, because this typically rapid equilibrium's dissociation rate can otherwise readily supply unbound agent fast enough to satisfy the equilibrium requirements for significant interaction with various proteins associated with all of the potential ADME-related processes. This situation typically prevails in a practical manner when deploying an assay method to assess a drug's plasma concentration, i.e., such determinations typically account for both free and bound drug, even though the assay measurement itself invariably detects only unbound drug. This is because the sample preparation method generally pulls bound drug off of the PPs prior to executing the detection method.

Note 2: Xenobiotics bound to PPs at such extremely high levels can be thought to have entered another compartment that, in turn, serves to immediately decrease the levels of free xenobiotic in the plasma while serving as a reservoir to more gradually release the agent back into the system. This scenario probably has evolutionary ties, where mammals came to enhance the PPB proteins to assist in accommodating lipophilic natural products in their diets that could have concentration-dependent toxicity.

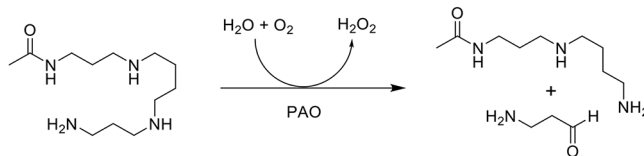
Note 3: More recently, it has become appreciated that the influence of PPB may be able to occur at less high levels of association with a xenobiotic. Given the dynamic nature of the flow-driven distribution for a xenobiotic, its initial unbound concentration, rather than a responsively increasable concentration, may be more important when the surface linked to a particular ADME event decreases in size, regardless of the specific ADME-related process's ability to capture unbound agent. Thus, a specific level where PPB generally becomes significant will not be cited as part of this term's definition. However, it should be clear that the more an agent undergoes PPB, the greater the chance for the latter to have an impact upon the agent's ADME profile: that impact would generally be to slow down the possible ADME processes that might occur on the agent.

See: Plasma and Serum; Plasma and Serum Concentration, Pharmacokinetics (PK), ADME, *AUC*, Clearance (*CL*), Compartment Model, Disposition, DMPK, Dose-Dependent Kinetics or Metabolism, Half-Life ($t_{1/2}$), Intrinsic Clearance, Metabolic Clearance, Metabolic Pathway/Pattern/

Profile, Non-Compartment PK Analysis, Partition Coefficient (P ; $\log_a P$ and $\text{clog}_a P$); Distribution Coefficient (D ; $\log_a D$), PBPK, Drug-Drug Interactions, and Pharmacodynamics.^{233,234}

177. Polyamine Oxidase (PAO)

This term is now known to encompass a group of related enzymes within the broader oxidoreductase family that cleave certain alkyl-amines to the des-alkyl amine and an aldehyde by using molecular oxygen with FAD and iron serving as cofactors. Specific members include: N^1 -acetylpolyamine oxidase EC 1.5.3.13; polyamine oxidase (propane-1,3-diamine-forming) EC 1.5.3.14; N^8 -acetylspemidineoxidase (propane-1,3-diamine-forming) EC 1.5.3.15; spermine oxidase EC 1.5.3.16; and non-specific polyamine oxidase EC 1.5.3.17. They are present in most mammalian tissues, where specific members play a role in the natural polyamines cycle, N^1 -acetylspermine and N^1 -acetylspemidine being particularly good substrates while spermidine itself is not. The PAO group works with MAO and DAO to generally deaminate xenobiotics where their structural requirements are similar to MAO, although as a group they can accommodate more complex and multiple amino-containing compounds. The example below depicts deamination of N^1 -acetylspermine to N^1 -acetylspemidine.



See: Catabolism, Deamination, Dealkylation, N -Dealkylation, Monoamine Oxidase (MAO), Diamine Oxidase (DAO), and Oxidation/Oxidase/Oxidoreductase.²³⁵

178. Polymorphism and Polymorphic Metabolism

Polymorphism reflects the existence of one or more alleles of a gene within a given population where the frequency of the rarer alleles is greater than can be explained by recurrent mutation alone and results in an observed phenotype with an incidence greater than 1 %. When the genes translate to metabolism-related machinery, the resulting polymorphic enzymes or other associated biomolecules can lead to differential metabolism or polymorphic metabolism among the individuals in this population. The

differences can be as large as distinct pathway changes, or as small as subtle alterations in the drug metabolism rates along the same biotransformation pathway.

See: Acetylation Phenotype, Aldehyde Dehydrogenase Polymorphism or Deficiency, Atypical Alcohol Dehydrogenase, Catechol-*O*-Methyl Transferase (COMT), Enzyme, Isoform or Isozyme (Isoenzyme), *N*-Acetyltransferases (NATs), Genotype/Genotyping, Pharmacogenetics and Pharmacogenomics, Phenotype/Phenotyping, Single Nucleotide Polymorphism (SNP), Extensive (or Rapid) Metabolizer and Poor (or Slow) Metabolizer.^{103,114-129}

179. Poor (or Slow) Metabolizer

A subpopulation within a given species that metabolizes a xenobiotic via a pathway under polymorphic influence at a significantly slower rate compared to the other subpopulations. The faster rate groups, in turn, can be classified as "extensive" or "rapid metabolizers."

Note 1: This profile can occur from a mutation or deletion in a drug metabolizing-related gene that results in lower expression of a fully functional metabolizing system; a similar expression of a less than fully functional metabolizing system; or a higher expression of a fully functional, even enhanced, catabolic system that degrades the metabolizing system.

See: Acetylation Phenotype, Aldehyde Dehydrogenase Polymorphism or Deficiency, Atypical Alcohol Dehydrogenase, Catechol-*O*-Methyl Transferase (COMT), Enzyme, Isoform or Isozyme (Isoenzyme), *N*-Acetyltransferases (NATs), Genotype/Genotyping, Pharmacogenetics and Pharmacogenomics, Phenotype/Phenotyping, Extensive (or Rapid) Metabolizer, and Polymorphism and Polymorphic Metabolism.^{103,114-129}

180. Pregnane X Receptor (PXR)

PXR is a nuclear receptor protein that serves as a transcription factor to regulate the expression of selected genes. In addition to being responsive to several endogenous ligands, such as many of the steroids, for which it is also sometimes referred to as a steroid sensing nuclear receptor (SXR), PXR is highly promiscuous in its formation of a complex with numerous xenobiotic partners. Exemplifying the latter is the complex formed with the

drug dexamethasone, which includes the formation of a heterodimer with the retinoid X receptor (RXR), which then binds to the response element of the CYP 3A4 promoter. Its interaction with the xenobiotic-responsive element (XRE) can likewise lead to upregulation of CYPs 3A4, 2C8, 2C9, and 2B6. Further underscoring its important role in the overall disposition of xenobiotics, the PXR complex can also upregulate certain of the drug conjugation enzymes, such as glutathione *S*-transferase, and even some of the efflux proteins, like P-glycoprotein (Pgp or MDR1).

See: Peroxisome Proliferator-Activated Receptors (PPARs), Aromatic Hydrocarbon Receptor (AHR), Glucocorticoid Responsive Element (GRE), Retinoid X Receptor (RXR), Constitutive Androstane Receptor (CAR) and CAR/RXR Heterodimers, and Xenobiotic Responsive Element (XRE).^{35,236}

181. Presystemic Elimination

Reduction in a xenobiotic's concentration prior to its distribution into the systemic circulation. Primarily used within the context of orally ingested agents that undergo first pass metabolism and excretion.

Note 1: In addition to elimination caused by the intestinal epithelium, hepatic tissues, and lungs, presystemic elimination can occur in the gut lumen due to degradation under the acidic conditions of the stomach, to breakdown by excreted peptidases, and from metabolism caused by the bacteria comprising the intestinal microflora.

See: Absorption, Bioavailability, Clearance, ADME, First Pass Metabolism, Hepatic Clearance, Hepatic Extraction, Metabolic Clearance, Presystemic Metabolism, and Enterohepatic Cycling.^{39,105,106}

182. Presystemic Metabolism

Reduction in a xenobiotic's concentration due to metabolism prior to its distribution into the systemic circulation. Primarily used within the context of orally ingested agents, which can undergo biotransformations caused by intestinal microflora, intestinal enterocytes, the liver, or the lungs prior to distribution within the general systemic circulation.

See: Absorption, Bioavailability, Clearance, ADME, First Pass Metabolism, Hepatic Clearance, Hepatic Extraction, Metabolic Clearance, Presystemic Clearance, and Enterohepatic Cycling.^{39,105,106}

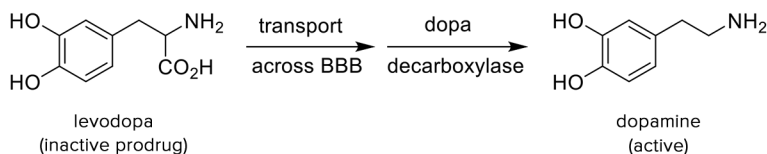
183. Primary Metabolite

A metabolite that is formed directly from the xenobiotic initially presented to the biological system (parent compound).

See: Metabolite versus Degradation and Decomposition Products, Metabolism, Drug Metabolism, Biotransformation, Secondary Metabolite, and Metabolic Pathway/Pattern/Profile.^{3,45,82,91}

184. Prodrug

As typically used: an inactive or weakly active pharmacological agent that undergoes conversion in the body to become an effective drug, the conversion generally involving a biotransformation or metabolic step. A more precise definition is: a compound that requires one or more structural changes to occur after its administration in order to elicit its desired pharmacological effects, such changes not including simple shifts in the equilibria of ionizable groups, nor the interchange of various salt or tautomeric forms that may be applicable to the compound within different environments. A classic example is shown below. While dopamine can be effective in treating Parkinsonism, it cannot cross the blood-brain barrier (BBB). Levodopa, itself inactive, is administered orally as a prodrug, because it can take advantage of an amino acid transporter system to gain entry into the body and the CNS. It then undergoes conversion to dopamine by brain dopa decarboxylase (although some is also prematurely converted by peripheral dopa decarboxylase, leading to the development of subsequent formulations also having a peripheral dopa decarboxylase inhibitor).



Note 1: The spelling and definition of this term have undergone an interesting evolution.¹⁶⁹ The term "pro-drug" was first introduced by Albert more than fifty years ago when he used it to describe compounds which require metabolic biotransformation in order to exhibit their pharmacological effects, such conversions being either an inherent property of the parent compound ("accidental" prodrug), or a property intentionally incorporated into an otherwise active drug by specific design.^{168a} Emphasizing the

latter, the term “drug latentiation” was also introduced at nearly the same time by Harper. This type of design strategy was defined as “chemical modification of a biologically active compound to form a new compound which upon *in vivo* enzymatic attack will liberate the parent compound.”^{169b} Kupchan et al. subsequently expanded the strategy to further include drug modifications that relied upon non-enzymatic processes to regenerate the parent compound within an *in vivo* setting, such as spontaneous hydrolysis of the modification as a function of pH.^{169c} Because “latentiation” implies a time-lag for the regeneration of the active substance and this type of event is not always required to be part of a prodrug’s most desirable pharmacokinetic profile, such terminology has not been sustained. Eventually, the term “prodrug” (without the former hyphen mark) became universally accepted.¹⁶⁹ Despite this consensus about what term to finally adopt, its intended meaning in a given usage has continued to vary, often being taken to include a much wider context of drug-related profiles and design strategies that, in some cases, are not appropriate. This situation has prompted the citation of a more precise definition in addition to the one that is typically encountered, as noted above.

Note 2: According to the more precise definition, prodrugs are inactive with regard to their desired effects within the range of their advantageous administration protocols until they undergo bioactivation by either a chemically driven conversion, an enzymatically driven biotransformation, or any multiple combination of these processes. However, this profile should not be taken to also encompass any undesired side-effects, which could be inherently present within the prodrug without the need for a preceding biotransformation. Also, according to this definition, efficaciously active agents whose metabolites are similarly active or contribute to the desired pharmacological profile by another mechanism should not be considered to be prodrugs. While such compounds have sometimes been referred to as “limited prodrugs,” they should retain their simple classification as “drugs,” no matter how short-lived the parent compound’s observable activity might be *in vivo*. That such drugs also happen to have active metabolites does not, then, alter their initial classification according to this term. Finally, although sometimes confused with prodrugs in the literature because they can also be used as a strategy to target selected biological compartments, “soft drugs” should not be regarded as prodrugs. The schematic relationships between a drug, prodrug, and soft drug can be found under the definition of a soft drug.

Note 3: Prodrug strategies can be attempted to enhance any of the ADMET properties associated with a drug candidate. A strategy that is particularly prominent today is to decrease toxicity by increasing selectivity for the desired, efficacious target, thus lowering the dose needed to interact with the latter and/or raising the dose needed to interact with the former, off-target site. To further appreciate the rich history of the prodrug field and its importance within today's drug design processes, readers are encouraged to consult the excellent review articles, monographs, and texts that have been dedicated specifically toward prodrugs by several distinguished experts across time.

Note 4: A classification system for different types of prodrugs has been proposed, but it has not been commonly adopted for general use. However, some system for the further subcategorization of prodrugs may be useful in the future due to the present rise in prodrug strategies that involve complicated molecular constructs to deliver therapeutic agents targeted toward specific tissues via connection to monoclonal antibodies, i.e., antibody-drug conjugates or ADCs. This possibility is further discussed in Appendix III, where interested readers can also consider the proposed classification system in an even further expanded manner suggested by the glossary's corresponding author.

