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An Introduction to Drug Disposition: The Basic Principles of Absorption, Distribution, Metabolism, and Excretion*

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ABSTRACT

A knowledge of the fate of a drug, its disposition (absorption, distribution, metabolism, and excretion, known by the acronym ADME) and pharmacokinetics (the mathematical description of the rates of these processes and of concentration–time relationships), plays a central role throughout pharmaceutical research and development. These studies aid in the discovery and selection of new chemical entities, support safety assessment, and are critical in defining conditions for safe and effective use in patients. ADME studies provide the only basis for critical judgments from situations where the behavior of the drug is understood to those where it is unknown: this is most important in bridging from animal studies to the human situation. This presentation is intended to provide an introductory overview of the life cycle of a drug in the animal body and indicates the significance of such information for a full understanding of mechanisms of action and toxicity.

Keywords. Xenobiotics; human and animal exposure; predictive value

INTRODUCTION

Humans and other animals are exposed on a daily basis to many xenobiotics, that is, compounds that are foreign to the normal energy-yielding metabolism of the body. Exposure to these xenobiotics may occur deliberately, as in the case of drugs and food additives; accidentally, as in the case of food contaminants and pesticides, or coincidentally, as in the case of industrial chemicals and environmental pollutants. In this paper, the terms drug, xenobiotic, and foreign compound will be used interchangeably. In the present context, the importance of ADME (absorption, distribution, metabolism, and excretion) principles in drug development will be emphasized, but it should be appreciated that these have comparable applicability in the safety assessment of all types of chemicals to which humans might be exposed.

To achieve its effect, whether therapeutic or toxic, a drug and/or its metabolites must be present in appropriate concentrations at its sites of action. The concentration of xenobiotic attained will depend on the dose, formulation, and route of administration, the rate and extent of absorption, its distribution through the body and binding to tissues, biotransformation, and excretion. It is the purpose of this presentation to give an overview of these processes and to comment upon the factors influencing them and their biological significance.

ABSORPTION

The processes of absorption are those that lead to the entry of a xenobiotic into the systemic circulation of the body. The most important site of absorption is the gastrointestinal tract, although absorption through the skin, the main barrier between the internal milieu and the external environment, and the respiratory tract, which is important for volatile compounds and materials present in aerosols and dust particles, can also occur. Regardless of the site of absorption, xenobiotics must cross cell membranes to enter the systemic circulation. Mechanistically this can occur in 1 of 2 ways (4). Small, lipophilic compounds can cross the cell membrane by passive diffusion along a concentration gradient. This transfer is directly proportional to the magnitude of the concentration gradient across the

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membrane and the lipid : water partition coefficient of the drug (3). Large, highly polar or charged xenobiotics cannot cross the cell membranes by simple diffusion and, hence, are dependent on the presence of active carrier-mediated transport mechanisms.

The Effect of pH and pKa on Absorption from the Gastrointestinal Tract

Many xenobiotics are weak acids or bases and are thus present in solution in both non-ionized and ionized forms. The non-ionized molecules tend to be lipid-soluble and cross membranes by passive diffusion, whereas the ionized forms have low lipid solubility and cannot cross the cell membrane (3). The partition of weak electrolytes across membranes will thus be a function of the pKa of the xenobiotic and the pH gradient across the membrane.

The low pH in the stomach favors absorption of weak acids. Weak bases are ionized and, thus, generally not absorbed from the stomach. In the intestine, absorption is rapid for weak acids ($pK > 3$) or weak bases ($pK < 7.8$). The longer transit time and increased surface area of the intestine mean that, for the majority of drugs, intestinal absorption is quantitatively more important even if it would be predicted to be less favorable on pH grounds (7).

First-Pass Elimination

Following absorption, drugs can be metabolized in the gut wall, prior to being transported to the liver via the hepatic portal vein (78). The hepatocytes of the liver are the major site of metabolism for the majority of drugs, and compounds can be extensively metabolized in the liver before reaching the systemic circulation. That portion of the dose that is absorbed from the lumen of the gastrointestinal tract but eliminated by metabolism in the gut wall and/or the liver on the way to the heart is said to have undergone first-pass elimination (3). The extent to which xenobiotics undergo first-pass elimination will have a major influence on the exposure to the compound following oral administration. The enzymes contributing to the metabolism of xenobiotics are also found in organs other than the liver, such as the lung and skin, albeit usually at a lower level. Thus, xenobiotics entering the body by routes other than the gastrointestinal tract can also be subject to first-pass metabolism.

Distribution

Following entry of a xenobiotic to the systemic circulation, its distribution into the various tissues of the body will be influenced by tissue hemodynamics, passive diffusion across lipid membranes, the presence of carrier-mediated active transport processes recognizing the xenobiotic, and protein binding in the blood and tissues.

The majority of tissue membranes behave as typical lipid barriers allowing small lipophilic molecules to cross cell membranes. Equilibrium drug concentration ratios are maintained by diffusion of drugs into and out of tissues. Drugs can accumulate in tissues at a higher concentration than predicted by simple diffusion under the influence of pH gradients, binding to intracellular constituents, or partitioning into lipid depots. Larger or more polar substances do not cross lipid membranes by passive diffusion and require specific transporters to enter the tissues (44). If a drug does enter a tissue by an active transport mechanism, its concentration in the tissue may be many times greater than its plasma concentration.

