12 **Biopharmaceutics**

Chapter Objectives

At the conclusion of this chapter the student should be able to:

1. Define ADME, disposition, elimination, bioavailability.
2. Understand the role of membrane transporters in ADME, and name two major transporter superfamilies and examples of members of those families.
3. Understand Overton's rule.
4. Describe concerted drug transport.
5. Define Phase 1, 2, and 3 drug metabolism and give examples.
6. Understand the concepts of inhibition and induction as they relate to drug transporters, metabolizing enzymes, ADME, and pharmacokinetics.
7. Describe clinically significant examples of induction and inhibition.
8. Describe the brain-barrier systems.
9. Relate rates of absorption, disposition, metabolism, and elimination to points on a curve of plasma level versus time.
10. Understand the role of the stomach and intestine in drug absorption and elimination; gastric emptying; intestinal transit; and regional differences in absorptive capacity, transporters, and enzymes.
11. Discuss the roles of the liver and the kidney in ADME and drug clearance.
12. Define apparent volume of distribution and/or clearance.
13. Relate K_a and K_{el} to ADME parameters (permeability, extraction ratios, clearance).
14. Describe causes of low bioavailability.

The purpose of this chapter is to provide the student with a biopharmaceutical foundation for studying the contemporary pharmaceutical sciences. The intended audience is predoctoral pharmacy (PharmD) and pharmaceutical science (PhD) graduate students. This information will be valuable to the practicing pharmacist because he or she is in a unique position to integrate and interpret the vast amounts of biologic, chemical, and physical information regarding drugs and drug products. The pharmacist can then convey practical advice to patients regarding the potential for drug interactions and for managing complex multidrug-treatment regimens. The pharmaceutical scientist also requires an understanding of this subject matter because of its emerging importance in drug discovery and development, including basic and applied research as well as in fields such as regulatory affairs.

The pharmaceutical sciences have been undergoing a revolution of sorts over the last two decades. A transition has occurred from focusing solely on the physical aspects of pharmacy such as dissolution, solubility, and compaction physics to the integration of these important disciplines with the biopharmaceutical sciences. First, in the 1970s and 1980s, there was an explosion of activity in the discovery and characterization of the cytochromes P-450 (CYP450s), an important group of enzymes responsible for metabolizing many endogenous and exogenous substances,1including 40% to 50% of all medications.2 CYP450s transform drugs and other xenobiotics into more hydrophilic substances in order to facilitate their elimination from the body. More recently, it has become increasingly recognized that membrane transporters play an important role in the absorption and elimination of drugs. Drug transporters are membrane-spanning proteins that facilitate the movement of endogenous or exogenous molecules across biologic membranes. Included in this broad category are proteins involved in active (i.e., energy- or adenosine triphosphate [ATP]-dependent) transport, facilitated transport, and ion channels. Membrane transporters and metabolizing enzymes are found throughout the human body and in all organs involved in the absorption and disposition of drugs. Whereas membrane transporters facilitate the movement of drugs and their metabolites into and out of specific organs, groups of organs make up subsystems in the human body with discrete functions. For example, “enterohepatic cycling” involves the movement of drugs from the intestine into the liver, back out into the bile, and then back into the intestine.

Although the complex interplay between these systems evolved for various physiologic purposes, the impact on drug–blood levels and the resulting therapeutic effect can be quite significant. We will explore the basic biopharmaceutical foundation for these complex systems and present the practical implications for pharmacotherapy throughout the chapter.

In an editorial in *Molecular Pharmaceutics*,3 G. L. Amidon concisely reflected the sentiment that was the basis for preparing this chapter: “Traditional scientific endeavors in drug delivery and drug product development have been rather phenomenological, more descriptive, and somewhat based on trial and error. That has been primarily due to a lack of tools and our limited understanding of the mechanisms involved at a cellular and molecular level. The rapid advances in the field of biological sciences, cell and molecular biology, and genomics and proteomics, in particular, have penetrated more than just the drug discovery phase of the pharmaceutical sciences. They are now rapidly changing the views and strategies in pharmaceutics and the pharmaceutical development sciences. While the impact is particularly evident in the membrane transporter and metabolism fields, advances

in the physical and material sciences, in parallel, are altering the pharmaceutical properties of drug candidates and delivery systems in new and innovative ways. The computational tool of bioinformatics, molecular property, biopharmaceutical property, and metabolism estimation have advanced rapidly in the past decade and are now having a significant impact on drug discovery and drug development. Traditional pharmacokinetics did not have molecular tools for understanding membrane transport and metabolism, or the effect of genetic polymorphism. Prodrug design and synthesis could not readily consider where and what enzymes convert the prodrug to the active drug. Receptors were primarily investigated for designing new chemical entities until molecular pharmaceutical scientists recognized that ligand-receptor interactions could be used to target drug to the receptor-expressing cells. Moreover, traditional dosage forms have not had to deal with high-molecular-weight ‘biopharmaceuticals,’ which generally have more complex pharmaceutical properties and sites of action hidden deep within the target cells.” In this chapter, the student will be introduced to the biopharmaceutical considerations of the pharmaceutical sciences.

Fundamentals

Absorption, Distribution, Metabolism, and Excretion

The molecular processes, tissues, and organs that control the absorption and disposition of drugs form the basis for the study of biopharmaceutics. The two primary processes relate to input into the body, that is, absorption and output from the body (i.e., disposition).

Absorption relates to the mechanisms of drug input into the body and into a tissue or an organ within the body. *Disposition* can be broken down into distribution and elimination. After a drug enters the systemic circulation, it is distributed to the body's tissues.*Distribution* depends on many factors, including blood perfusion, cell membrane permeability, and tissue binding. The penetration of a drug into a tissue depends on the rate of blood flow to the tissue, partition characteristics between blood and tissue, and tissue mass. When entry and exit rates are the same, distribution equilibrium between blood and tissue is reached. It is reached more rapidly in richly vascularized areas than in poorly perfused areas unless diffusion across membrane barriers is the rate-limiting step. After equilibrium is attained, bound and unbound drug concentrations in tissues and in extracellular fluids are reflected by plasma concentrations. *Elimination* relates to the chemical transformation and/or physical removal of drug from the body. Hence, elimination is the sum of the processes related to drug loss from the body, namely, *metabolism* and *excretion*. Metabolism and excretion occur simultaneously with distribution, making the process dynamic and complex. Excretion is the process by which a drug or metabolite is removed from the body without further chemical modification. Three primary routes of excretion occur through the bile (i.e., biliary excretion), intestine, and kidney (i.e., renal excretion).

Terminology

The following definitions are taken primarily from a variety of sources, which are indicated immediately after the defined term. In some cases, the original source has an expanded discussion on the topic, so the student is encouraged to utilize these sources for additional insights.

Bioavailability[4]

According to the U.S. Food and Drug Administration, bioavailability describes the rate and extent to which the active drug ingredient is absorbed from a drug product and becomes available at the site of drug action. Because pharmacologic response is generally related to the concentration of drug at the site of drug action, the availability of a drug from a dosage form is a critical element of a drug product's clinical efficacy. However, drug concentrations usually cannot be readily measured directly at the site of action. Therefore, most bioavailability studies involve the determination of drug concentration in the blood or urine. This is based on the premise that the drug at the site of action is in equilibrium with drug in the blood. This does not mean that the drug concentrations in blood and tissues are equal. Instead, it assumes that equilibrium is maintained and that blood concentrations are proportional to tissue and active-site concentrations. It is therefore possible to obtain an indirect measure of drug response by monitoring drug levels in the blood or urine. Thus, bioavailability is concerned with how quickly and how much of a drug appears in the blood after a specific dose is administered. The bioavailability of a drug product often determines the therapeutic efficacy of that product because it affects the onset, intensity, and duration of therapeutic response of the drug.

In most cases, one is concerned with the extent of absorption of drug (i.e., the fraction of the dose that actually reaches the bloodstream) because this represents the “effective dose” of a drug. This is generally less than the amount of drug that is actually administered in the dosage form.

In some cases, notably those where acute conditions are being treated, one is also concerned with the rate of absorption of a drug because rapid onset of pharmacologic action

is desired. Food can slow drug absorption and result in lower blood levels. This is particularly important for drugs that depend on certain levels for maximum effectiveness. Good examples of this are antibiotics that need to achieve minimum inhibitory concentrations to be effective. Conversely, there are instances where a slower rate of absorption is desired, either to avoid adverse effects or to produce a prolonged duration of action.

Key Concept
Biopharmaceutical Process: ADME

- Absorption
- Disposition
  - Distribution
  - Elimination
    - Metabolism
    - Excretion

Key Concept
Bioavailability Variability and Therapeutic Index

Therapeutic problems (e.g., toxicity, lack of efficacy) are encountered most frequently during long-term therapy when a patient who is stabilized on one formulation is given a nonequivalent substitute. This is well known for drugs like digoxin or phenytoin. Sometimes therapeutic equivalence may be achieved despite differences in bioavailability. For example, the therapeutic index (ratio of the maximum tolerated dose to the minimum effective dose) of amoxicillin is so wide that moderate blood concentration differences due to bioavailability differences in amoxicillin products may not affect therapeutic efficacy or safety. In contrast, bioavailability differences are important for a drug with a relatively narrow therapeutic index (e.g., digoxin).

Absolute Bioavailability4

"Absolute" bioavailability, *F*, is the fraction of an administered dose that actually reaches the systemic circulation and ranges from *F* = 0 (i.e., no drug absorption) to *F* = 1 (i.e., complete drug absorption). Because the total amount of drug reaching the systemic circulation is directly proportional to the area under the curve (AUC) of plasma drug concentration versus time, *F* is determined by comparing the respective AUCs of the test product and the drug administered intravenously.

Bioequivalence5 refers to chemical equivalents (i.e., drug products that contain the same compound in the same amount and that meet current official standards) that, when administered to the same person in the same dosage regimen, result in equivalent concentrations of drug in blood and tissues. Therapeutic equivalence refers to drug products that, when administered to the same person in the same dosage regimen, provide essentially the same therapeutic effect or toxicity. Bioequivalent products are expected to be therapeutically equivalent.

Genotype6

Genotype is the "internally coded, inheritable information" carried by all living organisms. This stored information is used as a "blueprint" or set of instructions for building and maintaining a living creature. These instructions are found within almost all cells (hence the word "internal" in the definition), are written in a coded language (the genetic code), are copied at the time of cell division or reproduction, and are passed from one generation to the next ("inheritable"). These instructions are intimately involved with all aspects of the life of a cell or an organism. They control everything from the formation of protein macromolecules to the regulation of metabolism and synthesis.

Membrane Permeability

Membrane permeability relates to the velocity with which a drug molecule moves across a membrane. The units of measurement for permeability are distance per time (e.g., cm/sec). Permeability is inversely related to the resistance of transport across membranes or tissues. Therefore, the higher the permeability, the lower is the resistance to movement across the membrane. Drugs can permeate membranes by passive diffusion through the cell membrane or between cells and by using transporters that "carry" drugs across the membrane. Passive permeability across membranes is determined by the solubility of the permeating molecule in the membrane, diffusion across the membrane into the cell, and the thickness of the barrier. This is covered in more detail inChapter 13.

Permeability as it relates to drug transporters is covered later in this chapter.

Phenotype6

Phenotype is the "outward, physical manifestation" of the organism. These are the physical parts, the sum of the atoms, molecules, macromolecules, cells, structures, metabolism, energy utilization, tissues, organs, reflexes, and behaviors: anything that is part of the observable structure, function, or behavior of a living organism. Rogers et al.7 defined phenotype as it relates to drug metabolism: "Phenotype is the observed characteristic (as influenced by dietary intake and environmental exposure) of a patient's enzyme activity, and includes such designations as 'poor metabolizer,' 'intermediate metabolizer,' 'extensive metabolizer,' and 'ultrarapid extensive metabolizer.'" Patients who express dysfunctional or inactive enzymes are considered poor metabolizers.8 Prodrugs, which require biotransformation to an active metabolite to elicit a therapeutic effect, are often not effective in these patients.

Example 12-1

Codeine and Analgesia

Drug toxicity can be observed in patients who are poor metabolizers because of impaired clearance of medications requiring biotransformation for elimination and excretion. Intermediate metabolizers are patients who demonstrate decreased enzyme activity and have diminished drug metabolism.2 Extensive metabolizers are patients who express enzymes that have normal activity,9 in whom the anticipated medication response would be seen with standard doses of drugs. Ultrarapid extensive metabolizers are patients who have higher quantities of expressed enzymes because of gene duplication.8 Normal doses of drugs in these patients may result in reduced or no efficacy (or toxicity with prodrugs) because of rapid metabolism.8 In this example, codeine, a commonly used analgesic for postoperative pain,

will be examined. It is well known that the quality of pain management varies among patients. Codeine is thought to be an effective analgesic because it is metabolized by the cytochrome P-450 2D6 (CYP2D6) pathway to morphine. This product is then quickly glucuronidated to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G), active analgesics. However, it has been shown that CYP2D6 is polymorphic in a number of different alleles, potentially causing a slow-metabolism phenotype, and may even be overexpressed (i.e., ultrarapid metabolizing phenotype) in certain people. This polymorphism results in patients having differing abilities to utilize that pathway for creating an active and effective analgesic. How common are these variations in drug metabolism? The frequency of poor metabolizers is 6% of the U.S. population, whereas only 1% of the Asian population is considered to be poor metabolizers. Similarly, the ultrarapid-metabolizing phenotype is also found worldwide, the greatest percentage being in Ethiopia (29%). In the case of codeine, a poor metabolizer will not receive as much pain management from a typical dose as a normal patient, and an ultrarapid-metabolizing patient may overdose at a similar dose.

P.261

Relationship Between Genotype and Phenotype

The “internally coded, inheritable information,” or genotype, carried by all living organisms holds the critical instructions that are used and interpreted by the cellular machinery of the cells to produce the “outward, physical manifestation,” or phenotype, of the organism.6

Relative Bioavailability4

“Relative” bioavailability refers to the availability of a drug product as compared to another dosage form or product of the same drug given in the same dose. These measurements determine the effects of formulation differences on drug absorption. The relative bioavailability of one product compared to that of another, both products containing the same dose of the same drug, is obtained by comparing their respective AUCs.

Pharmacokinetics4

Pharmacokinetics is the mathematics of the time course of absorption, distribution, metabolism, and excretion (ADME) of drugs in the body. The biologic, physiologic, and physicochemical factors that influence the transfer processes of drugs in the body also influence the rate and extent of ADME of those drugs in the body. In many cases, the pharmacologic action and the toxicologic action are related to the plasma concentration of drugs. Through the study and application of pharmacokinetics, the pharmacist can individualize therapy for the patient.

Pharmacodynamics

Pharmacodynamics is the study of the biochemical and physiologic effects of drugs and their mechanisms of action.

Pharmacogenetics

Pharmacogenetics is the study of how genetic variations affect drug response.

*Omics*10

The burgeoning fields of genomics and proteomics are spawning multiple “omic” subdisciplines and related areas. The suffix generally refers to the study of a complete grouping or system of biomolecules, such as a genome, containing all of an organism's genes, or its proteome, containing all of its proteins. For example, genomics is the scientific study of a genome and the roles that genes play, alone and together, in directing growth and development and in controlling and determining biologic structure and function. As the field has grown, it has been broken down into several major branches. Structural genomics focuses on the physical aspects of the genome through the construction and comparison of gene maps and sequences as well as gene discovery, localization, and characterization. At the same time, functional genomics attempts to move data from structural genomics toward biologic function by understanding what genes do, how they are regulated, and their activity. Pharmacogenomics looks at

genetic makeup or genetic variations and their connection to drug response. Variations in drug targets, usually proteins, and target pathways are studied to understand how the variations are manifested and how they influence response. The term pharmacogenetics is sometimes used instead, but it can also refer specifically to genetic profiles or tests that predict drug response.

Molecular and Cellular Biopharmaceutics

*Introduction**

A biologic membrane is a lipid bilayer, typically embedded with proteins, that acts as a barrier within or surrounds the components of a cell. The membrane that separates a cell from the surrounding medium is called a plasma membrane. Such membranes also define most organelles (i.e., structures with specialized functions suspended in the cytoplasm) within cells. The typical structure of a cell membrane is shown in Figure 12-1. The membrane is characterized by a lipid bilayer that is typically about 5-nm thick. The lipid bilayer is composed of two opposing layers of lipid molecules arranged so that their hydrocarbon tails face one another to form the oily bilayer core, whereas their electrically charged or polar heads face the watery or “aqueous” solutions on either side of the membrane. Most of the proteins found in biologic membranes are integral membrane proteins (i.e., they are anchored to the cytoskeleton). Examples of the functions that integral membrane proteins serve include the identification of the cell for recognition by other cells, the anchoring of one cell to another or to surrounding media, the initiation of intracellular responses to external molecules, and the transport of molecules across the membrane. In 1899, Overton[12,13] concluded that the entry of any molecule into a cell is the result of its “selective solubility” in the cell's boundary, and that the more soluble in lipids the molecule is, the greater is its permeability, a discovery that has since been called the Overton rule.[11] Overton's studies led to the hypothesis that cell

membranes are composed of lipid domains, which mediate transport of lipophilic molecules, and protein pores, which transport hydrophilic molecules. Eventually, these data were unified in the hypothesis that cell membranes are mosaics composed of lipid domains, through which lipophilic molecules permeate, and (water-filled) pore regions, presumably made up of proteins that allow the transport of hydrophilic molecules.[11,12,13,14,15,16]

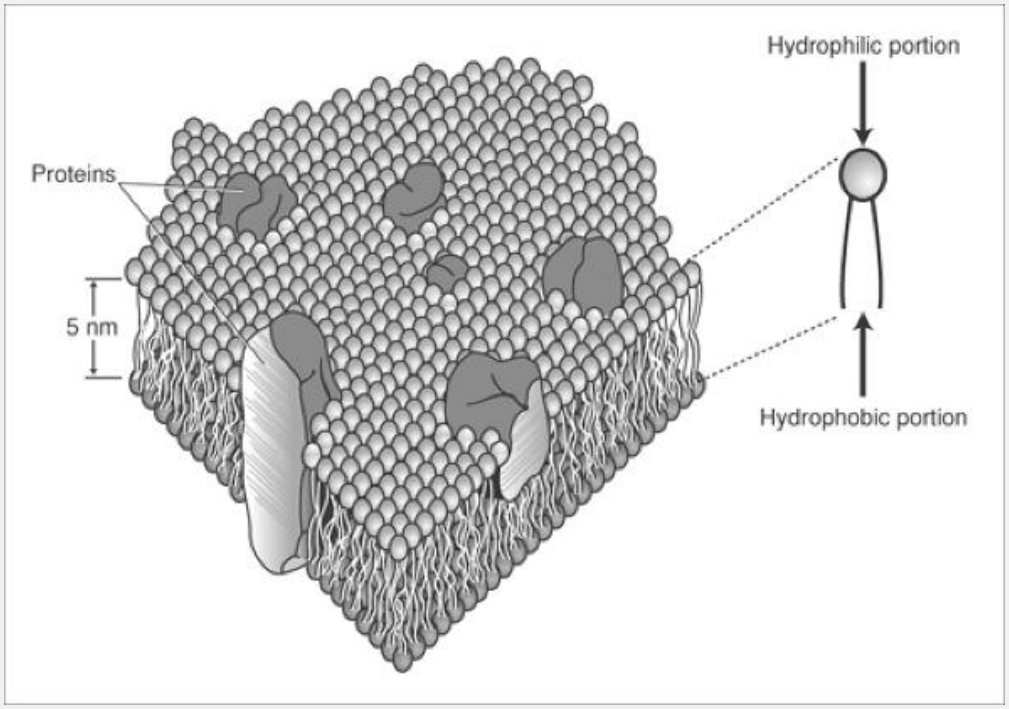


Fig. 12-1. Typical structure of a cell membrane. It is composed of a lipid bilayer that is 5-nm thick. Various types of proteins are part of the bilayer and serve a variety of physiological functions.

Overton also suggested that ions must use a different pathway across the membrane because the low dielectric constant of lipids prevented solvation of charged particles. However, large ions have significant lipid solubility because their charge is spread over a much larger area, providing an explanation for the rapid permeability of large charged particles, including many drugs. Because many drugs are organic acids or bases, the role of ionization in drug absorption has been the subject of much study over the years. Historically, in the pharmaceutical sciences, the role of ionization in membrane permeation has been described by the pH-partition hypothesis.17,18 As a general rule, the pH-partition hypothesis states that nonionized (i.e., lipid-soluble) drugs pass quickly through membranes, whereas ionized species are too polar to pass easily. Thus, it was expected that the rate of permeation of most drugs, which are organic acids and bases, is determined by the gradient for the nonionized form. The pH-partition hypothesis is covered in detail in Chapter 11.

Over the last several years, the mechanisms by which drugs and other xenobiotics are transported across biologic membranes have been reevaluated in light of the vast amount of information recently discovered during the mapping of the human genome and the identification of a vast number of proteins that may be involved in moving drugs across membranes. With the identification of aquaporins (in other words, water-conducting, protein-based channels), lipid transporters capable of transporting lipids such as the nonionized form of short-chain fatty acids, and drug transporters that can transport water and lipid-soluble drugs, the view of membrane transport is rapidly changing. In the words of Al-Awqati, in evaluating Overton's landmark work, “Needless to say, the [current analysis] … neither reduces the importance of Overton's insight into the lipid structure of the cell membrane nor nullify the likelihood that a few molecules may indeed travel through the lipid bilayer. However, what is certain today is that most molecules of physiological or pharmacological significance are transported into or out of cells by proteins rather than by a ‘passive’ solubility into the lipid layer and diffusion through it.”11 This adequately sums up the changes in our thinking about the membrane transport of drugs. The next section introduces drug-transporting proteins.

Drug Transporters, Cells, and Transport Pathways

Transporters are membrane proteins whose function is to facilitate the movement of molecules across cellular membranes. Although their primary function is to transport nutrients or other endogenous substances, many transporters also translocate drugs. For example, PepT1 is a transporter located at the brush border membrane of the human intestine responsible for the uptake of di- and tripeptides. However, it is able to transport many different drugs, such as valacyclovir, the L-valine ester prodrug of the acyclic nucleoside acyclovir,19 angiotensin-converting enzyme inhibitors,20 and cephalosporin antibiotics.21 The human genome sequence suggests that there are more than 700 known transport/carrier genes,22 and it has been estimated that at least 4% to 5% of the human proteome could be transporters23 (Fig. 12-2).

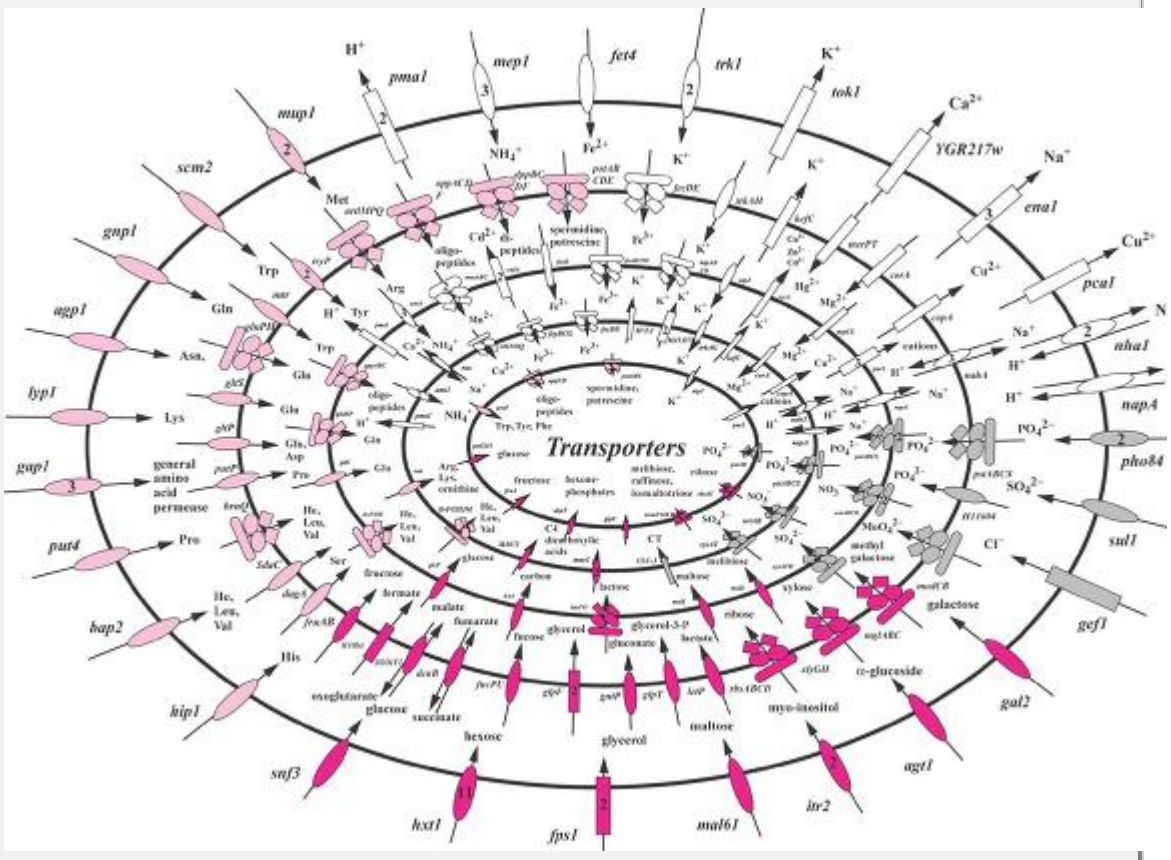


Fig. 12-2. Membrane transport functions identified by analysis of coding regions in five complete genomes. Circular representations from the center to the outer ring:*Mycoplasma genitalium, Methanococcus jannaschii, Synechocystis PCC6803, Haemophilus influenzae,* and *Saccharomyces cerevisiae*. Colors represent the four role categories: (1) amino acids, peptides and amines (light purple); (2) carbohydrates, organic alcohols and acids (dark purple); (3) cations (white); and (4) anions (gray). Ion-coupled permeases are designated by ovals, ABC transporters are shown as composites (circles, diamonds and ovals), and all other transporters are represented by rectangles. Arrows that point outward indicate efflux from the cell; those that point inward designate solute uptake from the environment. (From R. A. Clayton, O. White, K. A. Ketchum, and J. C. Venter, The first genome from the third domain of life, Nature **387**(6632), 459–462, 1997. With permission.)