See: DMPK, Biotransformation, Bioactivation, Blood-Brain Barrier (BBB), Transporters, Parent Compound, Pharmacokinetics (PK), ADME; ADMET and Soft Drugs.^{50-52,169,170,229}

185. Prosthetic Group

A non-protein substance whose tightly bound (sometimes covalent) association with an enzyme is required for the latter's function. It can be organic, such as a vitamin, or inorganic, such as a metal ion. An example particularly relevant for drug metabolism is the heme group, which has both an organic and inorganic component. Its association with the CYPs is requisite for their key role in the biotransformation of numerous xenobiotics. The heme group is composed of a protoporphyrin ring that utilizes its 4 pyrrole-ring nitrogen atoms to bind Fe^{+2} at its center.

See: Apoenzyme, Holoenzyme, and Cofactor.³⁴

186. Quantitative Structure-Metabolism Relationships (QSMR)

Multivariate data analyses that attempt to describe the relationship between quantifiable molecular parameters and the rates of a specific metabolic event for compounds within a series of substrates. Hansch is regarded as the “father” of conceptualizing quantitative structure-activity relationship (QSAR) studies applied to biological settings, including specific drug metabolism profiles, as well as toward observed efficacy.²³⁷ His tabulation of substituent constants for this use is landmark. Numerous molecular parameters have been additionally discerned for use during subsequent proliferation of such analyses and, for example, pertain to categories like: (i) size, volume, surface area, and molecular weight; (ii) aqueous solubility, polarity, and lipophilicity/hydrophobicity; (iii) point charge, charge distribution, and various electronic effects; (iv) hydrogen bonding; and (v) steric effects, particularly when assessed in close proximity to the site for the given metabolic event.

Note 1: The process is analogous to quantitative structure-activity relationship (QSAR) analyses that historically pertain to assessing the relationship between chemical structure and efficacy. Sometimes this phrase is retained for use within the context of drug metabolism, with “activity” then meant to additionally include metabolism as well as efficacy. Authors should then be careful to alert readers within the accompanying text that they are applying the phrase to mean either a given biotransformation event or a given efficacious event as may be appropriate for the situation undergoing analysis or discussion.

Note 2: In addition to defining meaningful and thus potentially useful molecular parameters to guide further structural modification, these types of analyses typically strive to predict the biological response that will be obtained for new compounds. To that end, reiterative improvements in the equation’s parameters are often derived by assessing several “test compounds” that have been selectively or randomly excluded from the initial analyses “training set” of compounds. With regard to both efficacy and drug metabolism analyses, predictive correlations have been most successful for large sets of compounds that are highly similar in overall structure and where the biological data has been derived from well-controlled *in vitro* assays, rather than from *in vivo* testing. Note that this is despite the fact that the original development of these types of analyses were from associations having *in vivo* data (see Random Walk).

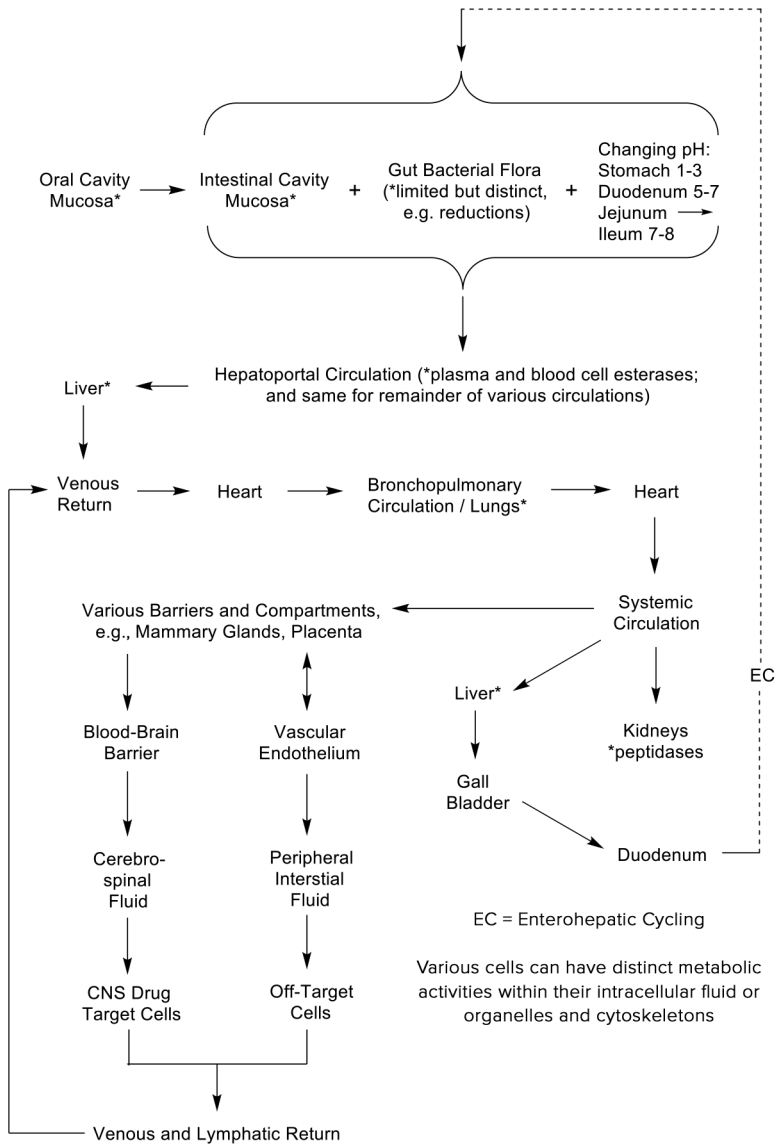
See: Metabophore, Metabophore Probe, Structure-Metabolism Relationship (SMR), Metabolic Prediction/Possibilities versus Probabilities, Metabolic Probe, Random Walk, and Rule of One. Landmark Refs.: 237, 238.

187. Random Walk

While this phrase originates within the principles of probability theory, Hansch was the first to apply it in the context of drug action, namely to emphasize the complexity of the path that a drug must traverse after its oral administration to an animal or human until the drug finally reaches its site of efficacious activity. For example, the path undertaken by a CNS drug after oral administration is depicted below. Sites regarded as being rich in drug metabolizing capability are denoted by an asterisk (*). More recently, this phrase has been used as part of the descriptive title for a quantitative structure-activity relationship (QSAR) method that attempts to predict drug-target interaction by random walk with restart on a heterogeneous network ("NRWRH").

Note 1: In probability theory, this is the stochastic process formed by the successive summation of independent, identically distributed random variables. Equations can be set up to appropriately accommodate the variables relative to sets of observed data. The situation for drugs, however, is not random, and their behavior derives from their chemical structures and the dictation by these of specific chemical properties, coupled to the largely identical distribution of the physiological variables that will be encountered within a given species. Hansch thus set up equations for this context that related the physicochemical properties of compounds to their observed biological properties, namely that of efficacious activity observed from an *in vivo* study. These were later termed Hansch equations and his assembly of them became the basis for considering him to be the "father of quantitative structure-activity relationship" (QSAR) studies.

Note 2: While ADME terminology had not yet become fashionable and the detailed study of each of their complexities was not yet feasible, these processes were at least appreciated in a gross manner, e.g., as more of a grey box. Thus, by analogy to probability theory, the ADME processes were essentially gathered and treated as a successive summation of identically distributed variables that then wrapped into the observable activity. Metabolism was not treated as a separate factor on the side of the equation



used to address the variable physicochemical properties. With time, however, metabolism has itself been treated as an observed activity, and for such analyses it is then placed on the other side of the equation, in place of observable efficacious activity.

See: ADME; ADMET, Absorption, Distribution, Metabolism, Biotransformation, Elimination, Excretion, Metabolic Pathway/Pattern/Profile, Blood-Brain Barrier (BBB), and Quantitative Structure-Metabolism Relationships. Landmark Refs.: 237, 238. For recent application of term, see Ref. 239.

188. Rapid (or Extensive) Metabolizer

See Extensive (or Rapid) Metabolizer.

189. Rate Constant (k or λ)

A constant which relates the rate of a linear kinetic process to the concentration or amount of the agent involved in the process.

See: Linear Kinetics, Pharmacokinetics (PK), Half-Life ($t_{1/2}$), Enzyme, Nonlinear Kinetics, and Michaelis-Menten Kinetics.^{39,40,104}

190. Reaction Phenotyping

In this context, the phrase typically refers to *in vitro* testing that attempts to determine which human metabolizing enzymes are involved in the biotransformation of a given xenobiotic.

Note 1: This is distinguishable from phenotyping an individual, which attempts to discern how that person will generally undertake biotransformations toward all types of xenobiotics.

See: Metabolic Pathway/Pattern/Profile, Metabolic Prediction/Possibilities versus Possibilities, Metabolic Profiling/Fingerprinting, Genotype/Genotyping, and Phenotype/Phenotyping.^{2,45,90,91,115,119,150-153,240}

191. Reactive Intermediates

Unstable (chemically reactive) species that have been postulated or observed in only a transient manner, which are rapidly formed in a catalyzed reaction and typically serve to decrease the energy barrier from reactant(s) to product(s), thereby increasing the overall reaction rate. For example, some of the biotransformations catalyzed by the CYPs produce reactive intermediates like a nitroso, carbene, ketene, quinone-imine, or quinone-methide while still on route to becoming metabolic products.

Note 1: Certain of the aforementioned examples can sometimes coordinate in a quasi-reversible fashion, with the iron present in the CYP's prosthetic heme leading to the transient formation of a catalytically inactive complex, or they can form a covalent bond with the CYP protein, thereby potentially deactivating the complex in a permanent manner. Should they escape the CYP catalytic site, they can quickly undergo reactions with neighboring macromolecules. Thus, reactive intermediates can be involved in mechanisms of toxicity, as well as accelerating the production of less active metabolites.

See: Non-Competitive Inhibition, Autoinhibition, Competitive Inhibition, Uncompetitive Inhibition, Mechanism-Based Product and Transition State Inhibitors, Covalent Binding, Suicide Inhibitor, Michaelis-Menten Kinetics, and Reactive Metabolites.^{42,62,146-148}

192. Reactive Metabolites

Unstable (chemically reactive) metabolic reaction products that are able to form covalent adducts with biomolecules. Depending on how aggressively promiscuous (reactive and nonselective) they are in pursuing a partner, their reactions can often extend beyond the CYPs and their immediately neighboring biomolecules. When electrophilic, they are instead candidates for bioinactivation via conjugation by the equally aggressive glutathione pathway. Likewise, the reactive aldehyde species produced by the CYPs during *N*-dealkylation, or by the oxidation of ethanol, are so commonplace that mammals have devised subsequent pathways to immediately deactivate them, e.g., by oxidation to their carboxylic acids or by reduction to their alcohols.

Note 1: CYP binding generally inhibits enzymatic activity; protein binding can lead to allergic responses, lipid binding to peroxidation or necrosis; and interactions with DNA can cause mutagenic, teratogenic, or carcinogenic effects; all depending upon the balance between toxic (reactive) metabolite formation versus the competing processes involving various detoxification mechanisms.

See: Non-Competitive Inhibition, Autoinhibition, Competitive Inhibition, Uncompetitive Inhibition, Mechanism-Based Inhibition, Covalent Binding, Suicide Inhibitor, Michaelis-Menten Kinetics, Reactive Intermediates, Bioactivation, Bioinactivation, Detoxification, *N*-Dealkylation, Dealkylation,

Alcohol Dehydrogenase (ADH), Aldehyde Dehydrogenase (ALDH), Aldehyde Oxygenase (AO), Glutathione (GSH), and Glutathione Conjugation.^{42,62,146-148}

193. Reactive Oxygen Species (ROS)

Endogenous ROS are components of oxygen metabolism that play important roles in cell signaling. However, environmental stress can increase their levels to a point where cell structures may be damaged, a syndrome known as "oxidative stress." Simple chemical species include: peroxide, superoxide, hydroxyl radical, singlet oxygen, and α -oxygen. The common pathway for reduction of molecular oxygen is depicted below, where several of these reactive species are shown. Dismutation is catalyzed by superoxide dismutase. Termination (full reduction) can be accomplished by both the glutathione and catalase pathways.

$O_2 + e^- \rightarrow O_2^-$ (superoxide; reactive radical anion)

Dismutation: $2 O_2^- + 2 H^+ \rightarrow H_2O_2 + O_2$ (hydrogen peroxide and molecular oxygen)

Partial reduction: $H_2O_2 + e^- \rightarrow HO^- + \cdot OH$ (hydroxide anion and hydroxyl radical; reactive radical)

Termination: $H_2O_2 + 2 e^- + 2 H^+ \rightarrow 2 H_2O$ (water; radical pathway terminated)

Note 1: Certain drug metabolism pathways can bioactivate a xenobiotic to produce related free radical species or xenobiotic metabolites that are themselves radicals, which may then undergo covalent interactions with enzymes and proteins or prompt lipid peroxidation. The formation of hydrogen peroxide can be particularly toxic, because its attenuated reactivity can allow it to distribute and interact with particularly sensitive biochemicals, such as DNA, within a cell's nucleus. The glutathione pathway protects cells from all of these events by either serving as a reducing agent (radical termination) or by undergoing a conjugation reaction with a reactive metabolite. Catalase protects cells from hydrogen peroxide. Toxicity results when oxidative stress overwhelms the local levels of these ubiquitous defensive pathways.