Active uptake processes tend to show stereoselectivity and can be particularly important for xenobiotics that may be analogs of nutrients (51). The operation of specific uptake mechanisms for xenobiotics may play an important role in the toxicity of some compounds. For example, amantanide and phalloidin are toxic cyclopeptides of the fungus *Amanita phalloides* (21). The toxins enter the liver via an active transport system involved in the transport of bile acids (23). Once inside the cells, the toxins bind to microfilamentous F-actin and destroy the mechanical stability of the liver cell membrane. This results in hemorrhagic liver swelling and animals die within 2–3 hr of intravenous dosing with the peptides (22). Co-administration of bile salts with the toxins reduces their hepatic uptake by this active transport mechanism and thereby limits the toxicity of the compounds. Distribution of xenobiotics can also be limited by binding to plasma proteins. Acidic drugs tend to bind to albumin, and basic drugs tend to bind to α_1-acid glycoprotein. As only unbound drug is in equilibrium across membranes, a drug that is extensively and strongly bound to plasma proteins has only limited access to the tissues.

Drug Reservoirs

Accumulation of a drug within a tissue can act as a reservoir serving to prolong its duration of action. If the stored xenobiotic is in equilibrium with that in plasma and is released as its plasma concentration falls, then the concentration of xenobiotic in plasma will be sustained and the pharmacological effect of the xenobiotic will be prolonged (3). Thus, the storage of a drug can prolong its action either within the tissue where the drug is held or at a distant site reached following rediffusion into the systemic circulation (29).

The concepts of drug reservoirs and how they influence the concentration of a xenobiotic at its

target tissue are well illustrated by the behavior of the lipophilic anesthetic thiopental, which is given by bolus intravenous injection (2). As a consequence of the high blood flow to the brain and its lipid solubility, thiopental reaches its maximum concentration in its target tissue within 1 min of intravenous injection. When the injection is stopped, the plasma concentration falls as the drug distributes into tissues such as muscle. As thiopental is not tightly bound to brain lipid, its concentration in the brain changes in parallel with changes in the plasma concentration, leading to a rapid termination of anesthesia by redistribution rather than elimination. A third distributive phase for thiopental occurs as the result of a slow, blood flow–limited uptake into poorly perfused tissues such as fat (3).

On repeated administration, fat and other poorly perfused tissues can accumulate large amounts of thiopental. These reservoirs are then capable of maintaining plasma and, hence, brain concentrations of thiopental at levels above those needed for anesthesia. Thus, a compound whose duration of action is limited by rapid redistribution from its site of action to storage sites can become long acting if storage deposits of sufficient size are established. At this point, termination of drug action becomes dependent on biotransformation and excretion of drug. The pharmacological consequence of these changes in tissue distribution is that the sleeping time after dosing of thiopental is changed from a few minutes following a single administration to a few hours following multiple dosing (29).

Toxicity testing is often performed using much higher doses of xenobiotics than humans are exposed to. As well as leading to saturation of metabolic pathways, it must be appreciated that these high doses can lead to changes in tissue distribution similar to those seen following multiple dosing of thiopentone.

METABOLISM

Drugs and other xenobiotics that gain access to the body may undergo 1 or more of 4 distinct fates, as follows (12):

1. Elimination unchanged
2. Retention unchanged
3. Spontaneous chemical transformation
4. Enzymic metabolism

Each of these fates are of importance but, in quantitative terms it is enzymic metabolism, often also referred to as biotransformation, that predominates.

The main site of metabolism of foreign compounds is the liver, although extrahepatic tissues, frequently the site of entry to or excretion from the body (e.g., lungs, kidneys, gastrointestinal mucosa), also play a role in the metabolism of xenobiotics (24 and references therein).

Compounds eliminated unchanged are generally either (a) highly polar such as strong carboxylic or sulfonic acids (e.g., sodium cromoglycate) or quaternary amines (e.g., pancuronium), which if absorbed are rapidly cleared into the urine or bile, or (b) volatile and hence readily lost via the lungs. In contrast, nonpolar, highly lipophilic compounds may be retained for long periods in tissue lipids, as occurs with chlorophenothane and many polyhalogenated aromatics. For a small number of compounds, spontaneous chemical transformation within the tissues of the body can be important: this may involve hydrolysis at the appropriate pH (e.g., thalidomide with its numerous breakdown products) or reaction with nucleophilic or electrophilic centers in tissue macromolecules, most notably the nucleophilic $-SH$ of glutathione (71).

The scope of drug metabolism is immense, and this is reflected in the range of chemical reactions that are involved in the metabolism of substrates, including oxidation, reduction, hydrolysis, hydration, conjugation, and condensation. Typically, the process of metabolism of xenobiotics is biphasic, whereby the compound first undergoes a functionalization reaction (oxidation, reduction, or hydrolysis), which introduces or uncovers a functional group ($-OH, -NH_2, -SH$) suitable for subsequent conjugation with an endogenous conjugating agent.

By far the most important enzyme system involved in Phase 1 metabolism is cytochrome P-450, the terminal oxidase component of the microsomal electron transfer system, which is responsible for the oxidation of many xenobiotics. The required electrons are supplied by the closely associated enzyme NADPH cytochrome P-450 reductase, a flavoprotein that transfers 2 electrons to cytochrome P-450 from NAD(P)H.