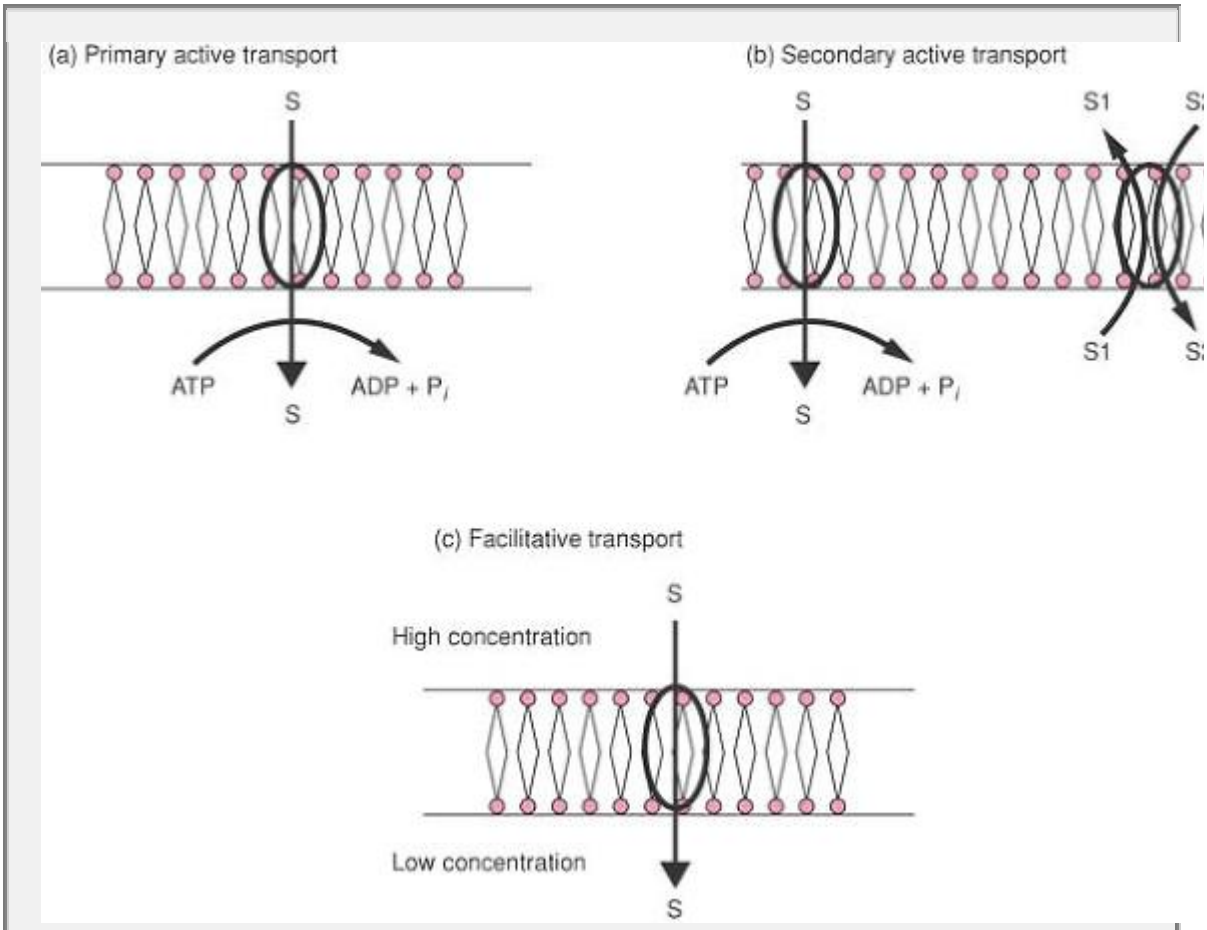


Fig. 12-3. Drug transport mechanisms across cell membranes fall into three general categories. In panel (A) primary active transport is depicted. In primary active transport, a drug or nutrient (S) is translocated across the membrane by means of a transport protein that spans the membrane. Energy in the form of ATP is required to drive the process. In panel (B) secondary active transport is depicted. In this case the drug or nutrient (S) crosses the membrane in the same manner as in (A). However, a second substrate (S1 or S2) is also moved into or out of the cell. In panel (C) facilitative transport is shown. Although transport is facilitated by proteins, the process is not energy dependent.

Drug transport mechanisms fall into three categories based on energetics and cotransport of other substances. These are primary and secondary active transport and facilitative transport. These mechanisms are depicted in Figure 12-3. Active transport involves the use of energy, usually ATP, to transport substrates across a biologic membrane. By using ATP, active transporters can move substrates to areas of high or low concentration. P-Glycoprotein (P-gp) is an example of a primary active transporter. Secondary active transport involves the cotransport of another substance such as an ion (e. g., H^+ or Na^+) along with the substrate. If the cotransported substance is transported in the same direction as the substrate, the process is called symport. If the cotransported substance is moved in the opposite direction, it is called antiport. An example of a symporter is the oligopeptide transporter PepT1. PepT1 transports a H^+ and a small peptide, typically a di- or tripeptide, into cells. Numerous drugs are also substrates for PepT1 including valacyclovir,19 angiotensin-converting enzyme inhibitors,20 and cephalosporin antibiotics.21 Glucose and Na^+ transport by means of the glucose transporter is another classic example of symport. An example of an antiporter is Na^+/K^+-ATPase, which transports $Na^+$and K^+ in opposite directions. Facilitative transport (also known as facilitated diffusion) is a non–energy-

dependent transporter-mediated mechanism. Because the transport mechanism is not energy dependent, these transporters cannot move substrates against a concentration gradient. In other words, substrates can only move from areas of high concentration to areas of low concentration. An example of a facilitative transport mechanism involves the equilibrative nucleoside transporters *es* and *ei*.

In this chapter, two major transporter superfamilies, the ATP-binding cassette (ABC) and the solute carrier family (SLC), will be introduced. The ABC transporter superfamily is the largest transporter gene family. ABC transporters directly use ATP hydrolysis as the driving force to pump substrates out of cells or prevent them from entering cells. The genes encoding ABC transporters are widely dispersed in the genome and show a high degree of amino acid sequence identity among eukaryotes.[24,25] Using phylogenetic analysis, we can divide the human ABC superfamily into seven subfamilies with more than 40 members. Several well-characterized drug transporting–related members are listed in Table 12-1.[26]Delineation of the topology of a transporter

P.265

P.266

is very important to gaining an understanding of its physiologic functions and substrate specificity. Knowing the transporter's structure may also enable the design of useful molecules to manipulate its transport activity and optimize the drug's pharmacokinetic behavior through enhanced absorption and targeted delivery. Membrane transporters may have various configurations across membranes. These configurations are shown in Figure 12-4 and are referred to as topologies. The topology of a membrane transporter relates to its physiologic function. Because of difficulties in crystallizing membrane proteins, the topology of ABC transporters has been proposed on the basis of computational simulations and confirmed by experimental data. In general, ABC transporters contain two ATP-binding domains, also known as nucleotide-binding domains, which are located intracellularly, and 12 membrane–spanning α-helices, which associate with each other to become specific membrane-spanning domains. Some transporters in the ABC superfamily, such as breast cancer resistance protein (BCRP, ABCG2), contain only one membrane-spanning domain and nucleotide-binding domain and are believed to associate with other proteins themselves to become functional. As a result of this, BCRP is also known as a half transporter.

Table 12-1 ATP-Binding Cassette Family Transporters

	Symbol	Alias	Rodent Ortholo gue	Tissue Distributi on	Subcellula r Localizati on	Functions
Subfa mily A	AB CA1	ABC -1	Abc a1	Many tissue s	–	A major regulator of cellular cholesterol and phospholip id homeostasi s. It effluxes phospholip

						ids (PS) and cholesterol from macrophages to apoA-I, reversing foam cell formation. Likely not involved in hepatic cholesterol secretion and intestinal apical cholesterol transport.
Subfamily B	ABCB1	P-gp, MDR1	Abcb1b	Many tissues (especially those with barrier functions such as L, BBB, P, K, I)	Apical	Efflux pump for xenobiotic compounds with broad substrate specificity, which is responsible for decreased drug accumulation in multidrugresistant cells and often mediates the development of resistance to anticancer drugs.

	ABCB4	MDR3	Abcb1a	L	Apical	Most likely involved in biliary phosphatidylcholine secretion from hepatocytes in a bile salt–dependent manner.
Subfamily C	ABCC1	MRP1	Abcc1a	Lu, T, I	Lateral	MRP1 transports glucuronides and sulfate-conjugated steroid hormones and bile salts. It also transports drugs and other hydrophobic compounds in presence of glutathione.
	ABCC2	MRP2, CMOAT	Abcc2	L, I, K	Apical	MRP2 excretes glucuronides and sulfate-conjugated steroid hormones and bile salts into

						bile. Other substrates include anticancer drugs such as vinblastine and anti-HIV drugs such as saquinavir. Contributes to drug resistance.
	ABCC3	MRP3	Abcc3	I, K	Lateral	MRP3 is inducible transporter in the biliary and intestinal excretion of organic anions.
	ABCC4	MRP4		Many tissues (especially L)	Basolateral	MRP4 transports prostaglandins out of hepatocytes back to blood circulation. It also transports cyclic nucleotides and some nucleoside monophosphate analogues including nucleoside-based

						antiviral drugs.
	ABCC5			L		Similar substrate specificity with MRP4
	ABCC6			K and L		MRP6 transports glutathione conjugates.
Subfamily G	ABCG2	BCRP, MXR, ABCP	Abcg2	P, B, L, I	Apical	BCRP functions as a xenobiotic transporter, which contributes to multidrug resistance. It serves as a cellular defense mechanism in response to mitoxantrone and anthracycline exposure. It also transports organic anions, steroids (cholesterol, estradiol, progesterone, testosteron

						e), and certain chlorophyll metabolites .

Key: L = liver; Lu = lung; T = testis; I = intestine; P = placenta; B = brain; K = kidney; BBB = blood–brain barrier.

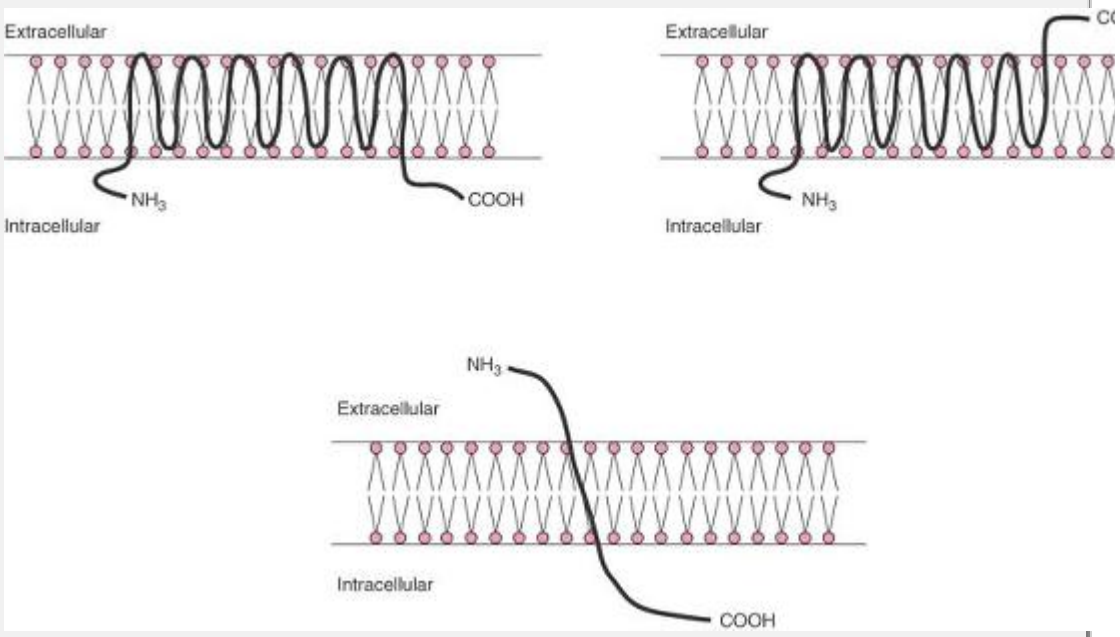


Fig. 12-4. Topologies of membrane-integrated proteins. (Drawn by G. You. With permission.)

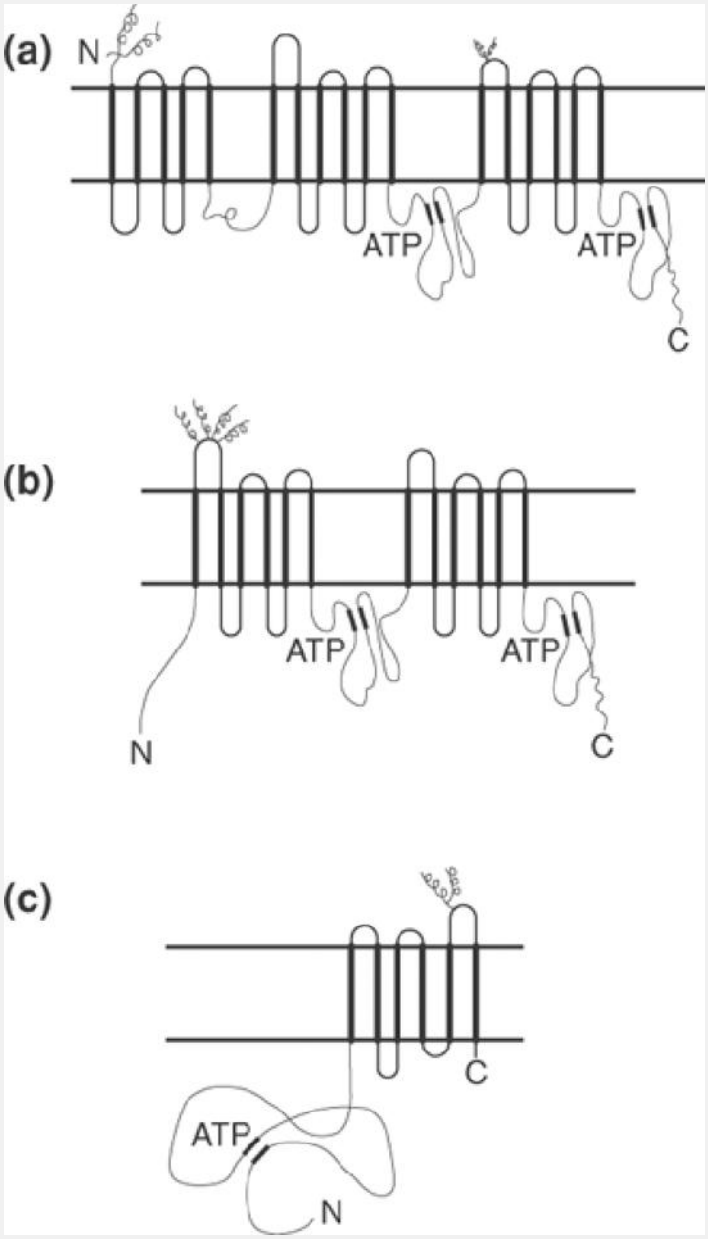


Fig. 12-5. Predicted structure of (*a*) multidrug resistance protein-1, (*b*) P-glycoprotein, and (*c*) breast cancer resistance protein. Shown is the linear secondary structure with putative transmembrane helices and the two ATP-binding domains. The potential glycosylation sites are denoted as C. (From T. Litman, T. E. Druley, W. D. Stein, and S. E. Bates, Cell. Mol. Life Sci. **58** (7), 931, 2001.

The commonly agreed topologies of three well-studied ABC transporters, P-gp, BCRP, and multidrug-resistance protein-1 (MRP1), are shown in Figure 12-5. It is important to realize that these structures are highly educated guesses and will remain somewhat controversial until the exact crystal structures can be determined.

Table 12-2 Solute Carrier Family Transporters

	Symbol	Alias	Rodent Orthologue	Tissue Distribution	Subcellular Localization	Functions
SLC15	SLC15A1	hPepT1	Slc15a1	I, K	–	Proton-coupled uptake of oligopeptides of 2–4 amino acids, beta-lactam antibiotics
SLC21	SLC21A3	OATP, OATP-A	Slc21a7	Many tissues (B, I, L, P, K)	Lateral	Mediates cellular uptake of organic ions in the liver. Its substrates include bile acids, bromosulfophthalein, some steroidal compounds, and fexofenadin.
	SLC21A6	OATP2, OATP-C, LST-1		L	Basolateral	Mediates the Na^+-independent transport of organic anions such as pravastatin, taurocholate, methotrexate, dehydroepiandrosterone sulfate, 17-beta-glucuronosyl estradiol, estrone sulfate,

						prostaglandin e2, thromboxane b2, leukotriene c3, leukotriene e4, thyroxine, and triiodothyronine. It may play an important role in the hepatic clearance of bile acids and organic anions.
SLC22	SLC22A1	OCT1	Slc22a1	L, K, I	Basolateral	Play a critical role in the elimination of many endogenous small organic cations as well as a wide array of drugs and environmental toxins.
	SLC22A6	OAT1, PAHT	Slc22a6	K, B, P	Basolateral	Involved in the sodium-dependent transport and excretion of endogenous organic anions, such as*p*-aminohippurate, cyclic nucleotides, dicarboxylates

						, and neurotransmitter metabolites, and xenobiotics such as β-lactam antibiotics, nonsteroidal anti-inflammatory drugs, and anti-HIV and antitumor drugs.

Key: L = liver; T = testis; I = intestine; P = placenta; B = brain; K = kidney.

ABC transporters interact with a wide variety of substrates, including sugars, amino acids, metal ions, peptides, proteins, and a large number of hydrophobic compounds and metabolites. ABC transporters play very important roles in many cellular processes, and several human genetic disorders such as cystic fibrosis, neurologic disease, retinal degeneration, cholesterol and bile transport defects, and anemia are the result of transporter dysfunction. ABC transporters are among the key players in multidrug resistance, a frequently observed phenomenon in cancer therapy, and they also significantly influence the pharmacokinetic behavior of many drugs. Because of this, drug transporters are becoming increasingly implicated in determining therapeutic outcomes.

Another transporter family that is involved in drug absorption and disposition is known as the SLC. More than 20 SLC subfamilies have been identified; they are responsible for transporting a variety of endogenous and exogenous substances, such as amino acids, glucose, oligopeptides, antibiotics, and nonsteroidal anti-inflammatory, antitumor, and anti-HIV drugs. In this chapter, only those well-characterized and drug transport–relevant SLC members are described. Their tissue distribution, subcellular localization, functions, and substrates are listed in Table 12-2. Computer modeling based on hydropathy analysis has enabled us to predict the linear secondary structures of organic anion transporters (OATs), organic cation transporters (OCTs), and organic anion-transporting polypeptides (OATPs) (Fig. 12-6). Unlike ABC transporters, these membrane-integrated transporters do not carry potential ATP-binding sites or an ATPase domain that can hydrolyze ATP. Most SLC members transport substrates with another ion in the same or opposite direction as the substrate. For example, the transport of oligopeptides and peptidomimetics by means of PepT1, the small peptide transporter (a symporter), has been shown to be proton and

membrane potential dependent on the apical surface of an epithelial cell (Fig. 12-7). Briefly, the Na^+/H^+ exchanger, an antiporter, located at the apical cell surface generates a lower pH in the microclimate of intestinal villi. Substrates are then taken up into epithelial cells by PepT1 coupled with the influx of proton. The influx of protons acts as the driving force for PepT1-mediated uptake, and this transport system is known as proton dependent.27 It was demonstrated that the lower pH in the lumen (pH 5–6.5) and the inside negative membrane potential established by Na^+/K^+-ATPase were critical

factors in the unidirectional uptake of substrates into cells by PepT1. Therefore, PepT1 functions as a net absorptive influx transporter rather than as secretory transporter in intestine. The effects of the proton on the orientation of PepT1 and symport events are depicted in Figure 12-8. In a similar manner, Na^+ and glucose cotransport effect the orientation and function of the glucose transporter (SLC2A1). These events are depicted in Figure 12-9.

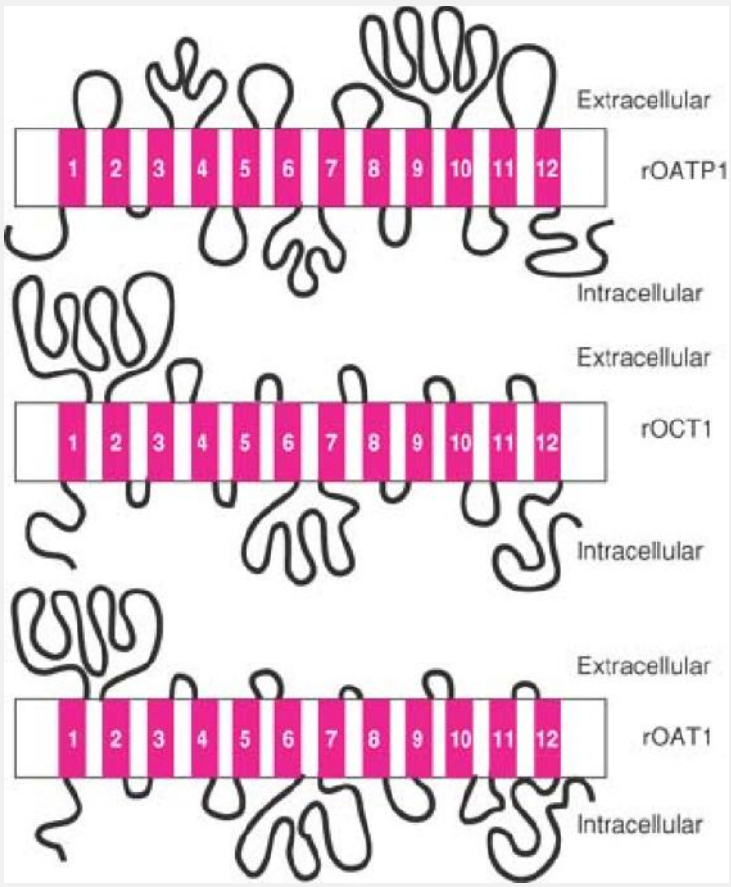


Fig. 12-6. Proposed topology of rat organic anion-transporting peptide-1 (rOATP1), organic cation transporter-1 (rOCT1), and organic ion transporter-1 (rOAT1). All transporters have 12 transmembrane helices. rOATP1 has a large extracellular loop between transmembrane helices 9 and 10, whereas rOCT1 and rOAT1 carry the large extracellular loop between helices 1 and 2.

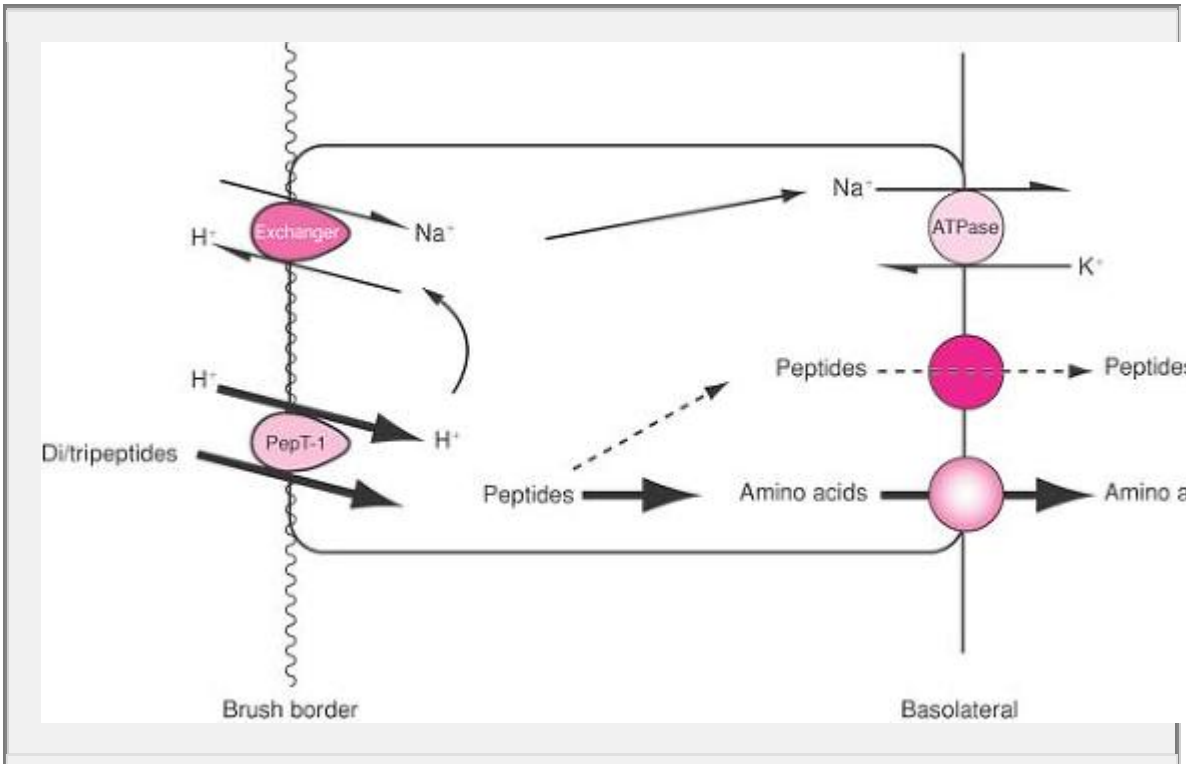


Fig. 12-7. A proposed model of PepT1-mediated transport. Di- or tripeptides enter the cells together with protons via PepT1 at the brush border membrane, and the proton and Na^+ gradients are maintained by the H^+–Na^+ exchanger and Na^+/K^+-ATPase, respectively.

