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Absorption, Distribution, Metabolism, and Elimination

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The four key physiological processes that govern the time course of drug fate in the body are absorption, distribution, metabolism, and elimination, the so-called *ADME processes*. Pharmacokinetics, the study of the time course of drug concentrations in the body, provides a means of quantitating ADME parameters. When applied to a clinical situation, pharmacokinetics provides the practitioner with a useful tool to design optimally beneficial drug dosage schedules for each individual patient. In the research and premarketing phase of drug development, it is an essential component in establishing effective yet safe dosage forms and regimens. An understanding of pharmacokinetic principles allows more rational therapeutic decisions to be made. In food animals, pharmacokinetics provides the conceptual underpinnings for understanding and utilizing the withdrawal time to prevent violative drug residues from persisting in the edible tissues of food-producing animals. A working knowledge of this discipline provides the framework upon which many aspects of pharmacology can be integrated into a rational plan for drug usage.

An Overview of Drug Disposition

To fully appreciate the ADME processes governing the fate of drugs in animals, the various steps involved must be defined and ultimately quantitated. The processes relevant to a discussion of the absorption and disposition of a drug administered by the intravenous (IV), intramuscular (IM), subcutaneous (SC), oral (PO), or topical (TOP) routes are illustrated in Figure 2.1. The normal reference point for pharmacokinetic discussion and analysis is the concentration of free, non-protein-bound drug dissolved in the serum (or plasma), because this is the body fluid that carries the drug throughout the body and from which samples for drug analysis can be readily and repeatedly collected. For the majority of drugs studied, concentrations in the systemic circulation are in equilibrium with the extracellular fluid of well-perfused tissues; thus, serum or plasma drug concentrations generally reflect extracellular fluid drug concentrations.

A fundamental axiom of using pharmacokinetics to predict drug effect is that the *drug must be present at its site of action in a tissue at a sufficient concentration for a specific period of time to produce a pharmacological effect*. Since tissue concentrations of drugs are reflected by extracellular fluid and thus serum drug concentrations, a pharmacokinetic analysis of the disposition of drug in the scheme outlined in Figure 2.1 is useful to assess the activity of a drug in the in vivo setting.

This conceptualization is especially important in veterinary medicine where species differences in any of the ADME processes may significantly affect the extent and/or time course of drug absorption and disposition in the body. By dividing the overall process of drug fate into specific phases, this relatively complex situation can be more easily handled. It is the purpose of this chapter to overview the physiological basis of absorption, distribution, metabolism, (biotransformation) and elimination. This will provide a basis for the chapter on pharmacokinetics that will deal with quantitating these processes in more detail.

Despite the myriad of anatomical and physiological differences among animals, the biology of drug absorption and distribution, and in some cases even elimination, is very similar in that it involves drug molecules crossing a series of biological membranes. As illustrated in Figure 2.2, these membranes may be associated with either several layers of cells (tissue) or a single cell, and both living and dead protoplasm may be involved. Despite the different biochemical and morphological attributes of each of these membranes, a unifying concept of biology is the basic similarity of all membranes, whether they be tissue, cell, or organelle. Although the specific biochemical components may vary, the fundamental organization is the same. This fact simplifies the understanding of the major determinants of drug absorption, distribution, and excretion.

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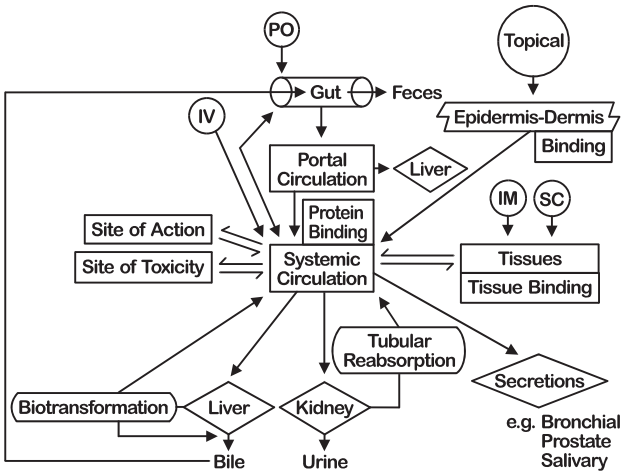

Figure 2.1 Basic schema by which drug is absorbed, distributed, metabolized, and excreted from the body. These processes are those that form the basis for developing pharmacokinetic models.

These membrane barriers often directly or indirectly define the nature of compartments or other mathematical modules in pharmacokinetic models. Biological spaces are defined by the restrictions on drug movement imposed by these barriers. The most effective barriers are those that protect the organism from the external environment. These include the skin as well as various segments of the gastrointestinal and respiratory tract, which also protect the internal physiological milieu from the damaging external environment. However, the gastrointestinal and respiratory barriers are modified deep within the body to allow for nutrient and gas exchange vital for life. The interstitial fluid is a common compartment through which any drug must transit, either after absorption on route to the blood stream or after delivery by blood to a tissue on route to a cellular target. Capillary membranes interfacing with this interstitial fluid compartment are relatively porous due to the fenestrae that allow large molecules to exchange between tissues and blood. Membranes define homogeneous tissue compartments and membranes must be traversed in all processes of drug absorption and disposition.

All cellular membranes appear to be primarily lipid bilayers into which are embedded proteins that may reside on either surface (intra- or extracellular) or traverse the entire structure. The lipid leaflets are arranged with hydrophilic (polar) head groups on the surface and hydrophobic (nonpolar) tails forming the interior. The specific lipid composition varies widely across different tissues and levels of biological organization. The location of the proteins in the lipid matrix is primarily a consequence of their hydrophobic regions residing in the lipid interior and their hydrophilic and ionic regions occupying the surface. This is thermodynamically the most stable configuration. Changes in the fluidity of the lipids alter protein conformations, which then may modulate their activity. This was one of the mechanisms of action proposed for gaseous anesthetics, although recently specific protein receptors have now been suggested. In some cases, aqueous channels form from integral proteins that traverse the membrane. In other cases, these integral proteins may actually be enzymatic transport proteins that function as active or facilitative transport systems. The primary pathway for drugs to cross these lipid membranes is by passive diffusion through the lipid environment.

Thus, in order for a drug to be absorbed or distributed throughout the body, it must be able to pass through a lipid membrane on some part of its sojourn through the body. In some absorption sites and in many capillaries, fenestrated pores exist, which allow some flow of small molecules. This is contrasted to some protected sites of the body (e.g., brain, cerebral spinal fluid) where additional membranes (e.g., glial cells) may have to be traversed before a drug arrives at its target site. These specialized membranes could be considered a general adaptation to further shelter susceptible tissues from hostile lipophilic chemicals. In this case, drug characteristics that promote transmembrane diffusion would favor drug action and effect (again unless specific transport systems intervene).

This general phenomenon of the enhanced absorption and distribution of lipophilic compounds is a unifying tenet that runs throughout the study of drug fate. The body's elimination organs can also be viewed as operating along a somewhat similar principle. The primary mechanism by which a chemical can be excreted from the body is by becoming less lipophilic and more hydrophilic, the latter property being required for excretion in the aqueous fluids of the urinary or biliary systems. When a hydrophilic or polar drug is injected into the bloodstream, it will be minimally distributed and rapidly excreted by one of these routes. However, if a compound's lipophilicity evades this easy excretion, the

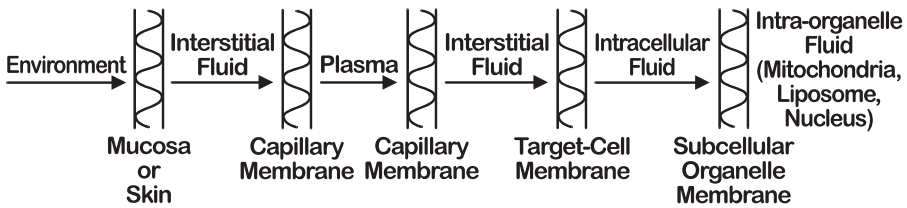

Figure 2.2 Illustration of how absorption, distribution, and excretion is essentially a journey of the drug through various lipoidal membrane barriers.

liver and other organs may metabolize it to less lipophilic and more hydrophilic metabolites that have a restricted distribution (and thus reduced access to sites for activity) in the body and can be more readily excreted. This basic tenet runs throughout all aspects of pharmacology and is a useful concept to predict effects of unknown compounds.

Drug Passage Across Membranes

Considerable evidence exists that lipid-based membranes are permeable to nonpolar lipid-soluble compounds and polar water-soluble compounds with sufficient lipid solubility to diffuse through the hydrophobic regions of the membrane. The rate of diffusion of a compound across a membrane is directly proportional to its concentration gradient across the membrane, lipid\water partition coefficient, and diffusion coefficient. This can be summarized by Fick's law of diffusion in Equation 2.1:

$$\text{Rate of diffusion (mg/sec)} = \frac{\text{D (cm/sec) P}}{\text{h (cm)}} \left(X_1 - X_2\right) \text{(mg)} \qquad (2.1)$$

where D is the diffusion coefficient for the specific penetrant in the membrane being studied, P is the partition coefficient for the penetrant between the membrane and the external medium, h is the thickness or actual length of the path by which the drug diffuses through the membrane, and $X_1 - X_2$ is the concentration gradient (ΔX) across the membrane. The diffusional coefficient of the drug is a function of its molecular size, molecular conformation and solubility in the membrane milieu, and degree of ionization. The partition coefficient is the relative solubility of the compound in lipid and water that reflects the ability of the penetrant to gain access to the lipid membrane. Depending on the membrane, there is a functional molecular size and/or weight cutoff that prevents very large molecules from being passively absorbed across any membrane. When the rate of a process is dependent upon a rate constant (in this case [DP/h] often referred to as the permeability coefficient P) and a concentration gradient, a linear or first-order kinetic process is evident (see Chapter 3 for full discussion). In membrane transfer studies, the total flux of drug across a membrane is dependent on the area of membrane exposed; thus the rate above is often expressed in terms of cm^2. If the lipid : water partition coefficient is too great, depending on the specific membrane, the compound may be sequestered in the membrane rather than traverse it.

Evidence also exists that membranes are more permeable to the nonionized than the ionized form of weak organic acids and bases. If the nonionized moiety has a lipid : water partition coefficient favorable for membrane penetration, it will ultimately reach equilibrium on both sides of the membrane. The ionized form of the drug is completely prevented from crossing the membrane because of its low lipid solubility. The amount of the drug in the ionized or nonionized form depends upon the pKa (negative logarithm of the acidic dissociation constant) of the drug and the pH of the medium on either side of the membrane (e.g., intracellular versus extracellular fluid; gastrointestinal versus extracellular fluid). Protonated weak acids are nonionized (e.g., COOH) while protonated weak bases are ionized (e.g., NH_3^+). If the drug has a fixed charge at all pHs encountered inside and outside of the body (e.g., quarternary amines, aminoglycoside antibiotics), they will never cross lipid membranes by diffusion. This would restrict both their absorption and distribution and generally lead to an enhanced rate of elimination. It is the nonionized form of the drug that is governed by Fick's Law of Diffusion and described by Equation 2.1 above. For this equation to predict the movement of a drug across membrane systems in vivo, the relevant pH of each compartment must be considered relative to the compound's pKa; otherwise, erroneous predictions will be made.

When the pH of the medium is equal to the pKa of the dissolved drug, 50% of the drug exists in the ionized state and 50% in the nonionized, lipid-soluble state. The ratio of nonionized to ionized drug is given by the Henderson–Hasselbalch equation (Equations 2.2 and 2.3).For acids:

$$\text{pKa} - \text{pH} = \log\left[(\text{HAcid})^0/(\text{HAcid})^-\right] \qquad (2.2)$$

For bases:

$$\text{pKa} - \text{pH} = \log\left[(\text{H Base})^+/(\text{H Base})^0\right] \qquad (2.3)$$

These equations are identical as they involve the ratio of protonated (H) to nonprotonated moieties. The only difference is that for an acid, the protonated form $(\text{H Acid})^0$ is neutral while for a base, the protonated form $(\text{H Base})^+$ is ionized. This topic is also presented in Chapter 5 (Equations 5.1 to 5.5; Figure 5.3).

As can be seen by these equations, when the pH is one unit less or one unit more than the pKa for weak bases or acids, respectively, the ratio of ionized to nonionized is 10. Thus each unit of pH away from the pKa results in a tenfold change in this ratio. This phenomenon allows for a drug to be differentially distributed across a membrane in the presence of a pH gradient, an effect that often dwarfs that obtainable by increasing dose to increase drug delivery to a specific tissue. The side of the membrane with the pH favoring ionized drug (high pH for an acidic drug; low pH for an alkaline drug) will tend to have higher total (ionized plus nonionized) drug concentrations. This pH partitioning results in so-called "ion trapping" in the area where the ionized drug predominates. Figure 2.3 illustrates this concept with an organic

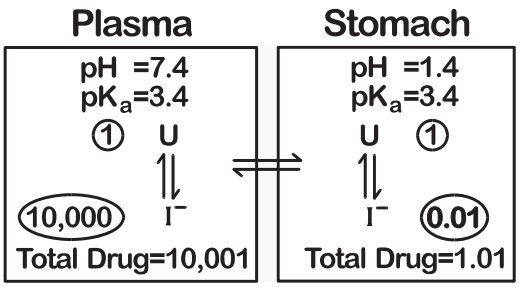

Figure 2.3 The phenomenon of pH partitioning and ion trapping of a weak acid.

acid of pKa = 3.4 partitioning between gastric contents of pH = 1.4 and plasma of pH = 7.4. Assuming that the nonionized form of the drug (U) is in equilibrium across the membrane, then, according to Equations 2.1 and 2.2, there will be a 100-fold (log 2; 3.4–1.4) difference on the gastric side and a 10,000-fold (log 4; 7.4–3.4) difference on the plasma side of the membrane, for a transmembrane concentration gradient of total drug (U plus I) equal to 10,001/1.01. Note that the unionized concentration on both sides of the membrane are in equilibrium. It is the total drug concentrations that are different. In this case, the gradient is generated by the difference in pH across an ion-impermeable barrier generated by the local milieu.

Such a gradient would greatly favor the absorption of this weak acid across the gastrointestinal tract into plasma. This is the situation that exists for weak acids such as penicillin, aspirin, and phenylbutazone. In contrast, a weak base would tend to be trapped in this environment and thus minimal absorption would occur. Examples of such weak bases are morphine, phenothiazine, and ketamine. Specific active transport systems may counter these predictions (e.g., β-lactam transporters in intestines), as well as the extreme surface area of the small intestines compared to gastric mucosa, which generally favors absorption of most drugs in the small intestines. With the weakly basic strychnine, pH-dependent absorption is toxicologically significant. If strychnine were placed into the strongly acidic stomach, no systemic toxicity would be observed. However, if the stomach were then infused with alkali, most of this base would become nonionized, readily absorbed, and lethal. In summary, weak acids are readily absorbed from an acid environment and sequestered in an alkaline medium. In contrast, weak bases are absorbed in an alkaline environment and trapped in an acidic environment.

This pH partitioning phenomenon is not only important in understanding absorption (as illustrated above), but also in any situation where the pH of fluid compartments across a biological membrane is different. It will occur for a drug distributing from plasma (pH = 7.4) to milk (pH = 6.5–6.8), to cerebrospinal fluid (pH = 7.3), or to intracellular sites (pH = 7.0). Thus weakly acidic drugs will tend not to distribute into the milk after systemic distribution (e.g., penicillin), while weakly basic drugs (e.g., erythromycin) will. If a disease process alters the pH of one compartment (e.g., mastitis), the normal equilibrium ratio will also be perturbed. In mastitis, where pH may increase almost one unit, this preferential distribution of basic antibiotics will be lost. The relatively acidic pH of cells relative to plasma is responsible for the relatively large tissue distribution seen with many weakly basic drugs (e.g., morphine, amphetamine). Similarly, in the ruminant, many basic drugs tend to distribute into the rumen, resulting in distribution volumes much larger than those in monogastrics. In fact, a drug that distributes into this organ may then undergo microbial degradation resulting in its elimination from the body.

This phenomenon is also very important for the passive tubular reabsorption of weak acids and bases being excreted by the kidney. For carnivores with acidic urine relative to plasma, weak acids tend to be reabsorbed from the tubules into the plasma while weak bases tend to be preferentially excreted. This principle has been applied to the treatment of salicylate (weak acid) intoxication in dogs where alkaline diuresis promotes ion trapping of the drug in the urine and hence its rapid excretion. Disease-induced changes in urine pH will likewise alter the disposition of drugs sensitive to this phenomenon.

Movement across the fenestrated capillaries of the body from plasma to tissue areas generally allows movement of most drugs. In these cases, relatively small molecules (molecular weights <1,000) can pass through independent of their lipid solubility, but larger molecules are excluded. In all of these scenarios, drugs move through these tissues as a solute dissolved in water and essentially are transported wherever the water goes. This process is termed *bulk flow* and is dependent on the concentration of drug dissolved in the plasma or tissue fluid. This is a linear process and thus is easily modeled by most pharmacokinetic systems. It is the subsequent uptake into cells and special tissue areas that are governed by the diffusion processes above.

There exist several specialized membranes that possess specific transport systems. In these cases, the laws of diffusion and pH partitioning do not govern transmembrane flux of drugs. These specializations in transport can best be appreciated as mechanisms by which the body can exert control and selectivity over the chemicals that are allowed to enter the protected domain of specific organs, cells, or organelles. Such transport systems can be rather nonspecific, as are those of the kidney and liver that excrete charged waste products.

In the gastrointestinal tract, relatively nonspecific transport systems allow for the absorption, and thus entrance to the body, of essential nutrients that do not

have sufficient lipophilicity to cross membranes by diffusion. In specific tissues, they allow for select molecules to enter cells depending upon cellular needs, or allow compounds that circulate throughout the body to have a biological response only in a tissue possessing the correct transport receptor. The primary example is the protein carrier-mediated processes of active transport or facilitated diffusion. These systems are characterized by specificity and saturability. In the case of active transport, biological energy is utilized to move a drug against its concentration gradient. In facilitated diffusion, the carrier protein binds to the drug and carries it across the membrane down its concentration gradient. The drugs transported by these systems normally cannot cross the membrane by passive diffusion because they are not lipophilic. These systems are important for the gastrointestinal absorption of many essential nutrients and some drugs (e.g., β-lactams), for cellular uptake of many compounds (e.g., glucose), for the removal of drugs from the cerebral spinal fluid through the choroid plexus, and for biliary and renal excretion of numerous drugs.

In some tissues, cells may absorb drugs by endocytosis or pinocytosis, processes where a compound binds to the surface of the membrane that then invaginates and interiorizes the compound. This is not a primary mechanism of transmembrane passage for most therapeutic drugs. It is the primary mechanism by which nanomaterials enter cells. Most inorganic ions such as sodium and chloride are sufficiently small that they easily can cross aqueous pores and channels through membranes. The movement of these charged substances is generally governed by the transmembrane electrical potential maintained by active ion pumps.

Finally, active transport may also occur in the opposite direction to remove a drug after it has been absorbed into specific cells or tissue sites. This is called the *P-glycoprotein system,* a class of drug transporters originally associated with multiple drug resistance (MDR) encountered in cancer chemotherapy. MDR transporters have been identified in intestinal epithelial cells, the placenta, kidney tubules, brain endothelial cells, and liver bile canaliculus. These will be addressed throughout this text for specific drug classes.

In conclusion, an understanding of the processes that govern the movement of drugs across lipid-based biological membranes is important to the study of drug absorption, distribution, and excretion. Lipid-soluble drugs are easily absorbed into the body and well distributed throughout the tissues. In contrast, hydrophilic drugs are not well absorbed and have limited distribution but are easily eliminated. Metabolism converts lipophilic drugs to more easily excreted hydrophilic entities. If membranes separate areas of different pH, concentration gradients may form due to pH partitioning or ion trapping. Membranes are the building blocks of biological systems and play a central role in defining the complexity of pharmacokinetic models.

Absorption

Absorption is the movement of the drug from the site of administration into the blood. There are a number of methods available for administering drugs to animals. The primary routes of drug absorption from environmental exposure in mammals are gastrointestinal, dermal, and respiratory. The first two are also used as routes of drug administration for systemic effects, with additional routes including intravenous, intramuscular, subcutaneous, or intraperitoneal injection. Other variations on gastrointestinal absorption include intrarumenal, sublingual, and rectal drug delivery. Many techniques are also used for localized therapy, which may also result in systemic drug absorption as a side effect. These include, among others: topical, intramammary, intraarticular, subconjunctival, and spinal fluid injections. Methods of utilizing these different routes of drug administration are also explored in Chapter 5.