The cytochromes P-450 are an enzyme superfamily consisting of a number of related isoenzymes, all of which possess an iron protoporphyrin IX as prosthetic group. The enzymes are named for the Soret band around 450 nm exhibited by the CO complex of the reduced form. The P-450 enzymes have been grouped together into families that share sequence identity. There are 10 mammalian gene families comprised of 18 subfamilies (52, 53). The most important enzymes involved in xenobiotic metabolism belong to the 1A, 2B, 2C, 2D, and 3A subfamilies. Although the individual enzymes are thought to metabolize substrates via the same catalytic mechanism (27), they tend to show selectivity toward substrates. For individual isoforms of P-450, the extent of this selectivity is highly variable with overlap of substrates and regio- and stereospecificities being

observed (26). In addition, substrates are often metabolized at more than 1 position, as in the case of testosterone. This is believed to be a function of both the binding characteristics of the enzyme and the ease with which the functional groups of the substrate undergo oxidation (66). A number of active site models have been proposed to explain the different substrate specificities of various P-450 isoenzymes.

CYP1A1

The substrate binding site of CYP1A1 has been proposed to consist of a hydrophobic cleft asymmetrically disposed to the heme iron atom. The asymmetric position of the binding site restricts the number of faces of the substrate that can be exposed to the active oxygen species (32). CYP1A1 has been implicated in the metabolism of a number of polycyclic aromatic hydrocarbon (PAH) compounds such as benzo(a)pyrene. The substrates tend to be large, rigid, planar molecules containing fused (hetero)aromatic rings that are good electron acceptors. Lewis et al (42) proposed that the binding site of CYP1A1 contains a number of aromatic amino acids that form a planar pocket to complement the (hetero)aromatic rings of the substrates. The metabolism of benzo(a)pyrene results in the preferential production of the bay region 7,8-diol-9,10-epoxide, which is a potent DNA-reactive ultimate carcinogen (33). In addition to PAH metabolism, CYP1A1 can metabolize a number of smaller non-PAH compounds in a regio- and stereoselective manner (62). It has been suggested that these substrates are positioned in the active site via hydrogen-bonding interactions between the substrate and an active site residue of CYP1A1 (38).

CYP2B1/2

The P-450 2B isozymes are involved in a number of biotransformations in the rat and are induced by phenobarbital. The substrates for CYP2B tend to be bulky, nonplanar molecules with greater conformational flexibility than CYP1A substrates (39). The substrates tend to have functional groups of similar size and hydrophobicity to isopropyl and to be poor electron acceptors (30, 39). It has been proposed that the binding site of CYP2B contains hydrophilic amino acids that are capable of forming hydrogen bonds with carbonyl and/or amine groupings of the substrate and hydrophobic nonaromatic residues that complement the isopropyl function (42).

CYP2C

The CYP2C subfamily appears to be important in metabolizing a number of xenobiotics particularly in humans (66). CYP2C8 effects the aromatic hydroxylation of warfarin and phenytoin, whereas CYP2C9 is involved in the metabolism of tolbutamide and a number of acidic nonsteroidal anti-inflammatory drugs and is potently inhibited by sulfaphenazole. CYP2C18 is subject to a genetic polymorphism manifest in the hydroxylation of *S*-mephenytoin and is not inhibited by sulfaphenazole (26). CYP2C substrates tend to have areas of strong hydrogen bond–forming potential positioned 5–10 Å from the site of oxidation, and a number are also charged at physiological pH (66). This has lead to the suggestion that hydrogen-bonding potential and possibly ion pair interactions are important in determining the substrate structure activity relationships of the P-4502C isozymes (66).

CYP2D

The CYP2D isozymes have been extensively investigated, as they are involved in the genetic polymorphic metabolism of debrisoquine, sparteine, and some 30 other substrates (17, 48). CYP2D1 (rat) and 2D6 (human) have similar substrate selectivities, but inhibition studies with quinidine (more potent in humans than rats) and its diastereoisomer quinine (more potent in rats than humans) demonstrate that differences in the enzyme active site must exist (65).

Substrates for CYP2D enzymes possess a basic nitrogen grouping that is mainly ionized at physiological pH, a hydrophobic region and a functional group capable of P-450 oxidation 5–7 Å from the basic nitrogen (69). Reactions catalyzed include aromatic hydroxylation (propranolol), aliphatic hydroxylation (metoprolol), and *N*-dealkylation (amiflamine) (17). The substrate binding site of CYP2D appears to contain a carboxyl group that binds and neutralizes the basic nitrogen of the substrate and a hydrophobic domain. The carboxylate group is assumed to serve as an anchoring site on the protein. Substrates can interact with either of the oxygen atoms of the carboxylate group (which are 2.2 Å apart), explaining why for some substrates the distance between basic nitrogen and site of oxidation is 5 Å, typified by debrisoquine, and for other substrates it is 7 Å, typified by dextromethorphan (38). The ionic bonding between substrate and enzyme means that the enzyme tends to have a high affinity for substrates and, thus, a low K_m (65). Many substrates also exhibit a coplanar conformation near the oxidation site and have a negative molecular electrostatic potential in a part of this planar domain approximately 3 Å away from the oxidation site (38).

The predictive value of the model was assessed by measuring the CYP2D6-mediated metabolism of 4 compounds, showing among them at least 14

TABLE I.—The 8 classical conjugation reactions.