The elimination of organic anions from blood into urine by OAT also involves the translocation of multiple ions across the basolateral membrane in the proximal tubule epithelium in the kidney. As shown in Figure 12-10, Na^+/K^+-ATPase establishes an inwardly directed Na^+ gradient. This Na^+ gradient then moves dicarboxylates into the cells via a Na-dicarboxylate cotransporter (SDCT2), which produces an outwardly directed dicarboxylate gradient. Finally, OAT transfers organic anions such as *para*-aminohippurate (PAH) into cells using the coupled efflux of dicarboxylate as the driving force.

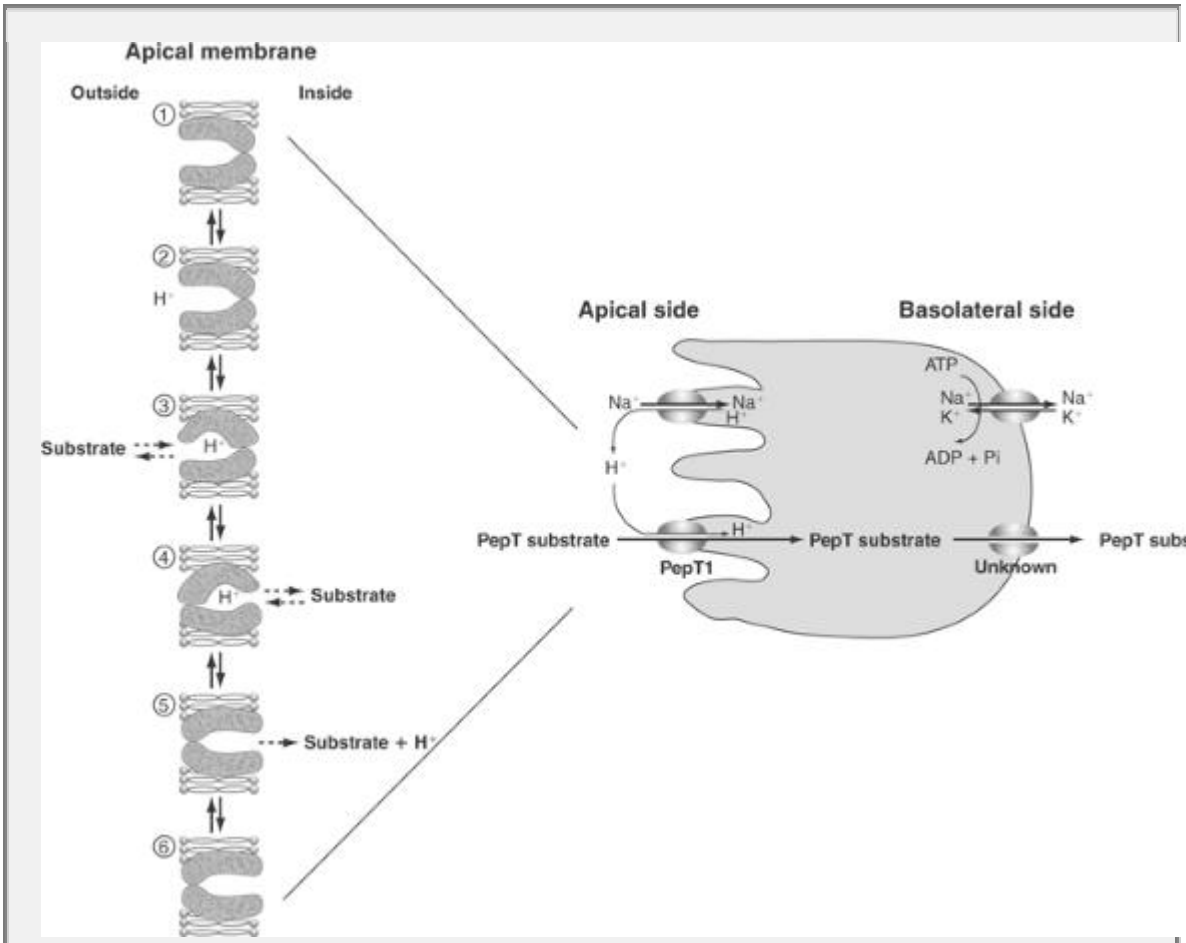


Fig. 12-8. Proposed mechanism of substrate transport by PepT1, the intestinal peptide transporter. (From D. R. Herrera-Ruiz and G. T. Knipp, J. Pharm. Sci. **92,** 691, 2003. With permission.)

In addition to transporting a wide variety of endogenous substance such as PAH, urate, cAMP, cGMP, tetraethyl ammonium, aliphatic quaternary ammonium compounds, and bile acids, SLC transporters also transport many clinically useful drugs including antibiotics and antiallergy, anti-HIV, and antitumor medications. A large body of data has shown that SLC transporters play a very important role in drug absorption and disposition and may be at the heart of numerous and significant drug–drug interactions. Most drug transporters are located in tissues with barrier functions such as intestine, kidney, liver, and the brain barriers. The cells at the border of these barriers are usually polarized. In other words, the plasma membrane of these cells is organized into at least two discrete regions with different compositions and functions. Figure 12-11 shows an example of a polarized cell and the transport pathways through and between cells. Enterocytes (i.e., intestinal absorptive cells) at the brush border membrane of intestine and epithelial cells at the renal proximal tubule have an apical domain (AP) facing the lumen and basolateral domain (BL) facing the blood circulation; hepatocytes are polarized into a canalicular (AP) membrane facing the bile duct and a sinusoidal (BL) membrane facing the blood circulation; syncytiotrophoblasts at the maternal–fetal interface of placenta have apical domain facing the maternal blood and a basolateral domain facing the fetus. The brain capillary endothelial cells that function as the blood–brain barrier (BBB) are also polarized into luminal and antiluminal membranes. In most cases, the expression of a drug transporter is usually restricted to one side, the apical or basolateral domain, of polarized cells (e.g., PepT1 is located only on the apical membrane).

Drug transporters can be categorized into efflux or influx transporters according to the direction that they transport substrates across cell membranes. Under this definition, transporters that pump substrates out of the cells are

P.270

called efflux transporters and those that transfer substrates into the cells are called influx transporters. This definition is widely used when drug transport studies are performed at the cellular level. For example, P-gp and multidrug-resistant proteins (MRPs) belong to the efflux transporter group because they pump substrates out of the cytosol and into the extracellular environment. On the other hand, PepT1, OCTs, OATs, and OATPs are categorized as influx transporters due to their ability to bring substrates into cells. Another way of classifying drug transporters is from a pharmacokinetic point of view. Based on this terminology system, transporters that transfer their substrates in the direction of the systemic circulation from outside the

P.271

body or into organs like the brain or the liver are called absorptive transporters, whereas transporters that transport drugs out of an organ or from the blood circulation into bile, urine, and gut lumen are called secretory transporters. For example, MRP1 (ABCC1) is an efflux transporter that can pump drugs such as saquinavir, an HIV protease inhibitor, out of cells. However, considering that MRP1 expression in enterocytes in the intestine is restricted to the basolateral membrane, efflux of saquinavir by MRP1 in the intestine leads to the movement of drug into the blood circulation. Therefore, MRP1 is considered an absorptive efflux transporter. Similarly, influx transporters could function as either absorptive or secretory transporters depending on the tissue and membrane domain where they are expressed. For example, intestinally expressed organic anion-transporting polypeptide-A (OATP-A) is localized on the apical domain of enterocytes. Orally administered fexofenadine, a histamine H_1-receptor antagonist, is transported into intestinal cells by OATP-A and then into the blood stream; therefore, OATP-A is considered an absorptive influx transporter.28 An influx transporter could also act as a secretory pump. For example, studies at the cellular level have demonstrated that organic anion transporter-1 (OAT1) is an influx transporter with substrates such as PAH, prostaglandin E2 (PGE2), decarboxylates, and various anionic drugs. In the kidney, OAT1 is found on the basolateral membrane of tubular epithelial cells responsible for eliminating certain endogenous and exogenous substances and their metabolites from the blood into the urine. Therefore, kidney OAT1 is thought to be a secretory influx transporter. In other words, OAT1 takes up substrates into kidney cells, but the process is oriented toward moving them out of the body.29

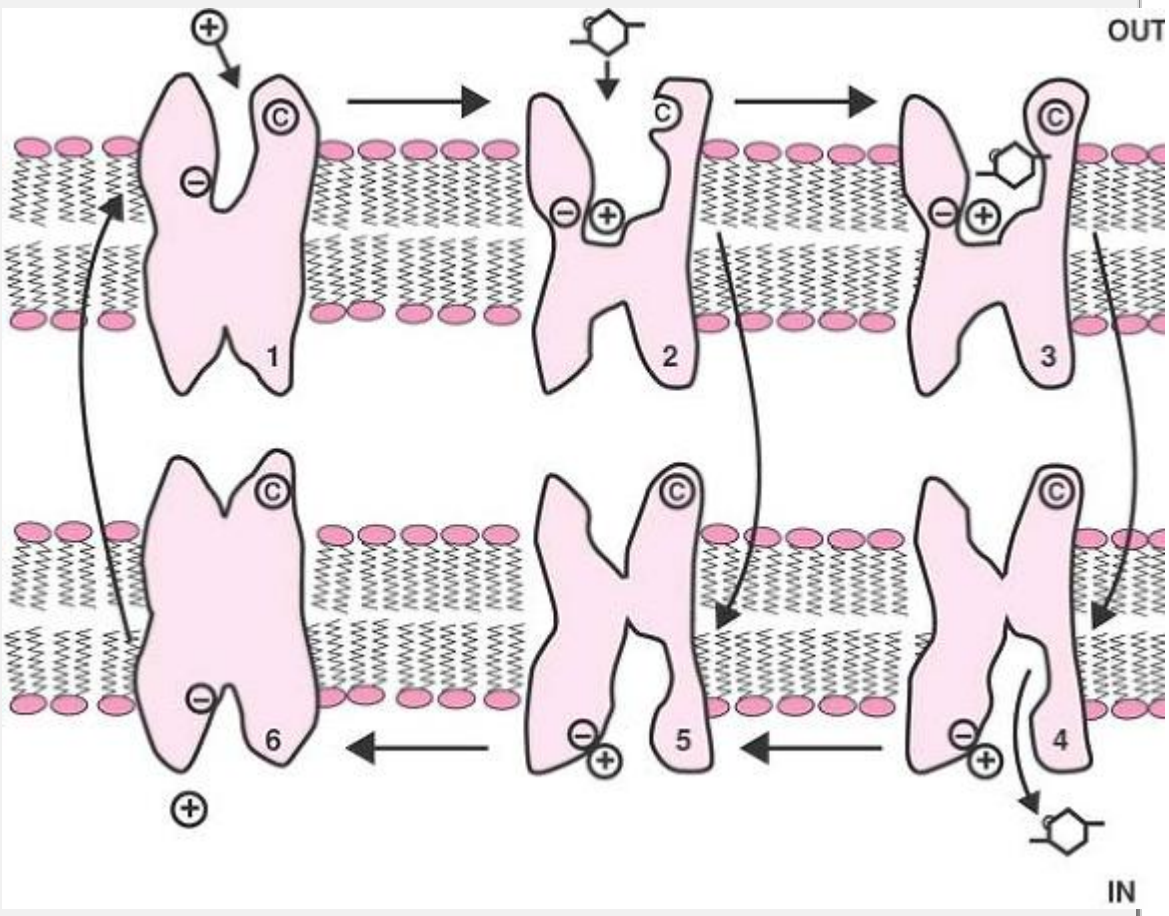


Fig. 12-9. Mechanism of glucose transport across a membrane by the glucose transporter.

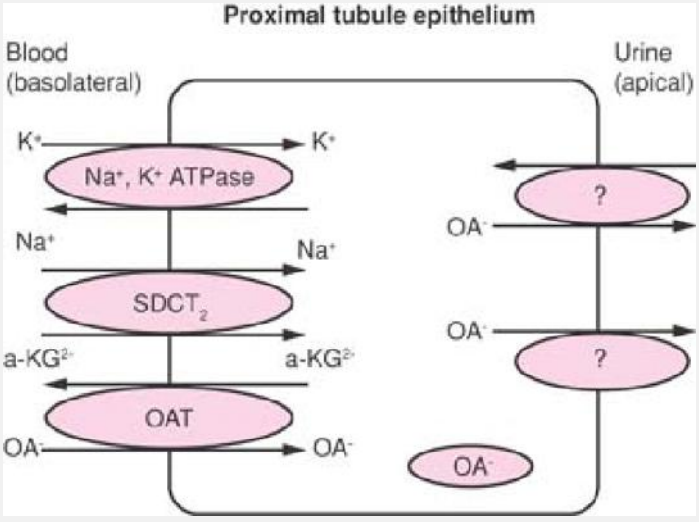


Fig. 12-10. Model of organic anion transporter (OAT)-mediated organic anion transport. Key: SDCT2 = Na^+-coupled dicarboxylate cotransporter-2; OA^- = organic

anion.

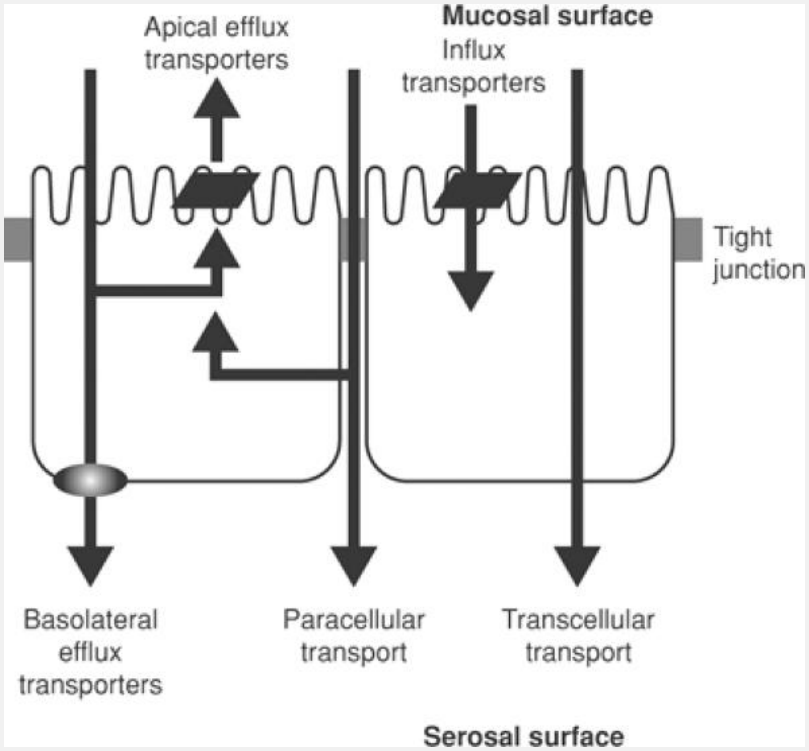


Fig. 12-11. Pathways across a cell monolayer. Drugs can cross between cells (i.e., paracellularly) or through cells (i.e., transcellularly). Drug transport out of cells is termed efflux and into cells is called influx.

Example 12-2
Concerted Transport Across Cells12

Concerted drug or xenobiotic transport occurs when the transport of a compound is facilitated across both membrane domains of a polarized cell by membrane transporters. Concerted transport implies that the transporters on the two domains move drugs in the same direction (Fig. 12-12). In this example, Madin–Darby canine kidney (MDCK) cells were cultured in a specialized device called a Transwell. A Transwell is a porous support made of a polymer and is used to grow cells as a continuous monolayer of polarized cell membranes (apical and basolateral) and functional cell–cell tight junctions. In this example, the human MRP2 or OATP8 genes were heterologously expressed in MDCK cells, and their expression was restricted to the apical and basolateral membranes, respectively (Fig. 12-13). When the basolateral-to-apical (B → A) transport of estradiol-17-β-glucuronide, a metabolite of a sex hormone, was measured, it was found that the rank order of B → A permeability of estradiol-17-β-glucuronide is MDCK–MRP2/OATP8 (MDCK expressing both MRP2 and OATP8) ≫ MDCK–MRP2 (MDCK expressing MRP2) > MDCK (Fig. 12-14). This observation indicated that estradiol-17-β-glucuronide was taken up by OATP8 at the basolateral membrane and extruded by MRP2 at the apical membrane in a concerted manner. In other words, OATP8 and MRP2 transport their common substrates in the same direction to achieve more efficient substrate movement across the cell monolayer. When MRP2 was not present, translocation (i.e., net transport) was reduced, suggesting that without the MRP2 transporter, estradiol-17-

β-glucuronide would accumulate inside the cells. Because many drugs are substrates for more than one transporter, it is likely that there are numerous concerted transport pathways for drugs across each membrane domain.

Drug Metabolism

Introduction5

The first human metabolism study was performed in 1841 by Alexander Ure, who observed the conversion of benzoic acid to hippuric acid and proposed the use of benzoic acid for the treatment of gout.30 Much has been learned about drug metabolism since that time, and the purpose of this section is to provide the biopharmaceutical background needed for the student to better understand drug metabolism. Because lipophilic drugs are efficiently deposited in tissues and cells, are readily reabsorbed across renal tubular cells, and tend to be highly bound to plasma proteins such as albumin, their clearance is very low. This can be partially explained by the fact that lipophilic drugs rapidly diffuse into hepatocytes or other cells containing various metabolic enzymes and have easy access to cytochrome P-450 anchored to endoplasmic reticulum (Fig. 12-15). To facilitate drug elimination and maintain homeostasis after the exposure to xenobiotics including drugs and environmental toxins, numerous biochemical transformations occur. These transformations are facilitated by two major groups of enzymes, and the process is called “drug metabolism.” Metabolic reactions generally have the effect of converting drugs into more polar metabolites than the parent drug. The conversion to a more polar form has important biologic consequences because it enhances the ability of the body to eliminate drugs.

Drug metabolism involves a wide range of chemical reactions, including oxidation, reduction, hydrolysis, hydration, conjugation, condensation, and isomerization. The enzymes involved are present in many tissues but generally are more concentrated in the liver. For many drugs, metabolism occurs in two apparent phases. Phase I reactions involve the formation of a new or modified functional group or a cleavage (oxidation, reduction, hydrolysis); these are nonsynthetic reactions. Phase II reactions involve conjugation with an endogenous compound (e.g., glucuronic acid, sulfate, glycine) and are therefore synthetic reactions. Metabolites formed in synthetic reactions are more polar and more readily excreted by the kidneys (in urine) and the liver (in bile) than those formed in nonsynthetic reactions. Some drugs undergo either phase I or phase II reactions; thus, phase numbers reflect functional rather than sequential classification. Drugs are metabolized to various degrees by oxidation, reduction, hydrolysis, and conjugation in the body. Some drugs are eliminated without any structural changes occurring at all. The process of elimination of a compound from the body without further chemical modification is known as excretion. Williams32classified all known metabolic

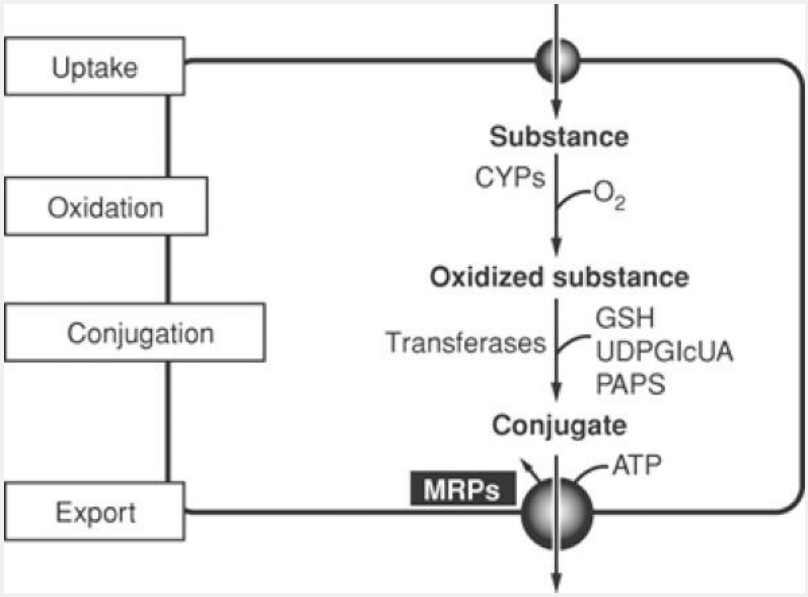
reactions as either phase I or phase II reactions. In recent years, a third phase of drug metabolism has been classified and is commonly referred to as phase III metabolism. The three “phases” of drug metabolism are shown in Figure 12-16. Phase I reactions include oxidation, reduction, or hydrolysis of the drug. In a phase II reaction, the drug or its polar metabolite is coupled to an endogenous substrate such as uridine diphosphate (UDP) glucuronic acid, sulfate, acetate, or amino acid. The third phase of drug metabolism involves transporting the drug, metabolite, or conjugated metabolite across a biologic membrane and out of the body. For example, one such mechanism, originally called phase III detoxification,33 utilizes the GS-X pump to transport xenobiotic metabolites out of the body. Because phase III reactions (i.e., membrane transporters) were covered in the previous section, only phase I and II reactions will be discussed here.

Key Concept

Concerted Transport12,31

Given the presence of various transporters on the two domains of polarized cells and the possible differences in transport direction (i.e., influx vs. efflux), it is natural to consider how these transporters may work with each other (i.e., in “concert”) or against each other.

Recently, the phenomenon of “concerted transport,” when membrane transporters on the AP and BL domains of a cell transport a drug substrate in the same direction (i.e., absorptive or secretory direction), has been studied. The liver is an important organ involved in the metabolism and clearance of endo- and xenobiotics. As seen in the accompanying figure, drugs are taken up by hepatocytes in the liver directly by passive diffusion or by means of influx transporters at the sinusoidal membrane (BL). They are then converted intracellularly to pharmacologically inactive, active, or sometimes toxic metabolites by the cytochromes P450 (CYPs). The metabolites are then conjugated with various endogenous compounds such as glucuronide and sulfate; consequently, they are excreted into the bile passively by diffusion or by means of transporters such as the MRP family at the canalicular membrane (AP). It has been found that influx transporters at the sinusoidal membrane (e.g., OATP-C and OATP8) and efflux transporters at the canalicular membrane (e.g., MRP2 and P-gp) work in concert to transport drugs and other substances into the bile. Therefore, the alliance between influx transporters on the basolateral/sinusoidal membrane and efflux transporters at apical/canalicular membranes of hepatocytes can efficiently eliminate endogenous wastes or toxic xenobiotics into bile.



Uptake, biotransformation, and multidrug-resistance protein (MRP)–mediated export of endogenous substances, drugs, and carcinogens. Key: CYPs, cytochrome P-450s; GSH = …; UDPGlc UA = …; PAPS = …; ATP = adenosine triphosphate. (From J. König et al., Biochim. Biophys. Acta **1461,** 377, 1999. With permission.)

Phase I Reactions

A major class of oxidative transformations was initially characterized by O. Hayaishi in Japan34 and H. S. Mason in the United States.35This class of oxygenases had requirements for both an oxidant (molecular oxygen) and a reductant (reduced nicotinamide-adenine dinucleotide phosphate [NADP]) and hence was given the name “mixed-function oxidases.” An understanding of the biochemical nature of these reactions grew out of early studies on liver pigments by Garfinkel36 and Klingenberg,37 who observed in liver microsomes an unusual carbon monoxide–binding pigment with an absorbance maximum at 450 nm. Omura and Sato38 ultimately characterized this pigment as a cytochrome. The function of this unique cytochrome, called P-450 (CYP450), was initially revealed in 1963 in studies by

Estabrook et al.,39 using microsomes from the adrenal cortex for the catalysis of the hydroxylation of 17-hydroxyprogesterone to deoxycorticosterone.

The most actively studied drug metabolism reaction is the CYP450-mediated reaction because the CYP450 family represents key enzymes in phase I reactions with several unique properties. This vast family is composed of more than 57 isoforms in humans alone (Table 12-3), mediates multiple oxidative reactions, and has very broad substrate

specificity (Table 12-4). Phase I reactions introduce a functional group (—OH, —NH_2, —SH, or —COOH) to drugs and usually results in a small increase in hydrophilicity. Various molecules with very diverse chemical structures and different molecular weights, ranging from ethylene (28 g/mole) to cyclosporine (1201 g/mole) are known to be substrates and/or inhibitors of CYP450. Catalysis by CYP450 is very slow compared to that of other enzymes such as catalase, superoxide dismutase, and peroxidase.

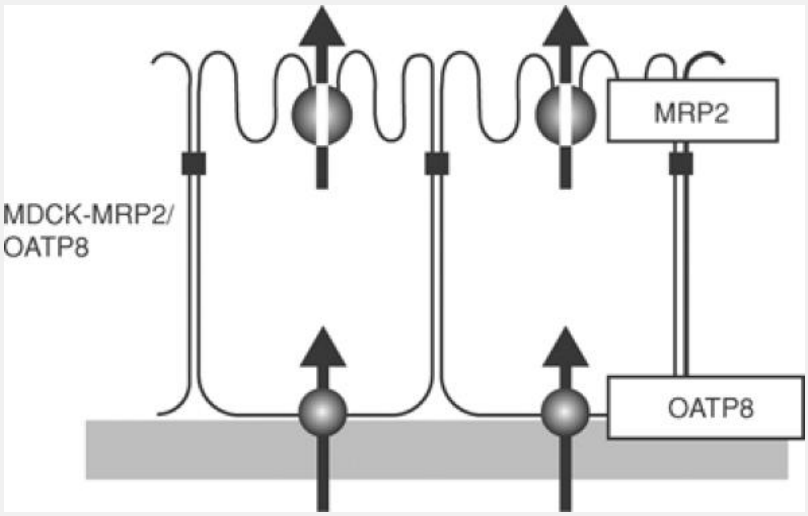


Fig. 12-12. Vectorial transport by human uptake transporter SLC21A8 (OATP8) and the apical export pump ABCC2 (MRP2). Key: MDCK = Madin–Darby canine kidney cell; MRP2 = multidrug-resistance protein-2; OATP8, organic anion-transporting peptide-8. (From Y. Cui, J. Konig, and D. Keppler, Mol. Pharmacol. **60** (5), 934, 2001. With permission.)