See: Active Metabolite, Bioactivation, Catalase, Covalent Binding, Glutathione (GSH), Glutathione Conjugation, Mercapturate/Mercapturic

Acid Conjugation, Oxidation/Oxidase/ Oxidoreductase, Oxidative Stress, Suicide Inhibitor, and Superoxide Dismutase.²⁴¹⁻²⁴³

194. Recombinant Enzyme

An enzyme expressed by a metabolically competent (host) cell line that has been transfected with a vector containing the enzyme's corresponding cDNA, so as to serve as a genetic insert: it is thus sometimes called a cDNA-expressed enzyme. It is common to simultaneously include a vector that can desensitize the host to an antibiotic, such that treatment of the cell culture with the latter allows only continued growth of the dually transfected cell line that will then produce the desired enzyme. The enzyme is either expressed in a functional form for direct use within the host cell, or, when separately isolated, can be re-activated by the addition of requisite cofactors or prosthetic groups. Examples relevant to drug metabolism include the expression of many of the CYPs, including their human (hCYPs) forms, flavin-containing monooxygenases, UDP-glucuronosyl transferases, and some glutathione *S*-transferases.

Note 1: These methods have become an important contributor to predicting what types of metabolic reactions (disposition) may occur for a new drug candidate once it becomes administered to humans (when it enters clinical studies).

Note 2: A wide range of isozymes produced in this manner are available from commercial sources, mainly from rodent and human genetic origins. It is also common for labs to be quite gracious in sharing vectors they have assembled for such purposes.

Note 3: The field has also moved toward the production of rodent animal models that have been transfected with numerous hCYPs and selected ADME-relevant transporters. A few of these cloned models are now commercially available as well.

See: Enzyme, Apoenzyme, Holoenzyme, Cofactor, Prosthetic Group, Metabolic Prediction/Possibilities versus Probabilities.^{244,245}

195. Reconstitution System

Medium required for catalytic activity by a given enzyme after its isolation or partial purification. In general, components are added so as to prompt the maximum achievable catalytic rate for a duration that is feasible according to the experiment of interest. For example, depending on the specific CYP, the following general conditions can typically promote a robust catalysis up to a ca. 15 to 30 min experimental timeframe: isolated CYP enzyme; NADPH-cytochrome *P*-450 reductase; cytochrome b5; a phospholipid mixture or isolated lipids; sodium cholate; divalent metal ions, such as Mg^{+2} ; reduced glutathione; and 50 to 100 mM pH 7.4 buffer at 37°C with exposure to air. An added NADPH-generating system is always also required. Many of these constituents are present in the standard microsomal fraction, where the CYPs can be regarded as being concentrated compared to the normal cellular milieu, if not partially purified.

See: Microsomal Fraction/Enzyme; Microsomes, Mixed-Function Oxidase, Cytochrome *P*-450 Enzymes (CYPs), Recombinant Enzyme, and Regenerating System.^{45,69-73,244}

196. Reductase

A broad term for enzymes that catalyze a reduction reaction, i.e., where the substrate receives one or more electrons or hydrogens from a donor molecule. One of the more common examples would be from the class of oxidoreductases, which typically require NAD(P)H as the hydrogen donor.

Note 1: The reductases are mainly located in the cytosol, especially from organs associated with biotransformations, such as the liver. They are also well-represented in the gut microflora and from there can often contribute significantly to the bioreduction of xenobiotics entering by the oral route.

See: Oxidation/Oxidase/Oxidoreductase, Gut Microflora, and Reduction.^{188,189,246}

197. Reduction

Biotransformation in which the substrate receives one or more electrons from a donor molecule, including via transfer of hydrogen, thus making a hydrogenation equivalent to a reduction reaction, but not always vice versa.

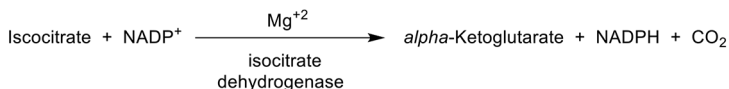
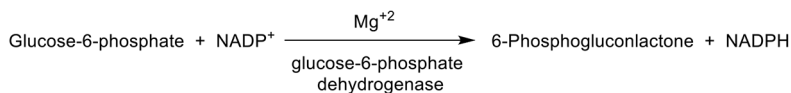
Note 1: Reductions are generally coupled to an oxidation where the electron-accepting substrate is reduced while the electron-donating partner is oxidized, characteristic of the oxidoreductase class of enzymes. Common partners during xenobiotic reductions are the reduced (H-containing) forms of glutathione, FAD, FMN, and NADP(H).

Note 2: Common substrate functionalities already present in xenobiotics or resulting from a xenobiotic's initial metabolism include: azo and nitro groups (as mainly catalyzed by the gut microflora and to a smaller extent by the liver); carbon-carbon double bonds; carbonyl groups, where the reduction of aldehydes (e.g., resulting from an initial *O*- or *N*-dealkylation biotransformation) to alcohols represents an important bioinactivation/detoxification pathway; certain disulfide linkages, which can lead to bioactivation; certain sulfoxide and *N*-oxide species; and certain carbon-halogen bonds, where reductive dehalogenation results in cleavage of the halide with replacement by a hydrogen atom.

See: Oxidation/Oxidase/Oxidoreductase, Reductase, Bioinactivation, Detoxification, Bioactivation, and Gut Microflora.^{188,189,246}

198. Regenerating System

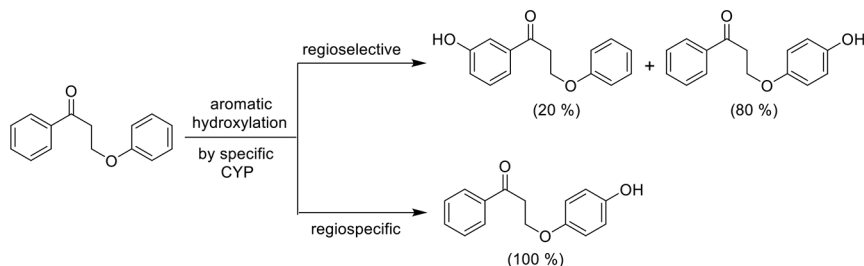
A method or set of conditions used to replenish a material that becomes depleted as part of an enzymatic reaction when conducted in an experimental setting where the normal process for the material's regeneration is no longer present. A common example relevant for xenobiotic metabolism would be the maintenance of NADPH while assessing CYP reactions utilizing a liver microsomal fraction. Two methods for the latter are shown below.



See: Microsomal Fraction/Enzyme; Microsomes, Mixed-Function Oxidase, Cytochrome *P*-450 Enzymes (CYPs), Recombinant Enzyme, and Reconstitution System.^{45,69-73,244}

199. Regioselective and Regiospecific Metabolism

Regioselective metabolism involves the differential formation of distinct metabolites that differ only in their regioisomeric locations of the same resultant modification by a single enzyme. When only one such possibility is observed, then the metabolic event can be considered to be regiospecific. A hypothetical example is shown below for the case of aromatic hydroxylation.



Note 1: Hydroxylation possibilities on the same aromatic ring, i.e., *ortho*- versus *meta*- versus *para*-, are almost always highly regioselective, if not completely regiospecific. Their selectivity/specificity follows the same electronic and steric factors contributing to the chemical reactivity observed for electrophilic aromatic substitution.

See: Aromatic Hydroxylation and Stereoselective Metabolism.^{247,248}

200. Retinoid X Receptor (RXR), Constitutive Androstane Receptor (CAR) and CAR/RXR Heterodimers

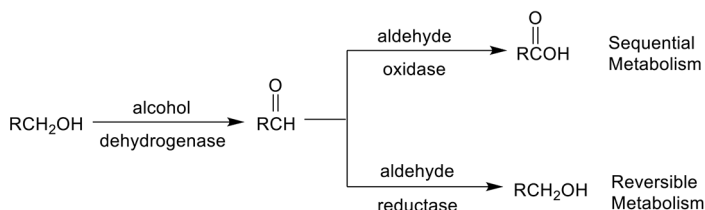
The retinoic acid receptors (RAR) and CAR are nuclear receptor proteins that serve as transcription factors to regulate the expression of selected genes. In addition to being responsive to endogenous ligands, where their signaling can regulate cellular differentiation, development, and basal-level endogenous metabolism (anabolism and catabolism), CAR can also interact with xenobiotic ligands, where its signaling can have an impact on drug metabolism and distribution. The interaction of endogenous ligands with the RXR- α , - β , and - γ members of the RAR family is not as well defined, such that the composite of their normal physiological roles remains unclear. However, like CAR, they can interact with xenobiotics and, in addition, can form heterodimers, such as RXR/PPAR and RXR/CAR, where subsequent signaling can impact pathways relevant to drug metabolism. The homodimers and RXR/CAR heterodimers can either promote or attenuate

signaling, depending on biological context. Examples include: RXR's induction of CYP 17A1, certain ABC-1 transporters associated with excretion, and down-regulation of certain CYP hydroxylases; CAR's activation or repression of genes coding for CYPs -2B, 2C, and 3A, sulfotransferases, and glutathione-S-transferases; and RXR/CAR's increased expression of CYP 2B6 and CYP 2B1 (aka "phenobarbital-responsive unit" or PBRU).

See: Aromatic Hydrocarbon Receptor (AHR), Glucocorticoid Responsive Element (GRE), Peroxisome Proliferator-Activated Receptors (PPARs), Pregnane X Receptor (PXR), and Xenobiotic Responsive Element (XRE).^{35,198,246,249-253} Drug Design Ref.: 254.

201. Reversible Metabolism

Occurs when the product from a given metabolic reaction is converted back to its initial structure, the return process generally mediated by another metabolic enzyme. An example of this within a closely proximal setting is shown below for the case of a simple alcohol. An example involving a distal arrangement is represented by the case where a phenol is converted to a glucuronide and then excreted via the bile, after which it undergoes hydrolysis by gut bacteria back to the original phenolic compound. This term is different from futile metabolism.



See: Futile Metabolism, Metabolic Pathway/Pattern/Profile, Enterohepatic Cycling, Gut Microflora, Glucuronic Acid Conjugation (Glucuronidation), Alcohol Dehydrogenase (ADH), Aldehyde Oxidase (AO), Aldehyde Dehydrogenase (ALDH), Oxidoreductases, Reduction, and Reductase.^{45,74,79-82,85,91,93}

202. Rule of Five

A molecule's ability to permeate membranes by passive processes will be reduced when: (1) its molecular mass is greater than 500 Da; (2) there are more than five hydrogen bond donor atoms; (3) there are more than

10 hydrogen bond acceptor atoms; and (4) its calculated octanol-water partition coefficient logarithmic value ($\epsilon\text{Log}_a P_c$) is greater than 5.²⁵⁵

Note 1: The “rules” derive from an assessment of about 2,000 drug molecules undertaken by Chris Lipinski,²⁵⁵ and they are often referred to as “Lipinski’s Rules.” While there are only four rules, their name stems from their common numerical theme that involves multiples of “5.”

Note 2: Large natural products and chemotherapeutic agents as a class were clearly stated to have been removed during the assessment. Likewise, the phrase “passive processes” intentionally emphasizes that when active transport systems or endocytosis are operative, they will supersede this type of determination. Finally, as a last proviso that draws from the context of the present, drug metabolism-related terminology, it should also be emphasized that any correlations to oral bioavailability are additionally subject to the “first pass effect.”

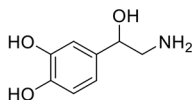
Note 3: Despite the provisos listed in Note 2, the rules are widely embraced and used as a guideline quite generally across a variety of chemical structures when undertaking drug design and decision-making, with the latter often also including considerations pertaining to oral bioavailability. This reliance tends to be directly proportional to the size of the compound library under investigation. Furthermore, during the early stages of a screening campaign, the molecular mass criteria is often lowered (e.g., to 400 Da), so that there will still be room to add substituents to any of the selected compounds during their subsequent structural optimization.

Note 4: Because the rules are particularly applicable when considering large numbers of compounds and because they can be used in a predictive manner for virtual compounds, $\epsilon\text{Log}_a P_c$ was set as the more useful parameter, rather than deriving experimental $\text{Log } P$ values.

Note 5: With regard to $\epsilon\text{Log}_a P_c$, while no lower limit was specified from the assessment, it is to be assumed that extremely polar molecules with values significantly less than 5 will likewise have difficulty. For example, a useful lower value would be $\epsilon\text{Log}_a P_c$ more negative than -1.0. Often not as apparent, however, is the reason for the cap at 5. For this, it is generally assumed that when lipophilicity rises above this value, the molecule will have trouble departing from the lipid bilayer into the aqueous layer on the

other side of the membrane, leading to low permeability into the cell despite ready uptake into the lipid membrane.

Note 6: Although Lipinski assigned a hydrogen bond donor ("HBD") count of 2 to an -NH_2 group in his original analysis, today's common practice of using the "rules" assigns a count of 1 to such a group,²⁵⁶ which is analogous to assigning a count of 1 for the hydrogen bond acceptor ("HBA") capability of an oxygen-containing group, even though the oxygen atom has two sets of lone-pair electrons. Thus, for counting purposes: common HBDs = $\text{OH} = \text{NH} = \text{NH}_2 = 1$ each; common HBAs = $\text{:N} = \text{:O} = 1$ each. An example is provided below by assessing norepinephrine.



Rule of 5 Assessment:

- (1) Molec Mass = $\text{C}_8\text{H}_{11}\text{NO}_3 = \text{ca. } 169 \text{ Da}$; $< 500 \text{ Da}$ so not a problem
- (2) $\text{HBD} = 4$; < 5 so not a problem
- (3) $\text{HBA} = 4$; < 10 so not a problem
- (4) $\text{Log}_p P = -1.2$ (from PubChem database); < 5 so seemingly not a problem but with the added corollary (this is more than -1.0 to the negative side) the extremely polar character could pose a problem; and likewise, when the basis amine is largely ionized at physiological pH, that particular rapid equilibrium form would not be expected to cross membranes in a passive manner.