Reaction	Conjugating agent
A. Reactions involving activated conjugating agents	
Glucuronidation	UDP glucuronic acid
Glucose conjugation	UDP-glucose
Sulfation	3'-Phosphoadenosine-5'-phosphosulfate
Methylation	*S*-adenosyl methionine
Acetylation	Acetyl coenzyme A
Cyanide detoxication	Sulfane sulfur
B. Reactions involving activated foreign compounds	
Glutathione conjugation	Glutathione
Amino acid conjugation	Glycine, ornithine, taurine

oxidative metabolic routes. From the model, 4 routes were predicted to be 2D6-mediated. *In vivo* and *in vitro* data from humans demonstrated that 3 of the 4 predicted metabolic routes were in fact mediated by CYP2D6 (40).

CYP3A

The CYP3A family tends to be involved in the metabolism of large, structurally diverse, fairly lipophilic compounds. Although substrates are bulky, metabolism tends to occur in small exposed functional groups that undergo reactions such as *N*-dealkylation and aliphatic hydroxylation. Substrates include the immunosuppressant cyclosporin A, nifedipine, and verapamil (66).

It has been suggested that the binding site for CYP3A is dominated by hydrophobic interactions and that, in contrast to CYP2D, which is governed by ionic bonding, this allows for a degree of flexibility in the position of substrate binding (67).

Conjugation Reactions

Phase 2 conjugation reactions may be divided into 2 distinct groups, depending on the source of energy for the process (10). In most instances, the energy is derived from the activated endogenous conjugating agent, as is the case for the glucuronic acid, sulfate, methylation, and acetylation reactions. In other examples, the energy is derived by prior metabolic activation of the xenobiotic, as is the case for glutathione and amino acid conjugations. Of the Phase 2 conjugation reactions listed in Table I, glucuronic acid conjugation ranks as highest importance, and many drugs (e.g., indomethacin, paracetamol, dapsone, clofibrate, morphine) are metabolized via this pathway. The conjugations are performed by a family of glucuronyl transferase enzymes located within the endoplasmic reticulum of the cells of the liver, intestine and kidney. These enzymes catalyze the conjugation of uridine diphosphate-α-1-glucuronic acid with nucleophilic O, N, C, and S atoms: during the reaction, C-1 of the sugar ring is inverted so that the products are 1-0-substituted B-D-glucopyranosiduronic acids. The enzymes have a molecular weight of between 50 and 60 kDa and exist as oligomers of between 1 and 4 subunits *in vivo* (60). At least 9 different isozymes in 2 different subfamilies are known to exist (8). Glucuronidation occurs in most mammalian species with the cat and related felines and the Gunn rat being notable exceptions.

Glutathione-*S*-transferases catalyze the conjugation of a number of functional groups (aryl and alkyl halides, lactones, epoxides, and quinones) with glutathione, the tripeptide γ-glutamylcysteinylglycine. The glutathione-*S*-transferases have a very extensive tissue distribution and are principally found in the cytosol of the cell. The proteins have a molecular weight of 24–28 kDa and exist as dimers *in vivo* (77). The dimeric proteins possess binding sites for glutathione and the electrophilic substrate, which brings the reactants close together (47). The mammalian glutathione transferase enzymes have been divided into 5 evolutionary classes: α, μ, π, θ, and microsomal (77). Typical substrates include parathion, urethane, ethacrynic acid, and 1-chloro-2,4-dinitrobenzene. The glutathione transferase enzymes are also very abundant in the liver cytosol (4–5% of total cytosolic protein). Thus, as well as having major significance in drug metabolism, these enzymes are also important in intracellular binding. This is particularly true for glutathione-*S*-transferase B (Ligandin). Compounds that bind to glutathione-*S*-transferases include bilirubin, estradiol, cortisol, testosterone, tetracycline, penicillin, and indocyanine green (43).

A number of catechol, phenol, and alcohol compounds are excreted as sulfate conjugates. This reaction between substrate and sulfate donor, 3'-phosphoadenosine-5'-phosphosulfate, is catalyzed by a family of sulfotransferase enzymes (18). The sulfotransferases have a cytosolic location and are found in many tissues including the liver, adrenals, lung, brain, jejenum, and blood platelets. The proteins

have molecular weights between 32 and 34 kDa and exist as homodimers *in vivo*. The sulfotransferase enzymes are normally classified into subfamilies based on their substrate specificity, although there is overlap among the different isozymes (18). Six different phenol transferases and 7 different steroid/bile acid sulfotransferases have been characterized in the rat. In humans, only 2 phenol transferases (M-PST and P-PST) and 1 steroid/bile acid sulfotransferase (DHEA-ST) have been characterized. Typical substrates of the human liver cytosolic transferases are dehydroepiandrosterone, pregnenolone, and testosterone (DHEA-ST), dopamine and acetaminophen (M-PST), and phenol and minoxidil (P-PST). As well as exhibiting species differences, it is known that phenol sulfotransferase activity varies among individuals over a 15-fold range (35).

N-acetyltransferase catalyzes the addition of an acetyl group to the amino group of amine, amino acid, and sulfonamide compounds using acetyl coenzyme A as a cosubstrate. *N*-acetyltransferase is a 26.5 kDa cytosolic enzyme found in the liver and intestine. Typical substrates include isoniazid and sulfanilamide. A well-defined polymorphism for *N*-acetyltransferase exists in many species including humans (61), which can have profound effects on toxicity. For example, isoniazid accumulates in slow acetylators and so may predispose these individuals to drug-induced neuropathy. On the other hand, the toxicity of isoniazid is related to the formation of its *N*-acetyl derivative, which is further metabolized to a reactive intermediate. Rapid acetylators are more susceptible to this type of side effect. The dog and related canine species do not possess this polymorphic enzyme.