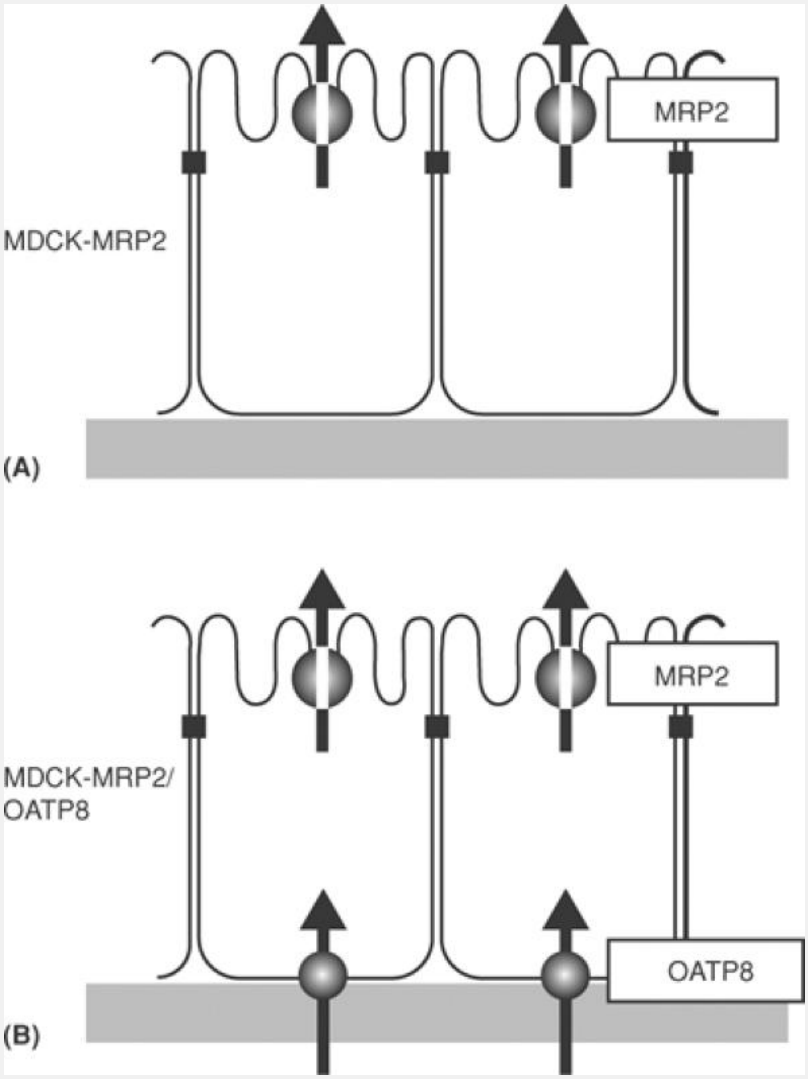


Fig. 12-13. Vectorial transport by (A) human uptake transporter SLC21A8 (OATP8) or (B) the apical export pump ABCC2 (MRP2). Key: MDCK = Madin–Darby canine kidney cell, MRP2 = multidrug-resistance protein-2; OATP8, organic anion-transporting peptide-8. (From Y. Cui, J. Konig, and D. Keppler, Mol. Pharmacol. **60** (5), 934, 2001. With permission.)

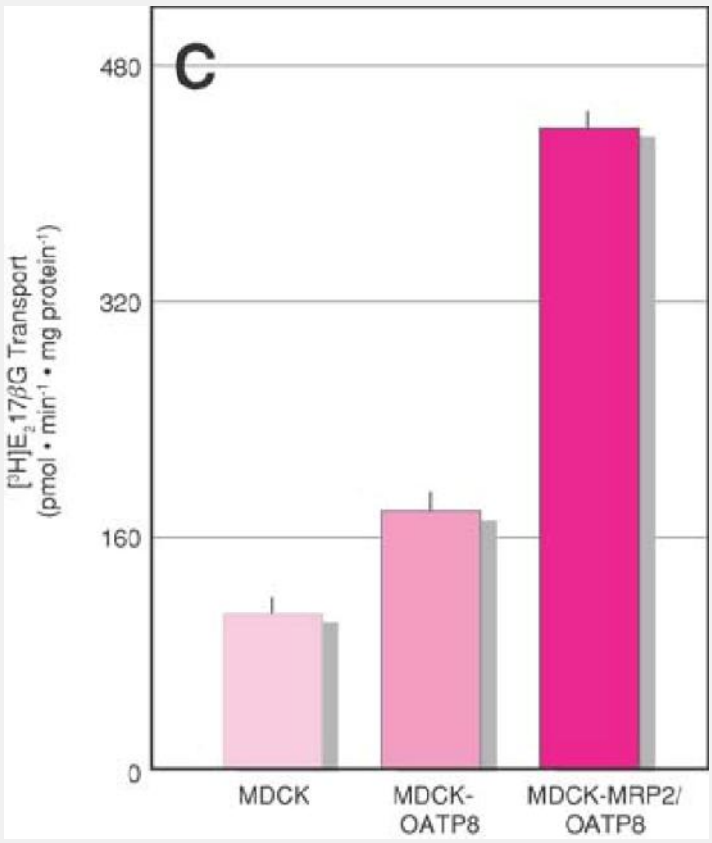


Fig. 12-14. Transcellular transport (from basolateral to apical) of estradiol 17-β-gluronide (E_2 17βG). (From Y. Cui, J. Konig, and D. Keppler, Mol. Pharmacol. **60** (5), 934, 2001. With permission.)

CYP450 actually consists of two enzymes, catalyzing two separate but coupled reactions:

Reductive half:	$O_2 + NADPH + H^+$	$\rightarrow$	$[O] + H_2O + NADP^+$
Oxidative half:	$RH + [O]$	$\rightarrow$	ROH
Coupled:	$RH + O_2 + NADPH + H^+$	$\rightarrow$	$ROH + H_2O + NADP^+$

More than 60 reactions are catalyzed by CYP450 (Table 12-5), and even a single CYP450 isoform can generate the several metabolites from a single substrate. The function of CYP depends largely on the presence of molecular oxygen and/or drugs as substrates. For example, CYP450 can act as both an

oxidative and a reducing enzyme (Fig. 12-17). These complex enzyme systems or mixed-function oxidases require NADPH, molecular oxygen, CYP450, NADPH–CYP450
P.274

reductase, and phospholipids. An overall scheme showing the catalytic cycle of CYP450 is shown in Figure 12-18.

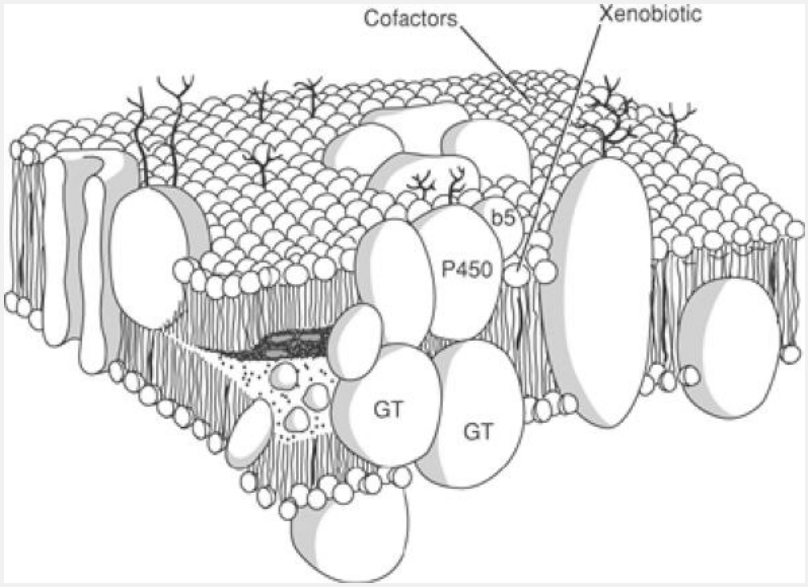


Fig. 12-15. Phase I and phase II enzymes integrated within or spanning across the lipid bilayer of the smooth endoplasmic reticulum (SER). Cytochrome P-450 enzyme complex, including cytochrome b5, and reduced nicotinamide-adenine dinucleotide phosphate (NADPH) as a cofactor are present in SER. Also present are glucuronyltransferase enzymes (GT). (From A. S. Kane, University of Maryland, College Park, Md., http://aquaticpath.umd.edu/appliedtox/metabolism.pdf, 2003.)

Phase II Reactions

The first reference to phase II drug metabolism was made more than 150 years ago, when Stadeler40 referred to the presence of conjugated phenol in urine, which was later isolated and characterized as phenyl sulfate by Baumann. A glucuronide conjugate was first discovered by Jeffe,41 who found that *o*-nitrotoluene gave rise to *o*-nitrobenzyl alcohol excreted as a conjugate in the dog: a few other sugar conjugates were also reported in the 1870s by von Mering's group42 and Schmiedeberg's group.43 Although the mercapturic acids were found in 1879 by Baumann and Preusse,44 the full mechanism of glutathione conjugation was not known until Barnes et al.45 characterized the relationship between glutathione and mercapturic acid in 1959. The enzymes responsible for phase II reactions are UDP-glucuronosyltransferases (UGTs) for glucuronidation, sulfotransferases (SULTs) for sulfation, and glutathione S-transferases (GSTs) for glutathione conjugation. Glucuronidation, the most common phase II reaction, is the only one that occurs in the liver microsomal enzyme system.5

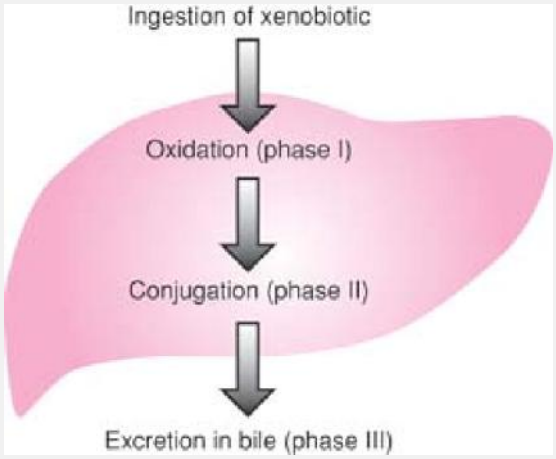


Fig. 12-16. Phase III elimination reactions in bile begin with oxidation in the liver (phase I), followed by conjugation (phase II). (From M. Vore, Environ. Health Perspect. **102,** 5, 1994. With permission.)

There are numerous enzyme families, and a variety of isoforms within families and different types of isozymes are found in the various animal species and humans. Three well-documented and important phase II enzyme families are shown in Table 12-6. Phase II reactions involve the conjugation of certain functional groups using conjugating cofactors as shown in Table 12-7. Polar groups introduced by phase I reactions are used as attachment sites for conjugation (phase II) reactions. For instance, a hydroxyl group added during a phase I reaction is a good target for glucuronide or sulfate conjugation. Conjugation reactions greatly increase the hydrophilicity and promote the excretion of drugs. Hydrophilic conjugates of drugs are typically less active than the parent compounds, with some notable exceptions, such as morphine-6-glucuronide, *N*-(4-hydroxylphenyl) retinamide glucuronide, and minoxidil sulfate, where the metabolites are more potent than their respective parent drugs. Glucuronides are secreted in bile and eliminated in urine. Chloramphenicol, meprobamate, and morphine are metabolized this way. Amino acid conjugation with glutamine or glycine produces conjugates (e.g., salicyluric acid formed from salicylic acid and glycine) that are readily excreted in urine but are not extensively secreted in bile. Acetylation is the primary metabolic pathway for sulfonamides. Hydralazine, isoniazid, and procainamide are also acetylated. Sulfoconjugation is the reaction between phenolic or alcoholic groups and inorganic sulfate, which is partially derived from sulfur-containing amino acids (e.g., cysteine). The sulfate esters formed are polar and readily

excreted in urine. Drugs that form sulfate conjugates include acetaminophen, estradiol, methyldopa, minoxidil, and thyroxine. Methylation is a major metabolic pathway for inactivation of some catecholamines. Niacinamide and thiouracil are also methylated.5

Table 12-3 Isoforms of Cytochrome P-450 Enzyme in Animals and Humans

Isoform	Mouse	Rat	Rabbit	Dog	Human
CYP1A	1, 2	1, 2	1, 2	1, 2	1, 2
CYP2A	4, 5, 12	1–3	10, 11	?	6, 7, 14
CYP2B	9, 10, 14, 19, 20	1–3, 8, 12, 15, 22–24	4, 5	11	6, 7
CYP2C	9, 10, 29, 37–40	6, 7, 11–14, 23, 24	1–5, 11–16, 30	21, 41, 42	8, 9, 18, 19
CYP2D	9–12	1–5, 18	23, 24	15	6
CYP2E	1	1	1, 2	1	1
CYP3A	11, 14, 16, 25	1, 2, 9, 18, 23	6	12, 26	3, 4, 5, 7, 43

Enzyme Induction and Inhibition

Most marketed drugs are metabolized by more than one CYP450 isoform, which means it is highly likely that drug–drug interactions are possible. Pharmacokinetic interactions related to drug metabolism can be categorized as either enzyme induction or inhibition.

Induction

Most drug-metabolizing enzymes are expressed constitutively, that is, they are synthesized in the absence of any discernible external stimulus. It has been shown that an increase in the activity of hepatic microsomal enzymes such as CYP450 can occur after exposure to structurally diverse drugs and xenobiotics. The stimulation of enzyme activity in response to an environmental signal is referred to as enzyme induction. Enzyme induction involves multiple mechanisms and usually occurs at the gene transcriptional level. Inducing agents may increase the rate of their own metabolism as well as those of other unrelated drugs by inducing various phase I and phase II enzymes (Table 12-8).

Table 12-4 Marker Substrates, Reactions, and Typical Inhibitors for Cytochrome P-450 Isozymes

CYP	Substrate (Reaction)	Inhibitor (Mechanism)
1A2	7-Methoxyresorufin (*O*-demethylation)	Furafylline (mechanism based)
	Caffeine (*N*3-demethylation)	7,8-Benzoflavone (competitive)
	Phenacetin (*O*-deethylation)	Fluvoxamine (competitive)
2A6	Coumarin (7-hydroxylation)	Methoxalen (mechanism based)
		Tryptamine (competitive)
		Trancylcypromine (competitive)
2B6	7-Benzoxyresorufin (*O*-debenzylation)	Orphenadrine (competitive)
	(*S*)-Mephenytoin (*N*-demethylation)	
2C8	Paclitaxel (6α-hydroxylation)	Quercetin
2C9	Tolbutamide (methyl hydroxylation)	Sulfaphenazole (competitive)
	Phenytoin (4α-hydroxylation)	Tienilic acid (mechanism based)
	Diclofenac (4α-hydroxylation)	
	(*S*)-Warfarin (7-hydroxylation)	
2C19	(*S*)-Mephenytoin (4α-	Ticlopidine (competitive)

	hydroxylation)	
	Omeprazole (oxidation)	Omeprazole
2D6	Debrisoquine (4-hydroxylation)	Quinidine (competitive)
	Bufuralol (1-hydroxylation)	Fluoxetine (competitive)
	Dextromethorphan (*O*-demethylation)	Paroxetine (competitive)
2E1	Chlorzoxazone (6-hydroxylation)	Diethyldithiocarbamate (mechanism based)
	4-Nitrophenol (3-hydroxylation)	4-Methylpyrazole (competitive)
	N-Nitrosodimethylamine (*N*-demethylation)	Disulfiram (mechanism based)
	Aniline (4-hydroxylation)	Pyridine
3A4	Nifedipine (oxidation)	Troleandomycin (metabolic intermediate complex)
	Erythromycin (*N*-demethylation)	Erythromycin (metabolic intermediate complex)
	Testosterone (6α-hydroxylation)	Ketoconazole, itraconazole (competitive)
	Midazolam (1-hydroxylation)	Gestodene (mechanism based)

Table 12-5 Phase I Drug-Metabolizing Reactions

Oxidation	Reduction	Hydrolysis
Aromatic hydroxylation	Nitro reduction	Amidine hydrolysis
Alipatic hydroxylation	Azo reduction	Ester hydrolysis
N-Oxidation (formation of N-oxide and N-OH)	Ketone reduction	Amide hydrolysis
S-oxidation (sulfoxidation)		
N-, O-, S-dealkylation	Reduction of α, β-unsaturated ketones	
Oxidation of cyclic amines to lactams	Aldehyde reduction	
Oxidative deamination	N-, S-oxide reduction	
Oxidation of methyl to carboxyl group		
Epoxidation		
Alcohol oxidation (conversion to aldehyde or carboxylic acid)		
Dehydrogenation, β-oxidation		

Example 12-3

Clinically Significant Enzyme Induction[46]

Administration of two or more drugs together may lead to serious drug interactions as a result of enzyme induction. Triazolam is a short-acting hypnotic drug that is extensively metabolized by CYP3A4. Rifampin is used with other medicines to treat tuberculosis and is known to be a potent inducer of CYP3A4. Coadministration of rifampin markedly reduces plasma concentrations and the pharmacologic effects of many drugs including triazolam. To potentially induce CYP3A4 enzymes, 600 mg of rifampin or placebo was administered once

daily to 10 healthy volunteers for 5 days. On the sixth day, 0.5 mg of triazolam was orally administered, and the plasma concentration profile of triazolam was monitored for 10 hr (Fig. 12-19). As expected, a significant drug–drug interaction between rifampin and triazolam was observed. The area under the plasma concentration versus time curve of triazolam in the rifampin phase was only 5% of that in the placebo phase and the maximum plasma concentration of triazolam was 12.4% of the control value. The conclusion of this study was that triazolam becomes pharmacologically ineffective after long-term rifampin treatment because the induction of microsomal enzymes by rifampin causes an increase in the metabolism of triazolam and a marked decrease in the plasma concentration and the efficacy of triazolam.

Inhibition

Enzyme inhibition generally occurs without delay and can result in the immediate loss of activity for one or more enzymes. Many drugs and xenobiotics are capable of inhibiting drug metabolism. With metabolism decreases, drug accumulation often occurs, leading to prolonged drug action and possibly serious adverse effects. Enzyme inhibition by drugs or xenobiotics can occur in several ways, including competitive inhibition, the destruction of preexisting enzymes, interference with enzyme synthesis, and inactivation of the drug-metabolizing enzymes by complexation. Drugs containing imidazole, pyridine, or quinoline groups, such as ketoconazole, metyrapone, and quinidine, are well-known reversible inhibitors. Inactivation of metabolizing enzymes by complexation is called quasi-irreversible inhibition and occurs when a noncovalent tight bond is formed between the metabolite and CYP450. Macrolide antibiotics such as troleandomycin and erythromycin, hydrazines such as isoniazid, and methylenedioxybenzenes such as isosafrole are all known as quasi-irreversible inhibitors. Lastly, xenobiotics containing specific functional groups can be metabolized by CYP450 to reactive intermediates that bind to the enzyme covalently. For example, compounds that contain olefins and acetylenes can alkylate the heme. It is also known that some S- or N-containing compounds such as tienilic and cyclopropylamine covalently bind to the apoprotein.

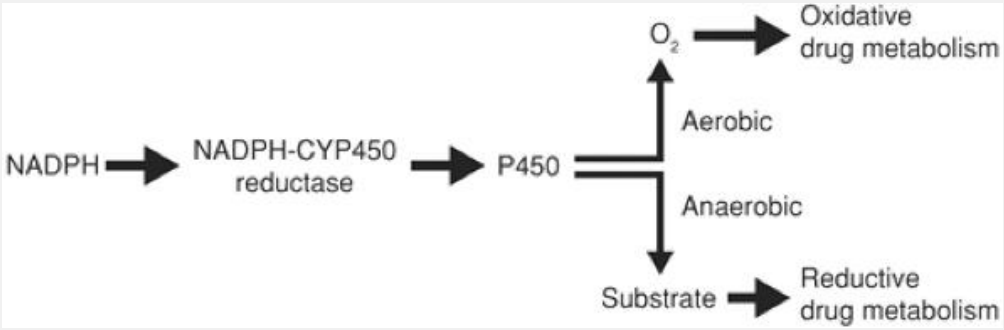


Fig. 12-17. Dual function of cytochrome P-450. Key: NADP = reduced nicotinamide-adenine dinucleotide phosphate. (From A. Y. H. Lu, Rutgers University, New Brunswick, N. J., Lecture Note, 2003.)

Example 12-4
Clinically Significant Enzyme Inhibition: Grapefruit Juice[47]

Many commonly consumed foods, drinks, and natural products or dietary supplements are known to alter the disposition of drugs. One particularly well-studied case, grapefruit juice, is

the subject of this example. Grapefruit juice is known to cause a considerable increase in the oral bioavailability of many drugs. This is because grapefruit juice is an inhibitor of CYP3A, a major player in the intestinal and hepatic metabolism of drugs, and when coadministered with substrates of CYP3A it causes a significant increase in drug–blood levels. The main mechanism of this drug–drug interaction is attributed to a decrease in intestinal CYP3A in humans (i.e., CYP3A4) by approximately 45% to 65%. In contrast, a single “dose” of grapefruit juice does not affect hepatic CYP3A4. Long-term multiple administrations of grapefruit juice inhibit CYP3A4 not only in the intestine but also in the liver. Verapamil is a calcium-channel blocker that undergoes extensive metabolism mainly by CYP3A4 in human. Verapamil was administered to 24 volunteers as a CYP3A4 substrate with grapefruit juice for 7 days. Grapefruit juice caused about a 50% increase in steady-state plasma concentrations of verapamil, showing a significant food–drug interaction (Fig. 12-20).

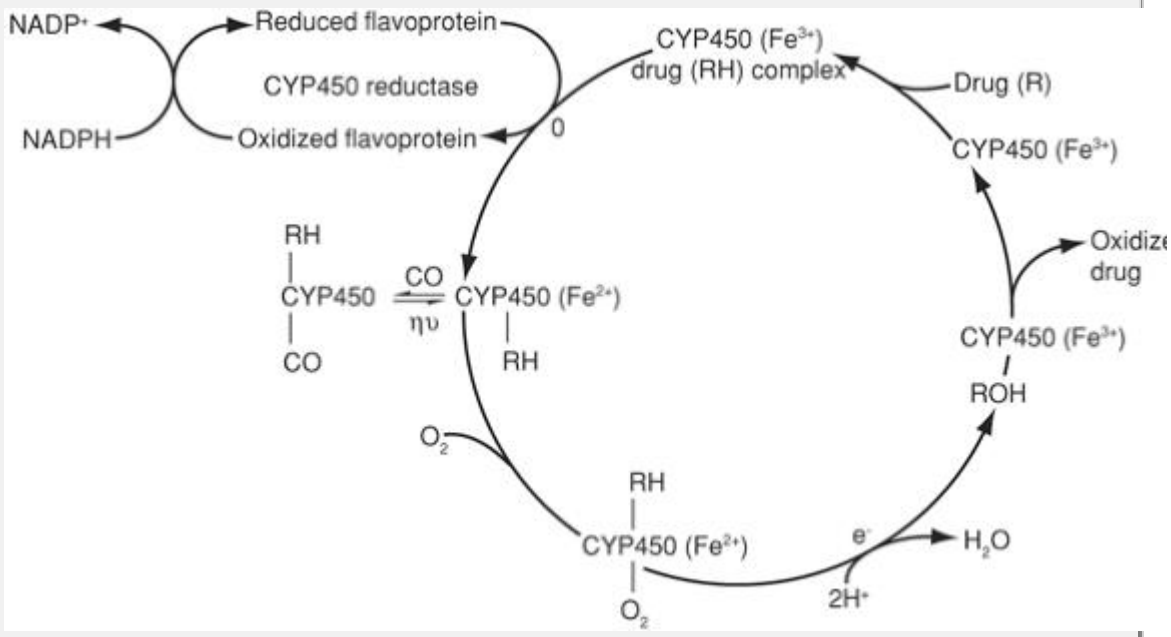


Fig. 12-18. Catalytic cycle of cytochrome P-450. Key: NADP = nicotinamide-adenine dinucleotide phosphate. *ηv* represents energy. RH represents the drug substrate. (From A. P. Alvares and W. B. Pratt, in W. B. Pratt and P. Taylor (Eds.), *Principles of Drug Action*, 3rd Ed., Churchill Livingstone, New York, 1990, p. 469. With permission.)