See: Absorption, Bioavailability (Absolute and Relative), Partition Coefficient, Passive Transport, Permeation/Permeability.²⁵⁵⁻²⁵⁸

203. Rule of One (Metabolism's Rule of One)

Applicable to small-molecule xenobiotics in general: (1) if there is a simple ester present in the compound, it will typically get hydrolyzed as the predominant metabolic pathway unless it is sterically hindered; (2) all metabolic processes are exquisitely sensitive to steric features, where the rate for any given event is inversely proportional to the immediate steric environment at its biotransformation site; (3) decreasing the electron density at the site of a CYP *P*-450-mediated biotransformation will generally attenuate aromatic hydroxylation, but have little impact on *N*- and *O*-dealkylation processes; (4) replacing all of the hydrogens on the carbon *alpha* to a heteroatom by fluorines can completely block CYP-mediated *N*- or *O*-dealkylation at that location, while replacement of an aromatic hydrogen by fluorine may have little impact on hydroxylation at that location beyond that of electron withdrawal; and (5) as an often excepted parameter, increasing the xenobiotic's overall polarity will tend to decrease its metabolism, particularly with regard to oxidative pathways, and vice versa.

Note 1: The “rules” derive from a more than ten-year review of small-molecule drug metabolism information from a wide variety of public and private sector sources, including unpublished communications with numerous experts in the field. While there are five rules, their “singular” name stems from the overwhelmingly high predictive probability associated with the second rule compared to the other four, for which exceptions are not uncommon.

Note 2: As a predictive tool pertinent for how a new drug and potential analogs might behave during *in vivo* drug metabolism studies, and ultimately on administration to humans, no additional provisos are needed, as their application attempts to add probability assessments on top of the relevant metabolic possibilities.

Note 3: Although not previously iterated in this manner, it is clear that the fields associated with prodrugs and drug targeting have completely embraced the theme of the first rule as the most prominent strategy toward preferentially allowing for quick release of the active agent.

Note 4: In addition to specific drug design strategies, however, the rules are also applicable in ranking the merits of compounds within large libraries and virtual settings, where assessment of metabolic probabilities, rather than just metabolic possibilities, becomes beneficial.

Note 5: Finally, it is also apparent that investigators sometimes are quick to assume that replacement of an aryl-H by F will block hydroxylation at that site, and especially so when they have been successful experimentally. As iterated in rules 3 and 4, even those successes are likely a result of the net decrease in electron density prompted by such a substitution, rather than an actual blockade of this particular metabolic event (e.g., see NIH-Shift).

See: Esterases, Hydrolysis, Metabolic Pathway/Pattern/Profile, Metabolic Prediction/ Possibilities versus Probabilities, Metabolic Switching, Prodrug, Soft Drug.^{2,3,15,50-52,90,167-170,259}

204. S9-Mix

A mix of the S-9 fraction and an NADPH regenerating system, which can be used to study *in vitro* drug metabolism similar to the microsomal fraction, where both types are rich in the CYPs. The S-9 fraction is the supernatant

obtained from a tissue (usually liver) homogenate after centrifugation for 10 min at 9,000 G.

Note 1: Typically 1 mL of this mixture contains: 0.3 mL of the S-9 fraction (itself made from three volumes of 0.15 M KCl per gram of wet liver, so as to have a fairly uniform protein concentration among preparations, where 1 mL of S-9 typically contains microsomes from 250 mg of wet liver); 8 mM MgCl_2 ; 33 mM KCl; 5 mM glucose-6-phosphate; 4 mM NADP^+ ; and 100 mM sodium phosphate (pH 7.4).

Note 2: Since all of the cofactors are present, this mix is generally deployed immediately and, like microsomal fractions, will provide robust enzymatic activity for periods up to 15 to 30 min at 37°C.

See: Microsomal Fraction/Enzyme; Microsomes, Reconstitution System, Regenerating System, and Phenobarbital Induction and Sleeping Time.^{45,69-73,159,229,244}

205. Saturation Kinetics

Nonlinear kinetics observed when the local concentration or the plasma concentration of the analyte (drug or metabolite) does not change proportionately with the dose or the amount of the analyte placed or being generated in the analytical matrix or test system.

Note 1: This type of relationship may be associated with several pharmacokinetic processes, but a saturable transporter or metabolism system are the most common.

Note 2: The list of factors that can lead to dose nonlinearity with high drug concentrations includes both saturation and induction of metabolic enzymes, saturation of active intestinal absorption pathways, active renal tubular secretion and reabsorption, plasma protein binding, tissue binding, dissolution, and distribution.

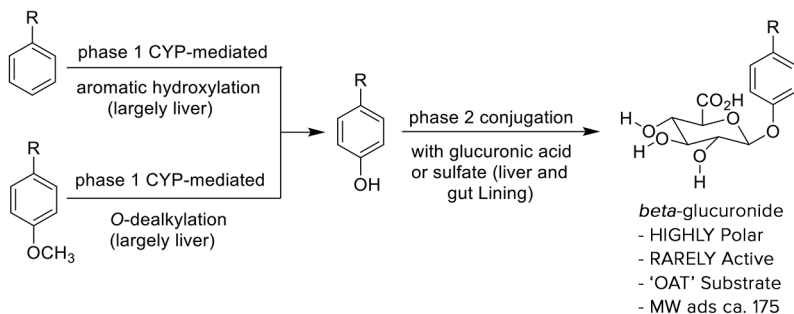
See: Clearance, Enzyme, Competitive Inhibition, Inhibition, Michaelis-Menten Equation/Kinetics, Half-Life ($t_{1/2}$), Linear Kinetics, and Plasma Protein Binding (PPB).^{38-40,104}

206. Secondary Metabolite

A sequential metabolite that is formed from a primary metabolite or any subsequent metabolites.

Note 1: There is a distinction between the various metabolites formed directly from the original xenobiotic or parent drug (all of which are "primary metabolites," regardless of their percentages and durations) versus metabolites subsequently formed from those or any other sequence of additional biotransformation products (all of which are "secondary metabolites," regardless of how many enzymatic steps may have occurred subsequent to the formation of the initial primary metabolite). There are no distinctions for tertiary or quaternary metabolites (all of these remain classified as "secondary metabolites"). Likewise, a secondary metabolite could be the major metabolite, as this classification is connected only with sequence and not with relative amounts.

Note 2: A common type of secondary metabolite is exemplified by a glucuronide when that conjugation occurs at an appropriate functional group that has either been generated or exposed by a preceding CYP-mediated aromatic hydroxylation or dealkylation of an aryl-ether. This is exemplified below.



See: Primary Metabolite, Metabolite versus Degradation and Decomposition Products, Metabolism, Drug Metabolism, Organic Anion Transporter (OAT), Biotransformation, and Metabolic Pathway/Pattern/Profile.^{3,45,82,91}

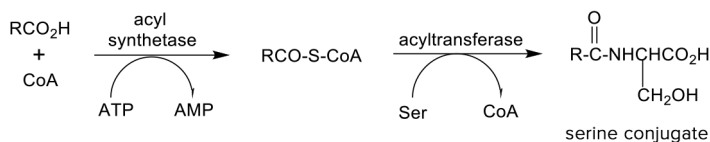
207. Second Pass Metabolism (Ψ First Pass Metabolism)

After a xenobiotic's absorption from the GI tract and "first pass metabolism" associated with the gut wall and dedicated hepatoportal circulation to the liver, the lungs can serve as a second metabolic compartment that is also well represented by the CYPs and analogously served by the dedicated bronchopulmonary circulation, all still occurring prior to returning to the heart's left ventricle for distribution into the systemic circulation. For administration routes that bypass the gut and liver, such as intravenous, intramuscular, subcutaneous, pulmonary, and topical, the lungs become a Ψ first pass metabolism pathway.

See: Absorption, ADME, Elimination, First Pass Effect/Metabolism (Pseudo-First Pass Effect), Extraction Ratio, Bioavailability (Absolute and Relative), and Oral Bioavailability.^{3,4,15,38-40,45,92,104-106}

208. Serine Conjugation

Conjugation of a xenobiotic's carboxylic acid group with endogenous serine according to the acylation pathway where the acid moiety is first activated by attachment to Coenzyme A.



Note 1: Glycine is one of the most commonly used amino acid partners; glutamine, ornithine, and taurine are also utilized.

Note 2: The conjugates resulting from the use of glycine are sometimes referred to as "hippuric acids" or "hippurates" after the historical characterization of this pathway with benzoic acid, the latter specifically called "hippuric acid." The formation of hippuric acid is still used today as a liver function test after administering benzoic acid.

See: Acylation, Amino Acid Conjugation, Acyltransferase, Glycine Conjugation, Hippuric Acid Conjugate (Hippurate), and Glutamine Conjugation.^{6,13}

209. Single Nucleotide Polymorphism (SNP)

A variation in a single nucleotide at a specific position within DNA that occurs within a genomic population to a significant degree, typically greater than 1 %. The two possible nucleotide variations are called “alleles” for this base position. SNPs can sometimes result in variations of the metabolism²⁶⁰ and/or pharmacokinetic profiles for certain xenobiotics observed in different individuals, e.g., extensive or poor metabolizers, and altered distribution or excretion patterns due to SNPs associated with the production of transporter proteins, such as Pgp.²⁶¹

Note 1: SNPs can occur within the coding sequence of genes, non-coding regions, or intergenic regions (sequences between genes). When they occur within the coding region, they can either be “synonymous,” in that they do not change the amino acid sequence of the resulting protein, or “nonsynonymous,” in that they change the resulting protein and alter the latter’s function. SNPs that are not in coding regions can still affect function, such as gene splicing, transcription factor binding, sequence of non-coding RNA, or messenger RNA degradation.

Note 2: Several maps (e.g., see Ref. 262) and databases are available that provide tracking information about the continuing identification of new SNPs and, most recently, at least one about their amino acid consequences. Examples for both include the National Center for Biotechnology Information’s *dbSNP*, and the *dbSNP* (e.g., see Ref. 263), both of which are intended for public use.

See: Acetylation Phenotype, Aldehyde Dehydrogenase Polymorphism or Deficiency, Atypical Alcohol Dehydrogenase, Extensive (or Rapid) Metabolizer, Genetic Polymorphism, Genotype/Genotyping, Phenotype/Phenotyping, Polymorphism, and Polymorphic Metabolism, Poor (or Slow) Metabolizer, and P-Glycoprotein (Pgp/MDR1/ABCB1).²⁶⁴⁻²⁶⁷

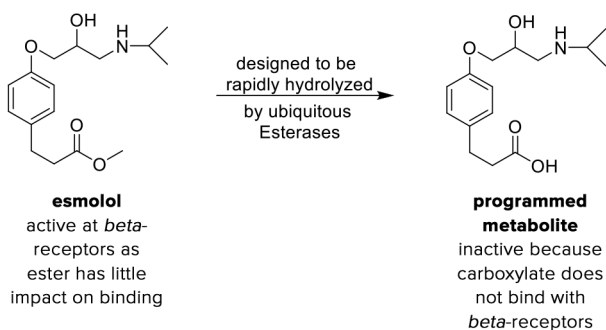
210. Slow (or Poor) Metabolizer

See: Poor (or Slow) Metabolizer.

211. Soft Drug

Pharmacologically active agent that has been designed to be inactivated to one or more nontoxic metabolites in a predictable and controlled manner

after achieving its therapeutic role. Note that a soft drug does not have to be a short-acting agent. Esmolol represents the prototypical soft drug, where a specific metabophore was appended to the classic pharmacophore for *beta*-adrenergic receptor blockade in order to program the resulting soft drug's metabolism according to a preselected metabolic pathway and biotransformation rate, into a metabolite that no longer would be acceptable to the *beta*-adrenergic receptor and thus inactive.^{268,269} In this case, the resulting PK profile is that of an ultra-short acting *beta*-blocker that provides therapeutic benefit by allowing for the moment-to-moment adjustment of adrenergic tone across the heart when it is administered by adjustable intravenous drip in either critical care or surgical settings. This technical definition should not be confused with the use of this same term in some countries as "slang" for certain narcotic agents, or in their legal systems' descriptions of the same (see Note 4 on the next page).



Note 1: While this definition stipulates a rational design of the soft drug, one can also state that a short-acting natural product is a "natural soft drug" when it is intentionally deployed in this type of therapeutic paradigm.

Note 2: Both prodrugs and soft drugs commonly incorporate esters to allow for hydrolytic bioactivation and bioinactivation, respectively, where the "rule of one" can then be used to effectively program reaction rates according to steric-driven structure-activity relationships.

Note 3: Bodor has published extensively in the soft drugs arena and has suggested a multi-layered classification system that further delineates several different types of soft drugs with separate tiers for tissue targeting and prodrug aspects.²⁷⁰ All of these classifications/sub-classifications, however, specify known metabolites as part of the initial design process

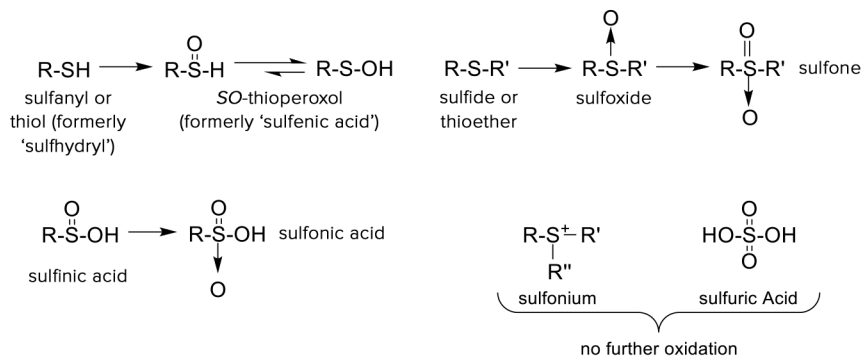
(i.e., Bodor's "retrometabolic drug design"), whereas the definition given above not only encompasses such demonstrable or actual literature-precedent features as rationale, but also further allows for *ab initio* design or unprecedented design strategies, the latter having proven to be useful by the clinically successful, marketed soft drug, esmolol. Furthermore, the principles elaborated for the esmolol strategy have been subsequently deployed in an *ab initio* design fashion by other investigators for several additional clinically successful soft drugs.