Gastrointestinal Absorption

One of the primary routes of drug administration is oral ingestion of a pill or tablet that is designed to deliver a drug across the gastrointestinal mucosa. The common factor in all forms of oral drug administration is a method to deliver a drug such that it gets into solution in the gastrointestinal fluids from which it can then be absorbed across the mucosa and ultimately reach the submucosal capillaries and the systemic circulation. Examples of oral drug delivery systems include solutions (aqueous, elixirs) and suspensions, pills, tablets, boluses for food animals, capsules, pellets, and sustained release mechanical devices for ruminants. The major obstacle encountered in comparative and veterinary medicine is the enormous interspecies diversity in comparative gastrointestinal anatomy and physiology, which results in major species differences in strategies for and efficiency of oral drug administration. This is often appreciated but overlooked when laboratory animal data is extrapolated to humans. Rats and rabbits are widely utilized in preclinical disposition and toxicology studies, although many investigators fail to appreciate that these species' gastrointestinal tracts are very different from one another and from humans.

From a pharmacologist's perspective, the gastrointestinal tract of all species can be simply presented as diagrammed in Figure 2.4. The GI tract is best conceptualized as actually being part of the external environment, which, in contrast to the skin, is protected and whose

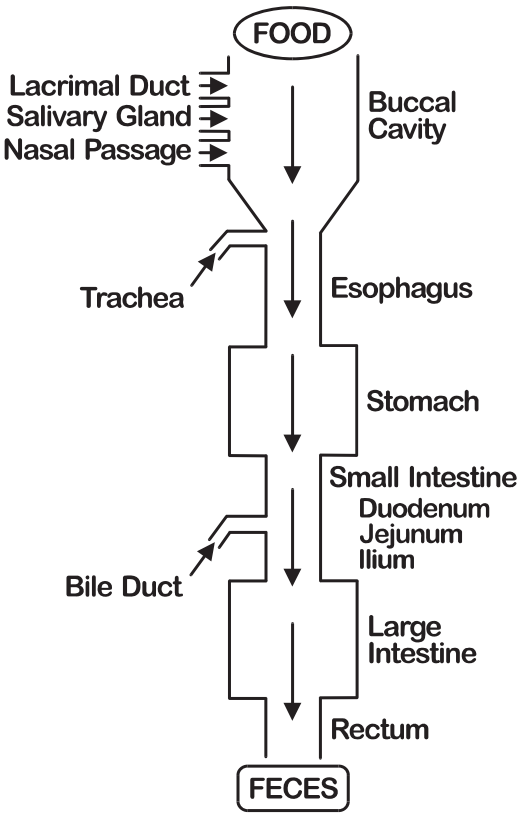

Figure 2.4 Functional structure of the gastrointestinal tract.

microenvironment is closely regulated by the organism. Because of the gastrointestinal tract's central role in digestion and nutrient absorption, there are many evolutionary adaptations to this basically simple mucosa structure that allow for physical, chemical, enzymatic, and microbial breakdown of food for liberation and ultimate absorption of nutrients. This tract is further adapted such that these digestive processes do not harm the organism's own tissues, which in carnivores may be identical to the food being eaten.

The gastrointestinal tract presents a significant degree of heterogeneity relative to morphology and physiology that translates to great regional variations in drug absorption. In the oral cavity, where food is masticated, some absorption may occur in sublingual areas. In fact, this site is actually utilized as a route for systemic drug (e.g., nitroglycerin) and nicotine (e.g., oral tobacco) delivery in humans. The esophagus and cranial portion of the stomach is lined by cornified epithelium, which provides an effective barrier that often decreases the chance of absorption for drugs formulated for intestinal drug delivery. A great deal of recent research activity has been focused on developing new transbuccal drug delivery systems. As mentioned, the prototype example was sublingual nitroglycerin tablets. Newer systems use novel adhesive technology, which allow actual polymer patches to adhere to the buccal mucosa. Such products are also being considered for some therapeutic applications in veterinary medicine (e.g., feline oral sprays). Compared to oral gastrointestinal absorption, buccal delivery bypasses the portal vein and thus eliminates the potential for first-pass hepatic biotransformation, discussed later.

The simple mucosal lining of the stomach allows absorption; however, the presence of surface mucus, which protects the epithelium from self-digestion secondary to acid and enzyme secretion, may be a barrier for some drugs. The acidity and motility of the stomach also creates a hostile environment for drugs and even influences the absorption of drugs farther down the tract. For oral drug absorption to be successful, the drug must be capable of surviving this relatively harsh environment. For some drugs (e.g., penicillin G) susceptible to acid hydrolysis, minimal absorption by the oral route will occur unless they are administered in a formulation that protects them in an acid environment but liberates them in the more alkaline environment of the intestines. Release of the drug from the stomach, a process controlled by gastric emptying, is a major rate-determining step in the onset and duration of oral drug activity. Species differences in the size of the pyloric orifice also limit use of some dosage forms in small animals versus humans.

The primary site for most drug absorption is the small intestine. In this region of the gastrointestinal tract, the pH of the contents are more alkaline and the epithelial lining is conducive to drug absorption. The blood flow to this region is also much greater than to the stomach. The small intestine is lined by simple columnar epithelium resting on a basement membrane and a submucosal tissue bed that is very well perfused by an extensive capillary and lymphatic network. This capillary bed drains into the hepatic portal vein. One of the major anatomical adaptations for absorption in this region is the presence of microvilli, which increase the surface area of the small intestine some 600-fold over that of a simple tube. The second anatomical adaptation are the villi of the intestine, which can be easily appreciated by examining a cross section (Figure 2.5). Since diffusion is the primary mechanism for drug absorption, the increase in area due to these two anatomical configurations significantly increases absorption, as can be seen from reviewing the area contribution to Equation 2.1. There are species differences in inherent permeability of the intestinal mucosa to chemicals, with the dog recently being recognized as having a higher permeability to many drugs than humans.

The viable epithelial cells of the intestines are also endowed with the necessary enzymes for drug metabolism that contributes to a second "first-pass" effect. Recent research has also indicated that the mechanism and extent of absorption, and magnitude of local

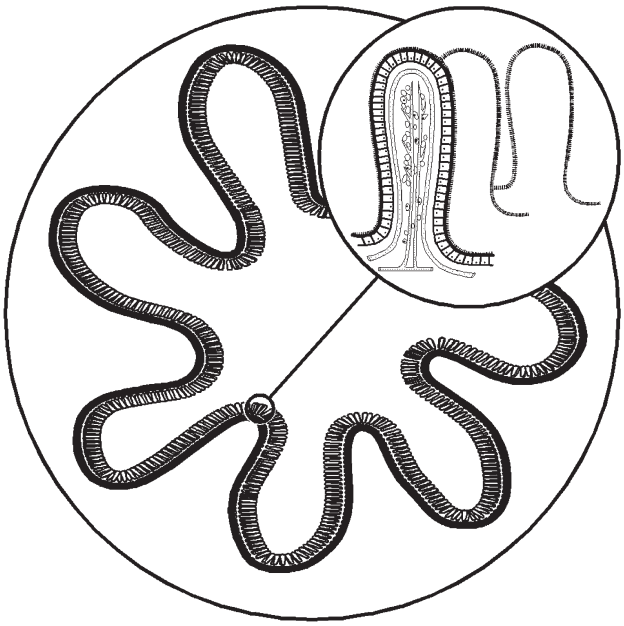

Figure 2.5 Cross-section of the small intestine showing villi adaptations, which serve to increase surface area available for absorption.

intestinal metabolism, varies between the tips and crypts of the villi. The final determinant of a drug's tortuous journey through the gastrointestinal tract is the resident microbial population that inhabits the intestinal contents. Many bacteria are capable of metabolizing specific drugs, resulting in a third component of the first-pass effect. This epithelial and bacterial biotransformation is generally categorized as *presystemic* metabolism to differentiate it from that which occurs following portal vein delivery of drug to the liver. However, from the perspective of pharmacokinetic analysis of plasma drug concentrations following oral drug administration, all three components are indistinguishable and become lumped into the aggregate process of oral absorption assessed as K_a.

Disintegration, Dissolution, Diffusion, and Other Transport Phenomenon

In order for a drug to be absorbed across the intestinal mucosa, the drug must first be dissolved in the aqueous intestinal fluid. Two steps, disintegration and dissolution, may be required for this to occur. *Disintegration* is the process whereby a solid dosage form (e.g., tablet) physically disperses so that its constituent particles can be exposed to the gastrointestinal fluid. Dissolution occurs when the drug molecules then enter into solution. This component of the process is technically termed the *pharmaceutical phase* and is controlled by the interaction of the formulation with the intestinal contents. These concepts are further elaborated on in Chapter 5.

Some dosage forms, such as capsules and lozenges, may not be designed to disintegrate, but rather to allow a drug to slowly elute from their surface. Dissolution is often the rate-limiting step controlling the absorption process and can be enhanced by formulating the drug in salt form (e.g., sodium or hydrochloride salts), buffering the preparation (e.g., buffered aspirin), or decreasing dispersed particle size (micronization) so as to maximize exposed surface area. This is extensively discussed in Chapter 5 (see equation 5.7). Alternatively, disintegration and dissolution can be decreased so as to deliberately provide slow release of the drug. This strategy is used in *prolonged-release* or *controlled-release* dosage forms and involves complex pharmaceutical formulations that produce different rates of dissolution. This may be accomplished by dispersing the dosage form into particles with different rates of dissolution or by using multilaminated dosage forms, which delays release of the drug until its layer is exposed. All of these strategies decrease the overall rate of absorption. Similar strategies can also be used to target drugs to the distal segments of the gastrointestinal tract by using enteric coatings that dissolve only at specific pH ranges, thereby preventing dissolution until the drug is in the region targeted. This strategy has been applied for colonic delivery of drugs in humans for treatment of Crohn's disease.

In slow-release or long-acting formulations, the end result is that absorption becomes slower than all other distribution and elimination processes, making the pharmaceutical phase the rate-limiting or rate-controlling step in the subsequent absorption and disposition of the drug. When this occurs, as will be seen in the pharmacokinetic modeling chapters to follow, the rate of absorption controls the rate of apparent drug elimination from the body and a so-called *flip-flop* scenario becomes operative.

There are significant species differences in the ability to use controlled-release oral medications designed in humans, by far the largest market, in other species. The first limitation involves the inability to use cellulose-based systems in ruminants due to the ability of rumen microbes to digest the normally inert cellulose matrix that controls rates of drug delivery. The second arises because of shorter gastrointestinal transit times in small carnivores, such as domestic cats and dogs, compared to humans. In this instance, drug release is designed to occur in the longer transit times seen in humans (approx. 24 hours). In dogs and cats, which have transit times half that of humans, drug release may still be occurring even after the tablet has been eliminated in the feces due to the shorter transit times. Other examples include the narrower pyloric opening in dogs, compared to humans, that may increase gastric retention of some larger dosage forms. These are but a few examples of significant species differences that, based on anatomical and physiological

factors, prevent the ready transferability of complex dosage forms across species.

After the drug is in solution, it must still be in a nonionized relatively lipid-soluble form to be absorbed across the lipid membranes comprising the intestinal mucosa. *It must be stressed that absorption across any membrane is a fine balance between adequate solubility on the donor side of the membrane with sufficient permeability (or active transport capacity) to actually transit the membrane*. For orally administered products, the pH of the gastrointestinal contents becomes very important, as is evident from the earlier discussion on pH partitioning. Specifically, a weak acid would tend to be preferentially absorbed in the more acidic environment of the stomach since a larger fraction would be in the nonionized form. However, the much larger surface area and blood flow available for absorption in the more alkaline intestine may override this effect. It is important to mention at this point why a weak acid such as aspirin is better absorbed in a bicarbonate buffered form, which would tend to increase the ionized fraction and thus decrease membrane passage. The paradox is that dissolution must first occur, a process favored by the ionized form of the drug. It is only the dissolved ionized aspirin that is available to the partitioning phenomenon described earlier. Thus, when more aspirin is dissolved in the buffered microenvironment, more is available for partitioning and diffusion across the mucosa. In contrast to the situation of a weak acid, a weak base would tend to be better absorbed in the more alkaline environment. However, it must be repeated that the very large surface area available in the intestines, coupled with high blood flow and a pH of approximately 5.3 in the immediate area of the mucosal surface makes it the primary site of absorption for most drugs (weak acids with pKa >3 and weak bases with pKa <7.8). Species differences in both gastric and intestinal pH further modulate this differential (e.g., canine gastric pH is much higher than humans). A further obstacle to absorption is that the compound must also be structurally stable against chemical or enzymatic attack. Finally, compounds with a fixed charge and/or very low (or very high) lipid solubility for the uncharged moiety, may not be significantly absorbed after oral administration. Examples include the polar aminoglycoside antibiotics, the so-called "enteric" sulfonamides, and quaternary ammonium drugs.

There are also specific active transport systems present within the intestinal mucosa of the microvilli that are responsible for nutrient absorption. However, these systems have a very high capacity and if a specific drug or toxicant has the proper molecular configuration to be transported, saturation is unlikely. There is some evidence that select therapeutic drugs (e.g., β-lactams such as ampicillin) may be absorbed by active transport systems in the small intestine. There are also transport systems (P-glycoprotein) that expel absorbed drug back into the intestinal lumen. This system is beginning to be studied more closely in veterinary species and will be discussed later in this chapter under distribution and elimination.

The complexity of these processes is clearly seen when one tries to extrapolate oral bioavailability of drugs between dogs and humans. In order to try and classify drug absorption in humans based on criteria of solubility and permeability, the Biopharmaceutical Classification System (BCS) was developed, which ranked drugs according to their extent and rate of absorption, with Class I drugs being both highly soluble and permeable resulting in generally very well absorbed compounds, in contrast to Class IV which had low solubility and low permeability and showed very poor oral absorption. Class II and III have mixed solubility and permeability characteristics. The advantage of such classifications is that manufactures changing formulations for Class I drugs only need to conduct in vitro dissolution studies since dissolution would be the rate-limiting process in absorption. Class IV drugs would require in vivo comparisons. Drugs classified using this system for humans did not correlate to what was observed in dogs, suggesting that the differences in GI physiology discussed above prevents easy interspecies extrapolations (Papich and Martinez, 2015).

Enterohepatic Recycling and Coprophagy

The gastrointestinal tract has also evolved into an excretory organ for elimination of nonabsorbed solid wastes and other metabolic byproducts excreted in the bile. The bile duct drains into the upper small intestine. For some drugs, this results in a phenomenon called *enterohepatic recycling* whereby a drug from the systemic circulation is excreted into the bile and is reabsorbed from the small intestine back into the bloodstream. In many cases, drugs that are metabolized by Phase II conjugation reactions are "unconjugated" by resident bacterial flora, which generates free drug for reabsorption. Thus compounds that are excreted into the bile may have a prolonged sojourn in the body because of the continuous opportunity for intestinal reabsorption. The cardinal sign of this process is a "hump" in the plasma drug concentration-time profile after administration (Figure 2.6). Bile also serves to emulsify fatty substances that are not capable of solubilizing in the primarily aqueous environment of the intestines. The result of this detergent-like action of bile is to form large surface area micelles having a hydrophilic surface and hydrophobic interior. These act as transport vehicles to deliver fat-soluble drugs to the intestinal brush border surface for diffusion across the lipid membrane into the cell. Without the interaction of bile acids, fatty substances would not be available for absorption since they could not traverse this "dissolution" barrier. Thus unlike most drugs, compounds that are absorbed

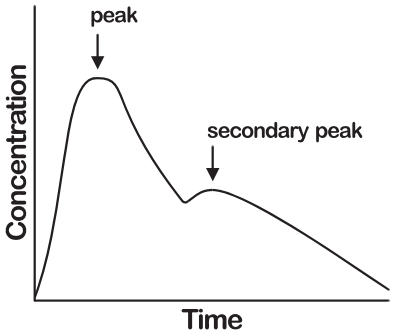

Figure 2.6 Concentration versus time profile demonstrating a secondary peak that could result from enterohepatic recycling.

by this route often must be administered with a meal to promote bile acid secretion and associated micelle formation.

Enterohepatic recycling has also been suggested as an important mechanism for enhanced activity of certain antiparasitic drugs such as the avermectins. As discussed in Chapter 41, the recycling of active drug is a major contributor to enhanced parasite exposure. Finally, some species such as rabbits, routinely ingest fresh feces for nutritional purposes, which provides another opportunity for drug to be reabsorbed into the body.

Species Effects on Gastrointestinal Transit Time and Food Interactions

Food may also interact with other aspects of oral drug absorption and have opposite effects for more hydrophilic drugs. Depending on the physicochemical properties of the specific drug, administration with food may significantly increase or decrease absorption. Such effects are not only drug dependent, but also are species dependent due to the continuous foraging behavior of ruminants and some other omnivores compared to the periodic feeding habits of predatory carnivores. These variables are difficult to incorporate into formal pharmacokinetic models yet they add to the variability in parameters derived from these studies or in drug response between species.

The first potential interaction relates to the rate of drug delivery to the small intestine that is governed by the rate of drug release from the stomach, the *gastric emptying time*. This process is dependent upon the eating habits of the species, with continuous foraging animals (e.g., herbivores such as horses and ruminants) having a steady input of drug and a relatively stable gastric pH compared to periodic eaters (e.g., carnivores like dogs and cats and omnivores such as pigs) who have more variable eating patterns with large swings in gastric pH depending on the presence or absence of food. In addition, the drug may directly interact with the ingested food, as is the case of chelation of tetracyclines with divalent cations such as Mg^{++} in antacids or Ca^{++} in milk products. Thus, the decision to administer a compound with or without food is species and drug dependent and may significantly alter the bioavailability (rate and extent of absorption) of the drug. In contrast, for very-lipid-soluble drugs, food is necessary in order to have bile release, which allows solubilization and absorption to occur.

The forestomachs of a ruminant provide a major obstacle to the delivery of an oral dosage form to the true stomach (abomasum) for ultimate release to the intestines, although a significant amount of drug absorption may occur from this site. The rumen is essentially a large fermentation vat (>50 liters in cattle, 5 liters in sheep) lined by stratified squamous epithelium, buffered at approximately a pH of 6 by extensive input of saliva, which maintains it in a fluid to soft consistency, designed primarily for the absorption of volatile fatty acids. If drugs dissolve in this medium and remain intact, they undergo tremendous dilution that decreases their rate of absorption. They then are pumped from the rumen and reticulum through the omasum for a rather steady input of drug into the true stomach. An understanding of the physiology of the ruminant has allowed for the development of some unique and innovative mechanical drug delivery technologies, which essentially are encapsulated pumps that "sink" to the bottom of the rumen and become trapped, much as many unwanted objects tend to when ingested by a ruminant (e.g., nails and wire in hardware disease). These "submarine-like" devices then slowly release drug into the ruminal fluid for a true sustained-release preparation. In preruminant calves, a drug may bypass the rumen entirely through the rumen-reticulo groove and essentially behave as if administered to a monogastric. In contrast, fermentation in the horse occurs after drug absorption by the small intestine and thus has less impact than in ruminants. However, a nonabsorbed drug that reaches the equine large intestines and cecum, the site of fermentation, may have disastrous effects (e.g., colic) if digestive flora or function is perturbed.

First-Pass Metabolism

Another unique aspect of oral drug absorption is the fate of the absorbed drug once it enters the submucosal capillaries. Drug absorbed distal to the oral cavity and proximal to the rectum in most species enters the portal circulation and is transported directly to the liver where biotransformation may occur. This is a major cause for differences in a drug's ultimate disposition compared to all other routes of administration. This may result in a significant first-pass biotransformation of the absorbed compound. For a drug that is extensively metabolized by the liver, this first-pass effect significantly reduces absorption of the active drug even when it is absorbed across the mucosa. This occurs for many opiate medications in dogs, reducing their efficacy after

oral administration. Finally, some drugs that are too polar to be absorbed across the gastrointestinal wall are formulated as ester conjugates to increase lipid solubility and enhance absorption. Once the drug crosses the gastrointestinal epithelium in this form, subsequent first-pass hepatic biotransformation enzymes and circulating blood and mucosal esterases cleave off the ester moiety releasing free drug into the systemic circulation.

There are selected drug administration sites that avoid first-pass hepatic metabolism by allowing absorption through gastrointestinal tract segments *not* drained by the portal vein. These include the oral cavity buccal and rectal routes of drug administration in some species, although this assumption hasn't been tested in many veterinary species.

Formulation Factors

The pharmaceutical literature is replete with formulation factors that may influence the dissolution and absorption of a drug preparation, assuming in the first place that one has an active component of known purity and potency. These are fully discussed in Chapter 5. The issue then becomes what are the potential interactions that can occur between the active ingredients and the excipients that make up the formulation. Additionally, what are the effects of the practitioner's compounding techniques (materials used, mixing efficacy, etc.) on the amount of active ingredients ultimately appearing in the formulation. Although this discussion is the focus of a biopharmaceutics text, the strategies are often encountered in pharmacokinetics as they may affect the parameters estimated after oral administration.