Enzyme Inhibition Kinetics

Although a thorough discussion of enzyme kinetics is presented inChapter 16, some pertinent points are highlighted here. If needed as a refresher, the student is referred to basic biochemistry textbooks for relevant background information. Enzyme inhibition (Fig. 12-21) can be classified by enzyme kinetics expressed by a change in K_m(the Michaelis–Menten constant) and/or V_m (maximal velocity). In competitive inhibition, the inhibitor binds to a free binding site on the enzyme. An increase in the inhibitor concentration results in a lower chance of binding between the substrate and enzyme, and thus theK_m value increases without a corresponding change in V_m,

Table 12-6 Isoforms of Phase II Enzymes in Animals and Humans

Enzyme	Mouse	Rat	Rabbit	Dog	Human
UGT1A	1, 5, 6, 9	1–3, 4P, 5–8, 9P, 10	?	6	1, 2P, 3–10, 11P, 12P
UGT2A	1	1	2	?	1
UGT2B	5	1–3, 6, 8, 12	13, 14, 16	?	4, 7, 10, 11, 15, 17
SULT1	A, B, C, E	A, B, C, E	?	A	A, B, C, E
SULT2	A	A	?	?	A, B
GST	A, P	A, M, S	?	?	A, M, P, T, Z

$$V = \frac{S \cdot V_m}{S + K_m(1 + I/K_i)} \qquad \textbf{(12–1)}$$

where I is concentration of inhibitor and K_i is inhibitory constant. In the case of noncompetitive inhibition, the inhibitor binds to its own binding site regardless of whether the substrate binding site is occupied. Because the degree of enzyme inhibition is dependent on the inhibitor concentration and is independent of substrate binding, the V_m values decrease with increasing inhibitor concentrations without changing K_m,

$$V = \frac{S \cdot V_m/(1 + I/K_i)}{S + K_m} \qquad \textbf{(12–2)}$$

Uncompetitive inhibition is observed when the inhibitor binds only to the enzyme–substrate complex. This results in a change in both $K_m$and V_m. Mixed-type enzyme inhibition

can also lead to changes in both parameters, and that could confuse the interpretation of the results.

Table 12-7 Phase II Drug-Metabolizing Reactions and Cofactors

Reactions	Cofactors
Glucuronidation N-, O-, S-, C-Glucuronidation Carbamic acid glucuronide	UDP-Glucuronic acid (UDPGA)
Sulfate conjugation	3-Phosphoadenosine 5′-phosphosulfate (PAPS)
Glycine conjugation	Glycine
Acetylation	Acetyl CoA
Methylation N-, O-, S-Methylation	*S*-adenosyl-L-methionine (SAM)
Glutathione conjugation	Glutathione

Metabolism and Drug Disposition

Orally dosed drug molecules are absorbed from the gastrointestinal (GI) tract through the GI wall and pass through the liver prior to reaching the systemic circulation. During the absorption process, drug molecules are exposed to various dispositional processes such as intestinal metabolism, intestinal secretion, hepatic metabolism, and biliary secretion. Among these processes, intestinal and hepatic metabolism are lumped together and are commonly referred to as “first-pass metabolism.” In the past, first-pass metabolism and hepatic first-pass metabolism were considered synonymous because of the dominating role of the liver in drug metabolism. However, recent studies have demonstrated that intestinal metabolism can be significant, especially if the role and potential impact of intestinal drug secretion is considered, and so it is best to refer to the process as “first-pass metabolism” unless specific mechanistic information is available that defines the role of the intestine and/or liver. The degree of the metabolism may vary greatly with each drug, and the resulting oral bioavailability can be very low. If a drug is completely absorbed and is not secreted, the oral fraction of dose absorbed or bioavailability, *F*, indicates the portion of drug that is absorbed intact:

Table 12-8 Drugs that induce Metabolism Clinically*

Inducing Agent	Induced Enzyme
Tobacco	CYP1A2
Phenobarbital, rifampin	CYP2B6
Rifampin, secobarbital	CYP2C9
Ethanol, isoniazid	CYP2E1
Carbamazepine, troglitazone, phenobarbital, phenytoin, rifabutin, rifampin, St. John's wort (hyperforin)	CYP3A4,5,7
Phenobarbital	Glucuronide transferase
Red grape (ellagic acid), garlic oil, rosemary, soy, cabbage, brussels sprouts†	Glutathione-*S*-transferase and glucuronide transferase

*Modified from Department of Medicine, Indiana University, Bloomington, Ind., http://medicine.iupui.edu/flockhart/clinlist.htm, 2003.

†In vitro or animal data.

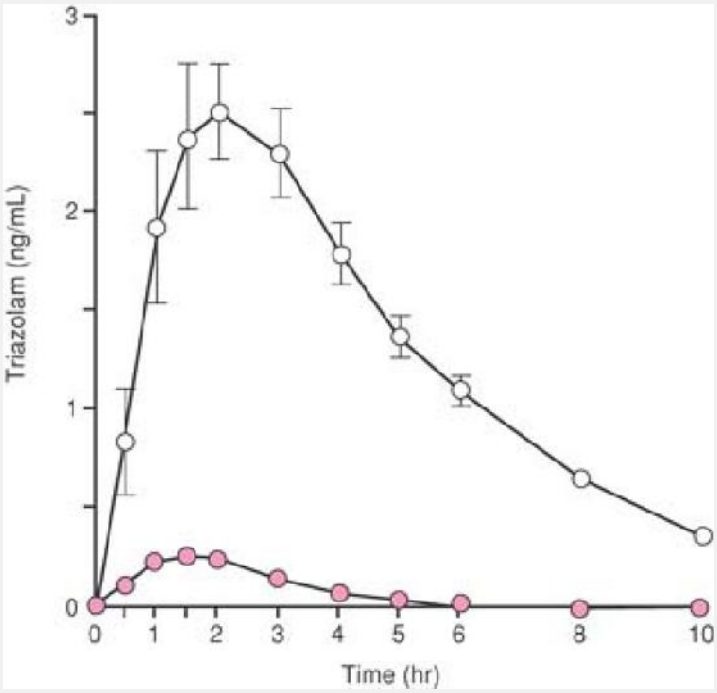


Fig. 12-19. Plasma concentration of triazolam in 10 individuals after 0.5 mg of oral triazolam, after pretreatment with 600 mg of rifampin once daily (•) or placebo (^) for 5 days.

$$F = \frac{\text{AUC}_{\text{oral}}}{\text{AUC}_{\text{IV}}} \qquad \textbf{(12–3)}$$

To get the precise value of first-pass metabolism from the *F* value, all of the foregoing assumptions must be satisfied. The fraction lost to metabolism would be equal to 1 - *F*. Of course, if absorption were incomplete, then 1 - *F* would represent the fraction of drug not absorbed due to incomplete absorption and/or lost to metabolism. Poor absorption could be due to many factors including low intestinal permeability; binding to intestinal tissue, mucus, or debris; or instability in the GI tract. The extraction ratio (ER) is commonly used to directly measure drug removal from the intestine or liver:

$$\text{ER} = \frac{C_a - C_v}{C_a} \qquad \textbf{(12–4)}$$

where C_a is the drug concentration in the blood entering the organ and C_v is the drug concentration leaving the organ. The relationship between bioavailability and intestinal and hepatic extraction ratios is expressed by the equation

$$F = F_{ABS}(1 - \text{ER}_{GI})(1 - \text{ER}_{H}) \qquad \textbf{(12–5)}$$

where F_{ABS} is the fraction of the dose absorbed through the intestinal mucosal membrane into the portal vein and ER_{GI} and $\text{ER}_{H}$are the gut and hepatic extraction ratios, respectively. When absorption is complete (i.e., F_{ABS} = 1) and

intestinal extraction is negligible (ER_{GI} [congruent] 0), the equation can be simplified to

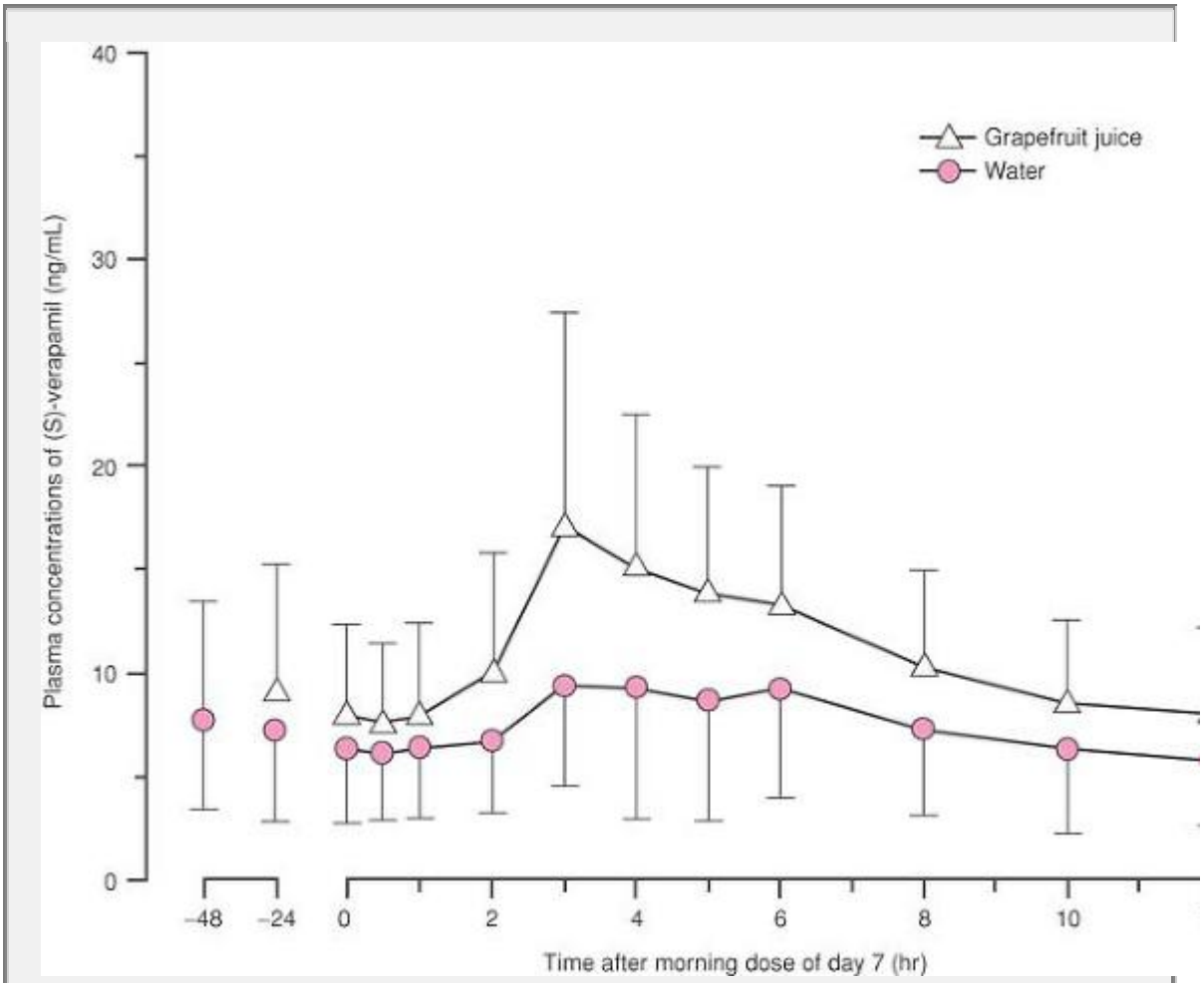


Fig. 12-20. Mean concentration versus time profile of (S)-verapamil within one dosing interval following oral administration of 120 mg of verapamil twice daily for 7 days during coadministration of water or grapefruit juice starting 48 hr prior to the dosing interval.

$$F = 1 - \mathrm{ER_H} \qquad \textbf{(12–6)}$$

Substituting equation (12-6) into equation (12-3) and rearranging results in

$$\mathrm{ER_H} = 1 - \frac{\mathrm{AUC_{oral}}}{\mathrm{AUC_{IV}}} \qquad \textbf{(12–7)}$$

which is used as an estimation of the liver extraction ratio.

Metabolism and Protein Binding

Hepatic clearance, CL_H, can be related to liver blood flow, Q, and the intrinsic clearance, CL_{int}, of liver using the following equation:

$$\mathrm{CL_H} = Q\frac{\mathrm{CL_{int}}}{Q + \mathrm{CL_{int}}} \qquad \textbf{(12–8)}$$

where the intrinsic clearance is the ability of the liver to remove drug without flow limitations. The Michaelis–Menten equation can be rearranged to give

$$\frac{V}{S} = \frac{V_m}{K_m + S} = \mathrm{CL_{int}} \qquad \textbf{(12–9)}$$

where the value of the rate of reaction, V, divided by the drug concentration, S, is conceptually the same as the intrinsic clearance, CL_{int}. Because the hepatic metabolizing enzymes are rarely saturated in a clinical situation, one can assume that K_m is much greater than S, and equation (12-9) reduces to

$$\mathrm{CL_{int}} = \frac{V}{S} = \frac{V_m}{K_m} \qquad (12\text{–}10)$$

In other words, the intrinsic clearance is constant, assuming the metabolizing enzymes are not saturated and that the protein binding of the drug is constant. Protein binding affects the intrinsic clearance of drugs because intrinsic clearance contains the free drug fraction, f_u, and the intrinsic clearance of free drug, CL_{int}:

$$\mathrm{CL_{int}} = f_u \cdot \mathrm{CL'_{int}} \qquad (12\text{–}11)$$

Equation (12-8) can be rewritten as

$$\mathrm{CL_H} = Q \frac{f_u \cdot \mathrm{CL'_{int}}}{Q + f_u \cdot \mathrm{CL'_{int}}} \qquad (12\text{–}12)$$

When the intrinsic clearance is much greater than hepatic blood flow, the hepatic clearance is dependent only on blood

flow. On the other hand, if the intrinsic clearance is much lower than hepatic blood flow, then hepatic clearance is dependent only on the intrinsic clearance. These two extremes are called flow-limited and metabolism-limited extraction, respectively. Drug protein binding does not affect hepatic clearance for drugs that demonstrate high intrinsic clearance. However, low-extraction drugs may be affected by protein binding, depending on the free fraction of drug. The studies of Blaschke48 demonstrate the relationship between protein binding and hepatic clearance (Fig. 12-22). Hepatic clearance of low extracted and medium- or low-binding drugs (less than 75% to about 80%) are not greatly affected by the changes in protein binding. These drugs are categorized as capacity-limited, binding-insensitive drugs. High-protein-binding and low-extraction drugs are considered capacity-limited, binding-sensitive drugs because a small change in the bound portion usually means large changes in the free drug fraction.

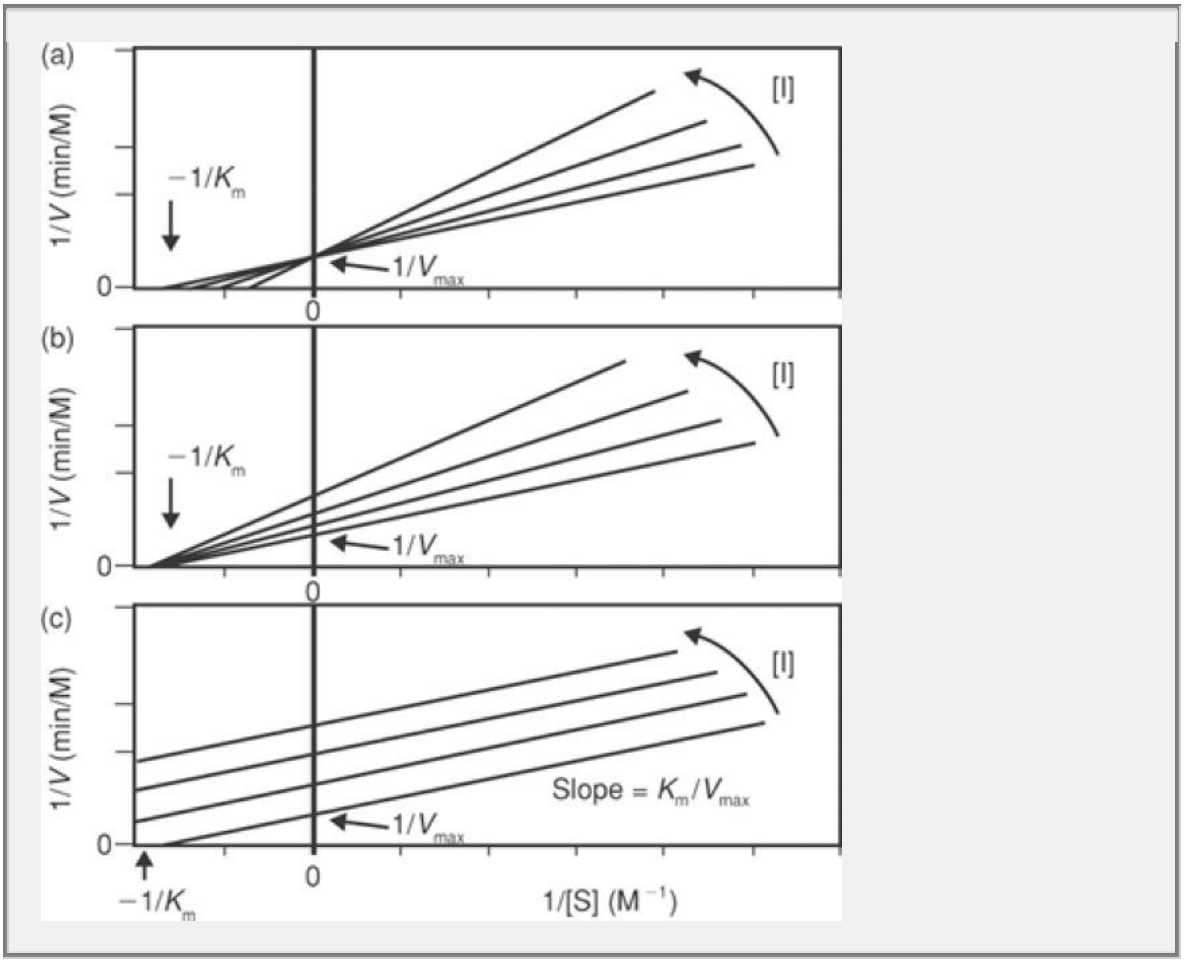


Fig. 12-21. The Lineweaver–Burk plot is used to distinguish the types of enzyme inhibition. Key: [I] = inhibitor concentration, [S] = substrate concentration. (*a*) Competitive inhibition, (*b*) noncompetitive inhibition, (*c*) uncompetitive inhibition. (Modified from D. P. Goldenberg, University of Utah, Salt Lake City, Utah. Lecture Slides inhibitors II. pdf, http://courses.biology.utah.edu/goldenberg/biol.3515/index.html, 2003.)

Drug Metabolism at the Subcellular Level

The endoplasmic reticulum (ER) is one of the most important cellular organelles in drug metabolism. Other fractions such as the mitochondria or cytosolic fraction play an important role in some cases (Table 12-9). The subcellular fractions comprising the S9 fraction, microsomes, and cytosolic fraction are the most widely used in vitro systems for studying drug metabolism. These fractions can be isolated by differential centrifugation techniques, which have permitted important advances in studies of drug metabolism. Because microsomal enzymes can oxidize a large portion of xenobiotics, incubation of a drug with liver microsomes is a widely used in vitro technique. Enzyme preparations have another advantage in that they are easy to prepare and can be obtained from small amounts of tissue. The level of drug-metabolizing enzymes is also readily determined. Enzyme kinetic parameters obtained from liver microsomes can be used to predict in vivo clearance. For instance, intrinsic clearance, CL_{int}, is calculated from K_m and V_m in equation **(**12-8**)**, and this intrinsic clearance is used again in calculating in vivo CL_H using equation **(**12-6**)** or **(**12-10**)** when the major elimination route of the drug is hepatic metabolism. Cellular fractions, like all experimental systems, have some limitations.

Organ-Level Biopharmaceutics

Organ-level biopharmaceutics is an important aspect of pharmacokinetics because the many transport and metabolism components that have already been introduced work together in a dynamic environment. In essence, it serves as a link between the molecular/cellular-level aspects and the “intact” system studied in pharmacokinetics. In this section, various organ systems and groups of organs that define key biopharmaceutical and pharmacokinetic processes will be described in detail. The key organ systems that will be discussed are the brain and choroid plexus, intestine, kidney, and liver. The kidney and liver are the primary organs of drug excretion (Fig. 12-23). Because the lung and the skin are minor organs of drug excretion, they will not be covered in depth in this chapter. Examples of groups of organs include the “first-pass” organ system, which includes the intestine, liver, and lungs, and the “enterohepatic” recirculation organs, which include the intestine, liver, and gallbladder. This chapter will not deal extensively with species differences in organ-level biopharmaceutics. However, for reference, Tables 12-10, 12-11 and12-12 are provided to show differences in organ weights, volumes, and blood flow for mice, rats, rabbits, rhesus monkeys, dogs, and humans. These values are used in correlative studies using physiologically based pharmacokinetic (PBPK) models (see the next section) in order to predict species differences and human dosing based on preclinical animal results.

Brain-Barrier Systems

In 1885, Paul Ehrlich,49 a German scientist, observed that many dyes can be distributed widely into body tissues but fail to stain brain parenchyma. This was attributed to a brain-barrier system. In fact, because the central nervous system is so well perfused, permeability is generally the major determinant of the drug distribution rate into the brain. Drugs reach the central nervous system by means of brain capillaries and the cerebral spinal fluid (CSF). Although the brain

receives about one sixth of the cardiac output, distribution of drugs to brain tissue is highly restricted. The restricted brain exposure to drugs and other xenobiotics is the result of two brain-barrier systems: (*a*) the BBB, which is formed by brain capillary endothelial cells, and (*b*) the blood–CSF barrier or the choroid plexus.

The brain-barrier systems are shown in Figure 12-24. Figure 12-24adepicts a brain capillary. It is composed of four kinds of cells: endothelial cells, pericytes, astrocytes, and neurons. The endothelial cells of the brain capillaries are more tightly joined to one another than are those of other capillaries. Another barrier to water-soluble drugs is the glial connective tissue cells (astrocytes), which form an astrocytic sheath close to the basement membrane of the capillary endothelium. The capillary endothelium and the astrocytic sheath form the BBB. Because the capillary wall rather than the parenchymal cell forms the barrier, the brain's permeability characteristics differ from those of other tissues. Drugs can also enter ventricular CSF directly by means of the choroid plexus. Figure 12-24b shows the choroid plexus. It acts as a BBB in the brain parenchyma because this capillary has a tight junction. There are two kinds of blood–CSF barriers. One is the arachnoid membrane and the other is formed by the epithelial cells of choroid plexus. Because these capillaries are permeable, only the arachnoid membrane and epithelial cells of choroid plexus function as a brain barrier.

Polar compounds cannot enter the brain by passive diffusion but can enter the interstitial fluids of most other tissues. Because membrane transporters are known to play a major role in the uptake of many compounds, it is likely that they also play a major role in the blood–brain and blood–CSF barriers. For example, drug uptake into brain endothelial cells is likely to be assisted by membrane transporters as described earlier in this chapter. However, secretory efflux transporters like P-gp may ultimately play a major role in limiting drug uptake into the brain parenchyma. As shown in Figure 12-25, a drug may permeate the apical membrane and be taken up into the brain endothelial cell. However, efflux transporters like P-gp are able to move the drugs back across the apical membrane and into the blood, protecting the brain from toxic substances or preventing drug absorption into the brain tissue. So, in addition to having a physically tight endothelium, membrane transporters play a major role in the brain's barrier properties. The following example with the HIV protease inhibitor amprenavir provides a good demonstration of the important role that membrane transporters play in the function of the BBB.

Key Concept

Blood Levels and Rates of Absorption/Elimination

The rates of absorption and elimination of drugs ultimately determine the resulting blood levels of drug that are achieved in the blood circulation, organs, tissues, and cells. Each point on a blood/plasma/serum drug concentration versus time curve reflects the rates of absorption and elimination at that time point (Fig. 12-28). From now on these curves will be referred to as plasma level versus time curves (PLTCs). If the rate of absorption is greater than the rate of elimination at that time point, the slope of the PLTC will be positive and the plasma concentrations are increasing. If the rate of absorption is slower than the rate of elimination at a given time point, the slope of the PLTC will be negative and the plasma concentrations are decreasing. When the rates of absorption and elimination are equal, the slope is zero and the corresponding (*x, y*) time point is known as (T_{max},C_{max}). As shown in Figure 12-28a, when the net rate of input into the body decreases, the slope of the absorptive phase also decreases and there is a shift of T_{max} (to a larger value) and C_{max} (to a lower value) as well. A slower input rate would result from a lower permeability, lower solubility, or slower gastric emptying rate. When the input rate is held constant but the rate of elimination is varied, a similar situation occurs (Fig. 12-28b). As the elimination rate constant increases, there is a shift in T_{max} to the left (i.e., shorter) and a decrease in C_{max}.

Example 12-5

Amprenavir Brain Penetration

To examine the role of P-gp in the effectiveness of the blood–brain barrier, Polli et al.50 examined the brain uptake of the HIV protease inhibitor amprenavir in mice. They examined the effect of the coadministration of ritonavir, another HIV protease inhibitor (GF120918), a specific P-gp inhibitor, or “genetic” P-gp–knockout (mdr1a/1b double knockout) mice on the brain uptake of amprenavir. Using whole-body autoradiography, they were able to visualize the brain uptake of amprenavir under these three conditions. In mice

treated with GF120918 and in the genetic knockout mice, they observed a 14- and 27-fold increase in brain amprenavir concentrations, respectively, due to the lack of P-gp. This can be visualized nicely in Figure 12-26. In Figure 12-26a the brain and CSF are shaded gray, indicating that amprenavir was able to penetrate the brain when P-gp was inhibited by GF120918. The control animal in Figure 12-26bshows is no amprenavir in the brain when P-gp is active and functional, suggesting that membrane transporters such as P-gp are an effective part of the blood–brain barrier. Ritonavir did not have an effect on amprenavir brain concentrations.