Note 4: This technical discussion should be distinguished from the use of this same term "on the street" as slang,²⁷¹ or in specific legal designations in certain European countries,²⁷² where in both situations "hard drugs" are associated with greater, and "soft drugs" with less, addicting properties. Those uses have nothing to do with the metabolic profiles of such compounds.

See: Prodrug, Bioactivation, Bioinactivation, Esterases, Metabophore, and Rule of One (Metabolism's Rule of One).^{50-52,167-170,229}

212. S-Oxidation

Any biotransformation where a sulfur atom is oxygenated. Somewhat analogous to the formation of *N*-oxides, although *S* has several more oxidation states to consider. Typically accomplished by the CYPs. Several examples pertaining to various *S* oxidation states are provided below.



Note 1: Sulfonium and sulfoxide forms can have asymmetric, tetrahedral-like coordinates. The sulfoxide stereoisomer consequences may need to be defined during the development of drugs undergoing such biotransformations.

Note 2: Carbon-sulfur systems can also undergo *S*-dealkylation reactions analogous to *O*-dealkylations so as to form sulfanyl groups, rather than hydroxy groups. The structure metabolism relationships for these pathways are similar.

Note 3: Carbon-sulfur double bonds can undergo an exchange of sulfur for oxygen.

See: *N*-Oxidation, *O*-Dealkylation, Oxidation/Oxidase/Oxidoreductase, and Stereoselective and Stereospecific Metabolism.²⁷³

213. Stereoselective and Stereospecific Metabolism

Stereoselective metabolism occurs when a biotransformation proceeds to a greater extent along preferred stereochemical pathways; stereospecific further indicates that the preference is exclusive for just one of the stereochemical options.

Note 1: When the choices involve only a pair of enantiomeric pathways, then the terms “enantioselective” and “enantiospecific metabolism” can also be used.

Note 2: The parent compound need not be chiral if it has one or more prochiral sites that can become chiral as a result of metabolism.

See: Enantioselective and Enantiospecific Metabolism, Substrate Specificity, and Regioselective Metabolism.^{247,248}

214. Structure-Metabolism Relationships (SMRs)

The correlation of one or more molecular parameters across a series of compounds versus the nature of the compounds' metabolic reactions. Molecular parameters can include net physicochemical properties and overall electronic surface potentials, as well as consideration of lipophilicity, electronic, or steric features in discrete regions of a molecule's three-dimensional structure.

Alternatively, the correlation of one or more chemical parameters for a given metabolic enzyme that relates to the possibility of compounds to serve as suitable substrates. These parameters can be the same as the above and most

often additionally include a characteristic functional group. For example, the SMR for a CYP capable of performing an aromatic hydroxylation might be defined within the context of preferentially doing so on a phenyl ring at the latter's most electron rich site and least sterically hindered position.

Note 1: This term is analogous to the more common phrase "structure-activity relationship" or "SAR." SAR is typically used relative to efficacious activity. However, it is also sometimes used to be inclusive of all types of biological responses, thus including SMRs as part of its overall meaning.

Note 2: In either case, SMRs can be summarized in terms of a "metabophore," which is analogous to the more common phrase "pharmacophore," where the latter is most often used with regard to efficacy but is sometimes used for other types of biological activities, including drug metabolism events.

See: Drug Metabolism, Biotransformation, Enzyme, Metabophore, Metabophore Probe, Rule of One, Substrate/Substrate Specificity, and Metabolic Prediction/Possibilities versus Probabilities.¹⁻³

215. Substrate/Substrate Specificity

In the present context, a substrate is an endogenous or xenobiotic compound that undergoes a given biotransformation reaction or active transport process because its structure contains the associated pharmacophore (metabophore or transportophore) required by the corresponding protein's active site. Specificity reflects the relative preference of an enzyme (or carrier protein) for specific substrates or a given substrate's preference for various enzymes (or carrier proteins). "Preference" translates functionally to a faster rate of metabolism (or transport) of the substrates.

Note 1: The affinity of a substrate for the protein is characterized by a binding constant (K_s or K_d) and the turnover efficiency by Michaelis-Menten constants $V_{\max}$ and K_m .

See: Enzyme, Apoenzyme, Holoenzyme, Cofactor, Prosthetic Group, Active Transport, Linear Kinetics, Nonlinear Kinetics, Michaelis-Menten Equation/Kinetics, Saturation Kinetics, Metabophore, and Structure-Metabolism Relationships (SMRs).^{1-3,38-40}

216. Substrate Inhibition/Product Inhibition

When an inhibitor of an enzyme is also a substrate/When the enzymatic products from a substrate's reaction cause inhibition.

Note 1: Since the presence of a substrate in the active site of an enzyme typically precludes another substrate from undergoing reaction at that same location at that same point in time, this is always taken as a transient, inhibitory situation. The same definition extends to the products from an enzymatic reaction. However, these terms are instead used to provide additional definition to the types of inhibitory pathways that any given compound might engage in.

Note 2: For example, an inhibitor can bind to an enzyme and either serve or not serve as a substrate. In either case, if binding is reversible, then these would be competitive inhibitors (one a substrate inhibitor and the other not), with their relative inhibitory efficiencies expressed as either IC_{50} or K_i values. Under normal operation, a substrate undergoes a reaction and the products are cleared from the active site. Thus, by definition a "substrate inhibitor" would always be a competitive inhibitor. If the products are not cleared as fast as would normally occur and themselves cause observable inhibition, then this situation would be called "product inhibition," where it would then be assumed that the initial compound must have served as a substrate in order to produce them.

Note 3: If binding is accompanied by the formation of a covalent bond such that it is no longer reversible, then this would be called non-competitive inhibition. Cases where the compound served as a substrate to produce products that bound covalently, or became bound itself during the reaction process, would be further classified as "suicide inhibitors." If the latter occurred for a species that resembled the transition state for the reaction, then these would be called "transition state inhibitors," with the same term used even when they do not form a covalent bond at that point.

See: Substrate/Substrate Specificity, Inhibition, Autoinhibition, Autoinduction, Competitive Inhibition, Non-Competitive Inhibition, Mechanism-Based, Product and Transition State Inhibitors, Covalent Binding, and Suicide Inhibitor. ^{42,62,146-148}

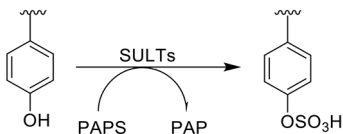
217. Suicide Inhibitor

A compound which undergoes a normal substrate's enzymatic reaction but instead produces a reactive intermediate or product that, in turn, forms a covalent bond in the active site and serves to inhibit the enzyme in a non-competitive manner.

See: Substrate Inhibitor/Product Inhibitor, Substrate/Substrate Specificity, Inhibition, Autoinhibition, Autoinduction, Competitive Inhibition, Non-Competitive Inhibition, Mechanism-Based, Product and Transition State Inhibitors, and Covalent Binding.^{42,62,146-148}

218. Sulfate Conjugation (Sulfation)

This biotransformation is important for a variety of endogenous molecules, as well as for xenobiotics. It involves the transfer of a sulfonate group to a hydroxy, and on occasion an amino group, when present within a substrate.



Note 1: The reaction is catalyzed by a family of sulfotransferases (EC 2.8.2), xenobiotics being particularly suitable for those members that are cytosolic and referred to as the "SULTs."²⁷⁴ The cofactor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) serves as a universal source of sulfate.²⁷⁵

Note 2: This pathway generally functions in parallel with the glucuronidation pathway, where each contributes according to the relative expression levels of their associated enzymes, the size of their cofactor pools at any given time, and their metabolophore matches with the substrate at hand. Among mammalian models, cats are distinct in their higher utilization of this pathway over that of glucuronic acid conjugation.

See: Cytochrome P-450 Sulfotransferases (Cytosolic Members or SULTs), Conjugation Reactions, Conjugate, Glucuronic Acid Conjugation (Glucuronidation), and Glucuronide.^{66,276,277}

219. Sulfotransferases (Cytosolic Members or SULTs)

A family of enzymes (EC 2.8.2) that transfer the sulfonate group from the cofactor 3'-phosphoadenosine-5'-phosphosulfate (PAPS), to hydroxyl or amino groups present on endogenous or exogenous substrates so as to form sulfate esters or sulfamates, respectively. Xenobiotics are particularly suited as substrates for the cytosolic family members or "SULTs."^{274,275}

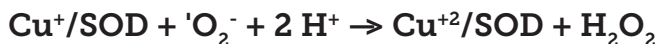
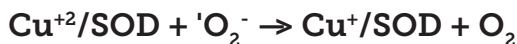
Note 1: The SULTs are cytosolic enzymes that function as homodimers having 32-35 kDa subunits. Thirteen distinct human SULTs have been identified and shown to be expressed differentially in several organs, including the liver, brain, lung, kidney, adrenal glands, and intestines.²⁷⁸

Note 2: Their nomenclature is similar to that delineated for the CYPs. It has been proposed that all SULTs constitute a gene superfamily. The 13 isoforms found in humans can be classified into four SULT families designated as SULT1, SULT2, SULT4, and SULT6. Within each SULT family, the constituent members are then further divided into subfamilies, such as SULT1A, B, C, and E.²⁷⁹

See: Sulfate Conjugation (Sulfation), Conjugation Reactions, Conjugate, Glucuronic Acid Conjugation (Glucuronidation), and Glucuronide.^{66,276,277}

220. Super Oxide Dismutases (SODs)

A family of metalloenzymes (e.g., EC 1.15.1.1) that catalyze the conversion of harmful superoxide radicals resulting from oxidative stress to less damaging species, namely molecular oxygen and hydrogen peroxide.



Note 1: The oscillating charge on the metal cofactor allows SOD to oxidize superoxide or, alternatively, to reduce it to hydrogen peroxide.

Note 2: This reaction represents the first step in detoxification within systems undergoing oxidative stress, catalase then further reducing the hydrogen peroxide to water.

See: Catalase, Oxidative Stress, and Reactive Oxygen Species (ROS).^{56,190-194,280}

221. Systemic/Systemic Circulation

The systemic circulation is that portion of the arterial flow that distributes blood to the entire body, i.e., both the peripheral tissues and central nervous system.

Note 1: The GI-tract, airways, skin surface, etc. are excluded from this definition, but would instead be encompassed by the phrase “whole body exposure.” Alternatively, these tissues, including their outer membranes, would still become accessed as part of the overall blood flow distributed by the systemic circulation and would be included in that context.

See: Whole Body Exposure, Hepatoportal Circulation, First Pass Effect/Metabolism, Second Pass Metabolism (ψ First Pass Metabolism), Enterohepatic Recycling, Blood-Brain Barrier (BBB), Presystemic Elimination, and Random Walk. General Medical Definition Refs.: 231, 232.

222. $T_{\max}$ or $t_{\max}$

The time after extra-vascular dosing when the drug concentration reaches a maximum within the fluid being measured, generally plasma, serum, or whole blood.

Note 1: The maximum concentration for intravascular dosing theoretically occurs immediately after administration, since there is no absorption process required. Hence, $T_{\max}$ is generally not reported for intravascular administration.

See: Pharmacokinetics (PK), ADME, AUC, Bioavailability (Absolute and Relative), DMPK, and PBPK.^{38-40,104}

223. Terminal Half-Life

The half-life for the slowest and final process of the parent drug's overall elimination.

See: Half-Life ($t_{1/2}$), Pharmacokinetics (PK), ADME, AUC, DMPK, PBPK, Elimination, Clearance, Excretion, and Phases of Drug Metabolism.^{38-40,104}

224. Therapeutic Window

The range of drug concentrations within a single subject or test group that can provide the desired therapeutic effect without prompting unacceptable side effects.

Note 1: While the most relevant concentrations are at the sites for the desired activity versus toxicities, a single value is most often obtained in plasma, serum, or whole blood due to practical limitations.

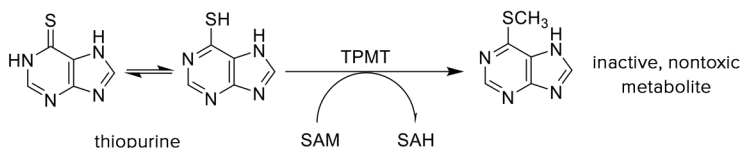
Note 2: The goal of drug therapy is to keep the active pharmaceutical agent concentration within the therapeutic window for as long as needed to alleviate the medical issue being treated.

Note 3: This term is related to the term “therapeutic index” (TI). The latter is typically the dose that prompts toxicity (TD_{50}) divided by the dose that is efficacious (ED_{50}), where each parameter has likely been obtained from two different models developed to optimize the assessment of each. The larger the TI, the “safer” the drug in terms of its potential side-effects.