Table 2.1 depicts the pharmaceutical processes involved in absorption that may be affected by formulation. Following oral administration of tablets, disintegration must first occur. The speed and efficacy of this process will determine how much drug is actually available for subsequent steps. The resulting particle size (and hence surface area) is an important determinant for the next dissolution phase where the drug enters solution, an absolute prerequisite for diffusion across the mucosal barrier. Dissolution also involves diffusion across the liquid boundary layers that are an interface between the particles and the absorption milieu. Many pharmaceutical factors may affect the efficiency of the disintegration and dissolution processes. For tablets, the nature and homogeneity of the excipients become important considerations. These factors are the primary determinants of differences in efficacy between so-called "pioneer" and generic drug products. Once the drug is in solution, binding or complexation to inert filler ingredients may occur. It is important to remember that all of this is happening while the particles are in transit through the gastrointestinal tract. Thus, if the formulation results in a decreased rate of disintegration or dissolution, the rate and extent of absorption may be decreased, especially in species with very short gastrointestinal transit times. Similar factors are involved with oral capsules and even liquid dosage forms where the drug may interact with the vehicle. In fact, these scenarios are probably most pertinent to practitioner compounding. For capsules, the breakdown of the capsule replaces tablet disintegration as the initial rate-determining step. After release of the capsule contents, all of the above factors come into play. It cannot be overstated that such pharmaceutical factors are critical determinants of the extent and rate of subsequent drug absorption.

Table 2.1 Pharmaceutical factors affecting absorption

Disintegration
Excipients
Compaction pressure
Enteric coatings, capsules
Homogeneity
Dissolution
Particle size/surface area
Binding
Local pH, buffers
Boundary layers
Barrier diffusion
Solubility
Transit time

Topical and Percutaneous Absorption

The skin is a complex, multilayered tissue comprising 18,000 cm^2 of surface in an average human male. The quantitative prediction of the rate and extent of percutaneous penetration (into skin) and absorption (through skin) of topically applied chemicals is complicated by the biological variability inherent in skin. Mammalian skin is a dynamic organ with a myriad of biological functions. The most obvious is its barrier property that is of primary concern in the absorption problem. Another major function is thermoregulation that is achieved and regulated by three mechanisms in skin: thermal insulation provided by pelage and hair, sweating, and alteration of cutaneous blood flow. Other functions of skin include mechanical support, neurosensory reception, endocrinology, immunology, and glandular secretion. These additional biological roles lead to functional and structural adaptations that affect the skin's barrier properties and thus the rate and extent of percutaneous absorption. Many of the topics discussed below are fully developed in Chapter 47 of this text.

The skin is generally considered to be an efficient barrier preventing absorption (and thus systemic exposure) of most topically administered compounds. It is a membrane that is relatively impermeable to aqueous solutions and most ions. It is, however, permeable in varying

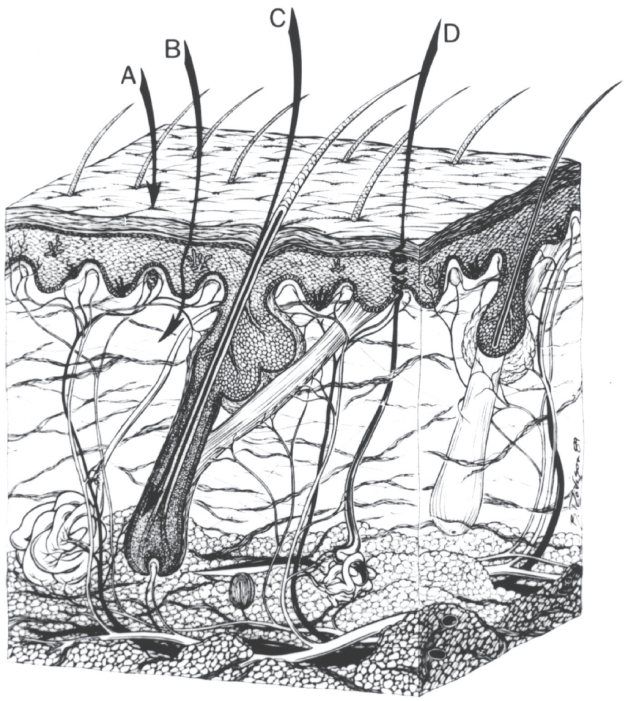

Figure 2.7 Microstructure of mammalian skin showing potential routes of penetration (A) intercellular, (B) transcellular, (C) intrafollicular, (D) via sweat ducts.

degrees to a large number of solid, liquid, and gaseous xenobiotics. Although one tends to think of most cases of poisoning as occurring through the oral or, less frequently, the respiratory route, the widespread use of organic chemicals has enhanced risk exposure to many toxicants that can penetrate the dermal barrier.

The gross features of mammalian skin are illustrated in Figure 2.7. Compared to most routes of drug absorption, the skin is by far the most diverse across species (e.g., sheep versus pig) and body sites (e.g., human forearm versus scalp). Three distinct layers and a number of associated appendages make up this nonhomogenous organ. The epidermis is a multilayered tissue varying in thickness in humans from 0.15 mm (eyelids) to 0.8 mm (palms). The primary cell type found in the epidermis is the keratinocyte. Proliferative layers of the basal keratinocyte (stratum germinativum) differentiate and gradually replace the surface cells (stratum corneum) as they deteriorate and are sloughed from the epidermis. A number of other cell types are also found interspersed in the epidermis including the pigmented melanocytes, Merkel cells which may play a sensory role, and Langerhans cells which probably play a role in cutaneous immunology.

In respect to drug penetration, the primary biochemical change is the production of fibrous, insoluble keratin that fills the cells, and a sulfur-rich amorphous protein that comprises the cell matrix and thickened cell membrane. In addition, the keratinocytes synthesize a variety of lipids that form the distinguishing granules in the stratum granulosum that release their contents into the intercellular spaces. The end result in the stratum corneum is dead proteinaceous keratinocytes embedded in an extracellular lipid matrix, a structure referred to by Elias as the "brick and mortar" model (see Figures 47.3 and 47.4).

The Stratum Corneum Barrier

It is this outermost layer, the stratum corneum, which provides the primary barrier to the penetration of foreign compounds. This barrier consists of flattened, stratified, highly keratinized cells embedded in a lipid matrix composed primarily of sterols, other neutral lipids, and ceramides. Although highly water retarding, the dead, keratinized cells are highly water absorbent (hydrophilic), a property that keeps the skin supple and soft. A natural oil covering the skin, the sebum, especially present in some species such as sheep, appears to maintain the water-holding capacity of the epidermis but has no appreciable role in retarding the penetration of xenobiotics. Disruption of the stratum corneum removes all but a superficial deterrent to penetration.

Dermis and Appendages

The dermis is a highly vascular area, providing ready access for drug distribution once the epithelial barrier has been passed. The blood supply in the dermis is under complex, interacting neural and local humoral influences whose temperature-regulating function can have an effect on distribution by altering blood supply to this area. The absorption of a chemical possessing vasoactive properties would be affected through its action on the dermal vasculature; vasoconstriction would retard absorption and increase the size of a dermal depot, while vasodilation may enhance absorption and minimize any local dermal depot formation.

The appendages of the skin are found in the dermis and extend through the epidermis. The primary appendages are the sweat glands (eccrine and apocrine), hair, and sebaceous glands, all of which show great interspecies and interregional variability. Since these structures extend to the outer surface, they potentially play a role in the penetration of certain compounds.

Topical Drug Delivery and the Definition of Dose

From the perspective of pharmacokinetic models of transdermal and topical drug delivery systems, there are significant differences from other routes of administration (e.g., oral, injection) as to what constitutes a dose. For most exposures, the concentration applied to the surface of the skin exceeds the absorption capacity. However, for therapeutic transdermal patches with a fixed concentration of the drug and rate-controlled release properties, it is the contact surface area that more accurately reflects dose and thus dose is expressed not in

mg/kg, but mg/cm^2 of dosing area. This surface area dependence also holds for any topical application even if absorption capacity is superseded. Yet, another source of nonlinearity results secondary to the effects of occlusive (water-impermeable) drug vehicles or patches. As the skin hydrates, a threshold is reached where transdermal flux dramatically increases (approximately 80% relative humidity). When the skin becomes completely hydrated under occlusive conditions, flux can be dramatically increased. Therefore, dose alone is often not a sufficient metric to describe topical doses, and application method and surface area become controlling factors.

Pathways for Dermal Absorption

Anatomically, percutaneous absorption might occur through several routes. The current consensus is that the majority of nonionized, lipid-soluble toxicants appear to move through the intercellular lipid pathway between the cells of the stratum corneum, the rate-limiting barrier of the skin. Very small and/or polar molecules appear to have more favorable penetration through appendages or other diffusion shunts, but only a small fraction of drugs are represented by these molecules. Simple diffusion seems to account for penetration through the skin whether by gases, ions, or nonelectrolytes.

The rate of percutaneous absorption through this intercellular lipid pathway is correlated to the partition coefficient of the penetrant. This has resulted in numerous studies correlating the extent of percutaneous absorption with a drug's lipid : water partition coefficient. Some workers further correlated skin penetration to molecular size and other indices of potential interaction between the penetrating molecule and the skin that are not reflected in the partition coefficient. For most purposes, however, dermal penetration is often correlated to partition coefficient. If lipid solubility is too great, compounds that penetrate the stratum corneum may remain there and form a reservoir. Alternatively, penetrated compounds may also form a reservoir in the dermis. For such compounds, slow release from these depots may result in a prolonged absorption half-life. Conditions that alter the composition of the lipid (harsh delipidizing solvents, dietary lipid restrictions, disease) may alter the rate of compound penetration by changing its partitioning behavior. Very similar to the situation with oral absorption previously discussed, the actual utility of a topical drug is a delicate balance between solubility and permeability which topical drug formulators attempt to exploit. Some of these concepts are further discussed in Chapters 5 and 47.

Recent studies have demonstrated that the skin may also be responsible for metabolizing topically applied compounds. Both Phase I and II metabolic pathways have been identified. For some compounds, the extent of cutaneous metabolism influences the overall fraction of a topically applied compound that is absorbed, making this process function as an alternate absorption pathway. Cutaneous biotransformation is used to promote the absorption of some topical drugs that normally would not penetrate the skin. Cutaneous metabolism may be important for certain aspects of skin toxicology when nontoxic parent compounds are bioactivated within the epidermis, for example benzo(a)pyrene to an epoxide. Finally, resident bacteria on the surface of the skin may also metabolize topical drugs, as was demonstrated with pentochlorophenol absorption in pig skin dosed in soil with and without antibiotics. This effect is potentiated under warm and wet occlusive dosing conditions that both promote bacterial growth and reduce skin barrier properties.

Variations in Species and Body Region

Penetration of drugs through different body regions varies. In humans, generally the rate of penetration of most nonionized toxicants is in the following order: scrotal > forehead > axilla = scalp > back = abdomen > palm and plantar. The palmar and plantar regions are highly cornified producing a much greater thickness that introduces an overall lag time in diffusion. In addition to thickness, the actual size of corneacytes and differences in hair follicle density may affect absorption of more polar molecules. Finally, differences in cutaneous blood flow that have been documented in different body regions may be an additional variable to consider in predicting the rate of percutaneous absorption. These factors are also important in animals, with the area of the inner ear known to be highly permeable and well perfused, thus an excellent site for drug absorption to occur.

Although generalizations are tenuous at best, human skin appears to be more impermeable, or at least as impermeable, as the skin of the cat, dog, rat, mouse, or guinea pig. The skin of pigs and some primates serve as useful approximation to human skin, but only after a comparison has been made for each specific substance. The major determinants of species differences are thickness, hair density, lipid composition, and cutaneous blood flow.

Factors that Modulate Absorption

Soaps and detergents are perhaps the most damaging substances routinely applied to skin. Whereas organic solvents must be applied in high concentrations to damage the skin and increase the penetration of solute through human epidermis, only 1% aqueous solutions of detergents are required to achieve the same effect. For a specific chemical, rate of penetration can be drastically modified by the solvent system used. In transdermal patches, specific chemical enhancers (e.g., solvents such as ethanol; other lipid-interacting moieties)

are included in the formulation to reversibly increase skin permeability and enhance drug delivery. Alternatively, drug release is formulated to be rate limiting from the patch system (membranes, microencapsulation, etc.) so that a constant (zero-order) release from the patch occurs, thereby providing controlled drug delivery. Since patches are designed with the permeability properties of specific species in mind, care must be taken when using a patch designed for one species in another. There are a number of topical veterinary drugs routinely used to achieve long-acting systemic therapeutic endpoints in animals. These include the topical pesticides formulated as "spot-ons" and "pour-ons." These products are further discussed in Chapters 43 and 47 of this text. All of these issues have also been discussed in a comprehensive review of this topic (Riviere and Papich, 2001).

Another strategy for transdermal delivery, which has not widely been employed in veterinary medicine, is to overcome the cutaneous barrier by using electrical (iontophoresis) or ultrasonic (phonophoreses) energy, rather than the concentration gradient in diffusion, to drive drugs through the skin. These techniques hold the most promise for delivering peptides and oligonucleotide drugs that now only can be administered by injection. In these cases, dose is based on the surface area of application and the amount of energy required to actively deliver the drug across skin. In iontophoresis, this amounts to a dose being expressed in $\mu Amps/cm^2$. Formulation factors are also very different since many of the excipients used are also delivered by the applied electrical current in molar proportion to the active drug. Finally, a recent but related strategy is to use very short-duration, high-voltage electrical pulses (electroporation) to reversibly break down the stratum corneum barrier, allowing larger peptides and possibly even small proteins to be systemically delivered.

Respiratory Absorption

The third major route for systemic exposure to drugs and toxicants is the respiratory system. Since this system's primary function is gas exchange (O_2, CO_2), it is always in direct contact with environmental air as an unavoidable part of breathing. A number of toxicants are in gaseous (CO, NO_2, formaldehyde), vapor (benzene, CCl_4), or aerosol (lead from automobile exhaust, silica, asbestos) forms and are potential candidates for entry via the respiratory system. There are no approved inhalational drugs for use in veterinary medicine. Each mode of inhalational exposure results in a different mechanism of compound absorption and for the purposes of this text, a different definition of dose. These concepts are also discussed In Chapters 11 and 48 of this text.

Opportunities for systemic absorption are excellent through the respiratory route since the cells lining the alveoli are very thin and profusely bathed by capillaries. The surface area of the lung is large (50–100 m^2), some 50 times the area of the skin. Based on these properties and the diffusion equation presented earlier (Equation 2.1), the large surface area, the small diffusion distance, and high level of blood perfusion maximize the rate and extent of passive absorption driven by gaseous diffusion.

The process of respiration involves the movement and exchange of air through several interrelated passages including the nose, mouth, pharynx, trachea, bronchi, and successive smaller airways terminating in the alveoli where gaseous exchange occurs. All of these anatomical modifications protect the internal environment of the air passages from the harsh outside environment by warming and humidifying the inspired air. The passages also provide numerous obstacles and baffles to prevent the inhalation of particulate and aerosol droplets. Thus the absorption of particulate and aerosolized liquids, such as those employed in nebulized drug therapy, is fundamentally different from that of gases. The absorption of such impacted solids and liquids along the respiratory tract has much more in common with oral and topical absorption, with the critical caveat that the precise dose of compound finally available for absorption is very difficult to determine. Great strides have been made in developing aerosol drug delivery devices for human use that take advantage of this mechanism of impaction; however, these may not be transferable to veterinary species since their efficacy is closely related to the geometry and physiology of the human respiratory tract.

Another unique aspect of respiratory exposure is the fact that the pulmonary blood circulation is in series with the systemic circulation. Thus, in contrast to cutaneous or oral exposure, compounds absorbed in the lung will enter the oxygenated pulmonary veins that drain to the systemic arterial circulation. Compared to oral administration, this reduces first-pass hepatic metabolism. However, the pulmonary circulation is adept at metabolizing peptides secondary to its role in inactivating peptide hormones.

Vapors and Gases

Since the rate of entry of vapor-phase toxicants is controlled by the alveolar ventilation rate, the toxicant is presented to the alveoli in an interrupted fashion whose frequency in humans is equal to the rate of breathing: about 20 times/min. Doses are generally discussed in terms of the partial pressure of the gas in the inspired air. Upon inhalation of a constant tension of a toxic gas, arterial plasma tension of the gas approaches its tension in the expired air. The rate of entry is then determined by the blood solubility of the toxicant. If there is a high blood : gas partition coefficient, a larger amount must be dissolved in the blood to raise the partial pressure. Gases with a high blood : gas partition coefficient require

a longer period to approach the same tension in the blood as in inspired air than it takes for less soluble gases. Similarly, a longer period of time is required for blood concentrations of such a gas to be eliminated, thus prolonging detoxification.

Another important point to consider in determining how much of an inhaled gas is absorbed into the systemic circulation is the relationship of the fraction of lung ventilated compared to the fraction perfused. Increased perfusion of the lung will favor a more rapid achievement of blood–gas equilibrium. Decreased perfusion will decrease the absorption of toxicants even those that reach the alveoli. Various "ventilation/perfusion mismatches" may alter the amount of an inhaled gas that is systemically absorbed. Similarly, pulmonary diseases that thicken the alveoli or obstruct the airways may also affect overall absorption.

Aerosols and Particulates

The absorption of aerosols and particulates is affected by a number of physiological factors specifically designed to preclude access to the alveoli. The upper respiratory tract, beginning with the nose and continuing down its tubular elements, is a very efficient filtering system for excluding particulate matter (solids, liquid droplets). The parameters of air velocity and directional air changes favor impaction of particles in the upper respiratory system. Particle characteristics such as size, coagulation, sedimentation, electrical charge, and diffusion are important to retention, absorption, or expulsion of airborne particles. In addition to these characteristics, a mucous blanket propelled by ciliary action clears the tract of particles by directing them to the gastrointestinal system (via the glottis) or to the mouth for expectoration. This system is responsible for 80% of toxicant lung clearance. In addition to this mechanism, phagocytosis is very active in the respiratory tract, both coupled to the directed mucosal route and via penetration through interstitial tissues of the lung and migration to the lymph, where phagocytes may remain stored for long periods in lymph nodes. Compared to absorption in the alveoli, absorption through the upper respiratory tract is quantitatively of less importance. However, inhaled toxicants that become deposited on the mucous layer can be absorbed into the myriad of cells lining the respiratory tract and exert a direct toxicological response. This route of exposure is often used to deliver pharmaceutics by aerosol. If a compound is extremely potent, systemic effects may occur.

The end result of this extremely efficient filtering mechanism is that most inhaled drugs deposited in nasal or buccal mucous ultimately enter the gastrointestinal tract. This can best be appreciated by examining the respiratory drainages depicted in Figure 2.1. Therefore, the disposition of aerosols and particulates largely mirrors that of orally administered drugs.

Nasal administration is a preferred route for many inhalant medications in humans. In these cases, great care is made to deliver aerosols of the specific size for deposition on the nasal mucosa and upper respiratory tract. The bioavailability of these compounds is assessed using the techniques developed for other routes, although a local effect is often desired. The problems with this strategy are the attainment of an accurately delivered dose and the inactivation and binding of administered drug by the thick mucous blanket. Drugs delivered by this route usually have a wide therapeutic window and large safety index. The final point to consider relates to some specific peculiarities of nasal absorption. In the region of the olfactory epithelium, there exists a direct path for inhaled compounds to be absorbed directly into the olfactory neural tissue and central nervous system, thereby bypassing both the systemic circulation and the blood–brain barrier. The mass of drugs involved in this uptake process is very small and thus would not affect a pharmacokinetic analysis. However, this route has obvious toxicological significance and unfortunately has not been carefully studied in veterinary species.

Other Routes of Administration

In order to complete this discussion of absorption, it is important to realize that there are other extravascular drug administration routes that are often encountered. Relative to pharmacokinetic analysis, these are dealt with in the same fashion as the primary routes discussed above. The important difference is that in all cases, the barrier to absorption is less than that encountered in oral or topical delivery. Second, all of these routes involve an invasive procedure to inject drug into an internal body tissue, thereby bypassing the epithelial barriers of the skin and gastrointestinal tract.

The primary therapeutic routes of drug administration are subcutaneous (SC or SQ) and intramuscular (IM). In these cases, the total dose of drug is known and injected into tissue that is well perfused by systemic capillaries that drain into the central venous circulation. Both of these routes as well as intravenous administration are termed *parenteral* to contrast primarily with oral (*enteral)* and topical dosing, which are classified as *nonparenteral* routes of drug administration. A primary difference between these two classes is that parenteral routes bypass all of the body's defensive mechanisms. Parenteral dosage forms are manufactured under strict guidelines that eliminate microbial and particulate contamination resulting in sterile preparations that must be administered using aseptic techniques. This restriction does apply to oral or topical dosage forms. As with all methods of drug administration, there are numerous variables associated with SC and IM dosing that can be

conveniently classified into pharmaceutical and biological categories.