*Gastrointestinal Tract*51

The GI tract is depicted in Figure 12-27. The role of GI tract in drug absorption is clearly evident. However, more recently the realization that the GI tract and, more specifically, the intestine play a role in drug metabolism and excretion has occurred. The stomach provides several major functions that affect the bioavailability of orally administered drugs. It processes food into chyme by vigorous contractions that mix ingested contents with gastric secretions and assist intestinal absorption. It regulates input of the liquefied nutrients into the small intestine. The stomach is a major site of chemical and enzymatic degradation. Because the stomach controls the rate of input into the intestine, where the majority of

drug and nutrient absorption takes place, it has a considerable impact on the pharmacokinetics of drugs. This is because it controls the concentration of drugs at the most important site of GI absorption—the small intestine. Therefore, if a drug's permeability and solubility are not low and do not limit their absorption, the gastric emptying rate will essentially control the blood concentration–time profile of the drug. If gastric emptying is slower, then the net absorption rate will be slower, the peak blood levels, C_{max}, will be lower, and the time to peak blood levels, T_{max}, will be longer.

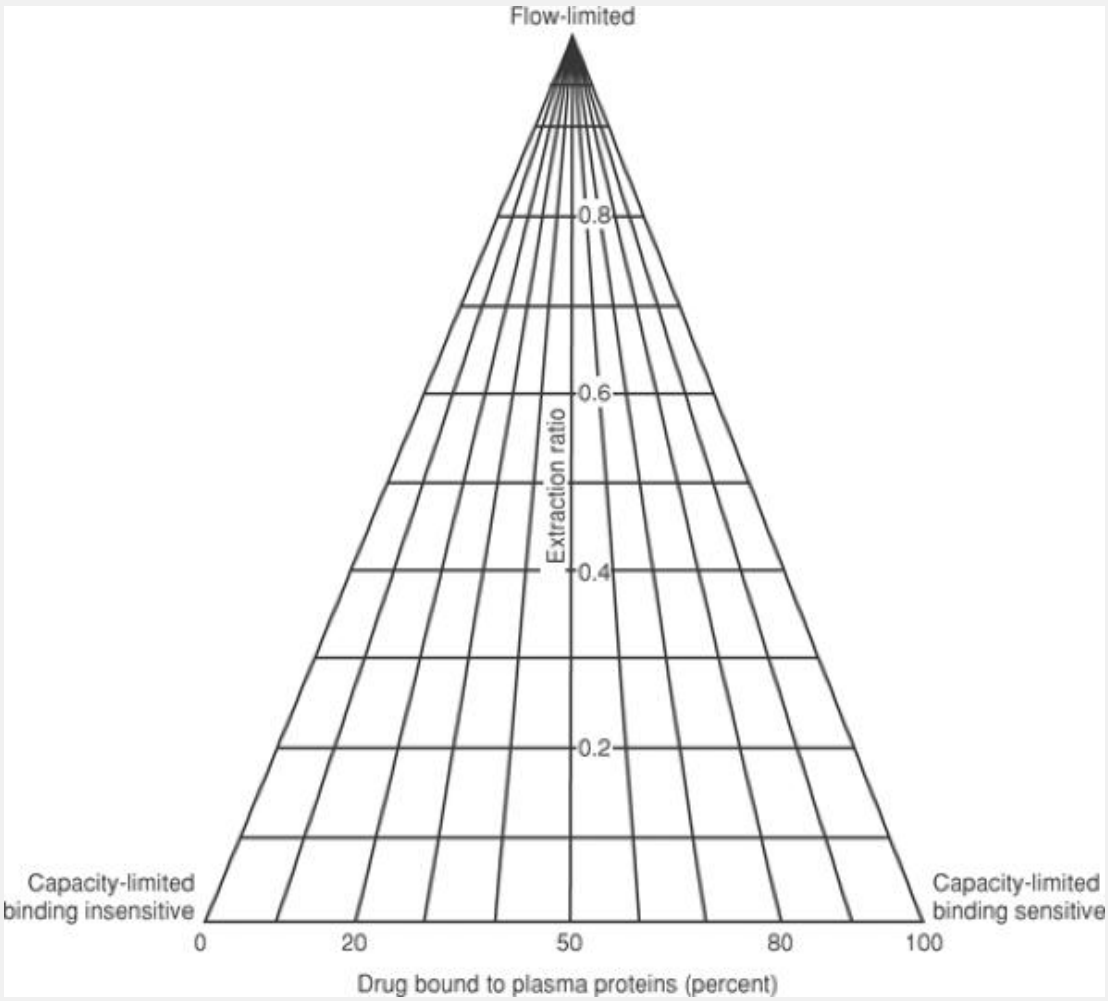


Fig. 12-22. The effect of hepatic extraction ratio and percent plasma protein binding on classification of hepatic clearance (flow limited; capacity-limited, binding sensitive and capacity-limited, binding insensitive). Any drug metabolized primarily by the liver can be plotted on the triangular graph. The closer a drug falls to a corner of the triangle, the more likely it is to have the characteristic changes in disposition in liver disease. (From T. F. Blaschke, Clin. Pharmacokinet. **2,** 40, 1977. With permission.)

Example 12-652

Gastric Emptying

Gastric emptying rate significantly affects drug absorption and the appearance of drug in the blood. Acetaminophen is a high-permeability and high-solubility drug. Therefore, the appearance of acetaminophen in the blood is strictly related to its emptying from the stomach and presentation to the absorbing site, the small intestine. In this study, acetaminophen was administered to five healthy male individuals. To stimulate gastric emptying, they also received metoclopramide. To reduce the gastric emptying rate, they received propantheline. As can be seen in Figure 12-29, altering the gastric emptying rate significantly altered the rate of acetaminophen absorption. In fact, Figure 12-29is very similar to the theoretical expectation as seen in the simulation in Figure 12-28a. Although this example demonstrates the role of gastric emptying, one should keep in mind that reduced permeability, reduced solubility, or even slower release from a drug product would result in qualitatively similar behavior. Examination of PLTCs has limited value because it is easy to conclude from Figure 12-29 that

absorption rate was slowed, but the cause of the reduced absorption rate cannot be understood without further information about the drug or its biopharmaceutics.

The stomach can be thought of as a two-part system, the upper part consisting of the fundus and upper body and the lower part consisting of the antrum and lower body. These two sections affect the motility of gastric contents and are very different. The upper section acts as a reservoir that can expand to accommodate ingested materials. This expansion does not cause a significant increase in internal pressure and helps generate a pressure gradient between the stomach and the small intestine. Gastric emptying is controlled by a gastric pacemaker, a group of smooth muscle cells in the midcorpus on the greater curvature of the stomach. Neural control of

gastric emptying occurs by means of extrinsic and intrinsic innervation. Contractions occur at a basal rate of three to four cycles per minute or as peristaltic waves initiated by the entry of solids into the stomach.53 Emptying occurs at a constant rate because the antrum maintains a relatively constant volume.54 The proximal stomach controls the emptying of liquids. It is directly related to the gastroduodenal pressure gradient.55 Noncaloric liquids such as sodium chloride empty from the stomach in a monoexponential pattern, the rate decreasing as intragastric volume and pressure decrease. If the intragastric fluid is caloric, acidic, or nonisotonic, initial emptying is retarded and then follows a more linear pattern.56The lower section of the stomach acts as a forceful grinder by developing powerful peristaltic contractions. These waves of contraction increase in force as they near the pylorus. When these forceful waves reach the pylorus, the membrane that separates the stomach from the duodenum is opened, and the contents of the stomach are administered as spurts of chyme.

Table 12-9 Subcellular Fractions and Metabolic Reactions

Fraction	Centrifugation	Metabolic Reaction
Nuclei and cell debris	500 × *g*	Little metabolic activity
Mitochondira	8000 × *g*	Glycine conjugation, fatty acid β-oxidation, monoamine oxidase
Lysosomes	15,000 × *g*	Ester hydrolysis, not so much involved in drug metabolism
Microsomes	100,000 ×*g*	Most of phase I reaction, glucuronidation, N-, *O*-methylation
Cytosol	100,000 ×*g*supernatant	Hydrolysis, alcohol and aldehyde dehydrogenase, sulfate and glutathione conjugation, acetylation

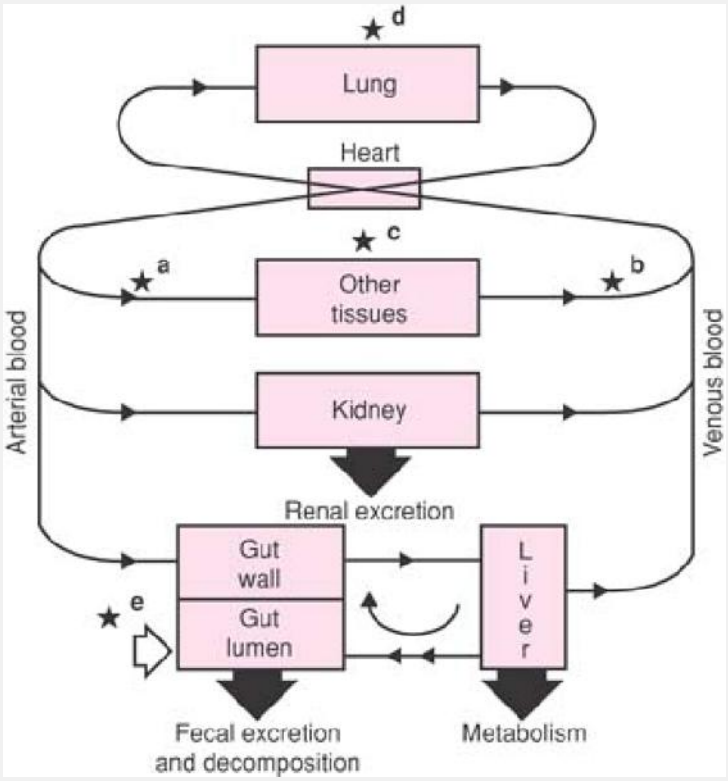


Fig. 12-23. Once absorbed from any of the many sites of administration, drug is conveyed by blood to all sites within the body including the eliminating organs. Sites of administration include: a, artery; b, peripheral vein; c, muscle and subcutaneous tissue; d, lung; and e, gastrointestinal tract. The dark- and light-colored lines with arrows refer to the mass movement of drug in blood and in bile, respectively. The movement of virtually any drug can be followed from site of administration to site of elimination. (From M. Rowland and T. N. Tozer, *Clinical Pharmacokinetics: Concepts and Application*, 3rd Ed., Lippincott Williams & Wilkins, Baltimore, MD, 1995, p. 12.)

Gastric motility is controlled by a very complex set of neural and hormonal signals. For instance, the system has a feedback loop in case the chyme is too acidic. Whereas gastrin is a hormone that stimulates gastric acid secretion, motilin is associated with housekeeping waves of motility that occur in the fasted condition. The fasted gastric motility cycle serves two functions and occurs as four “phases.” This cycle repeats about every 2 hr during the fasted state. Phase I typically lasts 40 to 60 min and consists of a gentle mixing period due to smooth muscle quiescence, during which there are only rare contractions. Phase II follows with peristaltic contractions occurring with an increase in frequency for approximately 25 to 40 min. These waves of activity originate in the stomach and propagate through the small intestine. Phase III is sometimes referred to as the “housekeeper” wave because the pylorus remains open to allow indigestible particles that are less than 12 mm in size to pass into the small intestine. Particles that are larger than 12 mm are rejected by the pylorus and remain in the stomach until they become small enough to pass. Phase III, which lasts 15 to 25 min, is characterized by powerful peristaltic contractions that occur three times a minute and empty the stomach into the small intestine. Phase IV lasts up to 7 min and is a transition between the forceful contractions of phase III and the gentle mixing contractions of phase I. The pH of fasting healthy adults is approximately 2 to 3, whereas fed-state pH is considerably higher, in the range of pH 5 to 6.

The volume and composition of ingested food determines the rate of gastric emptying.57 Gastric emptying of liquids is

P.284

rapid (half-time is about 12 min so that 95% is emptied within 1 hr).58 An increase in caloric content generally slows gastric emptying so that the rate of delivery of calories into the duodenum is relatively constant.59 It is estimated that nearly 50% of a solid meal remains in the stomach after ~2 hr. The temperature of the ingested meal is not important for liquids, which conduct heat rapidly, but may delay the emptying of hot or cold semisolid or solid meals, which have a higher thermal inertia. Gastric emptying occurs more rapidly in the morning than in the evening.60 Gastric emptying is slightly slower in healthy individuals older than 70 years of age of both sexes,61 even though the absorption of orally administered drugs does not seem to vary with age.61,62 The results of studies of the effect of body weight on gastric emptying of solids and liquids are inconsistent. Accelerated,63 delayed,64 and unchanged gastric emptying65 have all been reported. The differences in emptying rates are difficult to explain, but it appears that moderate obesity is not a major modifying factor, although the emptying of solids may be delayed in obese individuals who are at least 63% in excess of ideal weight.66 The influence of gender on gastric emptying is controversial. Whereas some authors have found similar gastric emptying rates for men and women,61,66 others have found slower gastric emptying in women than in men.67,68 The difference could be attributed to the phase of the menstrual cycle at the study time because the rate of solid gastric emptying decreases linearly during the menstrual cycle toward the luteal phase (days 19–28). The emptying of liquids does not differ between the two phases of the cycle.66,69 Pregnancy is believed to delay gastric emptying. However, the majority of studies have not shown delayed gastric emptying of liquids in women presenting during the first or second trimester for terminations of pregnancy, at elective caesarean section,70 and at first and third postpartum days.71 Absorption may occur from the stomach, but, typically, absorption is minimal. Although nonionized lipophilic molecules of moderate size may be absorbed, and even though the duration of exposure to epithelium is short, there is very little absorption

P.285

because of the small epithelial surface area and the physically thick, viscous mucus layer.

Table 12-10 Weights of Various Organs in the Mouse, Rat, Rabbit, Monkey, Dog, and Human*

Organ	Mouse (0.02 kg)	Rat (0.25 kg)	Rabbit (2.5 kg)	Rhesus Monkey (5 kg)	Dog (10 kg)	Human (70 kg)
Brain	0.36	1.8	14	90	80	1400
Liver	1.75	10.0	77	150	320	1800
Kidneys	0.32	2.0	14	25	50	310
Heart	0.08	1.0	5	18.5	80	330
Spleen	0.1	0.75	1	8	25	180

Adrenals	0.004	0.05	0.5	1.2	1	14
Lung	0.12	1.5	18	33	100	1000

*Organ weights are given in grams.
From B. Davies and T. Morris, Pharm. Res. **10,** 1093, 1993.

Table 12-11 Volumes of Various Body Fluids and Organs in the Mouse, Rat, Rabbit, Monkey, Dog, And Human*

	Mouse (0.02 kg)	**Rat (0.25 kg)**	**Rabbit (2.5 kg)**	**Rhesus Monkey (5 kg)**	**Dog (10 kg)**	**Human (70 kg)**
Brain	–	1.2	–	–	72	1450
Liver	1.3	19.6	100	145	480	1690
Kidneys	0.34	3.7	15	30	60	280
Heart	0.095	1.2	6	17	120	310
Spleen	0.1	1.3	1	–	36	192
Lungs	0.1	2.1	17	–	120	1170
Gut	1.5	11.3	120	230	480	1650
Muscle	10.0	245	1450	2500	5530	35,000
Adipose	–	10.0	120	–	–	10,000
Skin	2.9	40.0	110	500	–	7800

Blood	1.7	14.5	165	367	900	5200
Total body water	14.5	167	1790	3465	6036	42,000
Intracellular fluid	–	92.8	1165	2425	3276	23,800
Extracellular fluid	–	74.2	625	1040	2760	18,200
Plasma volume	1.0	7.8	110	224	515	3000

*Organ and other volumes are given in milliliters.
From B. Davies and T. Morris, Pharm. Res. **10,** 1093, 1993.

Table 12-12 Flow of Blood through the Major Organs and Flow of other Fluids in the Mouse, Rat, Rabbit, Monkey, Dog, and Human*

	Mouse (0.02 kg)	Rat (0.25 kg)	Rabbit (2.5 kg)	Rhesus Monkey (5 kg)	Dog (10 kg)	Human (70 kg)
Brain	–	1.3	–	72	45	700
Liver	1.8	14.8	177	218	309	1450
Kidneys	1.3	9.2	80	148	216	1240
Heart	0.28	3.9	16	60	54	240
Spleen	0.09	0.63	9	21	25	77
Gut	1.5	7.5	111	125	216	1100
Muscle	0.91	7.5	155	90	250	750

Adipose	–	0.4	32	20	35	260
Skin	0.41	5.8	–	54	100	300
Hepatic artery	0.35	2.0	37	51	79	300
Portal vein	1.45	9.8	140	167	230	1150
Cardiac output	8.0	74.0	530	1086	1200	5600
Urine flow	1.0	50.0	150	375	300	1400
Bile flow	2.0	22.5	300	125	120	350
GFR	0.28	1.31	7.8	10.4	61.3	125

*All blood flows are in mL/min; urine and bile flows and glomerular filtration rate (GFR) are in mL/day.

Absorption of drugs, fluid, and nutrients can occur from each section of the small intestine and colon. The absorption of fluids, nutrients, electrolytes, and xenobiotics occurs as chyme moves through the GI tract. The small intestine is partitioned into three sections of different sizes and function, the duodenum, the jejunum, and the ileum. Water is able to flow into or out of the lumen to maintain the isotonicity of the luminal contents with plasma. Approximately 8 to 9 liters

of fluid enter the upper GI tract every day—approximately 7 liters of secreted juices and 1.5 liters of ingested fluid. About 1 liter of fluid enters the colon, and only 100 mL of water leaves the body in the feces. For most drugs, the duodenum and the proximal jejunum are the best sites of absorption because they have the highest absorptive surface area. In general, absorptive surface area decreases as one travels down the intestine.72 The ratio of the absolute surface area of the human stomach to that of the small intestine is 1 to 3800; this shows why absorption of substances by the stomach is generally neglected. Similarly, the 570-fold difference73 between the small intestine and the colon suggests that the majority of absorption occurs in the small intestine. However, although this takes into account the surface area, the transit time of the colon is 4 to 24 times longer (i.e., 12–72 hr as compared to 3–4 hr) than in the small intestine. Therefore, a longer residence time could offset a lower absorptive surface area, making the colon as good site for the absorption of drugs as the small intestine.

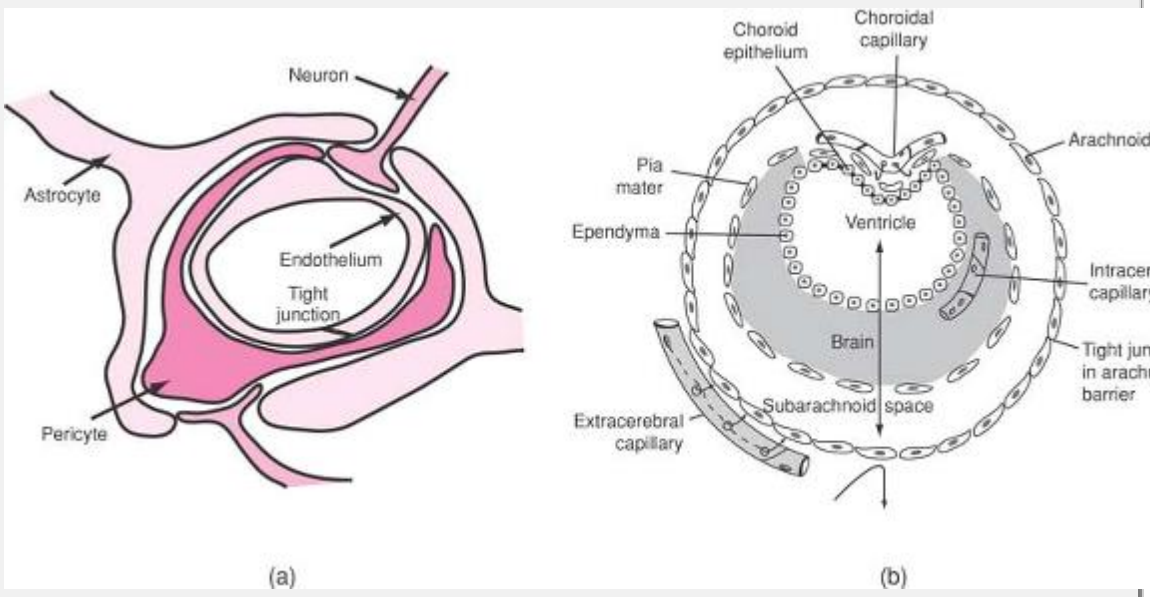


Fig. 12-24. (*a*) This is a schematic of brain capillary. It is composed of four kinds of cells; endothelial cell, pericyte, astrocyte, and neurons. Because of the close anatomical proximity of the cells, they stimulate endothelial cells to proliferate and differentiate. (*b*) This is a schematic of the brain-barrier system. The capillary has a tight junction. Hence, it acts as a blood–brain barrier in the brain parenchyma. There are two kinds of blood–CSF barriers. One is the arachnoid membrane and the other is the epithelial cell of choroid plexus. These capillaries are permeable. So, in these areas only arachnoid membrane and epithelial cells of choroid plexus can function as a barrier. (From B. Schlosshauer, Bioessays **15**, 341, 1993. With permission.)

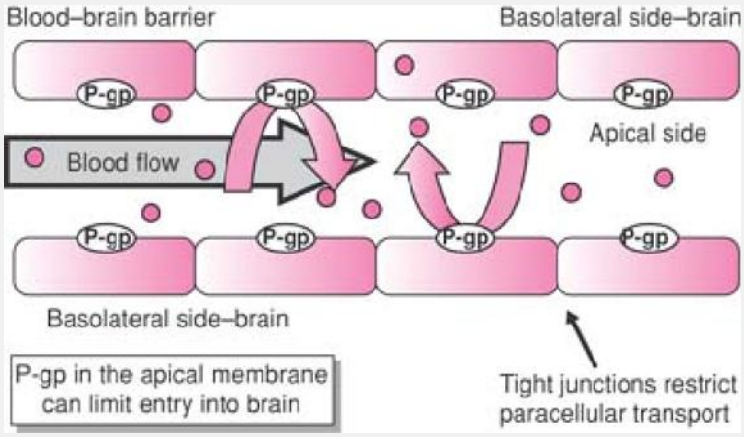


Fig. 12-25. P-glycoprotein, an efflux secretory transporter, is widely thought to limit entry of drugs into the brain, testis, intestines, and other organs and tissues. Drugs enter cells but are effluxed out of the cell by P-gp before they can enter the brain. This mechanism is responsible for minimizing brain exposure to toxic chemicals.

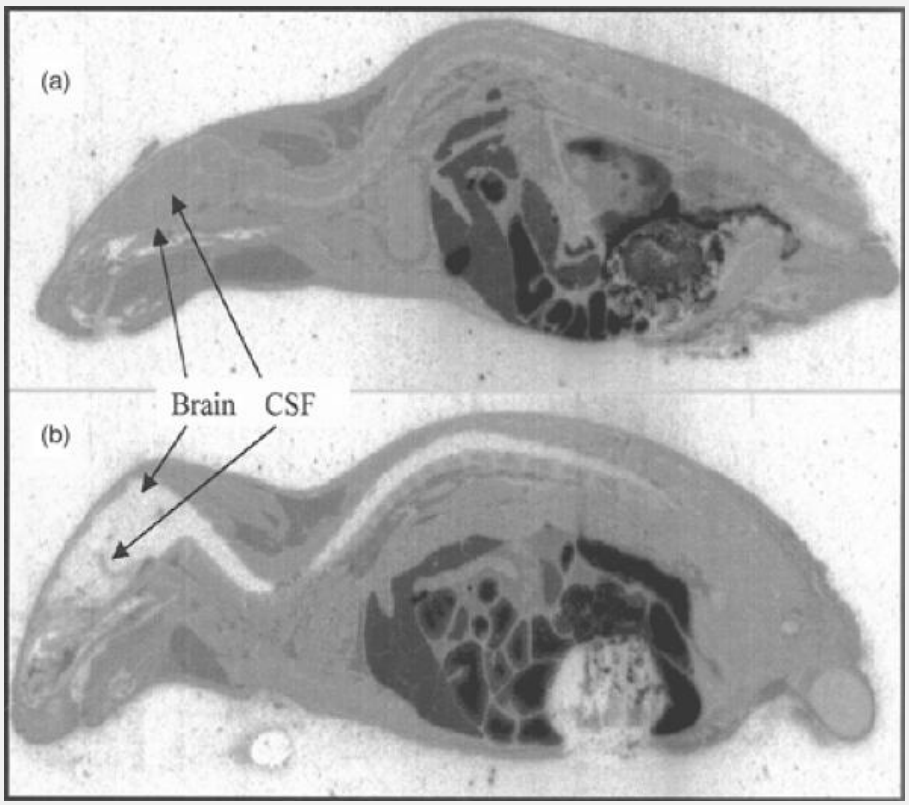


Fig. 12-26. Distribution of [14 C]-amprenavir in male CD-1 mice pretreated with the P-gp inhibitor GF120918. Animals treated with GF120918 (*a*) had a 13-fold increase in brain and 3.3-fold increase in CSF levels of amprenavir-related material over vehicle-treated mice (*b*).