See: Half-Life ($t_{1/2}$), Terminal Half-Life, Pharmacokinetics (PK), ADMET, AUC, DMPK, PBPK, Elimination, Clearance, Excretion, and Phases of Drug Metabolism.^{38-40,104}

225. Thiopurine S-Methyltransferase (TPMT)

TPMT (EC 2.1.1.67) catalyzes the methylation of aryl- and heteroaryl-sulfanyl groups using S-adenosyl-methionine (SAM) as the methyl donor. Detoxification of the anticancer drug 6-mercaptopurine (now “purine-6-thiol” or “thiopurine”) is exemplary:



Note 1: Genetic polymorphism of TPMT results in inter-individual variation in the sensitivity and toxicity toward thiopurine chemotherapeutic agents. Nearly 1/300 people are highly deficient for this enzyme. Measurement of TPMT status is recommended prior to administering thiopurine drugs to any population and prior to the use of cisplatin agents in children.

See: Conjugation Reactions, Conjugate, Transferase, Methylation, Methyltransferase, *N*-Methyltransferases, Catechol *O*-Methyltransferase (COMT), Genetic Polymorphism, Genotype/Genotyping, and Phenotype/Phenotyping.²⁸¹

226. Transferase

A general term for an enzyme that transfers an endogenous compound onto a polar functional group on another endogenous material (e.g., anabolism or control of function) or onto a xenobiotic compound (e.g., drug conjugation).

Note: The endogenous compound can either be polar, such as sugars, glucuronic acid, and amino acids, or non-polar, such as a methyl or acetyl.

See: Acetylation, *N*-Acetyltransferases, Acylation, Acyltransferases, Amino Acid Conjugation, Catechol-*O*-Methyl Transferase (COMT), Conjugate, Conjugation Reactions, Enzyme, *Gamma*-Glutamyltranspeptidase (γ -Glutamyltransferase; GGT), Glucuronic Acid Conjugation (Glucuronidation), Glucuronide, Glucuronosyltransferase, Glutamine Conjugation, Glutathione (GSH), Glutathione Conjugation, Glutathione-*S*-Transferase (GST), Glycine Conjugation, Mercapturate/Mercapturic Acid Conjugation, Methylation, Methyltransferase, *N*-Acetylation, *N*-Acetyltransferases (NATs), *N*-Methyltransferases, Serine Conjugation, Sulfate Conjugation (Sulfation), Sulfotransferases (Cystolic Members or SULTs), and Thiopurine-*S*-Methyltransferase (TPMT). General Dictionary Ref.: 282.

227. Transporters (Carrier Proteins)

Proteins which function to move ligands across membranes by processes that require energy (active transport).

Note 1: Analogous to enzymes, their ligands are referred to as “substrates,” even though no chemical change is made to the compounds that are transported. They can work against a concentration gradient and, while typically unidirectional, they can move substrates in either direction across the membrane, e.g., the Pgp transporter serves to pump compounds out of a cell.

Note 2: They can either be permanently embedded across the membrane (membrane spanning, e.g., Pgp) or move across the membrane while bound with one or more substrates (e.g., folate transporter).

Note 3: Several transporter molecules play critical roles during drug metabolism because they influence the uptake or export of xenobiotic compounds by cells possessing various drug metabolism enzymes. For example, within hepatocytes, the substrate carrier superfamily of proteins (SLC) are key basolateral (sinusoidal blood) uptake transporters that can serve to prolong the exposure of a given xenobiotic to the enriched array of metabolic possibilities present in the liver. Alternatively, the ATP-Binding Cassette superfamily of proteins (ABC), especially the Multidrug Resistance Protein (MDR or Pgp) and the Multiresistance Protein (MRP) subfamilies, represent key basolateral and canalicular (bile) export transporters that can serve to diminish the liver's metabolism of xenobiotics.

Note 4: Any possible metabolic event for a given xenobiotic at any locale within the body depends on: (i) the suitability of the xenobiotic's structure to serve as a substrate for the enzyme(s) associated with the event; (ii) the level(s) of the relevant enzyme(s) and competing enzymes present at that locale; and (iii) the concentration and residency time of the xenobiotic at that locale. Component (iii) is a function of the inherent physical properties of the substrate (e.g., $\log D$) and its suitability to serve as a substrate for transporters, where the latter's importance grows exponentially with the levels of the various transporters that may or may not be present and operative at that locale. While all three components represent a challenge for the prediction of a drug's metabolism *in vivo*, component (iii) arguably presents itself as the largest hurdle due to the presence of the transporters that additionally complicate the variables that need to be taken into account.

See: Absorption, ADME, Permeation/Permeability, Partition Coefficient (P ; $\log_a P$ and $\text{clog}_a P$), Distribution Coefficient (D ; $\log_a D$), Passive Transport, Rule of Five, Active Transport, Phases of Drug Metabolism, Random Walk, Blood-Brain Barrier (BBB), P-Glycoprotein (Pgp/MDR1/ABCB1), and Metabolic Prediction/ Possibilities versus Probabilities.²⁸³

228. Ultra-Rapid Metabolizer

An individual who has inherited multiple, functional copies of the same gene encoding a drug-metabolizing enzyme. This results in the over-

expression of the enzyme and a far greater than normal metabolism of its substrates.

See: Genetic Polymorphism, Genotype/Genotyping, and Phenotype/Phenotyping.^{103,114-129,230}

229. Ultra-Short-Acting Drug (USA Drug)

An ultra-short-acting (USA) drug is an agent whose duration of action (pharmacologic half-life) is significantly less than 15 minutes after its administration, to the extent that at least a pseudo-equilibrium (if not a true equilibrium) for an effective concentration has first been achieved within the relevant biocompartment. For comparative purposes (and not to be taken as a continuing strict definition), a short-acting drug might then reside in the range 15 to 30 min, a moderately long-acting drug in the range 0.5 to 5 h, a long-acting drug in the range 5 to 10 h, and an extremely long-acting drug in a range longer than 10 h. Thus, an agent's ultra-short duration is a consequence of quick bio-inactivation (e.g., drug metabolism) rather than being due to distribution out of the compartment associated with the drug's action. Alternatively, drugs exhibiting extremely long durations of action typically do exhibit a relationship with their distribution, regardless of their metabolism, namely that they may concentrate in and most certainly will leave only very slowly from the compartment associated with their biological activity (i.e., their observed duration is indirectly proportional to their rate of departure/furthered distribution). When the short duration for the agent has been engineered by drug design and synthesis, these agents are part of the broader family known as "soft drugs" (agents whose inactivating metabolism at any given rate, long or short, has been specifically programmed into the parent molecule, so as to achieve some desirable endpoint associated with the overall therapeutic efficacy-ADMET profile). Esmolol is the classic, prototypical soft drug (264, 265).

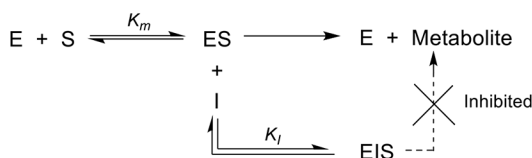
Note 1: Appreciating the distinctly different influences that metabolism and distribution play for these two extremes in a drug's duration of action and ADMET profile can allow for the two roles to be combined in a complimentary manner. For example, a delayed-release formulation placed within the target biocompartment that gradually releases a USA drug whose quick metabolism has been programmed to occur in the systemic circulation or liver, so as to produce innocuous metabolites, represents an ideal way to have a sustained (long, extremely-long duration) drug effect in

the desired compartment, while remaining free from off-target or systemic side-effect toxicity effects.

See: ADME, ADMET, Prodrug, and Soft Drug.^{50-52,167-170,229}

230. Uncompetitive Inhibition

A type of inhibition that occurs when an inhibitor binds reversibly only to the enzyme-substrate complex (ES complex) and does not bind to the enzyme alone. Both K_m and V_{max} decrease. The interactions between enzyme (E), substrate (S), and an uncompetitive inhibitor (I) are depicted below:



Note 1: Metabolism-independent inhibition occurs when a ligand inhibits an enzyme without undergoing biotransformation. Such direct inhibition has traditionally been divided into three categories: competitive, non-competitive, and uncompetitive.

See: Autoinhibition, Competitive Inhibition, Non-Competitive Inhibition, Mechanism-Based Inhibition, Covalent Binding, Suicide Inhibition, and Michaelis-Menten Kinetics.^{42,62,146-148}

231. Urinary Metabolic Ratio

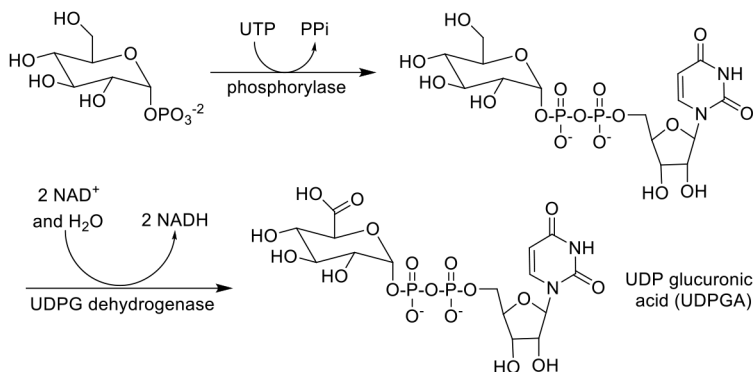
The amount of a particular metabolite found in the urine for a specified time period, as the numerator over one of the following in the urine for the same time period as the denominator: the amount of unchanged parent compound, the sum of the amounts of other metabolites, or the sum of unchanged parent and other metabolites.

See: Clearance (CL), Elimination, Excretion, Intrinsic Clearance, and Pharmacokinetics.^{38-40,104}

232. Uridine-diphospho-glucuronic Acid (UDPGA)

This cofactor for glucuronosyltransferase (GT; formerly UDP-glucuronosyltransferase or UGT) supplies the endogenous partner for the

glucuronidation pathway that is extremely common for xenobiotics. Its biosynthesis is shown below:



Note 1: Subsequent reaction with a drug is catalyzed by glucuronosyltransferase (GT) in an $\text{S}_{\text{N}}2$ manner to release UDP and a *beta*-glucuronide conjugate.

Note 2: The drug-conjugate has significantly increased polarity, generally little activity, and an increase in molecular mass of about 175 Da. Because of the added carboxylic acid group, the conjugate is also a candidate for excretion by organic anion transporters (OATs).

Note 3: In general, if the drug-conjugate's molecular mass is below 500 Da, it will be excreted via the urine; and if it is above 500 Da it will be excreted via the bile.

See: Glucuronosyltransferase (GT), Conjugation Reactions, Phases of Drug Metabolism, Aromatic Hydroxylation, Glucuronic Acid Conjugation (Glucuronidation), Glucuronide, Glucuronidase (β -Glucuronidase), and Sulfate Conjugation (Sulfation).^{45,63,66,130,131}

233. V_{max}

The maximum rate at which an enzyme can catalyze its reaction with a given substrate for a given set of experimental conditions. Increasing the concentration of substrate for a given amount of enzyme will eventually saturate the system at V_{max} ; no further increase in reaction rate will then occur.

See: Enzyme, Michaelis-Menten Equation/Kinetics, and Linear Kinetics.^{38-40,104}

234. V or V_d (Apparent Volume of Distribution)

Proportionality constant relating the drug concentration in the measured media, such as blood, plasma, or serum (C), to the amount of drug administered to the body (A), as expressed by the equation:

$$C = \frac{A}{V}$$

Note 1: The volume is termed “apparent” because it generally does not correspond to any true physiological volume in the body, except in rare special cases.

Note 2: V is only constant for pharmaceutical agents whose PK profile can be fit to a single compartment model. For agents requiring more than one compartment or PBPK models, V changes with time as the agent is distributed into other tissues.

See: Pharmacokinetics (PK), ADME; ADMET, Allometric Scaling, Allosteric Scaling, AUC , Bioavailability (Absolute and Relative), Clearance (CL), Compartment Model, Disposition, DMPK, Dose-Dependent Kinetics or Metabolism, Extraction Ratio, Half-Life ($t_{1/2}$), Intrinsic Clearance, IVIVE, Linear Kinetics, Metabolic Clearance, Metabolic Pathway/Pattern/Profile, Michaelis-Menten Equation/Kinetics, Non-compartment PK Analysis, Nonlinear Kinetics, Oral Bioavailability, Partition Coefficient (P ; $\log_a P$ and $\log_a P$); Distribution Coefficient (D ; $\log_a D$), PBPK, Pharmacodynamics, and Pharmacogenetics and Pharmacogenomics.^{38-40,104}

235. Whole Blood Concentration

Measurement used in PK analyses when significant amounts of the pharmaceutical agent can collect within red blood cells, but otherwise typically not as prevalent as using measurements of plasma or serum concentration.

Note 1: Collection requires mixing with an anticoagulant, such as heparin or EDTA, either during or immediately after drawing the whole blood sample.

See: Pharmacokinetics (PK), ADME; ADMET, Allometric Scaling, Allosteric Scaling, AUC , Bioavailability (Absolute and Relative), Clearance (CL), Compartment Model, Disposition, DMPK, Dose-Dependent Kinetics or

Metabolism, Extraction Ratio, Half-Life ($t_{1/2}$), Intrinsic Clearance, IVIVE, Linear Kinetics, Metabolic Clearance, Metabolic Pathway/Pattern/Profile, Michaelis-Menten Equation/Kinetics, Non-Compartment PK Analysis, Nonlinear Kinetics, Oral Bioavailability, Partition Coefficient (P ; $\log_a P$ and $\log_a P$); Distribution Coefficient (D ; $\log_a D$), PBPK, Pharmacodynamics, and Pharmacogenetics and Pharmacogenomics.^{38-40,104}

236. Whole Body Exposure

This phrase is used to indicate that all surfaces of the body, such as the GI-tract, airways, skin, eyes, etc., are included with the body's various compartments, as well as the venous and systemic circulation during the consideration of a xenobiotic's disposition after its exposure or administration to an individual.