Epoxide hydrolase enzymes catalyze the *trans* addition of water to a variety of epoxide compounds. Microsomal epoxide hydrolase is found in the liver, testes, kidney, ovary, and lung. It is an approximately 50-kDa protein, and typical substrates include styrene oxide, vinyl chloride epoxide, benzo(a)pyrene-4,5-oxide, phenytoin epoxide, and carbamazepine epoxide (63). Cytosolic epoxide hydrolase is a 60-kDa protein that exists as a homodimer of 120 kDa *in vivo*. Typical substrates of this enzyme include *trans*-stilbene oxide, epoxymethyl stearate, and arachadonic acid epoxide. Species differences occur in the activity of cytosolic epoxide hydrolase in that the mouse has high activity, the rabbit, guinea pig, and humans have intermediate activity, and the rat has very low activity (46).

Factors Affecting Metabolism of Xenobiotics

Factors influencing the rate and extent of metabolism via Phase 1 or 2 reactions *in vivo* can be physiological, endogenous, or exogenous (Table II). These are important in determining the biological effect of a xenobiotic. The intracellular concentration of a chemical is primarily dependent on dose size and its physicochemical and structural properties. Because the metabolism of most compounds is enzymatic, any factor that can influence the activity of these enzymes can alter metabolism.

Species Differences in Metabolism and Toxicity

Species differences in metabolism are of most significance, but variability can also occur in absorption, distribution, and excretion of foreign compounds. Species differences in drug metabolism reflect differences in the activities of the enzymes responsible for the various transformations. Such variations arise primarily from differences in the absolute activities of the enzymes, but the amounts of any endogenous inhibitors present, or the extent of any reverse reactions, are also relevant. Species differences occur in both Phase 1 and 2 metabolism and can be either quantitative (same metabolic route at different rates) or qualitative (different metabolic routes) (12). Given that species differences do occur, it is possible that they arise from 1 or more of 3 origins:

1. Deficiencies in certain enzymes that lead to a defect in a metabolism reaction that is otherwise widespread in occurrence. For example, the glucuronidation deficiency in the cat leads to increased toxicity of glucuronidogenic compounds in this and related species. The lack of acetylation of aromatic amines in dogs may explain why they are more susceptible to *p*-aminobenzoic acid and various hydrazines. The guinea pig has a deficiency in *N*-acetylation and is unable to *N*-acetylate *S*-substituted cysteines to form acid conjugates (10). This is not due to defective formation of glutathione conjugates (the first step in the mercapturic acid pathway), indicating that the defect lies in the final step of transformation of the glutathione conjugate to a mercapturic acid (15). The pig and opossum are defective in their ability to conjugate phenolic compounds with sulfate (10).

   Although most of the deficiencies involve conjugation reactions (12), there are a few examples of defects in oxidative metabolism. These include the inability of the guinea pig and steppe lemming to *N*-hydroxylate the arylacetamide, 2-acetylamidofluorene, and the inability of the rat and marmoset to *N*-hydroxylate the aliphatic amine chlorphentermine (10).
2. Restricted species occurrences. For instance, these are seen with the particular amino acid (glycine, glutamine, taurine, or ornithine) utilized in the

TABLE II.—Physicochemical, endogenous, and exogenous factors affecting the rate and extent of metabolism of xenobiotic chemicals *in vivo*.

Physicochemical	Endogenous	Exogenous
Electrophilicity	Age	Dose
Nucleophilicity	Sex	Nutrition
Lipophilicity	Species	Route of administration
Polarity	Strain	Time of day
Protein binding	Pathology	Enzyme inhibition
	Genetic deficiencies	Enzyme induction
	Cofactor availability	

conjugation of acids. Most species conjugate benzoic acid and heterocyclic and cinnamic acids with glycine, whereas bird species use ornithine. Of special interest are the reactions that are restricted to primates such as the glutamine conjugation of phenyl acetic acid (10).

3. Most common, the relative rates of competing pathways of metabolism rather than the route of metabolism. Amphetamine undergoes either aromatic hydroxylation, producing 4′-hydroxyamphetamine, or side-chain degradation to benzoic acid. The compound is metabolized extensively in most species, but the nature of metabolites formed is highly variable. In the rat, ring hydroxylation predominates, in the guinea pig chain breakdown is the major route, and in other species both routes are significant (10, 11, 13). Diazepam is extensively metabolized in humans, dog, and rat. In humans, the major metabolites are 3-hydroxydiazepam, N^1-desmethyldiazepam, and 3-hydroxydesmethyldiazepam (oxazepam). In the dog, oxazepam is the major product, and in the rat ring hydroxylations predominate with 4′-hydroxy-3-hydroxydiazepam, 4′-hydroxy-N^1-desmethyldiazepam, oxazepam, and 3-hydroxydiazepam all being formed. Oxazepam is further metabolized to the *O*-glucuronide in humans and dog but to a number of ring hydroxylated compounds in the rat (10). Phenol can be conjugated on the hydroxyl group with either sulfate or glucuronide. Humans and Old World monkeys excrete phenol principally as the sulfate, whereas New World monkeys excrete phenol principally as the glucuronide and the rat and mouse excrete approximately equal amounts of both conjugates (10).