Small intestinal absorption is also dramatically affected by regional differences in the distribution of transporters, metabolic enzymes, and so on. The practical implication of this is that even though the absorptive surface area in the duodenum is higher than in the ileum, absorption from the ileum is not necessarily lower for drugs and nutrients. For example, intestinal reabsorption of bile salts plays a crucial role in human health and disease. The small intestine absorbs 90% to 95% of the bile salts. Of the remaining bile salts, the colon converts the salts of deoxycholic acid and

lithocholic acid. Only 1% of the lithocholate is absorbed, and the colon excretes the rest. The bile salts lost to excretion in the colon are replaced by synthesis of new ones in the liver at a rate of 0.2 to 0.4 g/day, with a total bile salt amount of 3.5 g, which is constantly recycled by enterohepatic circulation. Enterohepatic circulation is discussed later in this chapter. Bile acid reabsorption is primarily localized in the terminal ileum and is mediated by a 48-kd sodium-dependent bile acid cotransporter known as ASBT, which is given the molecular designation SLC10A2.74

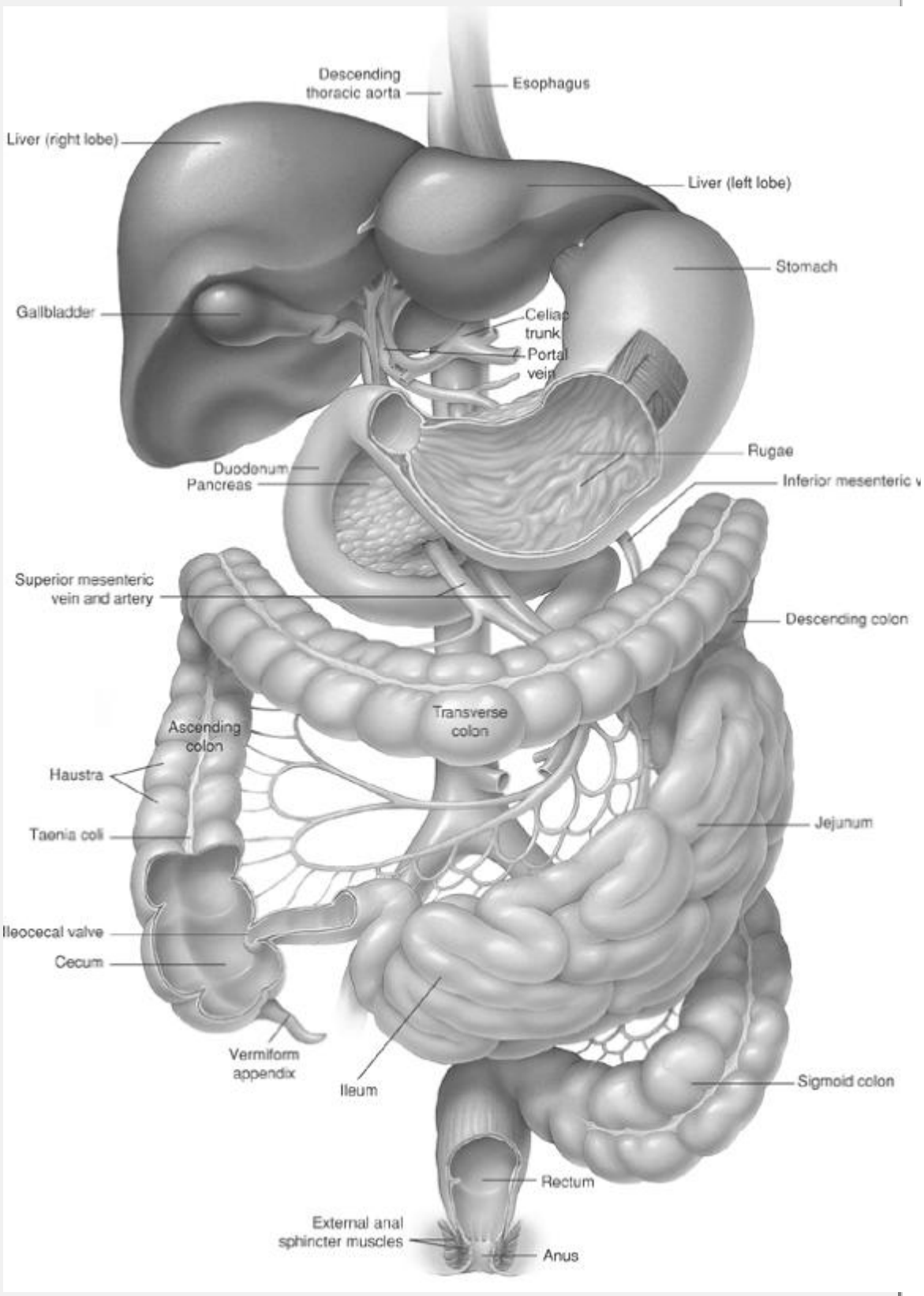


Fig. 12-27. Gastrointestinal system.

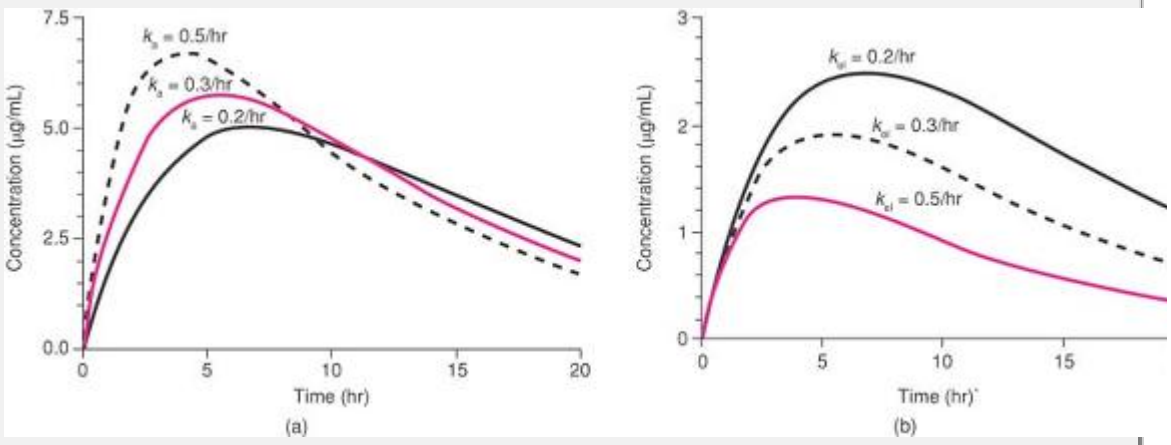


Fig. 12-28. Effect of absorption and elimination rate constant on the plasma concentration versus time profile. (*a*) Absorption (input) rate is increased from 0.2/hr to 0.5/hr while holding the elimination rate constant resulting in an increase in the slope of the absorptive phase. (*b*) The input rate is held constant while elimination rate constant is changed. Note that absorptive phase is unchanged (i.e., slopes are equal).

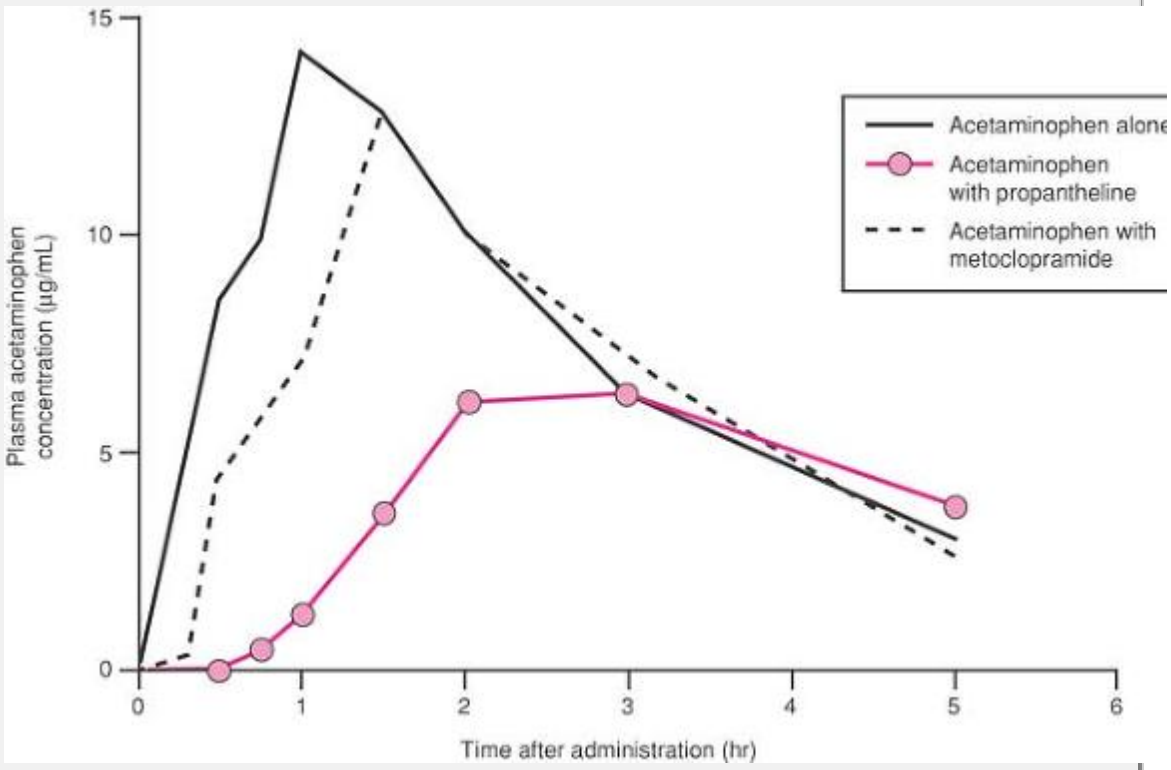


Fig. 12-29. Effect of propantheline and metoclopramide on acetaminophen absorption. Acetaminophen absorption is very rapid and is only limited by its

introduction into the intestine by the stomach. Metoclopramide increases the rate of acetaminophen gastric emptying resulting in a faster rate of absorption, higher $C_{max}$and shorter t_{max}. Propantheline has the opposite effect, slowing gastric emptying rate and delaying absorption. (From J. Nimmo, R. C. Heading, P. Tothill, and L. F. Prescott, Br. Med. J. **1,** 587, 1973. With permission.)

Region-specific absorption has been reported in animals and humans for a variety of drugs including allopurinol,75 amoxicillin,76benzoate,77 lefradafiban,78 oxyprenolol,79 talinolol,80 and thymidine analogues.81 Regional variation in the distribution of drug transporters also brings variatiability in absorption. Regional distribution of apical absorptive transporters including the apical bile acid transporter,82,83 monocarboxylic acid transporter-1,84 a nucleoside transporter,85 OATP3,86 and the peptide transporter PEPT187 has been reported. Segmental variability is also known to occur for metabolic enzymes and efflux/secretory transporters as well. The cytochrome P-450 3 A,88,89,90,91,92,93,94 SULTs, GSTs, and the UDP-glucuronosyltransferases95,96,97,98,99 are higher at the proximal than at the distal intestine. MRP2 intestinal secretion follows the distribution of the cytochrome P-450s and conjugation enzymes,100,101 whereas P-glycoprotein93,102,103,104,105 is higher in the jejunum/ileum than other parts of the intestine. Basolateral MRP3, in contrast to MRP2, is more prevalent in the ileum and colon.106

The impact of the varied regional distributions of drug transporters and metabolizing enzymes is difficult to predict because drugs can be substrates for numerous transporters and enzymes. For example, saquinavir, an HIV protease inhibitor, is known to be a substrate for the transporters P-gp, MRP1, MRP2, OATP-A, OATP-C, and the metabolic enzyme CYP3A.106,107,108,109,110 In addition to regional intestinal distribution, substrate affinity, enzyme/transporter capacity, turnover rate, and other factors ultimately determine the segmental absorption behavior and pharmacokinetics of drugs. Changes also occur in the characteristics of the paracellular spaces throughout the intestine. Intestinal pH is relatively constant and ranges from about pH 5 in the duodenal bathing region of the upper small intestine to pH 6.5 to 7.2 in other areas of the intestine and colon.

Kidney

Excretion is the process by which a drug or a metabolite is eliminated from the body without further chemical change. The kidneys, which transport water-soluble substances out of the body, are the major organs of excretion. The kidney performs two critical functions in the distribution and excretion of drug molecules. They excrete the metabolites formed by the liver or other organs/tissues and control the concentrations of many of the molecules found in the blood stream. The kidney does this by filtration of the blood. A depiction of a kidney is shown in Figure 12-30. Blood enters the glomerulus through the afferent arteriole and leaves through the efferent arteriole. About one fifth of the plasma reaching the glomerulus is filtered through pores in the glomerular endothelium; the remainder passes through the efferent arterioles surrounding the renal tubules. Drugs bound to plasma proteins are not filtered; only unbound drug is contained in the filtrate.5 After filtration in the glomerulus, the blood and waste/filtrate streams continue to be processed by the nephron, the individual working unit of the kidney. There are approximately 1 million nephrons in each kidney. The glomerular filtrate has essentially the same composition as the plasma that entered the glomerulus without a significant amount of protein and no red blood cells. Filtration occurs in the glomerulus by size and charge exclusion. However, secretion and reabsorption occurring in the tubules occur because of the permeability of the molecule being transported. The pore size of the glomerulus is large enough to allow molecules that are up to 8 nm in diameter to pass through.

As seen in Table 12-13, there is a steep molecular weight dependence on permeability in the kidney. The permeability of the solute is affected by size and charge if it is transported by passive diffusion;

however, in the kidney, solutes are transported out of the tubules by active transport. The primary reason is that passive diffusion occurs from regions of higher solute concentrations to lower concentrations. Because typical solute concentrations in the blood will be more dilute than those in the collecting duct and urine, diffusion out will not be favored. Another important factor is that the pores are lined with proteoglycans that have a very strong negative charge. It is this electrostatic repulsion that keeps albumin, which is only 6 nm in diameter, and most other proteins greater than molecular weight 69,000 from being filtered in the glomerulus. The kidney has a blood flow of 1200 mL/min, which creates a flow from the glomerulus into the proximal tubule of 125 mL/min. The bulk of this fluid flow is water, and if water was not actively reabsorbed, 180 liters of water would be lost each day. Fortunately, more than 99% of the water and varying amounts of its solutes are normally reabsorbed into the blood by way of the proximal tubules. This concentrates the filtrate greatly. The filtrate that comes from the glomerulus passes through the proximal tubule, where conservation of ions, glucose, and amino acids occurs by active and passive transport. In the proximal tubule, these molecules are reabsorbed from the glomerulus filtrate by the blood in the efferent arteriole. About 65% of the glomerular filtrate is reabsorbed before reaching the loop of Henle. The filtrate continues moving through the loop of Henle and distal tubule, where it is continuously reabsorbed. The maximal rate of reabsorption for various substances is shown inTables 12-14 and 12-15. These values indicate the maximum rate at which a species can be reabsorbed. The transport rate, however, may not be linear in concentration. This occurs when a system undergoes saturation kinetics. Although the efferent arteriole is in the process of reabsorbing water and other vital ions and solutes from the tubules, it also secretes molecules into the tubules. The remaining substance in the tubules enters a collecting duct and is considered urine. The ability of the kidneys to clean or clear the plasma of various substances is defined as plasma clearance:

$$\text{Plasma clearance (mL/min)} = \frac{\text{Urine flow (mL/min)} \times \text{Concentration in urine}}{\text{Concentration in plasma}} \quad \textbf{(12–13)}$$

There are several substances that are routinely measured to determine kidney function: creatinine, inulin, and

p-aminohippurate (PAH). These measure glomerular filtration rate and plasma flow through the kidneys. Inulin is not reabsorbed from the tubules and is not actively secreted into the tubules; therefore, any inulin found in the urine comes from glomerular filtration. As shown in Table 12-16, inulin is filtered in the glomerulus as easily as water is. Therefore, the plasma clearance of inulin is equal to the glomerular filtration rate. In terms of ADME and pharmacokinetics, the kidney is a primary organ of drug excretion. Drugs may be filtered by the glomerulus, reabsorbed into the blood stream by the proximal tubule, or secreted from the blood stream into the distal tubule. For proteins, general rules for glomerular filtration are as follows: (*a*) If the protein is bigger than immunoglobulin G (150 kd, 55-Å radius), it is rarely excreted; (*b*) if the protein is smaller than 40 kd and has a radius less than 30 Å, it is almost completely excreted; (*c*) negatively charged molecules are retained, even if small, because of charge repulsion with Bowman's space; and (*d*) elongated molecules have higher clearance than spherical molecules.

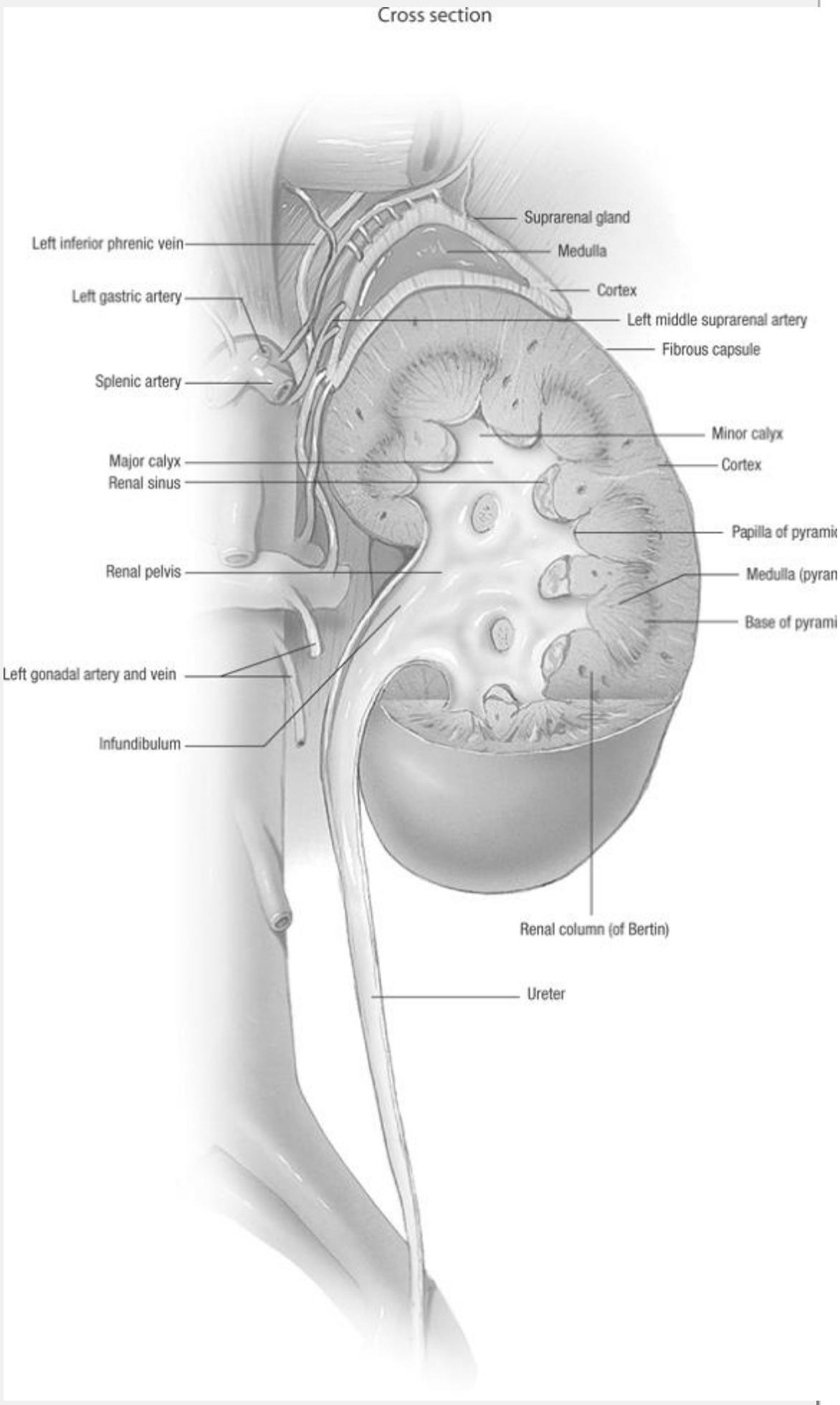

Cross section
Suprarenal gland
Left inferior phrenic vein
Medulla
Cortex
Left gastric artery
Left middle suprarenal artery
Fibrous capsule
Splenic artery
Minor calyx
Major calyx
Cortex
Renal sinus
Papilla of pyramic
Renal pelvis
Medulla (pyran
Base of pyrami
Left gonadal artery and vein
Infundibulum
Renal column (of Bertin)
Ureter

Fig. 12-30. Left kidney and adrenal gland.

Table 12-13 Permeability and Molecular Size

Molecular Weight	Permeability Compared to Water	Example Substance
5200	1.00	Inulin
30,000	0.5	Very small protein
69,000	0.005	Albumin

The limited ability of the kidneys to clear large materials from the body has been used as a way to increase the circulation time and decrease the clearance of drugs. Numerous studies have been published showing that polymers of similar size to proteins are cleared in a similar manner. For example, the molecular-weight threshold limiting glomerular filtration of an HPMA (N-(2-hydroxypropyl)methacrylamide) copolymer was found to be about 45 kd in rats.111 In mice, the molecular-weight cutoff was found to be about 30 kd for poly(ethylene glycol) (PEG).112 This size limitation has been exploited in drug delivery, with PEG being the most common polymer employed to date. PEG is advantageous as a protein-modifying agent because it is inert, water-soluble, nontoxic, and modular in size. Pegylation (i.e., chemically adding a PEG to a therapeutic agent) is now a well-established method of modifying the pharmacologic properties of a protein by, for example, delaying clearance and reducing protein immunogenicity.110,111,112,113,114,115 Among the various disease states that have been targeted for the study of drugs incorporating pegylation technology, the treatment of chronic hepatitis C with interferon-based compounds offers significant potential for clinical impact. Two compounds, peginterferon alfa-2a (PEGASYS) and peginterferon alfa-2b (PEG-Intron), are both approved for use alone and in combination with ribavirin for the treatment of chronic hepatitis C. However, the different PEG moieties attached to the native protein, the site of attachment, and the type of bond involved lead to vast differences with respect to the pharmacokinetics and pharmacodynamics of these two compounds.

Table 12-14 Tubular Transport Maximums of Important Substances Reabsorbed from Renal Tubules

Substance	Value	Units
Glucose	320	mg/min
Phosphate	0.1	mm/min
Sulfate	0.06	mm/min
Amino acids	1.5	mm/min
Urate	15	mg/min
Plasma protein	30	mg/min
Hemoglobin	1	mg/min
Lactate	75	mg/min
Acetoacetate	Variable, ~30	mg/min

Table 12-15 Tubular Transport Maximums of Important Substances Secreted into Renal Tubules

Creatinine	16 mg/min
p-Aminohippurate	80 mg/min
Glomerular filtrate	125 mL/min
Flowing into the loops of Henle	45 mL/min
Flowing into the distal tubules	25 mL/min
Flowing into the collecting tubules	12 mL/min
Flowing into the urine	1 mL/min

Example 12-7

The Design and Development of Pegfilgrastim (PEG-rmetHuG-CSF, Neulasta)
The following is the abstract of a paper by Molineux116:

- The addition of a polyethylene glycol (PEG) moiety to filgrastim (rmetHu-G-CSF, Neupogen) resulted in the development of pegfilgrastim. Pegfilgrastim is a long-acting form of filgrastim that requires only once-per-cycle administration for the management of chemotherapy-induced neutropenia. Pegylation increases the size of filgrastim so that it becomes too large for renal clearance. Consequently, neutrophil-mediated clearance predominates in elimination of the drug. This extends the median serum half-life of pegfilgrastim to 42 hr, compared with between 3.5 and 3.8 hr for Filgrastim, though in fact the half-life is variable, depending on the absolute neutrophil count, which in turn reflects of the ability of pegfilgrastim to sustain production of those same cells. The clearance of the molecule is thus dominated by a self-regulating mechanism. Pegfilgrastim retains the same biological activity as filgrastim and binds to the same G-CSF receptor, stimulating the proliferation, differentiation, and activation of neutrophils. Once-per-chemotherapy cycle administration of pegfilgrastim reduces the duration of severe neutropenia as effectively as daily treatment with filgrastim. In clinical trials, patients receiving pegfilgrastim also had a lower observed incidence of febrile neutropenia than patients receiving filgrastim.

Table 12-16 Relative Concentrations of Substances in the Glomerular Filtrate and in the Urine

	Glomerular Filtrate (125 mL/min)		Urine (1 mL/min)		Urine/Concentration in Plasma
Substance*	Quantity/ min (mEq)	Concentration (mEq/liter)	Quantity/ min (mEq)	Concentration (mEq/liter)	(plasma clearance per minute)
Na^+	17.7	142	0.128	128	0.9
K^+	0.63	5	0.06	60	12
Ca^{2+}	0.5	4	0.0048	4.8	1.2
Mg^{2+}	0.38	3	0.015	15	5.0
Cl^-	12.9	103	0.144	144	1.3
HCO_3^-	3.5	28	0.014	14	0.5

$H_2PO_4^-$	0.25	2	0.05	50	25
HPO_4^{2-}	0.25	2	0.05	50	25
SO_4^{2-}	0.09	0.7	0.033	33	47
Glucose	125†	100†	0†	0†	0
Urea	33	26	18.2	1820	70
Uric acid	3.8	3	0.42	42	14
Creatinine	1.4	1.1	1.96	196	140
Inulin	–	–	–	–	125
PAH	–	–	–	–	585

*PAH = *p*-Aminohippurate.

†Units for glucose are mg for quantity and mg/dL for concentration.
From *Textbook of Medical Physiology*, 8th Ed., W. B. Saunders, Philadelphia, PA, 1991, p. 304.