See: Systemic/Systemic Circulation, Hepatoportal Circulation, First Pass Effect/Metabolism, Second Pass Metabolism (ψ First Pass Metabolism), Enterohepatic Recycling, Blood-Brain Barrier (BBB), Presystemic Elimination, and Random Walk.^{38-40,104}

237. Wild-Type Enzyme

The enzyme isolated from the natural gene product of the allele with highest frequency or highest enzyme activity.

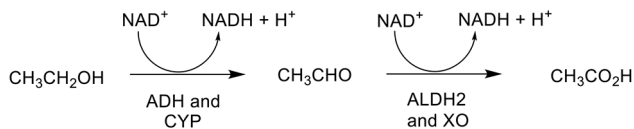
Note 1: Most commonly used in relation to an analogous form experimentally expressed and isolated using recombinant molecular biology methods and likely "humanized" while using a non-human host cell.

See: Genetic Polymorphism, Genotype/Genotyping, Phenotype/Phenotyping.^{103,114-129,230}

238. Xanthine Oxidase (XO)/Xanthine Dehydrogenase

This enzyme can interconvert between both activities (EC 1.17.3.2 and EC 1.17.1.4, respectively) depending on the protein's reversible sulfhydryl oxidation status, or will adopt only XO activity upon irreversible proteolytic modification. Important for the endogenous xanthine pathways, XO can also oxidize reactive aldehydes to their less toxic carboxylic acids, the latter role contributing to the second step in the clearance of ethanol. The metabolic disposition of ethanol is shown below, where alcohol

dehydrogenase (ADH) and certain CYPs perform the first oxidation and then aldehyde dehydrogenase (ALDH2) and XO become involved with the second oxidation.



See: Oxidation/Oxidase/Oxidoreductase, Aldehyde Dehydrogenase Polymorphism or Deficiency, Disulfiram-like Reaction, Alcohol Dehydrogenase, and Atypical Alcohol Dehydrogenase.²⁸⁴

239. Xenobiotic

A compound foreign to the living system under consideration. Thus, a natural product that is endogenous to a given plant becomes a xenobiotic when administered to humans. General Dictionary Ref.: 282.

240. Xenobiotic-Responsive Element (XRE)

The specific DNA sequence on the regulatory region of genes that can be bound by the aryl hydrocarbon receptor complex and usually causes an increased expression of that gene.

See: Pregnane X Receptor (PXR), Peroxisome Proliferator-Activated Receptors (PPARs), Aromatic Hydrocarbon Receptor (AHR), Glucocorticoid Responsive Element (GRE), and RXR and CAR/RXR.^{35,198,236,249-253}

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IUPAC standards for terminology technical reports. For the latter, final comments from Dr. Jürgen Stohner and Joshua Gannon proved particularly useful.

Appendix I: Defining the Term “Drug” in the Context of “Drug Discovery”

Given the numerous disciplines involved in drug discovery and the wide range of technical perspectives that exist among its participants, it is important to appreciate how using even a seemingly simple word like “drug” can become ambiguous among a group of interdisciplinary scientists. However, mandating a single, specific definition at the onset may not be the best way to enhance communication among drug discovery participants. An alternative approach is to provide a general or more fluid starting point that can allow the group of interacting scientists to devise a meaning suitable for their context that might be most beneficial to their particular discussion. In such cases, it will be the common knowledge base, potentially garnered from a tutorial about the general term, that will allow these practitioners to better understand each other’s perspectives and then devise their mutually beneficial working definition. This same situation arises when authoring a publication, where it may be necessary to provide some explanatory information for a term’s use/intended definition, depending on its specific context in the technical report and the differing perspectives of the intended audience. The following discourse about the term “drug” is taken from a textbook chapter pertaining to “Drug Discovery.”³ The text is intended for audiences consisting of both pharmacologists and medicinal chemists, primarily at the early stages of their careers, and so it became necessary during the writing of the chapter to devise a working definition applicable to the chapter’s educational thrust. However, the tutorial discourse leading to the final, rather succinctly stated singular definition, demonstrates how related knowledge can also allow a given term to remain dynamic and thus act as a bridge to other contexts across various disciplines and having individuals with varying technical perspectives.

The dictionary indicates that the word “drug” is derived from the Old English “dryge” which, in turn, was reconstructed from the Primitive Germanic “dreug” and hence the sound of its pronunciation even today. Because these older words meant to “dry,” it has been suggested that the word “drug” originally was used to convey how herbs were commonly processed so as to produce “*dried powders for administration as early medicinals*.” Even if historically correct, however, the field of medicine and the word “drug” have evolved well past this origin and a modern

usage must be sought. In today's broadest sense, the term "drug" is often taken to include any non-food substance that exhibits activity upon administration to any biological setting. The latter includes an *in vitro* test as well as an *in vivo* animal model or human subject. However, this definition also falls short of today's practice. It does not convey how the overall drug discovery process has so critically come to be a highly interdisciplinary effort all the way to its validated end-point within the clinic. Thus, the present chapter will specifically apply the term "drug" to include only single chemical substances that become marketed, albeit often then accompanied by added excipients, for use in human or veterinary medicine either by prescription ("ethical pharmaceuticals") or as "over-the-counter" products ("OTCs"). Inherent to this modern definition while still connecting to the past is the continuum of interdisciplinary activities needed to actually produce marketed substances that eventually become "*administered* as [today's] *medicinals*."

It can be noted that this definition does not exclude "recreational" or "abused substances." Because these chemicals involve non-conventional marketing strategies, to say the least, as well as non-therapeutic, if not harmful, applications, these "*drug shouldn't-be's*" will not be further considered within this chapter. Alternatively, the definition does exclude both herbals and dietary supplements or nutraceuticals even though some of these products may have a firmly established medicinal value. The latter are typically complex mixtures for which the complete range of components are often not well-understood and are sometimes not well-controlled in their lot-to-lot ratios. Thus, while dietary supplements and herbals can serve as excellent sources for new drugs, they are not themselves "drugs" by today's standards. Though they may sometimes represent very popular consumer products, they too will not receive further attention. Finally, in line with the working-definition and integral to fully appreciating how difficult it is to truly discover an actual drug substance, it will be important to keep in mind that all of the "compound libraries," "hit," "lead," "preclinical" and "clinical development compounds" that you will soon be reading about, should only be regarded as "*drug-wannabes*" since they are not yet marketed and thus all fall short of this strict definition.

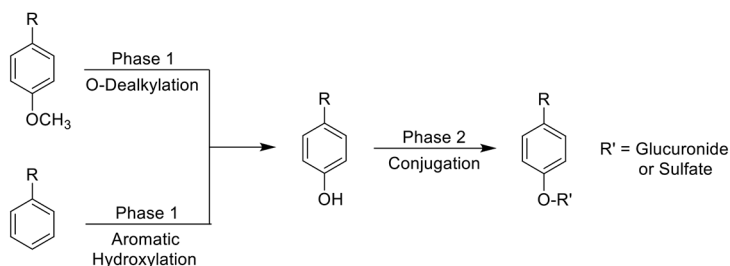
A similar view should be extended to the participants who work in the field of drug discovery. While investigators involved in studies at any given step in this process should be considered to be making very critical contributions, within the context of this definition the overall process of drug discovery entails numerous steps that need to be brought together as part of a much larger undertaking in order to eventually produce what we will be calling a true "drug." The huge challenge associated with this undertaking is illustrated in the next section of this chapter. Finally, in addition to the interdisciplinary emphasis afforded by this working-definition, the present chapter will primarily focus upon the process

of drug discovery as it specifically pertains to the pursuit of “small-molecule” agents. Although this will preclude in-depth consideration of biopolymers and vaccines, this division is commonly taken to separate these types of therapeutics from small-molecule drugs. The development and production of biopolymers are achieved in a distinctly different manner that relies heavily upon biotechnology-driven methods. Summarizing, for this chapter a drug is a single chemical substance marketed for use in human or veterinary medicine either by prescription or as an OTC.³

Appendix II: An Ongoing Terminology Controversy

The following discourse provides a historical backdrop of the differing views regarding the continued use of the terms “Phase 1” and “Phase 2” in the context of drug metabolism. **None of the definitions provided within this section should be taken as the final IUPAC recommendations for this glossary**, but rather as a basis for further consideration and comment related to how these particular terms might best be deployed in the future. Opinion on the future usage of these terms ranges from: “not to be used at all”; to “replaced by less sequential versions, such as Group 1 and Group 2 biotransformations”; to “as presently still being utilized by many”; to calls for “an even expanded version” that, although more complex, might become less controversial while potentially having some appeal for a much wider range of drug metabolism practitioners beyond those involved in drug discovery, e.g., toxicologists, nutritionists, and environmental scientists.

The terms “Phase 1” and “Phase 2” were introduced nearly 60 years ago by the late R. T. Williams,⁸¹ now recognized as one of the highly distinguished founders of the xenobiotic metabolism field. Dividing metabolic oxidation, reduction, and hydrolysis reactions (Phase 1) from “synthesis” reactions (Phase 2), which translate to what are now called “conjugation” reactions, his choice of such phraseology encompassed the general notion that this was also a common sequence of events, and further, that it typically yields inactive, more water-soluble metabolites. While the latter may often be applicable, there are many notable exceptions, such as *N*-acetylation (a Phase 2 or conjugation reaction), which would yield a less water-soluble metabolite than its amine precursor. Likewise, one of the major pathways that consistently behaves in this sequential manner is shown below. Today we further appreciate that even these types of xenobiotics would likely be simultaneously subject to several different types of biotransformation possibilities depending on the nature of their remaining structures (simplified to R for this example).



Nevertheless, these terms have been fully embraced over the years, often with what seems like an added emphasis on how important their sequential role is in the metabolic disposition of xenobiotics in general. Unfortunately, that a xenobiotic already having an aryl-hydroxyl-group (phenolic group) present is an immediate candidate for the indicated Phase 2 conjugation, without the need for a Phase 1 reaction, is sometimes not duly noted. Likewise, that a Phase 2 metabolite can still be subject to subsequent Phase 1 reactions may not be fully elaborated, e.g., the common case where an initial glutathione conjugate is further processed by a series of Phase 1 reactions to eventually produce a mercapturic acid. Finally, that an entire class of important conjugates are typically less polar, while others can retain activity, including in the latter certain glucuronides and sulfates, is often not noted either. In light of these exceptions and the resulting ambiguity, there are some practitioners who have advocated that the terms Phase 1 and Phase 2, despite their prestigious origin, should no longer be used, as they can be more confusing than helpful, especially for students new to the field.²²¹

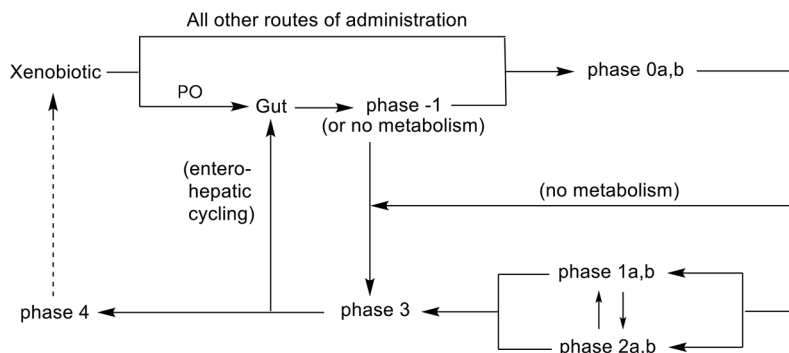
Amid such controversy, it appears that a majority of practitioners still feel that, in addition to their historical significance, there is merit to the retention of these terms when used today with appropriate tutorial material. For example, aside from the classic xenobiotic sequence noted above and from analogous pathways pertaining to key endogenous substrates (for example, see Ref. 222), there seems to be a compelling anatomical basis to consider these steps as having evolved to handle the indicated biotransformations in a tandem, enzymatic cascade fashion. The mutually high proportions of the enzymes associated with these pathways in the gut endothelium and liver suggest they work together toward biotransforming xenobiotics entering by the oral route. Especially noteworthy is the close physical proximity of many of the CYP enzymes and of uridine-diphospho-glucuronosyltransferase (UGT) along the endoplasmic reticulum lipid bilayer.^{223,224} The CYPs are

capable of catalyzing Phase 1 biotransformations that can generate an aryl-hydroxyl-group, and UGT is then capable of catalyzing the Phase 2 transfer of a glucuronic acid biochemical synthon to a hydroxyl-group. Even though this two-step pathway represents a small portion of the wide array of metabolic possibilities for a xenobiotic, the sheer number of natural products having aryl-methoxy-groups suggests that such chemical species had to be frequently dealt with as modern man evolved and ultimately developed a general diet and complimentary digestive system by sampling the edible materials available in Nature.