Finally, there are other occasional routes of drug administration employed that require absorption for activity. Administration of drugs by intraperitoneal injection is often used in toxicology studies in rodents since larger volumes can be administered. Peritoneal absorption is very efficient, provided adequate "mixing" of the injection with the peritoneal fluid is achieved. The majority of drug absorbed after interperitoneal administration enters the portal vein and thus may undergo first-pass hepatic metabolism. The disposition of intraperitoneal drug thus mirrors oral administration.

Some drugs are administered by conjunctival, intravaginal, or intramammary routes. In these cases achievement of effective systemic concentrations are often not required for what is an essentially local therapeutic effect. Prolonged absorption from these sites may result in persistent tissue residues in food-producing animals if the analytical sensitivity of the monitoring assay is sufficiently low. The systemic absorption of these dosage forms is quantitated using procedures identical to those employed for other routes of administration.

Bioavailability

The final topic to consider with absorption is the assessment of the extent and rate of absorption after oral, topical, or inhalational drug administration. The extent of drug absorption is defined as absolute systemic availability and is denoted in pharmacokinetic equations as the fraction of an applied dose absorbed into the body (F). Although this topic will also be discussed extensively in Chapter 3, it is important and convenient at this juncture to introduce the basic concepts so as to complete the discussion of drug absorption. If one is estimating the extent of drug absorption by measuring the resultant concentrations in either blood or excreta, one must have an estimate of how much drug normally would be found if the entire dose were absorbed. To estimate this, an intravenous dose is required since this is the only route of administration that guarantees that 100% of the dose is systemically available ($F = 1.0$) and the pattern of disposition and metabolism can be quantitated. Parameters used to measure systemic availability are thus calculated as a ratio relative to the intravenous dose.

For most therapeutic drug studies, systemic absorption is assessed by measuring blood concentrations. The amount of drug collected after administration by the route under study is divided by that collected after intravenous administration. When drug concentrations in blood (or serum or plasma) are assayed, total absorption is assessed by measuring the area under the concentration–time curve (AUC) using the trapezoidal method. This is a geometrical technique that breaks the AUC into corresponding trapezoids based on the number of samples assayed. The terminal area beyond the last data point (a triangle) is estimated and added together with the previous trapezoidal areas. Absolute systemic availability then is calculated as in Equation 2.4:

$$F\,(\%) = \frac{AUC_{route}\quad Dose_{iv}}{AUC_{iv}\quad Dose_{route}} \tag{2.4}$$

Calculation of F provides only an estimate of the extent, and not rate, of drug absorption. To calculate rate, pharmacokinetic techniques are required and presented in Chapter 3. Finally, so-called relative systemic availability may be calculated for two nonintravenous formulations where the data for the reference product is in the denominator and the test formulation in the numerator.

Distribution

A toxicant absorbed into the systemic circulation following any route of administration must reach its site of action at a high enough concentration for a sufficient period of time to elicit a biological response. Distribution processes determine this outcome. There are numerous tissues to which a chemical may be distributed, some of them capable of eliciting a pharmacological or toxicological (intended versus unintended) response while others serve only as a sink or depot for the chemical. Sinks may also be formed as a result of chemical binding to tissue or plasma proteins. The toxicological significance of such sinks is that chemicals will be distributed to, and in some cases stored in, these tissues and only slowly released back into the systemic circulation for ultimate elimination. Such tissue binding may actually protect against acute adverse effects by providing an "inert" site for toxicant localization. Storage may, however, prolong the overall residence time of a compound in the body and promote accumulation during chronic exposure, two processes that would potentiate chronic toxicity.

If the animal is a food-producing species, such tissue storage may result in residues in the edible meat products. Tissue concentrations thus become an endpoint in themselves, devoid of a biological or toxicological relevance in the tissue they are found. Their relevance is set by regulations that legally establish safe tissue tolerances or maximum residue levels for specific tissues and species. These are based upon extrapolations of safety to the consuming human population and food consumption patterns. These concepts are developed in Chapter 61.

Distribution of chemicals to peripheral tissues is dependent on four factors:

1) Physiochemical properties of the compound (pKa, lipid solubility, molecular weight)

2) Concentration gradient established between the blood and tissue
3) Ratio of blood flow to tissue mass
4) Affinity of the chemical for tissue constituents.

The physiochemical properties of the chemical are most important in determining its propensity to distribute to a specific tissue. For most molecules, distribution out of the blood into tissue is via bulk flow through the capillary pores or by simple diffusion down a concentration gradient; hence distribution is generally described by first-order rate constants. One can conceptualize distribution as "absorption" into the tissues from the blood. The complicating factors are that the driving concentration is now dependent upon blood flow, the surface area for "absorption into tissues" is dependent upon capillary density and tissue mass, the relevant partition coefficient is the blood/tissue ratio, and plasma/tissue protein binding complicates the picture. An understanding of distribution is a prerequisite to predicting pharmacological response.

Physiological Determinants of Distribution

Body fluids are distributed between three primary compartments, only one of which, vascular fluid, is thought to have an important role in the distribution of most compounds throughout the body. Human plasma amounts to about 4% of the total body weight and 53% of the total blood volume. By comparison, the interstitial tissue fluids account for 13%, and intracellular fluids 41%, of body weight. Use of recently developed microdialysis and ultrafiltration probes and catheters allow the concentration of drug to be directly monitored in the interstitial fluid and thus further open the window for pharmacokinetic analysis. The concentration that a compound may achieve in the blood following exposure depends in part upon its apparent volume of distribution. If it is distributed only in the plasma, a high concentration could be achieved in the vascular system. In contrast, the concentration would be markedly lower if the same quantity of toxicant were distributed to a larger pool including the interstitial water and/or cellular fluids.

The next major consideration is the relative blood flow to different tissues. Two factors will potentiate chemical accumulation into a tissue: high blood flow per unit mass of tissue and a large tissue mass. Tissues with a *high blood flow/mass* ratio include the *brain, heart, liver, kidney,* and *endocrine glands*. Tissues with an *intermediate ratio* include *muscle* and *skin,* while tissues with a *low ratio* (indicative of poor systemic perfusion) include *adipose tissue* and *bone*. These ratios are generalizations and some tissues may actually be categorized in two disparate groups. An excellent example is the kidney where the renal cortex receives some 25% of cardiac output and thus has a very high blood flow/mass ratio. However, the renal medulla receives only a small fraction of this blood flow and thus could be categorized in the intermediate to low group. If the affinity of the chemical for the tissue is high, it will still accumulate in poorly perfused tissues (such as fat), although it will take a long period of time to "load" or "deplete" these tissues. A relatively low blood flow/mass ratio is a major physiological explanation for depot formation.

Tissue Barriers to Distribution

Some organs have unique anatomic barriers to xenobiotic penetration. The classic and most studied example is the blood–brain barrier, which has a glial cell layer interposed between the capillary endothelium and the nervous tissue (illustrated nicely in Figure 9.4). In the schematic membrane scheme depicted in Figure 2.2, this amounts to an additional lipid membrane between the capillary and target tissue. Only nonionized lipid-soluble compounds can penetrate this barrier. Similar considerations apply to ocular, prostatic, testicular, synovial, mammary gland, and placental drug or toxicant distribution. In addition, pH partitioning phenomenon also may occur since the protected tissue (e.g., cerebrospinal fluid) may have a lower pH than the circulating blood plasma. Chemicals may also distribute into transcellular fluid compartments, which are also demarcated by an epithelial cell layer. These include cerebrospinal, intraocular, synovial, pericardial, pleural, peritoneal, and cochlear perilymph fluid compartments.

A few tissues possess selective transport mechanisms that accumulate specific chemicals against concentration gradients. For example, the blood–brain barrier possesses glucose, L-amino acid, and transferrin transporters. If the toxicant resembles an endogenous transport substrate, it may preferentially concentrate in a particular tissue. Recent work with the blood–brain barrier has demonstrated that some of these tissues also possess drug efflux transport processes that remove drug from the protected sites. Two such processes are P-glycoprotein associated with multidrug resistance (MDR) and the weak organic acid cell-to-blood efflux systems.

P-glycoprotein is a member of the so-called ATP-binding cassette proteins that include the cystic fibrosis transmembrane regulator and the sulfonyurea-sensitive ATP-dependent potassium channel. Drugs such as vinblastine, vincristine, or cyclosporine, which have the proper physiochemical characteristics (high lipophilicity) to enter the brain, do not achieve effective concentrations because of this active efflux mechanisms. This transport system has recently been shown to cause the unique breed sensitivity of Collies to ivermectin toxicity. These transporters are also responsible for decreased

bioavailability of some drugs due to active pumping of absorbed drug back into the intestinal lumen. A number of drugs also inhibit P-glycoprotein transport (e.g., ketoconazole, cyclosporine), which forms the basis for some complex drug–drug interactions. Similar processes and transport systems for peptides and other compounds are also found in other organs (e.g., liver). Chapter 50 in this text should be consulted for further details on P-glycoprotein.

Plasma Protein Binding

Following entry into the circulatory system, a chemical is distributed throughout the body and may accumulate at the site of toxic action, be transferred to a storage depot, or be transported to organs that will detoxify, activate, or eliminate the compound. Although many toxicants have sufficient solubility in the aqueous component of blood to account for simple solution as a means of distribution, the primary distribution mechanism for insoluble toxicants appears to be in association with plasma proteins. Although cellular components (e.g., red blood cells) may also be responsible for transport of drugs, such transport is seldom the major route. The transport of compounds by lymph is usually of little quantitative importance for many drugs, although it may be very important in delivering some lipophilic drugs and potentially nanoparticles to select organs. Both erythrocytes and lymph can play roles in the transport of some lipophilic drugs and toxins, in some instances to an important extent.

Studies of plasma proteins have shown albumin to be particularly important in the binding of drugs. This is especially true for weak acids, with weak bases often binding to acid glycoproteins. For certain hormones, specific high-affinity transport proteins are present. Studies of toxicant binding have been more limited, but there is evidence of a significant binding/partitioning role for lipoproteins in carrying very lipophilic chemicals in the blood. In the case of most drug–protein interactions, reversible binding is established, which follows the Law of Mass Action and provides a remarkably efficient means whereby drugs can be transported to various tissues. The strength of this association may be quantitated through the use of the dissociation constant, K_{diss}. Among a group of binding sites on proteins, those with the smallest K_{diss} value for a given drug will bind it most tightly. In contrast to reversible binding seen with most therapeutic drugs, agents like cisplatin and some potentially carcinogenic metabolites that are formed from chlorinated hydrocarbons (such as CCl_4) are covalently bound to tissue proteins. In this case, there is no true distribution of the drug as there is no opportunity for dissociation.

Once a molecule binds to a plasma protein, it moves throughout the circulation until it dissociates, usually for attachment to another large molecule. Dissociation occurs when the affinity for another biomolecule or tissue component is greater than that for the plasma protein to which the toxicant was originally bound. Thus, forces of association must be strong enough to establish an initial interaction, and they must also be weak enough such that a change in the physical or chemical environment can lead to dissociation. Dissociation could occur by binding to proteins of greater affinity (lower K_{diss} values), binding with a higher concentration of proteins of lower affinity, or changes in K_{diss} with changes in ionic strength, pH, temperature, or conformational changes in the binding site induced by binding of other molecules. As long as binding is reversible, redistribution will occur whenever the concentration of one pool (i.e., blood or tissue) is diminished. Redistribution must occur when the concentration is diminished in order to reestablish equilibrium.

Proteins complex with drugs by a variety of mechanisms. Covalent binding may have a profound direct effect on an organism due to modification of an essential molecule, but it usually accounts for a minor portion of the total dose and is of no importance in further distribution of drugs since such compounds cannot dissociate. As previously mentioned, when metabolites of some compounds are covalently bound to proteins, there may be no opportunity for subsequent release of the drug apart from release upon breakdown of the protein itself. The cancer chemotherapeutic drug cisplatin covalently binds to albumin through an aquation reaction. In incubation studies, "aging" occurs after a short period of time independent of drug concentration and the majority of circulating cisplatin is covalently bound.

Noncovalent binding is of primary importance with respect to drug distribution because of the opportunities to dissociate after transport. In rare cases, the noncovalent bond may be so tight (K_{diss} extremely small) that a compound remains in the blood for very lengthy periods. For example, 3-hydroxy-2,4,4-triiodo-α-ethyl hydrocinnamic acid has a half-life of about 1 year with respect to its binding to plasma albumin. The new cephalosporin antimicrobial cefovecin similarly is approximately 97% bound in dogs and has a half-life of 5.5 days, very long for this class of drugs.

Charged drugs may be bound to plasma proteins by ionic interactions. Electrostatic attraction occurs between two oppositely charged ions on a drug and a protein. Proteins are thereby capable of binding charged metal ions. The degree of binding varies with the chemical nature of each compound and the net charge. Dissociation of ionic bonds usually occurs readily, but some members of the transition group of metals exhibit high association constants (low K_{diss} values) and exchange is slow. Ionic interactions may also contribute to binding of alkaloids with ionizable nitrogenous groups and other

ionizable toxicants. Hydrogen bonds arise when a hydrogen atom, covalently bound to one electronegative atom, is "shared" to a significant degree with a second electronegative atom. As a rule, only the most electronegative atoms (O, N, and E) form stable hydrogen bonds. Protein side chains containing hydroxyl, amino, carboxyl, imidazole, and carbamyl groups can form hydrogen bonds, as can the N and O atoms of peptide bonds themselves. Hydrogen bonding plays an important role in the structural configuration of proteins and nucleic acids. Van der Waals forces produce weak interactions, which act between the nucleus of one atom and the electrons of another atom, i.e., between dipoles and induced dipoles. The attractive forces arise from slight distortions induced in the electron clouds surrounding each nucleus as two atoms are brought close together. The binding force is critically dependent upon the proximity of interacting atoms and diminishes rapidly with distance. However, when these forces are summed over a large number of interacting atoms that "fit" together spatially, they can play a significant role in determining specificity of toxicant–protein interactions. A final mechanism of binding is based on hydrophobic interactions. When two nonpolar groups come together, they exclude the water between them, and this mutual repulsion of water results in a hydrophobic interaction. The minimization of thermodynamically unfavorable contact of a polar grouping with water molecules provides the major stabilizing effect in hydrophobic interactions.

Methods for Assessing Protein Binding

A number of methods have been employed to study drug–protein interactions, including ultrafiltration, electrophoresis, equilibrium dialysis, solvent extraction, solvent partition, ultracentrifugation, spectrophotometry, and gel filtration. The most widely used techniques are ultrafiltration and equilibrium dialysis. The basic concept is that a semipermeable membrane is used, which restricts passage of protein but allows unbound drug to cross the barrier according the diffusion. Bound drug is placed on one side of the membrane and samples are collected from the protein-free side. Ultrafiltration allows rapid protein–drug separation while equilibrium dialysis requires time for the separation to occur. The fraction of free drug is then calculated based on the amount of total drug used.

Protein-binding data are frequently expressed in terms of percent of drug bound. Although useful, the limitations should be recognized, for as drug concentration is lowered, the percentage of binding increases. When a compound has a high affinity for a protein (e.g., albumin), percent binding falls sharply when the total drug concentration exceeds a certain value that saturates the binding sites available.

Displacement

If a toxicant or drug is administered after binding sites on a protein are occupied by another chemical, competition for the site occurs, and a higher concentration of free drug may be available. Competition for the same site on plasma proteins may have especially important consequences when one of the potentially toxic ligands has a very high affinity. If compound A has low fractional binding (for example, 30%) and compound B displaces 10% of A from the protein, the net increase of free A is from 70% to 73%, a negligible increase. However, if A were 98% bound and 10% is displaced, the amount of free A increases from 2% to 12%, a sixfold increase in free toxicant. A change in binding may also occur when a second drug produces an allosteric effect resulting in altered affinity of the protein for the originally bound drug (noncompetitive binding). There is great debate as to the clinical significance of such drug–protein displacements that increase drug free fraction, since, as will be seen in the next section on mechanisms of free-drug elimination, the increased free concentration of drug may result in its increased elimination from the body, negating any enhanced activity or toxicity secondary to the displacement.

Most pharmacokinetic models in both human and comparative medical literature assess only total drug concentrations. When the extent of protein binding differs dramatically between species, inappropriate extrapolations often occur if the drug is very highly protein bound in one species. Similarly, interpretation of the extent of tissue distribution when the extent of protein binding is not known may be misleading. The most precise predictions can often be made when the free fraction of drug is known over the concentration ranges of the study being conducted.

Other Factors Affecting Distribution

Among the factors that affect distribution, apart from binding to blood macromolecules per se, are the route of administration, molecular weight, rate of metabolism, polarity, and stereochemistry of the parent compound or metabolic products, and rate of excretion. Molecular weight, charge, and/or polarity have been previously discussed. Stereoselectivity in the disposition of a drug is an often ignored phenomenon which could influence many studies. Its impact on metabolism is obvious; however, any receptor-mediated binding or transport process, including high-specificity protein binding could be affected. Propranolol and ibuprofen have been shown to demonstrate stereoselective distribution.

A major factor determining distribution is the extent of tissue binding, a process identical to that of serum protein binding except that the results on drug disposition are opposite. Tissue binding is governed by the same

mechanisms as discussed above and tends to increase a drug's distribution, although not necessarily activity, if the drug is sequestered away from active drug receptors or target microorganisms. Covalent binding also occurs and is relevant to toxicology and tissue residue depletion. Depending on the pharmacokinetic model employed, irreversible covalent tissue binding may actually be mathematically detected as an increase in the drug's elimination, if only blood samples are used in the analysis, since there is no redistribution of drug back into the blood. For distribution to be quantitated, the basic assumption in most pharmacokinetic models is that the process is irreversible and thus ultimately an equilibrium will be achieved between drug movement into and out of tissue. When irreversible binding occurs, compound is extracted from blood and when excretory output (e.g., urine, feces, expired air) is not monitored, this is interpreted in many models as elimination. These model assumptions are often ignored.

As can be appreciated from this discussion, there are numerous factors that could affect distribution of a compound to tissues. Another is the methodology used to assess tissue distribution. Autoradiography is an excellent technique to anatomically localize distributed drug to the level of organs, cells, and even subcellular components. However, most pharmacokinetic studies rely on analytical techniques. When a tissue sample is collected from an animal, the sample is actually a homogenate of cells, extracellular fluid, and blood. The concentration measured cannot be uniquely assigned to any specific tissue or body fluid compartment. The use of microdialysis and ultrafiltration provides a direct estimate of extracellular fluid concentrations.

The extent of distribution of a compound is termed its volume of distribution (*Vd*) and is calculated by equations such as Equation 2.5:

$$\text{Vd}(1) = \text{Dose (mg)}/\text{Concentration (mg/l)} \qquad (2.5)$$

The *Vd* is actually a proportionality constant relating the plasma concentration to administered dose. Actual approaches to determine this will be presented in Chapter 3.

Renal Elimination

The ultimate route for drug elimination from the body is the kidney. Drugs can also be eliminated in bile, sweat, saliva, tears, milk, and expired air; however, for most therapeutic drugs these routes are generally not quantitatively important as mechanisms for reducing total body burden of drug. The degree of lipid solubility and extent of ionization in blood determines how much of drug will be excreted by the kidney. For drugs that are first biotransformed by the liver, the more water-soluble metabolites are then ultimately excreted through the kidney into the urine. The kidney has also been the most widely studied excretory organ because of the accessibility of urine to collection and analysis. Many of the principles utilized by pharmacologists in quantitating excretory organ function, especially clearance, were originally developed by renal physiologists to noninvasively assess kidney function. Dr. Homer Smith's classic reference on renal physiology is still instructive for the determination of renal clearance.

There are two components relevant to any discussion of renal drug excretion: physiology and quantitation. Renal drug excretion can be considered using the same principles of membrane transport developed earlier, except in this case the movement is from the vascular system to outside the body. Generally, only drugs that are either dissolved in the plasma or bound to circulating blood proteins are available for excretion. The pharmacokinetic parameter estimated by most of these approaches is the renal clearance of the drug.

Renal Physiology Relevant to Clearance of Drugs

For a perspective of drug excretion from the body, the kidney will be considered only as an excretory organ designed to remove foreign compounds (e.g., drugs) and metabolic by-products (e.g., creatinine, urea) from the blood. As will become evident, the major clinical indices of renal function such as blood urea nitrogen, serum creatinine, and creatinine clearance are actually pharmacokinetic parameters of creatinine and urea excretion!

The kidney receives approximately 25% of the cardiac output and thus processes a prodigious amount of blood. The kidney functions in a two-step manner to accomplish its function. The first step is passage through a filtering unit to retain formed cellular elements (e.g., erythrocytes, white blood cells) and proteins in the blood, only allowing the passage of plasma fluid into the remainder of the kidney. The second step utilizes a system of anatomically and physiologically segmented tubules to further modify the contents of the filtered fluid depending on a host of physiological needs including but not limited to fluid, electrolyte, and acid–base balance and the regulation of systemic blood pressure.