Sex Differences in Metabolism and Toxicity

Sex differences have been noted in the absorption (cephradine), protein binding (diazepam, warfarin), and biliary excretion (indocyanine green, tartrazine) of drugs and xenobiotics (9). However, the sex differences found in metabolic pathways have probably the most significance with regard to xenobiotic toxicity. Sex differences have been reported in both Phase 1 (e.g., *N*-demethylation of morphine and oxidation of pentobarbital) and Phase 2 metabolism (e.g., glutathione conjugation of 1,2-dichlorodinitrobenzene, glucuronic acid conjugation of *p*-nitrophenol, sulfation of *N*-hydroxy-2-acetylaminofluorene). The exact nature of the difference between the sexes is dependent on the enzyme activity, organ, strain, and species studied (9). In general, male rats metabolize drugs faster than the female, with the opposite seen in mouse (24).

The sex differences in each of these processes can lead to sex differences in toxicity. An example is the renal toxicity and tumors caused by the natural flavoring agent d-limonene. Administration of d-limonene and other aliphatic hydrocarbons results in a characteristic nephrotoxicity, a major feature of which is the accumulation of hyaline containing droplets in the proximal tubule cells and eventually renal tumors. These tumors are only observed in the male rat and not in the female rats or male and female mice (20).

The major low molecular weight protein excreted in the urine of male rats is α2u-globulin. It is excreted at a much lower rate in female rats. d-limonene or its metabolites bind specifically to α2u-globulin and prevent its *in vitro* degradation (41). Female rats are also 100 times less sensitive to the renal toxicity of decalin than males (1), which involves the same mechanism.

Male rats are more susceptible than females to liver damage induced by chloroform. This difference is thought to have a metabolic and hormonal basis and is determined by the effect of testosterone on microsomal activity in the liver (72). Treating female rats with testosterone lowers the LD_{50} of chloroform, whereas treating male rats with estradiol increases the LD_{50} of chloroform.

Age Differences in Metabolism and Toxicity

As well as being affected by sex and species differences, xenobiotic metabolism and disposition can also be affected by the aging process (37). For instance, the hepatobiliary transport of ouabain and

digitoxin metabolites decreases with increasing age in the male rat. For ouabain this is the result of a decrease in hepatic blood flow and decreased canalicular secretion, whereas for the digitoxin metabolites the decreased transport is the result of decreased biotransformation (37). A number of studies have shown that P-450 activities decline with age, although these changes are dependent on the sex, species, and metabolic pathway studied. For example, *N*-demethylation of aminopyrine and hydroxylation of hexobarbital decrease with age in male but not female rats (37). The information on Phase 2 reactions is less complete, but there is evidence indicating that between 6 and 20 mo of life glucuronidation and sulfation efficiency of paracetamol decreases in the rat (49, 50). This is the result of decreased levels of both UDP-glucuronic acid and UDP-glucuronyl transferase.

Variations in Metabolism and Toxicity Due to Differences in Dose Size

To maximize the sensitivity of toxicity tests, most studies involve dose regimes based on very high doses, which may be greatly in excess of the estimated human exposure. These doses are set with the assumption that the toxic response to a chemical shows a linear dose relationship. However, at the maximum tolerated doses, the capacities of the various primary mechanisms involved in ADME may become exceeded so that secondary mechanisms come into play. This phenomenon is commonly referred to as *metabolic switching* (19). Thus, before an extrapolation from high-dose animals to low-dose humans can be made, data on the influence of dose levels on metabolism in the test animal should be generated to determine whether absorption, metabolism, or excretion processes have thresholds.

At low doses, the metabolism of allylbenzenes largely follows the safe metabolic pathway via *O*-demethylation, but this pathway is saturated at higher doses, leading to a disproportionate increase in the metabolism via 1′-hydroxylation to the proximate carcinogen (14). The 1′-hydroxy allylbenzene is then conjugated with sulfate by sulfotransferase enzymes to form the sulfate ester (6). The sulfate ester is a good leaving group, and the resulting electrophilic carbonium ion reacts with nucleophilic sites on proteins and DNA leading to carcinogenicity. As this pathway becomes more important at higher doses, this may result in a hockey stick dose–response curve for the carcinogenicity of these agents.

The influence of dose size on metabolism and toxicity has been clearly demonstrated for acetaminophen, a very safe drug at low doses but a hepatotoxin at large doses, when its primary routes of metabolism become saturated. At low doses acetaminophen is detoxified by sulfate and glucuronic acid conjugation of the free OH group. These conjugates then undergo renal excretion. A small portion of the dose is metabolized to the reactive electrophile *N*-acetyl-*p*-benzoquinoneimine (NAPQI), which is trapped by conjugation with glutathione and subsequently excreted as the mercapturic acid and cysteine derivatives. At high doses, however, the sulfate and glucuronide pathways become saturated and the NAPQI produced quickly depletes cellular glutathione and then binds to cellular proteins. This correlates excellently with cell injury and hepatic necrosis (16, 34).

Role of Metabolism in Toxicity

The end products of metabolism are generally more polar and have greater molecular weight than the parent drug; they usually lack receptor activity and are more readily excreted. However, it is clear that many toxicological effects of xenobiotics are mediated through the formation of reactive intermediates ("toxication," "metabolic activation," or "bioactivation"). These reactive intermediates range from the very unstable such as 1-sulfooxysafrole and *N*-hydroxyacetylaminofluorene sulfate (6), which spontaneously lose the sulfate group to form carbonium or nitrenium ions, which react with DNA and proteins, to relatively stable reactive intermediates such as the metabolite of chloramphenicol causing blood dyscrasias (43). There is not, however, a direct relationship between stability of intermediates and their potential to induce toxicity, as even stable metabolites can be toxic.