Liver

The liver is an extremely important organ in biopharmaceutics and pharmacokinetics. After drug is absorbed from the gut, it potentially undergoes metabolism in the liver, secretion from the liver into bile, or reaches the systemic circulation intact. Of course, metabolites may also be secreted into bile, further metabolized, or make it into the systemic circulation. The liver is unique in its blood supply because it receives oxygenated blood from the hepatic artery and nutrient-rich but deoxygenated blood from the stomach, intestine, and spleen. The split between the two streams is approximately one fifth oxygenated and the remainder is nutrient rich. In most cases, the liver is thought of as containing lobules serviced/drained by a central vein in the center of each. However, the liver can functionally be thought of as being organized into acini, with two input streams, the hepatic artery and the portal vein, passing through the sinusoids and leaving through a terminal hepatic vein (Fig. 12-31). The sinusoids are lined with unique epithelial cells called hepatocytes. The hepatocytes have distinct polarity. Their basolateral side is lined with microvilli to take up nutrients, proteins, and xenobiotics. The apical side forms the

canalicular membranes through which bile components are secreted. It is the hepatocytes that perform functions essential for life. These functions include the production of bile and its carriers (bile acids, cholesterol, lecithin, phospholipids), the synthesis of essential serum proteins (albumin, carrier proteins, coagulation factors, many hormonal and growth factors), the regulation of nutrients and metabolism, and the conjugation of lipophilic compounds (bilirubin, cations, drugs) for excretion in the bile or the urine.117

Key Concept

Importance of Equilibria in Adme

It is very important to realize that ADME is filled with a number of dynamic equilibria that occur in a variety of organs and tissues. The net result of all of these processes is the observed plasma concentration versus time profiles. From the moment that a drug enters the body, the drug molecule strives to be in equilibrium between the tissues and blood. Other equilibria that occur are blood: active site concentration, parent: metabolite, blood: bile, blood: urine, and bound drug: unbound drug. These are just some of the equilibria that occur and the ones that play an important role in the blood or plasma concentration level measured in the study of pharmacokinetics. The rates of absorption, distribution, and elimination control drug–blood concentrations and are discussed further in the next section.

Earlier in the chapter, there was a discussion about metabolizing enzymes and transporters. These two systems are found in abundance within liver and play a major role in drug distribution and elimination. The liver is a major site of metabolism in the body, and it works with the kidney in removing waste from the blood stream. As mentioned previously, there are

three phases of drug metabolism. Phases I and II are involved in the biotransformation of drugs and, therefore, are strictly related to the ADME process of metabolism. In addition to metabolism, the liver also plays an important role in drug and metabolite excretion out of the body. This process is also known as phase III metabolism and involves the transport of drugs and metabolites out of cells by means of membrane transporters. In the liver this process is known as enterohepatic cycling and occurs by biliary excretion from the gall bladder and intestinal reabsorption of a solute (i.e., drug or metabolite), sometimes with hepatic conjugation (see phase II discussion earlier in the chapter) and intestinal deconjugation. Therefore, the liver's role in drug distribution or excretion occurs in conjunction with the intestine and the gall bladder. Drug is absorbed from the intestine and enters the liver, where the drug or metabolites can be secreted into the bile of the gall bladder. The gall bladder secretes bile, usually in conjunction with meals, and the drugs and metabolites reenter the intestinal tract. Therefore, the biliary “system” contributes to excretion to the degree that drug is not reabsorbed from the GI tract. In other words, the drug or metabolite is considered eliminated from the body as long as it is not reabsorbed from the intestine. On the other hand, the biliary system also contributes to drug distribution to the extent that intact secreted drug is reabsorbed from the intestine. In a fairly unique set of circumstances, in the enterohepatic cycling system even metabolized drug, usually a terminal step, can be reversed, adding to the distribution phase of drug disposition. For example, it has been shown that phase II metabolism (i.e., conjugation), particularly with glucuronic acid, typically leads to biliary excretion. Drug conjugates secreted into the intestine also undergo enterohepatic cycling when they are hydrolyzed and the drug becomes available for reabsorption. Metabolism is usually considered to be part of the elimination process (i.e., permanent removal from the body). However, the process of enterohepatic recycling could

also be considered as distribution because the metabolism step is reversible and drug can be absorbed over and over again into the body. Once again, secreted conjugates that are not converted back to the original drug and are excreted in the feces are considered to be “eliminated.”

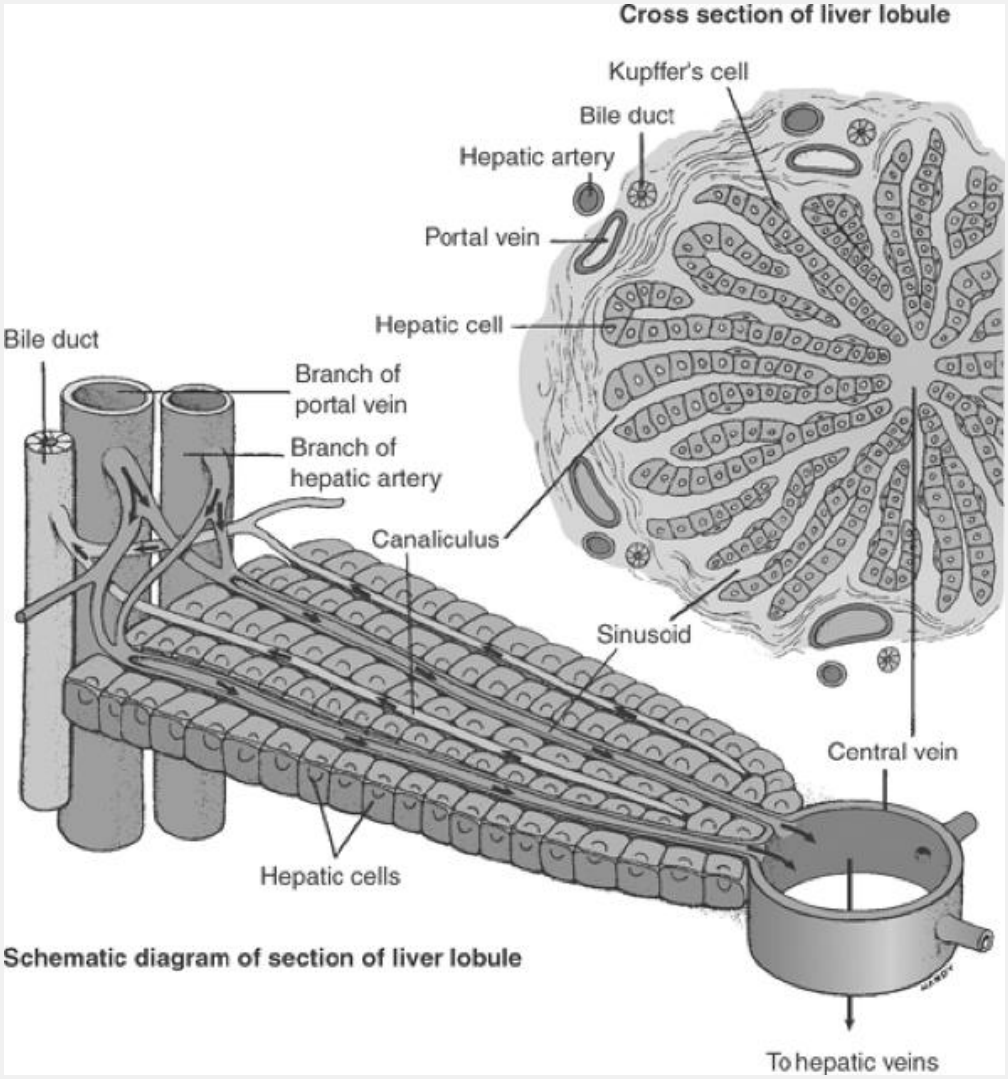


Fig. 12-31. A section of liver lobule showing the location of hepatic veins, hepatic cells, liver sinusoids, and branches of the portal vein and hepatic artery. (From S. C. Smeltzer and B. G Bare, *Textbook of Medical-Surgical Nursing*, 9th Ed., Lippincott Williams & Wilkins, Philadelphia, 2000.)

Drugs and their metabolites that are extensively excreted in bile are transported across the biliary epithelium against a concentration gradient requiring active secretory transport by membrane transporters. Secretory transport may approach an upper limit at high plasma concentrations of a drug, and substances with similar physicochemical properties may compete for excretion via the same mechanism. The drug transporters responsible for this behavior are those found in the liver and will not be reviewed here. Factors affecting biliary excretion include drug characteristics (chemical structure, polarity, and molecular size), transport across sinusoidal plasma membrane and cannicular membranes, biotransformation, and possible reabsorption from intrahepatic bile ductules. Intestinal reabsorption to complete the enterohepatic cycle may depend on hydrolysis of a drug conjugate by gut bacteria. Larger drugs (i.e., with a molecular weight greater than 300–500 g/mole) with both polar and lipophilic groups are more likely to be excreted in bile. Smaller molecules are generally excreted only in negligible amounts. The renal and hepatic excretion pathways are complementary to each other. In other words, a compound with high renal excretion, which is typical for a low-molecular-weight compound, will have low

biliary excretion and vice versa.[118,119] These values can be species dependent. For example, the excretion of organic anions greater than 500 g/mole is found to occur in the bile in humans, whereas the values are slightly lower for rats, guinea pigs, and rabbits, ranging from 325 to 475 g/mole.[120] Additionally, compounds which are usually excreted into the bile are more lipophilic; contain charged groups, such as carboxylic acid, sulfonic acid, or quaternary ammonium groups, are highly protein-bound anions bound to albumin, whereas cations are mainly bound to orosomucoid or a1-acid glycoprotein and have a high molecular weight. The opposite is true for substrates of renal excretion. These broad classifications should serve only as a guide. Levofloxacin, ofloxacin (Floxin), and ciprofloxacin are broad-spectrum antimicrobial agents for oral administration and are part of a class of fluorinated carboxyquinolones. These drugs are primarily excreted in the urine, yet they are carboxylic acids. For example, only 4% to 8% of Floxin (molecular weight 361.4) is excreted in the feces,[121] which would disprove the rule of carboxylic acids always being excreted in the feces.

Finally, dose dependencies are expected for enterohepatic circulation because membrane transporters play a major role and saturation at high doses or inhibition by competing substances may occur. This could lead to excretion by an alternative pathway or reduced drug excretion and significantly higher blood levels and, possibly, toxicity. In general, enterohepatic cycling may prolong the pharmacologic effect of certain drugs and drug metabolites. The pharmacokinetics (i.e., apparent volume of distribution and clearance) of a drug that undergoes enterohepatic cycling may be substantially altered. Enterohepatic cycling is also associated with the occurrence of multiple drug–blood level peaks and a longer apparent half-life in the plasma concentration–time profile. Of particular importance is the potential amplifying effect of enterohepatic variability in defining differences in the bioavailability. Bioavailability is also affected by the extent of intestinal absorption, gut-wall P-glycoprotein efflux, and gut-wall metabolism. Recently, there has been a considerable increase in our understanding of the role of transporters, gene expression of intestinal and hepatic enzymes, and hepatic zonation. Drugs, disease, and genetics may result in induced or inhibited activity of transporters and metabolizing enzymes. Reduced expression of one transporter, for example, hepatic canalicular multidrug resistance-associated protein-2 (MRP2), is often associated with enhanced expression of others, for example, the usually quiescent basolateral efflux MRP3, to limit hepatic toxicity.

Example 12-8
Biliary Excretion

Although the first impression about biliary excretion may be that it plays a role in orally absorbed medications, this example shows that drugs introduced into the body by other routes (e.g., intravenously) may also be excreted into the bile. These drugs may have poor oral absorption properties, so enterohepatic cycling is probably minimal for them. Both P-glycoprotein and MRP2, ATP-dependent membrane transporters, exist in a variety of normal tissues and play important roles in the disposition of various drugs. Sugie et al.[122] studied the contribution of P-glycoprotein and/or MRP2 to the disposition of azithromycin in rats. The disappearance of azithromycin from plasma after intravenous administration was significantly delayed in rats treated with intravenous injection of cyclosporine, a P-glycoprotein inhibitor, but was normal in rats pretreated with an intraperitoneal injection of erythromycin, a CYP3A4 inhibitor. When rats received an infusion of azithromycin with cyclosporine and probenecid, an MRP2 inhibitor, a significant decrease in the steady-state biliary clearance of azithromycin of 5% and 40% of the corresponding control values was observed, respectively. However, neither inhibitor altered the renal clearance of azithromycin, suggesting the lack of renal tubular secretion of azithromycin. Tissue distribution experiments showed that azithromycin is distributed largely into liver, kidney, and lungs, whereas neither inhibitor altered the tissue-to-plasma concentration ratio of azithromycin. Significant reduction in the biliary excretion of azithromycin was observed in Eisai hyperbilirubinemic rats, which have a hereditary deficiency in MRP2. These results suggest that azithromycin is a substrate for both P-glycoprotein and MRP2 and that the biliary and intestinal excretion of azithromycin is mediated via these two drug transporters.

Introductory Pharmacokinetics

Introduction

This section is not meant to replace pharmacokinetics textbooks, but rather to link the basic biopharmaceutical concepts introduced in this chapter to the simplest pharmacokinetic models, parameters, and behavior that relate to drug input and output into/from the body. We will cover the correlation

between in vitro and in vivo data using compartment models, permeability, and intrinsic clearance. Pharmacokinetics is the kinetic study of the ADME of drugs in the body. The compartment model assumes that the body is a simplified system of compartments and that drug transfer and elimination rates between/from compartments occur by a first-order process. Other transfer and elimination functions (e.g., nonlinear functions) have also been used in compartment models but will not be the focus of this chapter. A one-compartment model is the simplest and best-studied pharmacokinetic model even though few drugs truly follow these simplified kinetics. A number of in vitro and in situ models have been employed to predict in vivo drug absorption, including the parallel artificial membrane permeability assay, human colon carcinoma cells (Caco-2), Madin–Darby canine kidney (MDCK) cells, Ussing chamber using animal intestinal tissues, and in situ intestinal perfusion. The permeability data from these models, such as apparent permeability, P_a, and effective permeability, P_{eff}, can be used in the calculation of an absorption rate constant, K_a, in the one-compartment model. P_a and P_{eff} are typically synonymous terms and are considered "lumped" permeability coefficients because they represent a measure of all of the transport and metabolism processes occurring at a particular time. In other words, the apparent or effective permeability is the net permeability due to permeability by all pathways in the intended direction but also accounting for loss due to degradation, metabolism, binding, or transport in the opposite direction. In this section, we will also link the basic biopharmaceutical processes to the elimination rate constant, K_{el}, which can be calculated using the intrinsic clearance, CL_{int}, from in vitro metabolism experiments. The basic assumptions for each type of correlation will be listed and explained in this section with brief introduction of the one compartment model.

Key Concept

Apparent Volume of Distribution5

The volume of fluid into which a drug appears to be distributed or diluted is called the apparent volume of distribution (i.e., the fluid volume required to contain the drug in the body at the same concentration as in plasma). This parameter provides a reference for the plasma concentration expected for a given dose and for the dose required to produce a given concentration. However, it provides little information about the specific pattern of distribution. Each drug is uniquely distributed in the body. Some drugs go into fat, others remain in the extracellular fluid, and still others are bound avidly to specific tissues, commonly liver or kidney. Many acidic drugs (e.g., warfarin, salicylic acid) are highly protein bound and thus have a small apparent volume of distribution. Many basic drugs (e.g., amphetamine, meperidine) are avidly taken up by tissues and thus have an apparent volume of distribution larger than the volume of the entire body.

Compartmental Models and K_a/K_{el}

In the first model, we will not consider drug absorption but rather drug elimination. In the one-compartment model with rapid intravenous injection, a drug distributes into the body according to one-compartment-model "behavior." In other words, drug distribution in a one-compartment model is complete and instantaneous. The drug is eliminated by a first-order process,

$$\frac{dX}{dt} = -k_{el}X \qquad (12\text{–}14)$$

where X represents the amount of drug in the body at time t after administration and k_{el} is the elimination rate constant. Integration of equation (12-14) gives the following expression:

$$X = X_0 E^{-k_{el}t} \quad \text{or} \quad \log X = \log X_0 - \frac{k_{el}t}{2.303} \qquad (12\text{–}15)$$

where X_0 is the initial drug dose. The elimination rate constant, k_{el}, can be calculated from two fundamental pharmacokinetic parameters, total body clearance, CL_t, and apparent volume of distribution, V_d:

$$k_{el} = \frac{CL_t}{V_d} \qquad (12\text{–}16)$$

CL_t is defined as the volume of plasma or blood that is completely cleared of drug per unit time:

$$CL_t = \frac{-\left(\frac{dX}{dt}\right)}{C} \qquad (12\text{–}17)$$

V_d is a theoretical volume factor relating the amount of drug in the body and the concentration of drug in the plasma or blood:

$$V_d = \frac{X}{C} \qquad (12\text{–}18)$$

where C is the drug concentration in plasma or blood.

The elimination rate constant represents the sum of two processes:

$$k_{el} = k_m + k_e \qquad (12\text{–}19)$$

where k_m is the elimination rate constant by metabolism and k_e is the elimination rate constant by excretion. If the metabolism is dominant over excretion during elimination, the elimination constant can be replaced by k_m. If (*a*) the liver is the major metabolic organ, (*b*) hepatic drug metabolism shows no enzymatic saturation, and (*c*) the intrinsic clearance, CL_{int}, is much smaller than liver blood flow, Q, then total body clearance, CL_t, values, calculated with the one-compartment

P.296

intravenous model, correlates well with the intrinsic clearance, CL_{int}.

Key Concept

Drug Clearance and Organ Blood Flow

Two situations arise that show the relationship between drug clearance and liver blood flow. Organ clearance, CL, is given by $CL = QCL_{int}/(Q + CL_{int})$. In the case when Q much greater than CL_{int}, organ $CL = CL_{int}$. This occurs for drugs such as antipyrine, barbiturates, antiepileptics, and cumarin derivatives. In the second case, when Q is much less than CL_{int}, organ $CL = Q$. This occurs for various analgesics, tricyclic antidepressants, and beta-blockers. Protein binding may also have an effect, so, considering the free fraction of drug, f_B, one should use $f_B \cdot CL_{int}$ instead of CL_{int} in this situation. Liver blood flow in humans is 20.7 mL/min/kg or 1450 mL/min for a 70-kg person.

Example 1: Q much greater than CL_{int}. Antipyrine is negligibly bound to plasma proteins, eliminated exclusively through hepatic metabolism, and more than 99% of a given dose is excreted into urine as metabolites.123 The intrinsic clearance of antipyrine is 12.8 mL/min/person, which was calculated from in vitro intrinsic clearance, 1.62×10^{-4} mL/min/mg protein,118 and total liver microsomal protein, 7.88×10^{-4} mg/person.90 Human hepatic blood flow is reported as 1450 mL/min/70-kg person.124 In vivo systemic clearance of antipyrine is reported as 13.5 (9.3–22.8) mL/min/person in patients with liver cirrhosis and 49.3 (31.1–103) mL/min/person in healthy individuals. Calculated in vitro intrinsic clearance of antipyrine is close to the values for patients with liver cirrhosis, probably because in vitro experiments were done with liver samples obtained from patients who underwent partial hepatectomy.

Example 2: CL_{int} much greater than Q. In vivo systemic clearance propranolol is 1.21±0.15 liter/min for (+)-propranolol and 1.03±0.12 liter/min for (-)-propranolol.125The intrinsic clearance of racemic propranolol was 4180 mL/min/person, which was calculated from in vitro intrinsic clearance, 0.053 mL/min/mg protein,126 and total liver microsomal protein, 7.88 ×

10^4 mg/person.90 Human hepatic blood flow is 1450 mL/min/70-kg person as shown in*Example–1*.

In a one-compartment model with a drug absorption step such as oral administration, the drug enters the body by a first-order process. In this case, absorption is slower than the instantaneous injection that occurs during intravenous administration. Distribution of the absorbed drug molecules is instantaneous and elimination occurs according to one-compartment-model behavior as described previously:

$$\frac{dX_a}{dt} = -k_a X_a \qquad (12\text{–}20)$$

$$\frac{dX}{dt} = k_a X_a - k_{el} X \qquad (12\text{–}21)$$

where X_a is the amount of drug in the absorption site at time t after administration and k_a is the absorption rate constant. Integration of equations **(**12-20**)** and **(**12-21**)** gives the following expression:

$$X = \frac{k_a F X_0}{V_d(k_a - k)}(e^{-k_{el}t} - e^{-k_{el}t}) \qquad (12\text{–}22)$$

where F is the fraction of the dose, X_0, absorbed following oral administration.
The absorption rate constant as well as the elimination rate constant can be calculated from in vitro or in situ data in the oral absorption model. The absorption rate constant, k_a, can be related to the effective permeability,

$$k_a = \frac{SA \cdot P_{eff}}{V} = \frac{2P_{eff}}{r} \qquad (12\text{–}23)$$

where SA is the surface area, V is the volume of the intestinal segment, and r is the intestinal radius. If one assumes that a cylinder can be used to estimate the intestinal shape, then the SA/Vratio simplifies to 2/r. Others have examined the effect of other, more realistic intestinal geometries.127 However, for the purposes of this example, assuming cylindrical geometry keeps the mathematics straightforward.
One can “build” a model of the human body absorption and disposition of drugs by using compartmental models. Each compartment can represent an organ, tissue, or set of organs or tissues (Fig. 12-23). For example, sometimes a two-compartment model is appropriate. Here, fast-perfused and slow-perfused tissues are grouped together into separate compartments. Typically, when organs or tissues are lumped together it is difficult to examine the behavior of specific individual organ systems. When the goal is to examine specific organ systems, PBPK models are constructed (Fig. 12-23). Using flow rates (e.g., blood flow, intestinal transit), volumes, and input and output rate constants, one can construct a PK model of an organ system. The PBPK models have a long and rich history that is covered in much more detail in a course in pharmacokinetics. We leave those details to those courses.

Bioavailability4

Introduction

The words absorption and bioavailability are used in many ways. The purpose of this section is to introduce the student to the biopharmaceutical basis and practical meanings of the word bioavailability. “Bioavailability,” as defined by the U.S. Food and Drug Administration in the *Code of Federal Regulations* (21 CFR 320.1[a]), means the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. Because pharmacologic response is generally related to the concentration of drug at the receptor site, the availability of a drug from a dosage form is a critical element of a drug product's clinical efficacy. However, drug concentrations usually cannot

be readily measured directly at the site of action. Therefore, most bioavailability studies involve the determination of drug concentrations in the blood or the urine. This is based on the premise that the drug at the site of action is in equilibrium with the drug in the blood. It is therefore possible to obtain an indirect measure of drug response by monitoring drug levels in the blood or the urine. Thus, bioavailability is concerned with how quickly and how much of a drug appears in the blood after a specific dose is administered. The bioavailability of a drug product often determines the therapeutic

efficacy of that product because it affects the onset, intensity, and duration of therapeutic response of the drug. In most cases one is concerned with the extent of absorption of drug (i.e., the fraction of the dose that actually reaches the blood stream) because this represents the “effective dose” of a drug. This is generally less than the amount of drug actually administered in the dosage form. In some cases, notably those where acute conditions are being treated, one is also concerned with the rate of absorption of a drug because rapid onset of pharmacologic action is desired. Conversely, these are instances where a slower rate of absorption is desired, either to avoid adverse effects or to produce a prolonged duration of action.

*Causes of Low Bioavailability*5

When a drug rapidly dissolves and readily crosses membranes, absorption tends to be complete, but absorption of orally administered drugs is not always complete. Before reaching the vena cava, a drug must move down the GI tract and pass through the gut wall and liver, common sites of drug metabolism; thus, a drug may be metabolized (first-pass metabolism) before it can be measured in the systemic circulation. Many drugs have low oral bioavailability because of extensive first-pass metabolism. For such drugs (e.g., isoproterenol, norepinephrine, testosterone), extraction in these tissues is so extensive that bioavailability is virtually zero. For drugs with an active metabolite, the therapeutic consequence of first-pass metabolism depends on the contributions of the drug and the metabolite to the desired and undesired effects. Intestinal secretion of drugs by transporters such as MRP2 and P-gp and enterohepatic recirculation may also cause low oral bioavailability. Low bioavailability is most common with oral dosage forms of poorly water-soluble, slowly absorbed drugs. More factors can affect bioavailability when absorption is slow or incomplete than when it is rapid and complete, so slow or incomplete absorption often leads to variable therapeutic responses. Insufficient time in the GI tract is a common cause of low bioavailability. Ingested drug is exposed to the entire GI tract for no more than 1 to 2 days and to the small intestine for only 2 to 4 hr. If the drug does not dissolve readily or cannot penetrate the epithelial membrane (e.g., if it is highly ionized and polar), time at the absorption site may be insufficient. In such cases, bioavailability tends to be highly variable as well as low. Age, gender, activity, genetic phenotype, stress, disease (e.g., achlorhydria, malabsorption syndromes), or previous GI surgery can affect drug bioavailability. Reactions that compete with absorption can reduce bioavailability. They include complex formation (e.g., between tetracycline and polyvalent metal ions), hydrolysis by gastric acid or digestive enzymes (e.g., penicillin and chloramphenicol palmitate hydrolysis), conjugation in the gut wall (e.g., sulfoconjugation of isoproterenol), adsorption to other drugs (e.g., digoxin and cholestyramine), and metabolism by luminal microflora.

Chapter Summary

A shift has occurred in the pharmaceutical sciences from focusing solely on the physical and chemical aspects of pharmacy such as dissolution, solubility, and compaction physics to the integration of these important disciplines with the biopharmaceutical sciences. The purpose of this chapter was to provide the student with a biopharmaceutical foundation for studying the contemporary pharmaceutical sciences. At this point you should be able to define ADME and understand the differences between the two possibilities for Ds (distribution and disposition) and Es (excretion and elimination) in ADME. Two major membrane transporter superfamilies play an important role in ADME and if they work together (concerted drug transport), drugs and metabolites can be moved into or out of the body with great efficiency. Phase 1, 2, and “3” drug metabolism was also introduced in this chapter. The student should have a good understanding of the concepts of inhibition and induction as they relate to drug transporters, metabolizing enzymes, ADME, and pharmacokinetics. Graphical representations of the rates of absorption, disposition, metabolism, and elimination and blood (plasma) level versus time curves were also introduced. The very important concept of bioavailability was introduced. Finally, various organ systems were covered to give the student a better understanding of the complexity of ADME and the interplay of molecular, cellular, and organ level functions on pharmacokinetics.

Practice problems for this chapter can be found at thePoint.lww.com/Sinko6e.

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Chapter Legacy

Fifth Edition: published as Chapter 14 (Biopharmaceutics). Updated by Patrick Sinko.
Sixth Edition: published as Chapter 12 (Biopharmaceutics). Updated by Patrick Sinko.