The primary functional unit of the kidney is the nephron depicted in Figure 2.8. Depending on the species, there may be 500,000 nephrons per kidney. The sum of their individual function is the observed organ function. Their specific anatomical arrangement is species dependent, often determined by the evolutionary adaptation of the animal to its environment relative to the need to conserve body fluids. The filtration unit is the glomerulus, while the remainder of the fluid processing is accomplished by the extensive tubular system, whose segments are named in relation to their relative distance

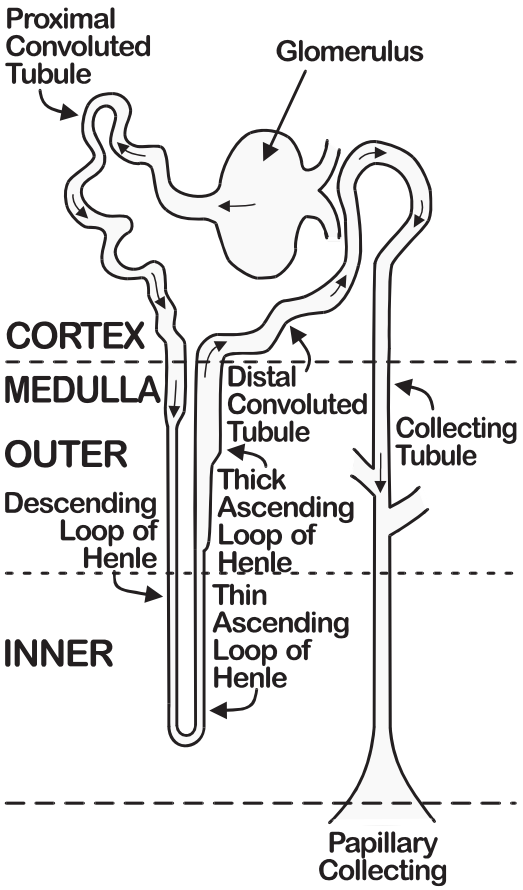

Figure 2.8 Structure of a nephron.

(proximal versus distal) measured *through* the tubules from the glomerulus. The junction between these is a unique anatomical adaptation called the loop of Henle that is designed to use countercurrent exchangers to efficiently produce a concentrated urine since most of the water that is filtered by the glomerulus must be reabsorbed back into the body. The loop of Henle also forces the distal tubules to return toward the surface of the kidney to interact with the glomeruli. Grossly, the region of the kidney containing the glomeruli as well as the proximal and returning distal tubules are on the outside toward the surface and comprise the renal cortex. This region of the kidney is very well perfused by blood and is primarily characterized by oxidative metabolic processes. The interior region is the medulla that is occupied by the penetrating loops of Henle, is poorly perfused, and is characterized by anaerobic metabolism. The amount of tubular fluid filtered by the glomeruli is acted upon by the various nephron segments to reabsorb wanted materials (primarily water and sodium) back into the blood and to let the remainder be excreted into the urine.

The kidney is also the site where acid–base balance is metabolically tuned by controlling acid and base excretion. Some of these processes are coupled to electrolyte secretion (e.g., potassium and sodium) and thus are further modulated by hormones such as aldosterone. These nephron functions may inadvertently alter the amount of drug eliminated in the tubules by changing tubular fluid pH and consequently the ionized fraction of weak acids and bases according to the Henderson–Hasselbach equation presented earlier. This modification in tubular fluid may affect the value of renal clearance determined in pharmacokinetic studies.

There are specific tubular transport systems that excrete products directly into the tubular fluid, which are not filterable because of plasma protein binding. Other transport systems reabsorb essential nutrients (e.g., glucose) back into the blood that were filtered into the tubular fluid. Drugs are also processed by these same transport systems making drug excretion dependent upon the physiological status of the animal. This is especially true when a drug biochemically resembles an endogenous substrate. As is similar to all transport processes, saturation and competition may occur, which may impart nonlinear behavior on a drug's kinetics.

Mechanisms of Renal Drug Excretion

Drugs are normally excreted by the kidney through the processes of (i) glomerular filtration, (ii) active tubular secretion and/or reabsorption, and/or (iii) passive, flow-dependent, nonionic back diffusion. These processes can be considered as vectorial quantities, each possessing magnitude and direction relative to transport between tubular fluid and blood. Their sum determines the ultimate elimination of a specific drug by the kidney as illustrated in Figure 2.9. *The total renal excretion of a drug equals its rate of filtration plus secretion minus reabsorption.* If a drug is reabsorbed back from the tubular fluid

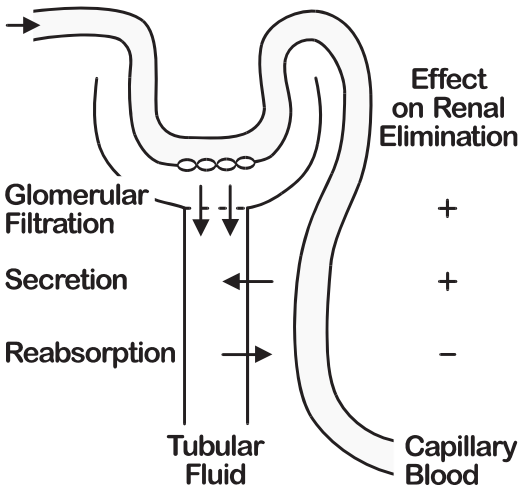

Figure 2.9 Vectorial processes of nephron function and their net effect on overall renal drug elimination.

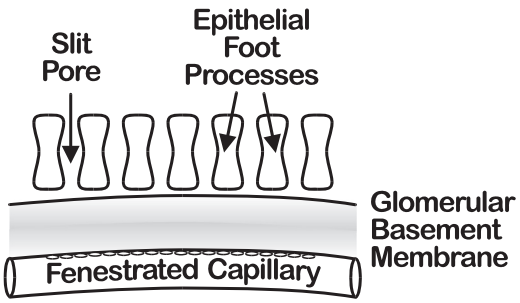

Figure 2.10 The glomerular filtration barrier.

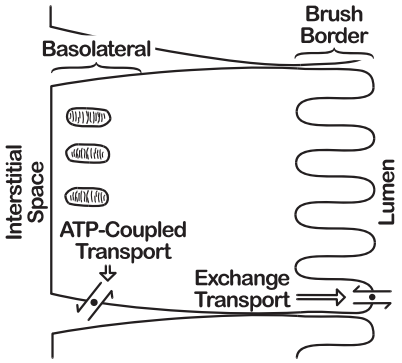

Figure 2.11 Schematic of a renal tubular cell illustrating location of active and exchange transport systems.

into the blood, its net renal excretion will be reduced. In contrast, if a drug is secreted from the blood into the tubular fluid, its net excretion will be increased. These events will be subsequently quantitated.

Excretion by glomerular filtration is unidirectional with drug removal from the blood by bulk flow. Only non-protein-bound drugs are eliminated by this process, a characteristic that is important when predicting elimination pathways for drugs. The rate of drug filtration, therefore, is dependent upon both the extent of drug protein binding and the glomerular filtration rate (GFR) whose calculation will be developed below.

Glomerular filtration is essentially ultrafiltration through the relatively permeable glomerular filtration barrier, which consists of the epithelial cells of Bowman's capsule, the glomerular basement membrane, and the slit-pores formed from juxtaposing epithelial foot processes (Figure 2.10). These possess a fixed negative charge that is a major contributor to the rate-limiting aspect of this barrier. When damaged, filtration selectivity is impaired and proteins may pass into tubular fluid. This is the primary manifestation of glomerular diseases that affect drug excretion.

The magnitude of active tubular secretion is not affected by the extent of plasma drug protein binding. These saturable, carrier-mediated processes are energy dependent and described by the laws of Michaelis–Menten enzyme kinetics. In order to promote absorption from the tubular filtrate into blood, tubule cells have microvilli, much like the intestinal mucosal cells, which maximize the surface area to cell volume ratio presented to the tubule. For secretion from the interstitial space into the tubule lumen, the basolateral surfaces of these cells (side facing the capillaries) have intensive membrane invaginations that also increase the surface area for interaction with the perfusing capillaries to facilitate active secretion. To provide the energy to drive these processes, proximal tubule cells have high mitochondrial densities to generate ATP, which fuels the Na^+-K^+ ATPase-coupled transport systems. This high level of oxidative metabolism is the primary reason for the sensitivity of the kidney to hypoxic or anoxic conditions, which result in renal damage if blood perfusion is interrupted even for short periods of time.

The cellular structure of transport systems across tubule cells involves two separate pairs of transport proteins, which creates an overall "polarity" of tubule cell function relative to the interstitial fluid and tubular lumen (Figure 2.11). One set is located in the brush border of the interface with tubular fluid and the other is located in the basolateral membrane. Energy coupling with ATP generally occurs in the basal portion of the cell (proximity to mitochondria), which, in secretion, builds up intracellular drug concentrations that are then transported to the tubular fluid by concentration-driven facilitated transport carriers. In reabsorption, the reverse occurs as the basolateral active "pumps" create low intracellular drug concentrations, which promote facilitated carrier-mediated reabsorption through the brush-border tubular membrane. Most transport systems are also stoichometrically coupled to the transport of an electrolyte (e.g., Na^+, K^+, Cl^-, H^+), which assures electrical neutrality and provides a mechanism for modulating the systemic concentrations of these elements. The primary ion, which drives these transporters and which regulates overall renal function, is sodium. Thus, all drug transport systems are usually coupled to a Na^+ ATPase transmembrane system whose structure and polarity will determine the nature and direction of drug movement.

There are two distinct secretory pathways in the later sections of the proximal renal tubule that are relevant to a discussion of drug and toxicant excretion: one for acidic and one for basic compounds; the so-called organic anion and organic cation transporters. There are multiple types within each class and most do not show absolute substrate specificity. The primary orientation of this system is from blood to tubular filtrate, removing drugs and/or metabolite conjugates from the blood. Active reabsorption systems are also present that act on a drug already present in the filtered load. These systems are generally present to recover essential nutrients (e.g., glucose) that

have been filtered by the glomerulus. Some drugs reach their target sites by this mechanism making their tubular fluid concentration more important for predicting activity than their blood concentrations. An excellent example is the diuretic furosemide that is first secreted by the tubules into the tubular fluid and then is actively reabsorbed back into the tubular cells to gain access to its receptors for activity. Thus, the best concentration–time profile to predict the diuretic action of furosemide is that of the urine rather than blood.

Drugs (and other endogenous substrates) may compete for tubular transport sites, thereby functioning as reversible, competitive inhibitors. This interaction has been classically studied with the organic acid transport system. Weak acids such as probenecid or phenylbutazone will inhibit secretion of the weak acid penicillin, thereby prolonging penicillin blood concentrations. Thus, when more than one drug in the same ionic class is administered, and they compete for the same organic ion transporter, their rate and extent of renal excretion will be affected. Many drug metabolites are conjugates (e.g., glucuronides) produced by Phase II hepatic biotransformation reactions and secreted by the transport system for weak acids, which may further complicate the pattern of drug excretion.

There are direct pharmacokinetic implications to the carrier-mediated mechanism of renal tubular drug secretion. The limited capacity of carrier-mediated processes means that above certain blood drug concentrations, transport will proceed at a maximal rate independent of concentration in blood; that is, so-called *nonlinear zero order kinetics* will become controlling, which will have adverse effects on the utility of normal linear pharmacokinetic models. These factors may become more important in renal disease states where renal capacity is already diminished. Under these circumstances, drug renal clearance will approach the glomerular filtration rate because additional drug concentrations in blood will not now be secreted into the urine. At subsaturation concentrations, renal clearance of an actively secreted substance is dependent on and limited by renal plasma flow and thus flow-limited mechanisms discussed below will become important considerations.

The final determinant of a drug's renal disposition is the mechanism of nonionic passive tubular reabsorption, or back diffusion, a process dependent upon urine flow rate, lipid solubility of the nonionized drug moiety, and urine pH. At low urine flow rates, there is greater opportunity for diffusion of drug from the distal tubular fluid back into the blood. Diffusion is facilitated by the high concentration of drug in the tubular fluid. Polar compounds having low lipid solubility, such as many drug metabolites, are not reabsorbed since they cannot cross the lipid membrane. In contrast, lipid-soluble, nonionized drugs are reabsorbed into the blood. The ratio of ionized to nonionized molecules determines the concentration gradient that drives the drug into the fluid. The extent of reabsorption is again a function of the drug's pKa and the pH of the tubular fluid, as described by the Henderson–Hasselbach equations (see Equations 2.2 and 2.3). The pH of the urine can undergo drastic changes as a function of diet and coadministered drugs (e.g., urine acidifiers and alkalizers). Tubular reabsorption of organic acids occurs with pKa values between 3.0 and 7.5 and for basic drugs with pKa values between 7.5 and 10.5. Weak acids thus are reabsorbed at low urinary pH (acidic), while weak bases are reabsorbed at high urinary pH (alkaline). Therefore, the renal excretion of an acidic drug decreases in acidic urine but increases in alkaline urine.

This principle is employed in treating salicylate intoxication in dogs. A brisk, alkaline diuresis is induced to decrease salicylate reabsorption into the blood and hasten excretion into the urine by trapping the salicylic acid in an ionized form in the alkaline urine. Reabsorption is further decreased by the elevated urinary flow rate. In contrast, induction of an alkaline diuresis will enhance the toxicity of basic drugs by increasing the amount of tubular reabsorption. Drugs often employed in critical care situations, such as procainamide or quinidine, have increased reabsorption and thus systemic activity in this alkaline state.

Species differences in urinary pH can have a major influence on the rate of renal excretion of ionizable drugs. Carnivores tend to have a more acidic (pH 5.5–7.0) urine than herbivores (pH 7.0–8.0). Thus, with all other disposition factors being equal, a weakly acidic drug will have a higher renal excretion in herbivores than in carnivores and a weakly basic drug will have a greater renal excretion in carnivores than in herbivores. In healthy animals, small changes in urinary pH or urine flow rate do not significantly contribute to altered drug clearance. However, with decreased function in renal disease, there is a decreased tubular load of drug. Altered urinary pH theoretically could further decrease overall drug clearance.

There are two other peculiarities of renal tubular transport that must be discussed before quantitating these processes. Some drugs are reabsorbed into the tubules by pinocytosis. This occurs by interaction of filtered drug in the tubular fluid with the brush border membrane. This is a very low-capacity and slow process that is easily saturated. Pinocytozed drugs are then transferred to lysosomes and generally digested in the cell (e.g., peptides and filtered proteins such as β_2 microglobulin). However, for some compounds, such as the aminoglycosides, enzymatic breakdown does not occur and the drug is essentially stored in the kidney. Therefore, although the drug is reabsorbed from the tubular fluid, it is not transported through the cell into the blood. Thus unlike other tubular reabsorption processes, reabsorption with storage or metabolism

does decrease elimination of drug from the body. Such reabsorption has toxicological significance because the drug does accumulate in the tubular cells and could produce an adverse effect. Finally, this phenomenon has a major influence on the prediction of tissue residue profiles in the kidney resulting from drugs with prolonged elimination half-lives (e.g., aminoglycosides).

The final confounding influence on determination of renal drug clearance is when a drug is metabolized by the kidney. Most of the Phase I and Phase II enzymatic systems present in the liver also exist in the kidney, although different isozymes may be expressed. Oxidative processes generally occur within the proximal tubule cells. Two scenarios may occur. The first is when the drug is solely metabolized by the kidney and not the liver, or a combination of both processes occurs. The second is when *relay* metabolism occurs and the kidney further metabolizes a drug already biotransformed by the liver. These interactions are complex and generally are of toxicological significance.

Renal drug biotransformation may also occur in the medulla by anaerobic metabolic processes (e.g., prostaglandin endoperoxide synthetase). This process is small relative to reducing overall body burden because only 1% of renal blood flow delivers compounds to this region, but it has toxicological significance to the renal medulla where drug and/or metabolite may accumulate. Finally, brush border enzymes are present that metabolize peptides in the filtered tubular load to amino acids for reabsorption. Stereoselectivity in both active tubular secretion and metabolism in the kidney may occur with specific drugs (e.g., quinidine). The implications to assessment of renal drug excretion is similar to that of drugs metabolized by the kidney and is usually not taken into account.

The Concept of Clearance and its Calculation

Clearance is a concept widely used to measure the efficiency of drug elimination from an organ or the whole body. The concept was developed for use in assessing kidney function by renal physiologists. The problem with simply measuring the concentration of drug in urine as an index of its renal excretion is that the kidney also modulates the volume of urine produced in association with its primary mission of regulating fluid balance. Thus, the concentration of drug alone may be higher or lower depending on the ultimate urine volume. To accurately assess how much drug is eliminated, the product of volume of urine produced and the concentration of drug in urine (mass/volume) must be determined to provide the amount excreted (mass). If timed urine samples are collected, an excretion rate (mass/time) is determined. Similarly, to assess how efficient this process is, one must know how much drug is actually presented to the kidney for excretion. This is related to the concentration of drug in the arterial blood. The physiological concept of clearance was developed by early workers to generate a parameter that measured the true efficiency of renal excretion processes by assessing the total mass of compound ultimately excreted and relating it to the concentration of drug presented to the kidney for excretion.

There are two definitions of renal clearance that are used to define equations to calculate this parameter from real data. The first is *the volume of blood cleared of a substance by the kidney per unit of time*, that is, the volume of blood required to contain the quantity of drug removed by the kidney during a specific time interval. This will be derived when we have developed pharmacokinetic parameters in Chapter 3 to quantitate Vd and fractional excretion rates. The second definition is *the rate of drug excretion relative to its plasma concentration*. In both cases, the actual value for a drug's renal clearance is the vectorial sum of {filtration + tubular secretion − tubular reabsorption}, making it a parameter that estimates the entire contribution of the kidney to drug elimination. Similarly, any change in renal drug processing will be reflected in renal clearance *if* it is not compensated for by more distal components of the renal tubules.

There are two types of data needed to calculate clearance: (i) an estimate of blood drug concentration presented to the kidney and (ii) the amount of drug removed by the kidney. The latter can be estimated either by measuring the amount of drug excreted by the urine or comparing the difference between the renal arterial and venous drug concentrations to assess how much drug was extracted while passing through the organ.

To begin, we will use the classic approach (Equation 2.6), which directly measures extraction, based on Fick's Law:

$$\mathrm{Cl\ (ml/min)} = (\mathrm{Q})\ (\mathrm{E}) = (\mathrm{Q})\ \frac{\left(\mathrm{C_{art}} - \mathrm{C_{ven}}\right)}{\left(\mathrm{C_{art}}\right)} \quad (2.6)$$

where Q is renal arterial blood flow (ml/min), E is the extraction ratio and C_{art} and C_{ven} are arterial and venous blood concentrations. The obvious difficulty with this approach is that arterial and venous blood samples must be collected. However, renal physiologists realized that this approach could be modified to more easily assess renal function if a few assumptions were made.

The first is that the amount of substance removed or extracted by the kidney is equivalent to that which is excreted into the urine. If one makes a timed collection of urine and measures the urine concentration and volume, the amount (X) of drug extracted by the kidney over a specific time interval, that is, its *rate* of renal excretion denoted $\Delta X/\Delta t$, is the following (Equation 2.7):

$$\Delta \mathrm{X}/\Delta \mathrm{t\ (mg/min)} = \left[\mathrm{U_X\ (mg/ml)}\right]\ [\mathrm{V\ (ml/min)}] \quad (2.7)$$

where U_x is the concentration of drug in urine and V is the urine production. Now the only component needed is the concentration presented to the kidney. Workers used constant rate intravenous infusion of chemicals to insure that so-called *steady state* blood concentrations were achieved. With this experimental design, the renal clearance of substance X is calculated as the following (Equation 2.8):

$$Cl_{(renal)}\,(ml/min) = (\Delta X/\Delta t)/C_{art} = \mathbf{U_X}V/C_{art} \tag{2.8}$$

This expression is equivalent to the definition for clearance above that relates the rate of drug excretion to its plasma concentration. This expression serves as the basis for many of the pharmacokinetic techniques to be developed in subsequent chapters.

Some minor discrepancies may result when drug clearances are calculated by use of blood or plasma data alone versus techniques such as those that employ urine collection. As discussed earlier, tubular reabsorption with storage (e.g., aminoglycoside antibiotics) will result in a lower $Cl_{(renal)}$ calculated from urine data rather than blood-based methods because tubular reabsorption would not be reflected in the venous blood concentrations since the substance is now trapped in the tubular cells. A similar discrepancy may occur with intrarenal drug metabolism since this process does not return parent drug to venous blood. In a research setting, the difference is often used as conclusive evidence that either of these two phenomena actually occur.