Bioactivation of chemicals can result from almost all the enzymes of drug metabolism, even the conjugations. For example, conjugation with glutathione generally produces inactive metabolites, but the conjugation of dibromoethane with glutathione, catalyzed by glutathione-*S*-transferases, results in the formation of a reactive thiiranium ion (73), responsible for the carcinogenicity of this compound in rats and mice. Thus, in such cases biological activity is not related to the absolute dose but is, rather, a function of the concentration of the ultimate reactive species that binds to the cellular target. Factors important in determining such toxic response are the following:

1. The nature of the ultimate reactive species, in many cases a reactive intermediate, and the availability of target molecules at the site of formation. Toxic reactive intermediates are typically electrophiles such as epoxides, quinones and nitrenium ions, free radicals, and other reactive oxygen species. Certain sites in cellular macromolecules will be favored depending on the properties of the electrophiles. One of the most suc-

cessful theories in this respect is the theory of hard and soft acids and bases (59). This theory predicts that hard electrophiles (low polarizability and small atomic radii) will preferentially react with nucleophiles (e.g., benzo[a]pyrene-7,8-diol-9,10-oxide and aflatoxin B_1-8,9-epoxide), whereas the soft species (high polarizability and relatively greater atomic radii) will react best with each other (e.g., cinnamaldehyde, *N*-acetyl-*p*-benzoquinoneimine and *trans*-anethole epoxide, which all react with glutathione).

2. The role of the target molecules in cell function. Of primary importance in cell function are the energy supply and the integrity of cellular membranes. The cell needs to have sufficient adenosine triphosphate (ATP) to adequately perform transport, synthesis, and repair processes. A reduction in ATP synthesis can lead to uncoupling of the oxidative phosphorylation and subsequently cell death. Other critical targets are enzymes [e.g., ATPases (*N*-acetyl-*p*-benzoquinoneimine)] and cytochrome oxidase a_3 (cyanide) and nucleic acids [e.g., N-7 of guanine (aflatoxin), N-2 of guanine (safrole, estragole), N-6 of adenine (safrole, estragole)].
3. The effectiveness of cellular defense mechanisms in detoxifying the active species and repairing initial damage. Detoxication reactions will compete with the reaction of the reactive intermediate with tissue macromolecules and convert them into harmless metabolites for elimination from the body. Epoxides, for example, are detoxified by epoxide hydrolases. Other electrophiles may be detoxified by the action of glutathione-*S*-transferases, or directly by glutathione, and sometimes sulfur amino acids such as methionine may constitute a first line of defense. The relative concentration and activity of the activating and detoxifying enzyme systems will determine the extent of toxicity. Any factor influencing these activities such as DNA repair or glutathione resynthesis will also influence the toxic response.

EXCRETION

There are 2 main elimination routes from the body for xenobiotics and their metabolites. In both the kidneys and the liver, polar compounds are excreted more efficiently than lipophilic compounds. Thus, lipid-soluble compounds are not readily excreted from the body unless they are first metabolized to more polar, more water-soluble compounds.

Renal Excretion

The mechanisms involved in renal excretion are filtration, secretion, and reabsorption.

Filtration. Substances that are polar or charged and have little binding to plasma proteins are eliminated primarily by glomerular filtration. Examples include the aminoglycoside antibiotics and vancomycin. In contrast, compounds extensively bound to plasma proteins tend to remain in the blood and do not undergo extensive filtration (e.g., indocyanine green, bilirubin.

Secretion. Some drugs are removed from plasma and secreted into the proximal tubules by the cells of the tubular wall. Secretion occurs via active transport mechanisms that can differentiate among compounds on the basis of charge. The first system transports weak acids, including numerous drug conjugates produced in the liver, penicillins, and a number of thiazide diuretics. The second system transports basic substrates including cimetidine, histamine, and choline (29). The carrier systems are relatively nonselective, and xenobiotics of similar charge compete for transport.

Reabsorption. Compounds that are lipid-soluble undergo extensive reabsorption within the tubules and, hence, are poorly eliminated by the kidneys. Reabsorption occurs down a concentration gradient from tubular fluid to plasma. Reabsorption of weak electrolytes is highly pH-dependent. If the tubular urine is made more alkaline, weak acids are more ionized and, hence, are excreted to a greater extent. Conversely, if the tubular urine is made more acidic, the weak acids are less ionized and undergo reabsorption and renal excretion is reduced. Alterations in urine pH have the opposite effect on the renal excretion of weak bases (3). In the normal population, urine pH is highly variable and so are the urinary excretion rates of weak electrolytes.