Likewise, from a systems biology perspective, there may be merit in an even further expanded use of the Phase 1 and 2 terminologies. For example, the term "Phase 3" has sometimes been deployed to designate the excretion of xenobiotics and their metabolites from the body, particularly emphasizing in this usage the importance of active transport pathways.²²⁰ More recently, the term "Phase 0" has seen some use to convey events like a xenobiotic's absorption and distribution prior to undergoing a biotransformation event.¹³⁰ Both terms have merit in that they draw attention to the importance of ADME parameters in conjunction with xenobiotic metabolism per se. This type of systems biology approach is, indeed, inherently fundamental to the overall process of xenobiotic metabolism.¹⁵⁸ Extending this paradigm in both directions, i.e., to include the role of gut microflora as part of the exposure process,²²⁵ and of metabolic events within the environment subsequent to excretion,²²⁶ actually forms a continuum or loop back into the human diet that can take on relevance across even more technical disciplines, while also prompting new ways to drive drug discovery and development.²²⁷

Taking the view that the Phase 1 and Phase 2 designations may be useful as process classifications, but not as strict sequential pathways, can allow for their continued usage. Furthermore, retaining their specific phraseology as a "phase number" pays tribute to the historical significance conveyed above, while underscoring that there are classical sequences of events which are often traversed by a substrate. Adopting an "a" designation to convey that an absorption or distribution process is occurring prior to any metabolic event, or that a biotransformation is occurring for the first time at a specific location on a parent xenobiotic or metabolite, can instead be used to track the actual sequence of events and molecular modifications. For the latter, it follows that a "b" then conveys that a metabolic event has already occurred anywhere on the compound in relation to tracking absorption or distribution

behavior, or at the same structural locale that is next undergoing another metabolic reaction at that location or on a Phase 2 appendage. Taking all of the above into consideration, a potential expansion of this nomenclature is illustrated below for the human case of exposure to a xenobiotic. Note that the term “phase,” as defined here in the context of drug metabolism, is no longer capitalized and uses Arabic numerals, so that these terms are more readily distinguished from the analogous terms used for the stages of drug clinical testing, which instead are capitalized and use Roman numerals, i.e., they are denoted as Phase I, Phase II, etc.



The depicted model and its designated nomenclature begin by distinguishing the potentially important role that gut microflora can play in the metabolic fate of a xenobiotic after its oral ingestion (PO) versus other routes of administration. Thus, **phase -1** (minus one) designates all of the possible biotransformations (some of which are quite unique to the microorganisms) that can be catalyzed by the gut microflora. As such, phase -1 also encompasses the role that these microorganisms may play during enterohepatic cycling, e.g., hydrolysis of a phenolic-glucuronide metabolite that has undergone biliary excretion back to the phenol, which can then be reabsorbed, rather than proceed toward fecal elimination.

Phase 0 designates all of the non-metabolic dispositional processes to which a xenobiotic or metabolite will traverse, such as absorption passively or by transport across membranes and association with biomolecules not involved in drug metabolism, while undergoing fluid-driven distribution through the living system. The important relationships of a drug's transport coupled to its metabolism, and a metabolite's transport coupled to its excretion, are thus recognized by this nomenclature. For this term,

the "a" designates that these phenomena occur prior to a xenobiotic's first mammalian metabolic event (as shown in the scheme), while the "b" designates that a biotransformation reaction has occurred and intends to further convey that these processes continue throughout the entire timeframe after a xenobiotic's arrival until it and all of its metabolites are cleared from the living system.

In line with the historical definition, **phase 1** includes all hydrolytic, oxidative, and reductive biotransformations. The "a" then provides a sub-classification for such events occurring at a given point on the substrate for the first time, while the "b" indicates that the reaction event occurs at a point on the substrate which has already participated in a preceding biotransformation. Typical phase 1a examples include ester hydrolysis, aromatic hydroxylation, arylether *O*-demethylation, and the first of possible *N*-dealkylations. Aside from the hydrolytic reactions, the most common phase 1a biotransformations are generally catalyzed by the CYP family. The phase 1b events utilize a broader range of enzymes to catalyze the same types of chemical reactions. Typical phase 1b examples include: the rapid reduction or further oxidation of the aldehyde metabolites resulting from CYP-mediated *N*- or *O*-dealkylations; further processing of an initial glutathione conjugate, so as to form a mercapturic acid metabolite; and successive β -oxidations of aliphatic carboxylic acid chains. It can be noted that phase 1b metabolism is often critical for avoiding metabolite toxicity (i.e., further processing of aldehydes) or for reducing a metabolite's molecular mass as a prelude to the latter's excretion (i.e., further processing of a glutathione conjugate reduces its initial molecular mass increase from 307 to only 162, which is then similar to the increase of 175 that results from formation of a common glucuronide conjugate).

Also in line with the historical definition, while additionally incorporating similar "a" and "b" subclassifications, **phase 2** metabolism includes all of the conjugation reactions that can take place, either as the first metabolic event at a given point on the substrate ("a"), or as a subsequent reaction at a point on the substrate having already undergone a biotransformation ("b"). Typical examples include: glucuronidation and sulfation of aromatic hydroxyl groups either present on the parent xenobiotic (phase 2a) or having been instilled there (phase 2b) by a preceding phase 1a, CYP-mediated aromatic hydroxylation or an aryl- *O*-demethylation biotransformation; glutathione entrapment of highly reactive electrophilic centers present in

the substrate; *N*-acetylation or *N*-, *O*-, and *S*-methylation reactions; and glycine conjugation of carboxylic acid moieties either present on the parent xenobiotic (phase 2a) or having resulted (phase 2b) from a preceding phase 1a, esterase-mediated hydrolysis reaction.

The term **phase 3** encompasses the various excretion-associated events that either the unmetabolized xenobiotic (note first line in the diagram immediately after the phase 0a,b processes) or its metabolites will eventually engage. Key examples include: physicochemical-driven partitioning behavior between biological milieu associated with excretion, such as the loop of Henle within the kidneys' nephrons; transport into the bile canaliculi at the hepatobiliary interface, especially for negatively charged metabolites by the organic anion transporters (OATs) present within the liver's hepatocytes; and transport across the GI epithelium into the gut lumen, especially for metabolites that are good substrates for P-glycoprotein (Pgp). Also accounted for in this expanded nomenclature's diagram is the possibility for enterohepatic cycling, a key example afforded by the steroids that, upon their glucuronide conjugation and excretion into the gut, can be hydrolyzed by the microflora and thus subject to reabsorption.

Finally, **phase 4** encompasses all of the processes that can continue to contribute to an excreted compound's environmental fate. Typical examples include: the possibility for both continued biodegradation (catabolism) or potential anabolism by a variety of microbial, plant, or animal species and "distribution" both by waterways or by edible plants and animals so as to enter the nutritional cycles of mammals, where reingestion by humans, as suggested by the dotted line in the nomenclature's accompanying scheme, then comes full circle along a systems biology theme.

After relating the differing views about the value of using the "phase 1" and "phase 2" terms, in the end the above diagram, along with a tutorial, has been placed in the list of definitions generated for this glossary. Far from providing a concise definition, it is certainly not intended as any type of mandate for usage. Instead, the goal for its inclusion is to provide a fertile ground for a wide variety of technical participants to consider and to discuss the theme of xenobiotic metabolism in the most broad scope possible while retaining all of its components connected within a rational framework that emphasizes a systems biology approach.

Appendix III: A Possible Classification System for Prodrugs

Today, a wide range of interdisciplinary technologies contribute toward prodrug strategies, such that the complexity for their design options has risen significantly.²⁸⁵ This encourages efforts to further classify prodrugs into subcategories that can be useful for clearly assigning their given type of technology and for potentially clarifying the associated regulatory processes needed for bringing prodrugs through preclinical development, clinical studies, and eventually, into the marketplace.²⁸⁵ While the definitions given above for prodrugs and soft drugs are in line with the most recent IUPAC nomenclature recommendations, no system of subcategories has yet been uniformly adopted by practicing technicians, nor have any been officially mandated by drug regulatory bodies and thereby prompting their recognition, let alone their formal adoption by IUPAC. Thus, the following discussion and suggested subcategorizations are, at this time, intended only for interested readers to consider, and perhaps to devise alternative systems that could be useful to the prodrug field of the future.

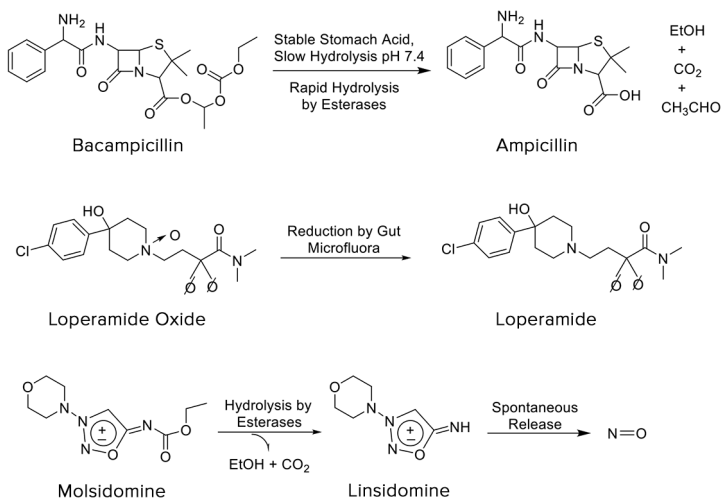
One potentially useful classification has proposed two distinct categories:^{286,287} Type I prodrugs, which undergo bioactivation within cells; and Type II, which undergo bioactivation within biological fluids outside of cells. The proposed system elaborated below also starts from this fundamental division. A few structural examples are provided in order to demonstrate how the various categories might be able to be adopted.

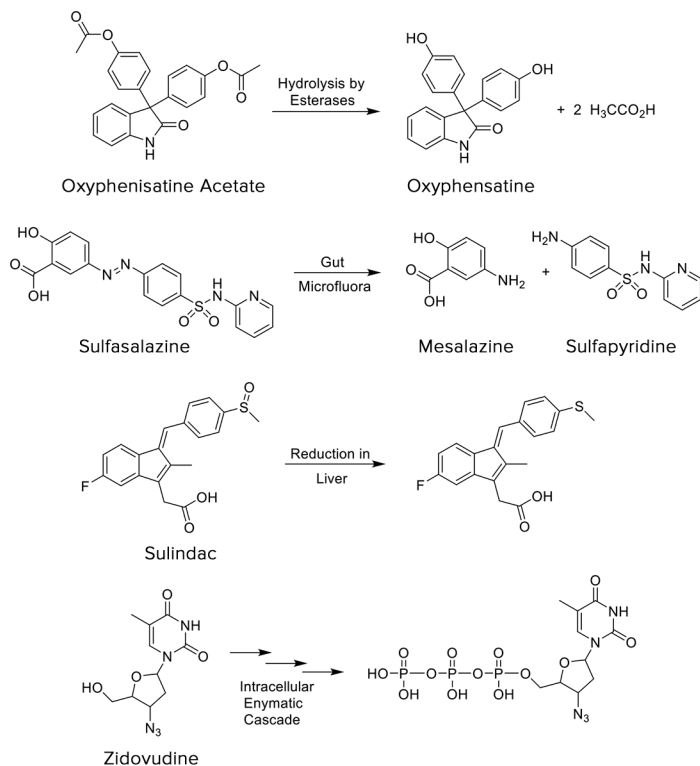
Expanding the system initially introduced by Wu et al.,^{286,287} the following table attempts to provide further definitions for several subtype categories, starting from this same classification system, but with minor alterations.²⁸⁵ Overall, this system is based on which region of a prodrug's "random walk"²³⁷⁻²³⁹ is primarily responsible for its bioactivation. This strikes a certain historical cord with medicinal chemists' early thoughts about the importance of drug distribution and how it can impact both therapeutic efficacy and offtarget toxicity. The table's title and descriptive phraseology have been modified from the original publication in order to further emphasize this theme. Some of the subcategories have also been modified to better accommodate the latest molecular biology-associated technologies, such as those like the antibody-drug conjugates (ADCs), which have recently become very popular as prodrug constructs.^{32,33} The accompanying figure then shows some of the structures and their key biotransformations for the examples listed on the table.

"Regional" Classification System for Prodrugs²⁸⁵ (a)

Type	Bioactivation Zip Code	Bioactivation Subtype	Street Address	Category Examples
I	Intracellular	IA	Therapeutic target tissue or cells	Acyclovir, L-DOPA, Zidovudine
		IB	Metabolic tissues (e.g., GI mucosa, liver, lungs)	Enalapril, Molsidomine, Sulindac
		IC	Molecular biology enhanced localization (mAb for select antigens; exogenous enzymes) (b)	ADCs, ADEPTs, GDEPTs, VDEPTs
II	Extracellular	II	GI fluids (includes gut microbiome) (c)	Loperamide Oxide, Oxyphenisatin Acetate, Sulfasalazine
		IIB	Systemic circulation and other extracellular fluid compartments	Bacampicillin, Bambuterol, Fosphenytoin
		IIC	Molecular biology enhanced localization (mAb for select antigens; exogenous enzymes) (b)	ADCs, ADEPTs, GDEPTs, VDEPTs

(a) This table has been adapted from an original version published by Wu and Farrelly.²⁸⁶ **(b)** ADCs whose mAbs have enhanced localization at the target cells, can be engineered to first enter the cells prior to releasing the active drug cargo (Type IC), or to first release the cargo in the immediate extracellular matrix so it can enter the cells without having the mAb still attached (Type IIC). Both approaches have distinct technical challenges. The same is true for mAb-delivered exogenous enzymes (ADEPTs). Similarly, once gene altered "exogenous-like" enzymes have been expressed (GDEPTs or VDEPTs), they can be either retained within the cell (Type IC) or shuttled to the extracellular matrix (Type IIC) in order to biotransform their subsequently administered prodrug partners that have been tailored to be ideal substrates. **(c)** While the microbiome's (i.e., gut bacteria or microflora) composite of metabolic activity is largely accomplished in their own "intracellular" region, this compartment is being regarded as "external" to that of all the human cells making up the body's tissues, organs etc.





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