Nonlinearity of Tubular Secretion and Reabsorption

The discussion thus far has been limited to determining clearances of substances that are primarily eliminated through glomerular filtration. The pharmacokinetics of this process are linear since saturation does not occur and only non-protein-bound drugs are filtered through the glomerular basement membrane complex. When the renal clearance of a drug eliminated by glomerular filtration is estimated, only the filtration of the free or unbound drug is assessed; thus changes in protein binding will change the net excretion of the drug. Drugs and toxicants that undergo passive tubular reabsorption obey Fick's Law of Diffusion since concentration gradients described again by linear first-order rate constants provide the driving force across the tubular epithelium. In contrast, compounds that are actively secreted from postglomerular capillaries across the renal tubules and into the tubular fluid show saturation at high concentrations, competition with drugs secreted by the same pathways, and dependence on the magnitude of renal blood flow; all hallmarks of nonlinear pharmacokinetic behavior. For such compounds, clearance will not be constant but rather will be dependent upon the concentration of drug presented to the kidney.

As tubular secretory pathways become saturated, a drug's clearance will decrease. To develop this concept, we will revisit our definition of a drug cleared by GFR and acknowledge that only the free or unbound drug concentration (C_f) is eliminated by filtration. Protein bound drug (C_b) cannot be filtered. Therefore, the rate of renal excretion ($\Delta X/\Delta t$) can be expressed as simply this (Equation 2.9):

$$\Delta X/\Delta t = C_f \times GFR \tag{2.9}$$

As C_f becomes greater, $\Delta X/\Delta t$ will increase in direct proportion (e.g., linearly increase). However recalling Equation 2.8, its clearance will be $\Delta X/\Delta t$ *divided* by C_{art}. In this case, C_{art} is the total blood concentration presented to the kidney ($C_f + C_b$). Clearance thus equals the following (Equation 2.10):

$$Cl_{(renal)} = (\Delta X/\Delta t)/C_{art} \tag{2.10}$$

These relations have two implications. The first is that as total blood concentrations of drug increase, so does $\Delta X/\Delta t$; however, $Cl_{(renal)}$ remains constant (Equation 2.11) because

$$Cl_{(renal)} = (C_f \times GFR)/(C_{art}) \tag{2.11}$$

and C_{art} will increase in direct proportion to $C_f + C_b$ as long as the fraction bound does not change. However, if the extent of protein binding of a drug is increased ($C_f \downarrow$, $C_b \uparrow$), its rate of renal excretion, $\Delta X/\Delta t$, will decrease as will its clearance since the C_{art} ($C_f + C_b$) will be constant. Therefore, drugs cleared by filtration have constant clearance with changing total drug concentrations but are sensitive to the extent of protein binding. This is one reason mentioned above in the discussion of plasma protein binding, that displacement of a protein bound drug that increases C_f will result in increased renal clearance, which then reduces its concentration to normal. For such a drug with high protein binding, only the small fraction presented for filtration can ever be extracted and cleared by the kidney. Since the total renal clearance of a compound is the sum of filtration plus secretion, a drug *solely cleared by filtration* will have a relatively low clearance compared to one that is also actively secreted. If one considers this in terms of the extraction ratio (E) defined above, Cl_B will always be less than the renal blood flow (Q) since the extraction ratio is less than one and dependent on the glomerular filtration fraction. Such drugs are termed *low extraction* drugs and their $Cl_{(renal)}$ will be sensitive to the extent of protein binding. Examples of such drugs include inulin, the aminoglycoside antibiotics, tetracyclines, digoxin, and furosemide.

In contrast, consider a drug that also undergoes active tubular secretion. In this case, even a drug that is protein bound (C_b) or distributed into red blood cells will

be secreted into the urine since the affinity for specific tubular transport proteins will be greater than that for the relatively nonspecific protein-binding sites or partitioning in erythrocytes. The extraction ratio will thus approach 1.0 and $Cl_{(renal)}$ will approach the renal blood flow Q. Such drugs are termed *high extraction* or *perfusion limited* to acknowledge the relationship of clearance to blood flow. The classic example is para-amino hippurate (PAH) because it is almost completely extracted as it passes through the kidney, making its clearance almost equal to renal plasma flow. In fact, PAH renal clearance had once been used in clinical situations to estimate renal blood flow. Other such drugs include many of the β-lactam antibiotics (e.g., penicillin) and many sulfate and glucuronide conjugate products of hepatic drug biotransformation. Another implication of active tubular secretion is that at sufficiently high concentrations, saturation of the secretory pathways may occur. Finally, the maximal renal clearance possible is renal blood flow.

The final pathway modulating renal excretion is when a drug undergoes passive tubular reabsorption. The dependency of this process on urinary pH has already been discussed. In this case, C_{art} will be constant but $\Delta X/\Delta t$ and thus $Cl_{(renal)}$ will vary depending on the urinary pH. Since this is an equilibrium process, time is required for this diffusion to occur. Thus if the renal clearance of a drug is dependent on urine flow, it is presumed to undergo passive tubular reabsorption. When high tubular loads are presented, reabsorption is overloaded as equilibrium cannot be achieved and nonreabsorbed drug is eliminated into the urine.

We have focused this discussion on renal drug elimination. However, clearance is used throughout physiology and pharmacokinetics to quantitate drug elimination through any organ as well as from the body. The relevant equation (Equation 2.12) defining the whole body clearance (Cl_B) of a drug is the sum of all elimination clearances:

$$Cl_B = Cl_{(renal)} + Cl_{(hepatic)} + Cl_{(other)} \quad (2.12)$$

Calculation of Cl_B provides an efficient strategy for estimating how a drug or toxicant is eliminated from the body as it indirectly compares systemic clearance to renal and hepatic clearances.

Hepatic Biotransformation and Biliary Excretion

Hepatic disposition is one of the final keys in the ADME scheme needed to describe the disposition of many drugs and chemicals in the body. The liver is responsible for both biotransformation and biliary excretion. In many ways, the liver should be considered as two separate organs, one encompassing metabolism and the other biliary excretion.

Drug localization and biotransformation in the liver are dependent on many factors associated with both the biological system and drug itself. These factors include the biological properties of the liver (chemical composition, relative activity of major drug metabolism enzymes, hepatic volume/perfusion rate, and drug accessibility to and extraction by hepatic metabolic sites) as well as the physicochemical properties of the drug (pKa, lipid solubility, molecular weight). In a quantitative sense, the liver is the major drug metabolism organ in the body.

Species differences in drug metabolic fate are, in most cases, the primary source of variation in drug disposition and, therefore, in drug activity or toxicity, across species. It has been estimated that 90% of drugs administered to humans are metabolized and polymorphisms in human metabolizing enzymes are responsible for the vast majority of adverse drug events. Extrapolation of metabolism data between animal species is an important issue as is the ability to correlate in vivo pharmacokinetic and metabolic data with in vitro metabolic findings.

Recalling our earlier discussion about the phenomenological role of metabolism in drug distribution and excretion, it would be hard to imagine what would happen in biological systems without xenobiotic metabolism. Absorbed compounds would stay in the body for a much longer period of time and have prolonged activity, tissue accumulation, and, potentially, toxicity. Metabolism is necessary for the animal or human body to rid itself of lipophilic xenobiotics as an effective defense mechanism against adverse effects. In general, the intensity of drug action is proportional to the concentration of the drug and/or its active metabolite(s) at the target site. On the other hand, drug-associated toxicity is also dependent on the chemical form (active or inactive) and concentration at the same or other relevant target site. Therefore, any process or factor that modifies the drug/metabolite concentration at a target site will cause an altered activity or toxicity profile. Drug metabolism may often result in metabolite(s) with altered chemical structures, which change the receptor type affected, drug–receptor affinity, or pharmacological effect. Most parent drugs can be deactivated to inactive metabolites. In contrast, some drugs can also be activated either from an inactive form (prodrug) to an active drug, or from an active form (e.g., meperidine) to an active metabolite (normeperidine) with similar activity/toxicity. Therefore, drug metabolism can either reduce or enhance parent drug's effect, create another activity, or even elicit toxicity, depending on both the drug and the biological system in question.

Therefore, the pharmacological and pharmacokinetic properties of a drug can be changed by metabolism in one or several of the following ways: pharmacological

Table 2.2 Drug metabolism reactions

Phase I	Phase II
Oxidation	Glucuronidation/glucosidation
Cyt P-450 dependent	Sulfation
Others	Methylation
Reduction	Acetylation
Hydrolysis	Amino acid conjugation
Hydration	Glutathione conjugation
Dethioacetylation	Fatty acid conjugation
Isomerization	

activation or deactivation; change in disposition kinetics of drug uptake (absorption from application site), distribution, and excretion (e.g., bile excretion, enterohepatic circulation, and renal excretion). The remainder of this chapter focuses on hepatic metabolism and drug hepatobiliary excretion in animal species and introduces some basic biochemical and pharmacokinetic concepts relevant to this role. Although these discussions are focused on the liver, the principles elucidated may also be applicable to extrahepatic sites of drug biotransformation.

Phase I and Phase II Reactions

Various metabolic pathways are involved in drug metabolism including oxidation, reduction, hydrolysis, hydration, and conjunction. These processes can be divided into Phase I and Phase II reactions (Table 2.2). Phase I includes reactions introducing functional groups to drug molecules necessary for the Phase II reactions, which primarily involve conjugation. In other words, Phase I products act as substrates for Phase II processes, resulting in conjugation with endogenous compounds, which further increase their water solubility and polarity, thus retarding tissue distribution and facilitating drug excretion from the body. Specific examples of drug metabolism are included in chapters throughout this text. The focus of this introduction will be to briefly overview the general processes involved in drug metabolism relative to how they might affect pharmacokinetic parameters and the disposition of drugs in the body. Interested readers should consult standard texts in drug metabolism or biochemical pharmacology/toxicology for specific detailed examples illustrating the chemistry and genetic control of these processes.

Our knowledge regarding the molecular mechanisms of drug metabolism has been predominately gained from studies on the liver at different experimental levels including in vivo intact animals; ex vivo liver perfusion; and in vitro liver slices, hepatocyte cell cultures, isolated/purified subcellular hepatocyte organelles, and isolated enzyme or enzyme components. Two subcellular organelles are quantitatively the most important; the endoplasmic reticulum (ER) (isolated in the microsome fraction) and the cytosol (isolated in the soluble cell fraction). Phase I oxidation enzymes are almost exclusively localized in the ER, along with the Phase II enzyme of glucuronyl transferase. In contrast, other Phase II enzymes are mainly present in the cytoplasm. Microsomal fractions of the hepatocyte retains most, if not all, of the enzymatic activity in drug metabolism.

Phase I metabolism includes four major pathways: oxidation, reduction, hydrolysis, and hydration, among which oxidation is the most important. Attention is usually focused on oxidation mediated by the microsomal mixed-function oxidase system (e.g., cytochrome P450, etc.) due to its central role and significance in governing the metabolic disposition of many drugs and xenobiotics. An understanding of this pathway is often critical to making interspecies extrapolations.

Phase II conjugating enzymes play a very important role in the deactivation of the Phase I metabolites of many drugs as well as in direct deactivation of some parent compounds when their specific structure doesn't require Phase I modification. For example, the analgesic drug paracetamol can be deactivated directly by Phase II reactions using glutathione, glucuronide, and sulfate conjugation mechanisms. Phase II deactivation can be achieved by both gross chemical modification of the drug thereby decreasing their receptor affinity, and by enhancement of excretion from the body, often via the kidney.

Among the reactions catalyzed by drug metabolism enzymes in the hepatic ER, cytochrome (Cyt) P450-dependent mixed-function oxidation is the most intensively studied. This reaction catalyzes the hydroxylation of hundreds of structurally diverse drugs and compounds, whose only common feature appears to be a relatively high lipophilicity. The enzyme consists of a family of closely related isoenzymes embedded in the ER membrane. Its name is based on the fact that the cytochrome is a pigment that exhibits a maximal absorbance wavelength of 450 nm when reduced and complexed with carbon monoxide. With the advent of gene cloning and sequencing, and the application of molecular biology techniques to Cyt P450 structure analysis, tremendous progress was made in the last decade in the isolation and sequencing of the cDNAs encoding multiple forms of the hemoprotein. The rapid determination of full-length Cyt P450 amino acid sequences enabled the development of a coherent nomenclature system describing hundreds of different and unique Cyt P450s.

A great deal of work has been fostered in this area through development of a nomenclature system for cytochrome P450 enzymes (CYP) based on DNA/amino acid sequence. This allows enzymes to be unambiguously classified. An enzyme is coded by its family (1,2,…) followed by subfamily (A–D) and then gene (1,2,…), and if necessary, allelic variant (*1, *2,…). Thus, a common

Table 2.3 Example of cytochrome P450 enzymes and substrates in the dog

Subfamily	Gene code	Sample substrates
1A	1A1, 1A2	Theophylline, phenacetin, 7-ethoxyresorufin, caffeine
2B	2B11	Ketamine, propofol, pentobarbital, warfarin
2C	2C21, 2C41	Diclofenic, midazolam
2D	2D15	β blockers, celecoxib, dextromethorphan
3A	3A12, 3A26	Macrolides, steroids, cyclosporine

enzyme found in dogs is termed CYP3A12, which is responsible for steroid oxidation. These enzymes are classified on the basis of sequence and not function. There is thus a great deal of overlap between substrate specificities and enzymes with different CYP identifications. The numbers are also species specific, thus preventing direct comparison between species. Not surprisingly, most work has been done for human enzymes, although recently work has also begun to define the primary enzymes involved in veterinary species. Table 2.3 lists some of the cytochrome P450 enzymes identified in the dog. This area of cytochrome P450 pharmacogenomics in the dog has recently been reviewed (Martinez et al., 2013).

Impact of Metabolism

One can precisely predict the impact of drug metabolism on therapeutic drug effect if one can identify the precise enzymes by which a drug is metabolized. The same enzymes are not involved in metabolizing the same drugs in different species and, in many cases, substrate specificities overlap, making precise prediction difficult. A variety of Phase I and Phase II reactions can take place simultaneously or sequentially in the body. For example, parathion can be catalyzed by Cyt P450 to an intermediate, which in turn can either be further oxidized to paraoxon or hydrolyzed to *p*-nitrophenol followed by conjugation reactions. Finally, as discussed earlier, a compound metabolized in the liver may be subsequently metabolized in the kidney prior to excretion, making it possible for these various metabolic steps to be carried out in multiple organs. Stereochemistry also plays a major role in drug metabolism since most enzyme system can be stereoselective. Examples include the enantiomers of amphetamine, cyclophosphamide, pentobarbitone, phenytoin, verapamil, and warfarin. Large differences in enzyme expression and function occur between species. Many of these were first identified in broad differences in the ability of some species studied to perform Phase II reactions (e.g., cats deficient in glucuronidation, pigs in sulfation). We now know that this is even more complex, especially in species and breed differences in specific CYP isoenzymes.

To further complicate this scenario, there is often overlap between cytochrome P450 substrate specificities and those for P-glycoprotein. That is, the same drug may be handled by both systems, classic examples being cyclosporine and ketoconazole. Such phenomena are often detected by complex and species-specific drug–drug interactions. For compounds handled in such a fashion, one can be assured that large differences will be seen between animal species making interspecies extrapolations very difficult.

In summary, Phase I metabolism is primarily responsible for drug deactivation, although Phase II plays an important role in deactivation of some drugs. Phase I reactions prepare drugs or toxicants for Phase II metabolism; that is Phase I modifies the drug molecule by introducing a chemically reactive group on which the Phase II reactions can be carried out for the final deactivation and excretion. This increased water solubility after metabolism restricts a drug's metabolite distribution to extracellular fluids, thereby enhancing excretion. Specific pathways for drug metabolism and transport are discussed in the individual drug chapters as well as their pharmacogenomics in Chapter 50.

Hepatic Clearance

As presented in our discussion on renal excretion, clearance of a drug by an organ (Cl_{org}) can be ultimately defined as a function of its blood flow (Q_{org}) and its extraction ratio (E_{org}) expressed in Equation 2.6 as $Cl_{org} = Q_{org}\, E_{org}$. The ability of the liver to remove drug from the blood, defined as *hepatic clearance,* is related to two variables: intrinsic hepatic clearance (Cl_{int}) and rate of hepatic blood flow (Q_h), as defined in Equation 2.13:

$$Cl_h = Q_h \left[Cl_{int}/\left(Q_h + Cl_{int}\right)\right] = Q_h E_h \qquad (2.13)$$

where Cl_h is the hepatic clearance, Q_h is the hepatic blood flow, and $Cl_{int}/(Q_h+Cl_{int})$ is the hepatic extraction ratio or E_h. Intrinsic clearance (Cl_{int}) is conceptualized as the maximal ability of the liver to extract/metabolize drug when hepatic blood flow is not limiting. It represents the inherent metabolic function of all enzyme systems in the liver to metabolize the drug in question. As seen in Equation 2.13, when $Cl_{int} \gg Q_h$, hepatic extraction ratio ≈1.0 (*flow limited or high extraction*, usually seen with $E_h > 0.8$), Cl_h is dependent only on the blood perfusion rate Q_h. The more blood passing through the liver, the more drug molecules will be extracted by the liver for metabolic elimination. A hepatic blood perfusion-dependent hepatic clearance will then be seen. Drugs with such high extraction ratios will show significant first-pass metabolism after oral administration to the

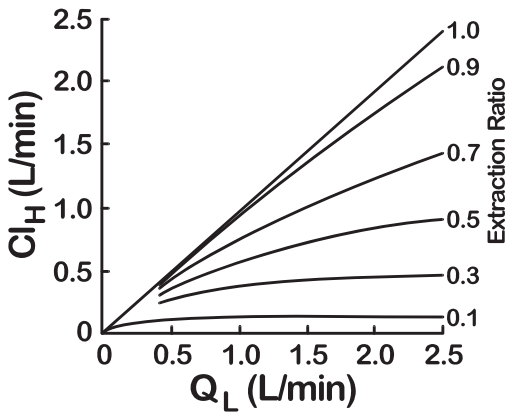

Figure 2.12 Relationship between liver blood flow (Q) and hepatic clearance (Cl_H) for drugs with different hepatic extraction ratios. Extraction ratio values are at a blood flow of 1.5 l/min.

extent that this route of administration may not produce effective systemic drug concentrations. Finally, the highest hepatic Cl possible is Q_h.

In contrast, if $Cl_{int} \ll Q_h$, the E_h is close to zero and thus the Cl_h is dependent only on the Cl_{int}; that is the liver extracts as many drug molecules as it can from the blood flow presented (*metabolism limited or low extraction*, usually with E_h <0.2). These two extremes occur with propranolol and antipyrine, respectively. Intermediate values of extraction ratio of 0.2–0.8 give hepatic clearance rates that can be dependent to varying extents on both hepatic blood perfusion rate and intrinsic clearance. Based on earlier discussions, one can appreciate that such classifications are very species specific since they depend upon the presence of specific enzyme systems for the specific drug.

To estimate hepatic drug clearance, one must consider the drug's physicochemical properties, hepatic drug metabolism enzyme activity, and rate of hepatic blood perfusion. This relationship between hepatic clearance and liver blood perfusion rate for drugs with different extraction ratios can be appreciated when Equation 2.13 is plotted in Figure 2.12. With a lower hepatic extraction drug, the blood perfusion rate is less important to $Cl_{hepatic}$. For a high hepatic extraction drug, the $Cl_{hepatic}$ is proportional to the blood flow, as discussed earlier. The reader should note the similarity of this discussion to that introduced earlier concerning capacity or flow-limited renal tubular clearance. The concepts are identical for both organs; however, they are more often employed when hepatic clearance is modeled due to the much greater difference in species-inherent metabolic capacities.

Metabolism Induction and Inhibition

Drug metabolism is substantially influenced by enzyme induction or inhibition that occurs secondary to the deliberate or passive intake of a number of chemicals that animals are increasingly exposed to either in the environment, for medical reasons, as dietary supplements, or in humans simply as a result of lifestyle (smoking, alcohol consumption, etc.). In laboratory animals, contaminants and natural constituents of diet have been shown to affect the pattern of drug metabolism observed. In many cases, the compound itself may alter its own metabolic fate by induction or inhibition.

Induction

Many currently used drugs, food additives, household chemicals, and environmental contaminants (including pesticides) possessing diverse chemical structure, pharmacological or toxicological activity are well known to induce their own metabolism and/or that of other compounds in humans and animals. Induction of metabolism may arise as a consequence of increased synthesis (at different transcriptional/translational levels), decreased degradation, activation of preexisting components, or a combination of these processes. With so many compounds able to alter hepatic metabolism, a great deal of effort has been spent in recent years to understand the mechanisms behind these processes. This is important from a therapeutic perspective since the intrinsic hepatic clearance of a drug will change if the enzymes responsible for metabolizing it are induced, thereby increasing metabolic capacity. Similarly, the pattern of Phase I and Phase II metabolism may be changed if one enzyme component's activity has been modified by inducers. These interactions introduce a significant complexity to pharmacokinetic models describing the disposition of drugs extensively metabolized by the liver. However, they have also prompted research efforts aimed at elucidating the mechanisms behind these processes, which, when understood, should provide a strategy for developing mechanistically meaningful models for simulation of drug metabolic disposition.