Biliary Excretion

Whereas small, polar compounds with low protein binding are excreted in the urine, larger, lipophilic compounds, which may be extensively protein-bound, are excreted into the bile (43). A number of factors are known to influence the extent of biliary excretion of xenobiotics. For instance, molecular size (commonly expressed as molecular weight), the presence of polar groups, and the chemical structure of xenobiotics have all been shown to influence the extent of biliary excretion (67). There are at least 3 ATP-dependent transport systems intimately involved in the excretion of xenobiotics across the hepatocyte canalicular membrane.

gp170 (P-glycoprotein). This transport protein is responsible for the biliary excretion of a number of lipophilic and cationic compounds including digoxin, vinblastine, and quinidine (28, 36, 74). *P*-glycoprotein is an integral 170-kDa membrane protein

that is identical to the protein that confers multidrug resistance in cell lines resistant to chemotherapy (76). Multidrug resistance is a consequence of the rapid transport of anticancer drugs out of cells, thus preventing them from exerting their pharmacological action.

gp110. This transport protein is a 110-kDa glycoprotein responsible for the biliary excretion of a number of unipolar steroid compounds including bile acids such as taurocholate (55, 64).

MOAT (or Multispecific Anion Transporter). This transport protein is responsible for the ATP-dependent transport of a number of compounds (56, 57). Most but not all of these xenobiotics possess 2 anionic groups and a hydrophobic domain within their chemical structure (58). The physiological substrate for this transporter is bilirubin and its conjugated metabolites (31). Xenobiotic substrates include oxidized glutathione, ouabain, indocyanine green, ampicillin, and ceftriaxone (31).

The active transport processes in the liver tend to excrete large, lipophilic compounds, whereas the active transport processes in the kidney tend to excrete smaller, more polar compounds. It has been shown that for many structurally related series of compounds there is an inverse relationship between the extents of biliary and renal excretion (5, 45) so that the liver and kidney appear to work in a complementary fashion to eliminate many xenobiotics from the body.

Enterohepatic Circulation

A number of drugs are excreted into the bile and then reabsorbed into the systemic circulation from the intestine (25). This leads to further excretion in bile, and a cycle is set up. The usual enterohepatic circulation involves excretion of drug conjugates, hydrolysis of the conjugates in the intestine, and reabsorption of the parent compound. It is not unusual for compounds to be finally eliminated from the body in the urine despite undergoing extensive biliary cycling [e.g., fenofibrate (75)].

Other Routes of Excretion

Pulmonary excretion is important for the elimination of anesthetic gases and vapors. Small quantities of some other drugs and metabolites are also excreted by this route. Unlike in the kidney and liver, lipophilic compounds can be excreted via the lungs. Some xenobiotics are also excreted in saliva, sweat, and breast milk (3). The latter route, although not a major excretory route, is important, as the xenobiotics may exert unwanted effects on the nursing infant.

Metabolism and Toxicity Studies in Relation to Human Exposure

The safety assessment of drugs and other chemicals to which human populations may be exposed depends largely on the extrapolation of findings in animal experiments to the human situation. It is therefore critical that we have an adequate understanding of the predictive value of animal tests as indicators of human effects. Despite the commonality of fundamental mechanisms of drug action, it is now appreciated that there are numerous situations where the effects of a drug or a chemical on the body depend on the animal species in question. Principally, but not exclusively, these situations involve the species-specific expression of adverse reactions. Again, numerous examples may be quoted, such as the teratogenicity of thalidomide in humans and rabbits but not rats and the pneumotoxicity of phenylthiourea in rat but not rhesus monkey or, apparently, humans.

In determining the predictive value of animal tests as indicators of human risk, it is obviously important that we understand the reasons why chemicals exert such effects in a species-specific fashion. The responses of the animal body to a toxic chemical depend on (a) the particular target mechanisms present and their sensitivity to the chemical in question and (b) the processes of the metabolism and disposition of the chemical that will govern both the nature of the chemical compounds present in the body and their concentration–time profiles in the body in general and in specific targets of interest. There are examples where species variations in the effect of a toxic chemical are a consequence of qualitative differences in the target mechanisms. This is the case with peroxisome proliferation, which is a phenomenon essentially restricted to rats and mice and closely related with hepatocarcinogenesis in those species but which has no significance for human populations (68), the α2u-globulin nephropathy now established as being a male rat–specific problem (70), and the well-known association between hepatic enzyme induction and liver and thyroid neoplasia in rats and mice (54). Nevertheless, the similarities of structure and function of higher organisms at the molecular level mean that the mechanisms of toxicity, to a large extent, are identical in animals and humans.

An entirely different situation applies when we consider the metabolism and disposition of xenobiotics. Although the basic pattern of metabolism is common to all species, there do occur important quantitative and qualitative differences in the individual components of the overall sequence. It is only rarely that species differences in absorption and

distribution of compounds through the body are encountered, while species variation in the rate and route of excretion is commonly a consequence of species differences in metabolism.

For the vast majority of compounds to which humans may be exposed, judgments about their safety have to be made on the basis of animal studies. It is thus important to have a proper understanding of the predictive value of such studies, and it is a matter of experience that interspecies differences in xenobiotic metabolism represent an important confounding factor in such extrapolation. In general, the similarities of structure and function between animal species and humans at the functional and molecular levels mean that the mechanisms of toxicity are to a large extent identical across species. Nevertheless, a number of important examples are documented where the toxicity of a compound is dependent on the species chosen for the test. Although many factors can contribute toward such variations, species differences in metabolism underlie the great majority of these examples. In designing toxicity tests, it would be desirable to use an ideal animal model species, chosen on the basis of its similarity to humans, but it is perhaps not surprising that no such species exists. Nevertheless, it is important to have an awareness of the degree of similarity between the animal chosen and the human situation in terms of these criteria. Ideally, this information should be available prospectively to aid in the design of toxicity tests, but even now it is often the case that such information is only used retrospectively, in the interpretation of results of animal studies.

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