As discussed above, the activity of the Cyt P450 system is of particular importance to the overall hepatic metabolic clearance of a drug. In the mid-1960s, both Cyt P450 and its associated flavoprotein reductase were found to be induced by phenobarbitone pretreatment that was accompanied by induction of drug metabolism. Induction was generally accompanied by increases in liver microsomal Cyt P450 content. Diverse drug metabolism responses to different inducers, which all induce hepatic Cyt P450, can be dependent on the substrate of interest (substrate specificity) with stero- and regioselectivity, confirming that subpopulations of Cyt P450 (isoenzymes) might be present. This now widely accepted concept has had a profound influence on drug discovery, design of metabolism studies, and the resulting structure of pharmacokinetic models. With the advent of the CYP nomenclature system discussed above, enzymes

can now be classified both as to substrate specificity, but also as to which compounds induce or inhibit their function. Pharmacogenomic studies have begun to identify the specific genes responsible for isoenzyme induction and also identify species differences. Unfortunately, these studies are just beginning to be applied in veterinary species and a complete picture is still lacking on their impact on clinical therapeutics. A major cause of this problem is that, as mentioned above, there are not specific homologs between human and veterinary species enzymes, making marker substrates different. The important concept is that induction will occur and could have a significant impact on therapeutic efficacy. Specific examples of such interactions are presented within the drug-specific chapters of this text.

Metabolism Inhibition

Similar to the induction of metabolism, inhibition is a well-recognized phenomenon secondary to serial drug dosing, coadministration of drugs, endogenous compounds, environmental xenobiotics, and complex multiple-ingredient drug formulations. Several mechanisms for metabolism inhibition have been noted, including the destruction of preexisting enzymes (by porphyrinogic drugs and xenobiotics containing olefinic (C = C) and acetylenic (C ≡ C) functions), inhibition of enzyme synthesis (by metal ions), or complexing with the hemoprotein thereby inactivating enzymes. Many drug–drug interactions may be explained at the level of Cyt P450 destruction. In contrast to the porphyrinogic drugs, metal ions such as cobalt exert their inhibitory effects by modulating both the synthesis and degradation of the heme prosthetic group of Cyt P450. Formation of inactive Cyt P450-inhibitor complex is another mechanism for drug metabolism inhibition. Inhibitors are usually substrates of Cyt P450 and require metabolic conversion to exert their full inhibitory effects, in a manner similar to porphyrinogic drugs and xenobiotics. However, inhibitors forming complexes with hemoprotein are metabolized by Cyt P450. These inhibitors can form metabolic intermediates or products that tightly bind to the hemoprotein, thereby preventing its further participation in drug metabolism. As can occur with induction, coadministration of inhibitor drugs may result in clinically important drug interactions. Specific examples of such interactions are presented within the drug-specific chapters of this text.

Biliary Drug Elimination

As an exocrine function of the liver, bile excretion is thought to be present in almost all vertebrates. The three basic physiological functions of the bile are (i) to serve as the excretory route for products of biotransformation, (ii) to facilitate the intestinal absorption of ingested lipids such as fatty acids, cholesterol, lecithin, and/or monoglycerides due to the surfactant properties of bile forming mixed micelles, and (iii) to serve as a major route for cholesterol elimination in order to maintain normal plasma cholesterol levels. In addition to its physiological functions, bile is also pharmacologically and toxicologically important since some heavy metals and enzymes are also excreted via the biliary system. Bile secretion is very important to chemical/drug transport and elimination under both physiological and pathological conditions. However, bile secretion has proven difficult to study mainly due to the inaccessibility of the biliary tree for direct sampling.

The Mechanism of Bile Formation

Bile is continuously produced by liver cells and then stored in the gall bladder, except for those species (rat, horse) lacking it. The pH of bile ranges from 5.0 to 7.5 depending mainly on the animal species. Biliary excretion is a major route for some drugs with MW >300 and a high degree of polarity. This occurs by active transport of drug and metabolites into bile, thus saturation and competition are important issues to consider. Passive diffusion of drug into bile is insignificant. Most of the compounds secreted into bile are finally excreted from the body in feces where they may be subject to enterohepatic circulation and degradation by intestinal microflora.

Bile is formed at two sites: the ramifications of the bile duct within the portal triads and the anastomosing network of the narrow bile canaliculi in the hepatic parenchyma. The bile canaliculi are the primary secretory units of the liver. These small channels or furrows are lined by the apical membranes of the hepatocytes and thus do not have their own epithelium or basement membrane. Because hepatocytes form a canalicular lumen wherever they abut, most canaliculi communicate with each other, forming an anastomosing network. Similar to the relationship of the nephron to the kidney, the volume and composition of canalicular bile are often determined by the activity of several cords of hepatocytes.

The overall bile flow is in the opposite direction to sinusoidal blood flow and thus solute transfer from plasma to bile involves a counterflow process (Figure 2.13). Such a blood–bile flow pattern reduces rediffusion of biliary solutes such as drugs and metabolites back into sinusoidal plasma in the portal area, which is richer in solute concentration, bathes periportal hepatocytes, and is exposed to higher canalicular concentration of any given solute. Three routes of fluid and drug transfer from the sinusoid to the bile canaliculus have been postulated: transcellular, paracellular, and vesicular. These multiple mechanisms contribute to the large interspecies differences seen in biliary drug excretion.

Drug uptake into hepatocytes by passive diffusion is so efficient that it is rate limited by the delivery of the

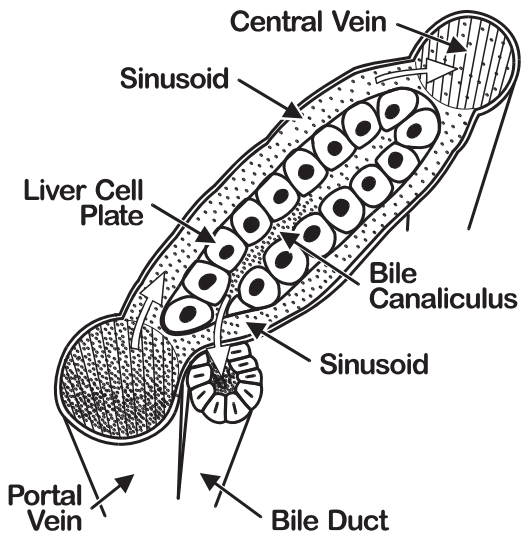

Figure 2.13 Gradient concept of bile secretion in a liver lobule.

drug to the liver (i.e., blood flow) rather than membrane transport, thereby exhibiting flow-dependent clearance. However, for highly polar molecules, passive diffusion is not an efficient mode of hepatocellular uptake, and there is an increased reliance on carrier-mediated transport systems. Drug metabolites, particularly conjugated metabolites (e.g., sulphates and glucuronides), are invariably more polar than their precursors and thus are more likely to experience hepatocyte membranes as diffusional barriers. With such a barrier, the hepatocellular export of a locally formed metabolite will depend on the presence and activity of carrier-mediated transport systems for sinusoidal efflux and biliary excretion. Transport systems of current interest include the P-glycoproteins, which are responsible for the biliary excretion of a range of organic cations, and the canalicular multispecific organic anion transporter. Intracellular trapping of metabolites formed in the liver, secondary to low membrane permeability, is clinically important because many are potentially hepatotoxic and/or capable of interfering with the hepatic transport of endogenous compounds or other drugs and metabolites. Again this phenomenon is conceptually similar to renal tubular sequestration and has similar pharmacokinetic implications. Finally, if the metabolite is unstable, intracellular accumulation can lead to the regeneration of the precursor and so-called "futile cycling" within hepatocytes.

Biliary Drug Transport

Some parent drugs and numerous drug metabolites derived from hepatic metabolism are excreted in the bile into the intestinal tract. The excreted metabolites can be excreted via feces although, more commonly, they are subject to reabsorption into the blood and are eventually excreted from the body via urine. There are at least three different biliary transport pathways for organic anions, cations, and neutral compounds, although metals may also have their own transport carriers/systems. Both *organic anions* and *organic cations* can be actively transported into bile by carrier systems again similar to those involved in the renal tubule. Such transport systems are nonselective, and ions with similar electrical charge may compete for the same transport mechanisms. Additionally, a third carrier system, whose activity is sex dependent, may be involved in the active transport of *steroids* and *related compounds* into bile. In contrast to renal excretion, amphiphatic drugs (those having both polar and nonpolar properties) are preferentially excreted in the bile. The drug (or metabolite) excreted into the small intestine can be reabsorbed into blood forming the so-called drug enterohepatic cycle. This is an important factor changing the blood : liver or liver : bile drug concentration ratios during studies of the hepatobiliary transport mechanisms and drug hepatic elimination.

The biliary excretion of weak acids is the most important mechanism in drug hepatic elimination. Bromosulfophtalein (BSP) and analogues are dyes used as diagnostic probes of liver function and model substances in studies of the hepatic uptake of organic anions. Antibiotics such as ciprofloxacin can be actively excreted into bile in the presence of a biliary tract obstruction. Tetracyclines, mainly excreted into urine via glomerular filtration, are also concentrated in the liver and accordingly excreted into the small intestine via the bile, and then partially reabsorbed.

Glucuronides of endogenous compounds and drugs can be actively transported from hepatocytes into bile via transport systems similar to those for organic anions. Glucuronide conjugates are very important in hepatic drug metabolism and biliary excretion. The effectiveness of biliary excretion for glucuronide conjugates can be greatly limited by enzymatic hydrolysis after the bile is mixed with the small intestine contents, thereby releasing the parent drugs to be reabsorbed and enter the enterohepatic cycle. The reabsorbed drugs and metabolites can be ultimately excreted in urine. Some drug metabolites further undergo either biotransformation in the liver or other organs or are subjected to microbiological and physicochemical degradation in the small intestine before being excreted in the feces.

Weak bases can be actively transported into bile via carrier systems similar to the renal transport processes. Atropine, isoproterenol, and curare are eliminated by this mechanism, with atropine being almost equally excreted by the kidney (unchanged form) and hepatic metabolism followed by biliary excretion. Organic cation transport is not as important as the organic anion pathway. Neutral compounds may employ the third transport systems. Ouabain, a cardiac glycoside, is used as a model uncharged and nonmetabolized (by rat liver) compound

Table 2.4 Mean bile flow in selected species

Species	Bile flow (ml/min/kg body weight)
Cat	11
Chicken	20
Dog	4–10
Guinea pig	200
Hamster	50
Human	5–7
Monkey	10
Mouse	78
Opossum	20
Pig	9
Pony	19
Rabbit	90
Rat	50–80
Sheep	43

in hepatobiliary transport studies. Organic anions and neutral steroids such as ouabain may share common mechanisms in their excretory pathways.

Molecular weight is a key determinant of the extent to which drug/metabolite molecules are transported into bile. The molecular weight cutoff required for biliary excretion is much greater than that for renal tubular secretion, being from 300 to 500 in most species. If the molecular weight is lower, the compound may be preferentially excreted in urine. Excretion of molecules larger than 850 Da occurs mainly via the biliary active transport system. However, molecular weight is not the sole factor determining the route of drug excretion. Physicochemical properties of the drug (polarity/lipophilicity, structure) are also very critical to the extent of biliary excretion of a drug/metabolite, with amphiphatic drugs being well secreted by the biliary route.

The specific animal species being studied is also an important factor, as reflected in different molecular weight cutoff thresholds. Table 2.4 lists species differences in bile flow rate that may also contribute to the great species specificity seen in biliary drug transport. Such variations in both a species' enzyme profile for biotransformation and inherent ability to excrete drugs in bile result in great difficulty in predicting hepatic clearance across drugs in different species.

Conclusion

This chapter presents an overview of some essential principles of ADME that are needed to understand drug- and species-specific behavior of drugs, examples of which are discussed in most chapters of this text. The next chapter focuses on the pharmacokinetic tools used to quantitate these processes so that safe and effective dosages of drugs can be administered to animals. There is a tremendous amount of research being conducted on the determinants of ADME processes because they are so important in assuring safety and efficacy of drugs across all species. They are the primary source of differences in drug action across species.

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3

Pharmacokinetics

Jim E. Riviere

Pharmacokinetics is best defined as *the use of mathematical models to quantitate the time course of drug absorption and disposition in man and animals*. With the tremendous advances in medicine and analytical chemistry, coupled with the almost universal availability of computers, what was once an arcane science has now entered the mainstream of most fields of human and veterinary medicine. This discipline has allowed dosages of drugs to be tailored to individuals or groups to optimize therapeutic effectiveness, minimize toxicity, and avoid violative tissue residues in the case of food-producing animals. This subject and its concepts have become especially important as a consequence of the dramatic and almost radical changes that occurred at the end of the last decade relative to the regulations surrounding drug use in veterinary medicine in the United States. For most of the recent past, the operative concept was that a single dose of drug listed on a product label was optimal for all therapeutic uses. Recently, however, the legal concept of "flexible or professional labeling" and the passage by the US Congress in 1994 of the Animal Medicinal Drug Use Clarification Act (AMDUCA) legalizing extralabel drug use challenged this simplistic ideal of a single optimal dose. The veterinarian must now select a drug dose based on numerous factors inherent to the therapeutic scenario at hand to maximize therapeutic efficacy and minimize the likelihood of drug-induced toxicity or induction of microbial resistance. Furthermore, linking pharmacokinetic models to models of drug action, presented in the next chapter, allows for developing a quantitative link between dose and efficacy or toxicity. Unlike human medicine and companion animal practices, food animal veterinarians face the further restriction that proper withdrawal times must be determined to ensure that drug residues do not persist in the edible tissues or by-products (milk, eggs) of treated animals long after they have left the care of the veterinarian (Figure 3.1). As will be demonstrated and further discussed in Chapter 61, the "withdrawal time" is in reality a pure pharmacokinetic parameter since it can be calculated solely from a knowledge of the legal tissue tolerance and the drug's half-life or rate of decay in that tissue.

Yet it is not only the food animal veterinarian that faces these challenges. The laboratory animal and exotic/zoo animal worker must often extrapolate drug dosages across species with widely differing body sizes and physiology since there are very few approved drugs for the treatment of such animals. Pharmacokinetic principles and techniques are ideally suited for this application. Practitioners are often faced with disease processes (e.g., renal failure) that are known to affect the disposition of a drug. Knowledge of how such a pathological process affects a drug's clearance is sufficient knowledge to adapt a dosage regimen appropriate for this condition.

The previous chapter of this text presented the underlying physiology of drug fate. The processes involved in absorption, distribution, metabolism, and elimination are the primary phenomena that must be quantitated to predict the fate of a drug or toxicant in an animal. The two primary characteristics needed to adequately describe these processes are their *rate* and *extent*. In fact, this can be appreciated in the origin of the word *kinetic,* which is defined as: "*of or resulting from motion*." Many mathematical approaches to this problem have evolved over the course of the history of pharmacokinetics. The reader is suggested to consult the selected readings for more in depth presentations of pharmacokinetic modeling. In addition, hybrid as well as novel strategies are constantly being developed to quantitate these processes. However, all approaches share certain fundamental properties that are based upon estimating the rates of chemical movement.

A Primer on the Language of Pharmacokinetics

The roots of pharmacokinetics lie in the estimation of rates. The language is that of differential calculus. It is instructive to briefly overview the basic principles of rate

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Companion Website: www.wiley.com/go/riviere/pharmacology

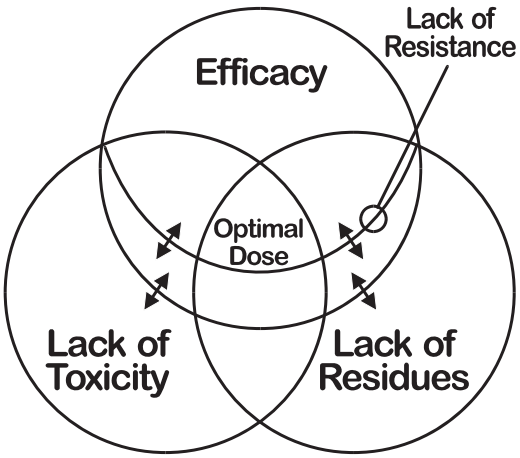


Figure 3.1 The food animal veterinarian's dilemma in optimizing the dose of a therapeutic drug.

determination since the logic imbedded in its syntax forms the basis of pharmacokinetic terminology.

To begin, a *rate* in pharmacokinetics is defined as *how fast the mass of a compound changes per unit of time*, which is expressed mathematically as the change (represented by the Greek letter delta, Δ) in mass per small unit of time (Δt). This is synonymous with the flux of drug in a system. Units of rate are thus mass/time. For the sake of convenience only, we will express this in terms of mg/min. We will begin this discussion using mass of a compound (X), which in clinical terms would be related to the dose, rather than using concentration. As will be developed shortly, mass and concentration are easily convertible using the proportionality factor of volume of distribution.

The rate of drug excretion ΔX/Δt actually has two components, a constant that reflects the rate of the process and the amount of compound available for transfer (Equation 3.1):

$$\Delta X/\Delta t = KX^n \tag{3.1}$$

where K is the fractional rate constant (1/min), X (mg) is the mass or amount of a compound available for transfer by the process being studied, and n is the order of the process.

For a first-order process, n = 1. Since $X^1 = X$, this equation simplifies to Equation 3.2:

$$\Delta X/\Delta t = KX \tag{3.2}$$

By definition, in this first-order (n = 1) or linear processes, K is *constant* and thus *the actual rate of the process ($\Delta X/\Delta t$) varies in direct proportion (and hence linearly) to X*. K can be viewed as the fraction of X that moves in the system being studied (absorbed, distributed, or eliminated) per unit of time. Therefore, as X increases, ΔX/Δt increases in direct proportion. In linear models, the rate constant is fixed but the rate of the process changes in direct proportion to the mass available for movement.

As can again be appreciated by examining the equation for Fickian diffusion (Equation 2.1 in Chapter 2), compounds that are either absorbed, distributed, or eliminated in direct proportion to a concentration gradient are by definition first-order rate processes. The rate constants (K_n) modeled in pharmacokinetics are actually aggregate constants reflecting all of the membrane diffusion and transfer processes involved in the disposition parameter being studied. This includes pH partitioning phenomena in the body, which exist when blood and a cellular or tissue compartment have a pH gradient that alters the fraction of drug available for diffusion. Recall that it is only the unionized fraction of a weak acid or base that diffuses down its gradient across a lipid membrane. The rate constant also reflects the degree of plasma protein binding since only the free fraction of drug is available for distribution. The actual value of a K in a pharmacokinetic model thus reflects all of these variables whose relationship defines the biological system that we are attempting to quantitate.

For a nonlinear or zero-order process, by definition n = 0. Since $X^0 = 1$, the rate equation now becomes

$$\Delta X/\Delta t = K_0 \tag{3.3}$$

In this scenario, the *rate of excretion is fixed and thus independent of the amount of compound available, X*. K_o now has the units of rate (mg/min) and is *not* a mass-independent fractional rate constant. Although this would appear to simplify the situation, in reality nonlinear kinetics actually complicate most models. Nonlinear behavior becomes evident when saturation of a process occurs. The focus of most pharmacokinetic studies is on drugs with linear pharmacokinetics since the majority of therapeutically active compounds are described by these models.

The use of ΔX/Δt to describe the rate of a process is experimentally and mathematically cumbersome, since ΔX/Δt changes as a function of concentration. Figure 3.2 graphically depicts this scenario. Calculus has been used to describe these same processes using the concept of a derivative. Instead of describing rates in terms of some small, finite time interval (Δt), differential equations express rate in terms of the change in compound mass over an infinitesimally small time interval termed dt. Equation 3.1 could now be written as the following (Equation 3.4):

$$dX/dt = -KX \tag{3.4}$$

The biological interpretation is identical. Note that K and X are the same in both equations; the only change is a conceptual one in that dX/dt now describes the instantaneous rate of change in mass over time. By convention, if the amount of drug is increasing, dX/dt is positive (e.g.,

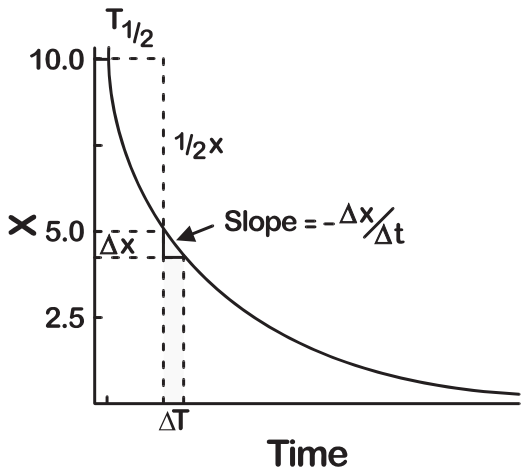


Figure 3.2 Plot of the decay in drug (X) versus time. The T1/2 is defined as the time required for X to deplete to 1/2 X. Slope at any time is ΔX/ΔT.

absorption of drug into the blood); if it is declining (e.g., elimination or distribution from the blood), the rate is negative or −dX/dt.

One may solve a differential equation through the process of integration ($\int$), which transforms the equation back into terms of t rather than dt. Integration is in reality a process by which the area under the curve (AUC) defined by ΔX/Δt is taken. By repeatedly summing these areas for the entire experimental period, the area under the curve will be obtained. We introduced this concept in Equation 2.4 when the concept of AUC was introduced. Analogous to the relation of a derivative to a slope, integration sums the areas under infinitesimally small regions defined by dX/dt.

We can use the technique of integration to solve the rate Equation 3.4. We must integrate the equation from X at time zero (X_0) through X at time t (X_t) to obtain a formula for the mass of drug at any time (Equation 3.5):

$$\int_0^t \left(\frac{dX}{dt}\right) dt = \int_0^t (-KX)\, dt \tag{3.5}$$

There are numerous techniques to accomplish this integration and the interested reader should consult a calculus textbook for further details. The result is the following (Equation 3.6):

$$X_t = X_0 e^{-Kt} \tag{3.6}$$

where *e* is the base of the natural logarithm (e = 2.713). *It is important to realize that the process of integrating the differential equation describing rate generates the exponential term found in most linear pharmacokinetic models.* Exponentials can easily be eliminated from an equation by taking their natural logarithm (*ln*) since the logarithm is defined as the power to which a base (in this case *e*) is raised. Taking the natural logarithms of Equation 3.6 yields the following (Equation 3.7):

$$\ln X_t = \ln X_0 - Kt \tag{3.7}$$

If one plots these data, a straight line results, as seen in Figure 3.3, which is much easier to deal with than the curve in Figure 3.2. Recalling the algebraic expression for a straight line on x–y coordinates, in this case the y intercept becomes X_0 and the slope of the line is −K. The equation has been linearized providing a simple graphical method to calculate the rate constant.

This equation can be linearized because it is a first-order rate function. This type of plot, which is widely used throughout pharmacokinetics, is termed a semilogarithmic plot (in contrast to the Cartesian plot) since the logarithm of mass is plotted against time. Again, *when a straight line results on a semilogarithmic plot, one can assume that a linear first-order process is operative and the slope of the line is the exponent of an exponential equation.* Alternatively, a linear regression program on a computer or calculator could be used to calculate K by regressing the ln X against time; the slope is −K and the y intercept is $\ln X_0$. In earlier work, graph paper was used that plotted logarithms to the base 10 (log x) $\{10^x\}$ rather than the base e (ln x) $\{e^x\}$ where x is the logarithm, the transformation of bases can be accomplished as the following (Equation 3.8):

$$\log X = \ln X/2.303 \tag{3.8}$$

which transforms Equation 3.7 to

$$\log X_t = \log X_0 - Kt/2.303 \tag{3.9}$$

If base 10 semilogarithmic graph paper is used to plot Figure 3.3, the slope becomes −K/2.303. As this technique was in widespread use before the advent of digital computers, this is still encountered in older manuscripts and texts.

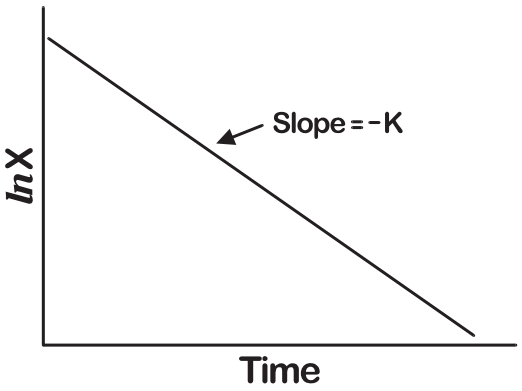


Figure 3.3 Semilogarithmic plot of drug decay versus time with slope equal to −K.

The Concept of Half-Life

The exponential equations in pharmacokinetics have another property that is important to biological applications. This is the concept of half-life (T1/2) whose logic is central to much of this discipline. The astute biologist reading this text will have realized that Equation 3.6 is the same as that used to describe population doubling times in microbiology or ecology and used to generate population growth curves, defined as the time needed for a population of organisms to double their total numbers when they are in their so-called *logarithmic* growth phase. The only difference is that since growth is described, the exponent is positive in this application. In pharmacokinetics, our perspective is decay with a T1/2 instead being the time required for the amount of drug to decrease by one-half or 50%. The concept of T1/2 is applicable only to first-order rate processes.

Using Equation 3.7, one can derive a simple equation for T1/2. We first rearrange terms to solve for T, which yields

$$T = (\ln X_0 - \ln X_t)/K \tag{3.10}$$

We now solve for the time at which X_t is equal to 1/2 the initial amount X_0, that is, where $T = \text{T1/2}$. Substituting these values, the equation reduces to

$$K = 0.693/T^1/_2. \tag{3.11}$$

It is this transformation of K with T1/2 that introduces the ln 2 or 0.693 into many pharmacokinetic equations.

What does T1/2 really mean? Assume that we start with X, decrease it by half, and repeat this process 10 times. Table 3.1 compiles this data and lists how much drug is remaining and how much has been excreted over each Δt corresponding to one T1/2. Note that if you sum these columns, you would have accounted for 99.9% of the original dose X. After 10 T1/2s, 99.9% of the drug has been eliminated or the rate process being studied has been completed. This also illustrates the logic that must be used when dealing with doses. For example, if you double the dose to 2×, then after one T1/2 you would be back to the original dose! Many rules of thumb used in pharmacokinetics and medicine are based on this simple fact. For therapeutic drugs, most workers assume that after five T1/2s, the drug has been depleted or the process is over since 97% of the depletion has occurred. This also illustrates a very simple way to calculate T1/2, by simply determining the time required for drug concentration to decrease by 50%. This was depicted in Figure 3.2. Equation 3.11 can then be used to obtain K. It now is time to develop our first pharmacokinetic model using mathematical rather than physiological concepts.

Table 3.1 Relationship of T1/2 and amount of drug (A) in the body

Number of T1/2s	% of Drug remaining	% of Drug eliminated
1	50	50
2	75	25
3	87.5	12.5
4	93.75	0.625
5	96.88	0.312
6	98.44	0.156
7	99.22	0.078
8	99.61	0.039
9	99.80	0.019
10	99.90	0.0097

One-Compartment Open Model

The most widely used modeling paradigm in comparative and veterinary medicine is the compartmental approach. In this analysis, the body is viewed as being composed of a number of so-called equilibrium compartments, each defined as representing nonspecific body regions *where the rates of compound disappearance* are of a similar order of magnitude. Specifically, the fraction or percent of drug eliminated per unit of time from such a defined compartment is constant. Such compartments are classified and grouped on the basis *of similar rates of drug movement* within a kinetically homogeneous but anatomically and physiologically heterogeneous group of tissues. These compartments are theoretical entities that allow formulation of mathematical models to describe a drug's behavior over time with respect to movement within and between compartments. Since pharmacologists and clinicians sample blood as a common and accessible biological matrix for assessing drug fate, most pharmacokinetic models are constructed with blood or plasma drug concentrations as the central reference to which other processes are related.

The simplest compartment model is when one considers the body as consisting of a single homogeneous compartment; that is, the entire dose X of drug is assumed to move out of the body at a single rate. This model, depicted in Figure 3.4, is best conceptualized as instantly dissolving and homogeneously mixing the drug in a beaker from which it is eliminated by a single rate process described by the rate constant K, now termed K_{el}. Since the drug leaves the system, the model is termed *open*. Equation 3.6 is the pharmacokinetic equation for the one-compartment open model. Although expressed in terms of the amount of drug remaining in the compartment, most experiments measure concentrations. This requires the development of the volume of distribution (Vd) (recall Equation 2.5 when distribution was discussed). In terms of the one-compartment model, this would be the volume of the compartment into which the dose of drug (D) instantaneously distributes. *Vd*

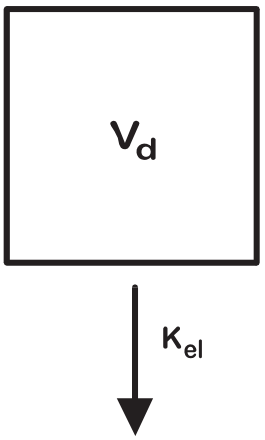


Figure 3.4 One-compartment open pharmacokinetic model.

thus becomes a *proportionality factor* relating D to the observed concentration Cp by

$$Vd\ (ml) = X\ (\cancel{mg})/Cp\ (\cancel{mg}/ml) = D/Cp \qquad (3.12)$$

Using this relation, we can now rewrite Equation 3.6 in terms of concentrations, which are experimentally accessible by sampling blood, instead of the total amount of drug remaining in the body.

$$Cp = X_0/Vd\ e^{-Kelt} = Cp_0 e^{-Kelt} \qquad (3.13)$$

A semilogarithmic plot seen after intravenous administration using this model is depicted in Figure 3.5. Vd quantitates the apparent volume into which a drug is dissolved, since, recalling the discussion in Chapter 2, the true volume is determined by the physiology of the animal, the relative transmembrane diffusion coefficients, and the chemical properties of the drug being studied. A drug that is restricted to the vascular system will have a very small Vd; one which distributes to total body water will have a very large Vd. In fact, it is this technique that is used to calculate the plasma and interstitial spaces.

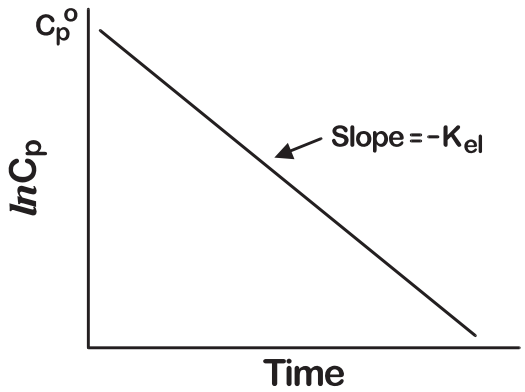


Figure 3.5 Semilogarithmic concentration-time profile for a one-compartment drug with slope $-K_{el}$ and intercept Cp_0.

From this simple analysis, and using the model in Figure 3.4, a number of useful pharmacokinetic parameters may be defined. Assuming that an experiment such as depicted in Figure 3.5 has been conducted using a dose of D and values for K_{el} and Vd have been determined, T1/2 can easily be calculated from Equation 3.11 above.

Clearance

Recalling the development of clearance concepts in Chapter 2, we now can easily determine Cl_B using this information. Clearance was defined as *the volume of blood cleared of a substance by the kidney per unit of time.* If one considers the whole body, this would read as *the volume of distribution of drug in the body cleared of a substance per unit of time.* Translating this sentence to the syntax of pharmacokinetic terminology and considering whole body elimination, Vd represents the volume and K_{el} the fractional rate constant (units of 1/time). Thus clearance is

$$Cl_B(ml/min) = Vd)ml)\ K_{el}(1/min) \qquad (3.14)$$

There is another method available to calculate Cl_B. In Chapter 2, clearance was also defined in Equation 2.8 as *the rate of drug excretion relative to its plasma concentration*. We can also express this sentence in the syntax of pharmacokinetics and get this relation:

$$Cl_B = (dX/dt)/Cp \qquad (3.15)$$

If we integrate both the numerator and denominator of this relation from time $0 \rightarrow \infty$, the numerator is the sum of the total amount of drug which has been excreted from the body; that is, assuming intravenous administration, the administered dose D. The denominator is the integral of the plasma concentration time profile the area under the curve (AUC). The relation thus becomes:

$$Cl_B = D/AUC \qquad (3.16)$$

There are two approaches to calculate AUC. A common approach is to use the trapezoidal method depicted in Figure 3.6. However, for the one-compartment model that generates the semilogarithmic C-T plot depicted in Figure 3.5, the problem is simply determining the area of the right triangle. The area of this triangle (AUC) is height divided by the slope of the hypotenuse, or:

$$AUC = Cp_0/K_{el} \qquad (3.17)$$

Interpretation of Pharmacokinetic Parameters

With these equations, we now have the three so-called *primary pharmacokinetic* parameters describing drug disposition in the body: *T*1/2, Cl_B, and *Vd*. The data required to calculate them is a knowledge of dose and an experimental derivation of either K_{el} or T1/2.

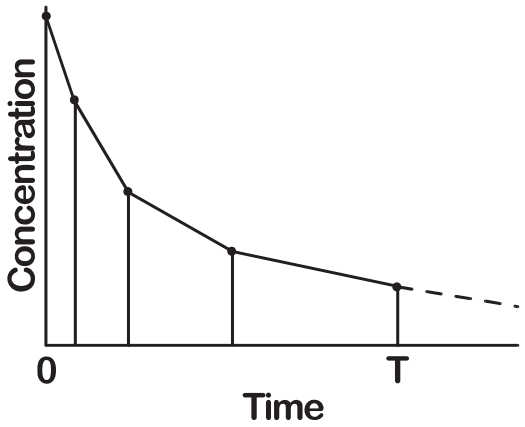


Figure 3.6 Trapezoids formed from sampled concentration versus time data for calculation of areas. To estimate the total AUC, the curve must be extrapolated beyond the ultimate sample time, T, to infinity (—).

This is a good place to discuss the limits of calculating parameters from such simple concentration-time profiles. Only two parameters are actually being "measured" from this analysis: the slope K_{el} and intercept Cp_0 of the semilogarithmic plot, which – using Equation 3.12 directly – determines Vd. The third parameter Cl is "calculated" from the two measured parameters. Based on the mathematical method used to calculate these, some workers suggested that K_{el} and Vd are the independent parameters in a pharmacokinetic analysis and Cl is a derived parameter. This assertion is usually made when the statistical properties of the parameters are being defined since errors for these can be easily obtained. However, this belief is an artifact of the use of a compartmental model as a *tool* to get at values for these physiological parameters. Biologically, the truly independent parameters are the Vd and Cl, with K_{el} and thus T1/2 becoming the dependent variables. From this biological perspective, the true relationship is

$$T1/2 = (0.693 \times Vd)/Cl \tag{3.18}$$

The observed half-life of a drug is dependent upon *both* the extent of a drug's distribution in the body *and* its rate of clearance. If the clearance of a drug is high (e.g., rapidly eliminated by the kidney), the T1/2 is relatively short. Logically, a slowly eliminated drug will have a prolonged T1/2. Not obvious at first is that if a drug is extensively distributed in the body (e.g., lipid-soluble drug distributed to fat), Vd will be large *and* the T1/2 will also be relatively prolonged. In contrast, if a drug has restricted distribution in the body (e.g., only the vascular system), the Vd will be small, a large fraction of the drug will be available for elimination, and thus the T1/2 relatively short. In a disease state, T1/2 may be prolonged by either a diseased kidney, a reduced capacity for hepatic drug metabolism, or an inflammatory state, which increases capillary perfusion and permeability, thus allowing drug access to normally excluded tissue sites. Therefore, T1/2 is physiologically dependent on both the volume of distribution and clearance of the drug.

Cl_B is the sum of clearances from all routes of administration:

$$Cl_B = Cl_{Renal} + Cl_{Hepatic} + Cl_{other} \tag{3.19}$$

There is another strategy that can be used to estimate clearance in an intravenous study. This is based on the basic principle of mass balance. The strategy is to infuse a drug into the body at a constant rate R_o (mass/time) and then measure plasma drug concentrations. By definition, when a steady-state plasma concentration is achieved, C^{ss} (mass/volume), the rate of drug input must equal the rate of clearance from the body, Cl_B:

$$R_o\ (mg/\text{min}) = C^{SS}\ (mg/\cancel{ml}) \times Cl\ (\cancel{ml}/\text{min}) \tag{3.20}$$

Rearranging this equation gives a simple formula for determining Cl:

$$\begin{aligned} Cl\ (ml/\text{min}) &= R_o\ (\cancel{mg}/\text{min})/C^{SS}\ (\cancel{mg}/ml) \\ &= R_o/C^{SS}\ (ml/\text{min}) \end{aligned} \tag{3.21}$$

The Cl calculated in this manner is identical to that determined using Equations 3.14 and 3.16 above, and requires only knowing the rate of infusion and assaying the achieved steady-state concentration. One may also calculate the Vd from an intravenous infusion study by the relation:

$$Vd = R_0/C^{SS}K_{el} \tag{3.22}$$

Many of the pharmacokinetic parameters above may also be obtained by analysis of urine data alone, an approach beyond the focus of the present introduction.

Absorption in a One-Compartment Open Model

The analysis above assumes that the drug was injected into the body, which behaves as a single space into which the drug is uniformly dissolved. The first real-world complication is when the drug is administered by one of the extravascular routes discussed in Chapter 2. In this case, the drug must be absorbed from the dosing site into the bloodstream. The resulting semilogarithmic concentration–time profile, depicted in Figure 3.7, now is characterized by an initial rising component that peaks and then undergoes the same log-linear decline. The proper pharmacokinetic model for this scenario is depicted in Figure 3.8. The rate of the drug's absorption is governed by the rate constant K_a. When the absorption process is finally complete, elimination is still described by K_{el} as depicted in Figure 3.5. The overall elimination half-life can still be calculated using K_{el} if this terminal slope is taken after the peak (C^{max}) in the linear portion of

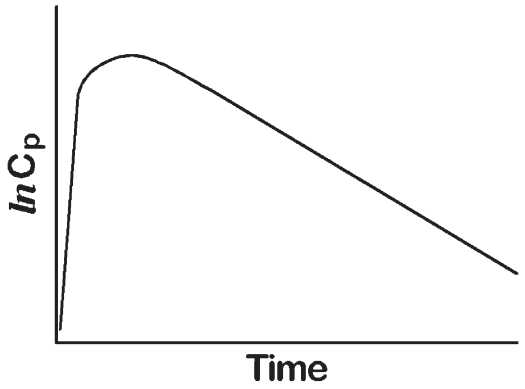


Figure 3.7 Semilogarithmic plot of plasma concentration versus time with first-order absorption.

the semilogarithmic plot (providing $K_a \gg K_{el}$). However, calculation of Vd and Cl becomes more complicated since K_a is present and, unlike an intravenous injection, one is not assured that all of the drug has been absorbed into the body. In order to handle this, we must now write the differential equations to describe this process by including rate constants for absorption and elimination:

$$dX/dt = K_a D - K_{el} X \tag{3.23}$$

where D is the administered dose driving the absorption process and X is now the amount of dose absorbed and available for excretion. The relationship between D and X is the absolute systemic availability F originally introduced in Equation 2.4 [X = FD]. In the language of differential equations, rates are simply additive, which allows the same data sets to be described in components

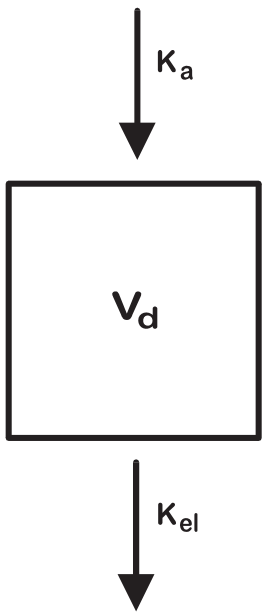


Figure 3.8 One-compartment open pharmacokinetic model with first-order absorption.

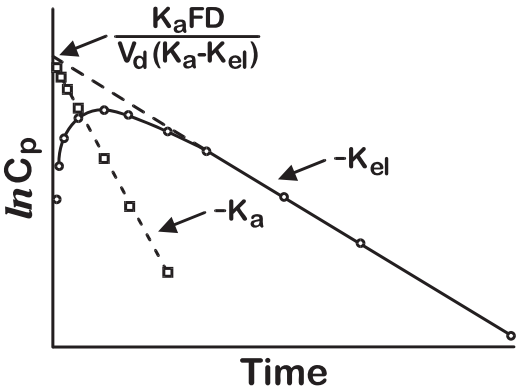


Figure 3.9 Semilogarithmic plot of plasma concentration versus time using a one-compartment open pharmacokinetic model with first-order absorption. The profile is decomposed into two lines with slopes $-K_a$ and $-K_{el}$.

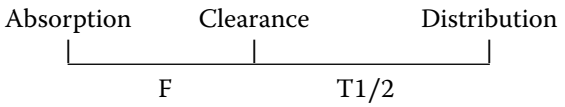
reflecting the different processes. As above, integrating this equation and expressing it in terms of concentrations, gives the expression that describes the profile in Figure 3.9:

$$C = \frac{K_a FD}{Vd(K_a - K_{el})}\left[e^{-K_{el}t} - e^{-K_a t}\right] \tag{3.24}$$

This is an excellent point in the discussion to appreciate the validity of the use of multiexponential equations to describe blood C-T profiles as the exponential terms, which, like the rates above from which they were derived, are simply additive. A C-T profile is the sum of the underlying exponential terms describing the rate processes involved. This property of superposition is the basis upon which observed C-T profiles may be "dissected" to obtain the component rates. Figure 3.9 illustrates this process where an observed semilogarithmic profile is plotted as a composite of its absorption phase (controlled by K_a) and the elimination phase (controlled by K_{el}). In contrast to the intravenous scenario, the time zero intercept is now a more complex function, which is dependent upon the fraction of administered dose that is systemically available and thus able to be acted on by the elimination process described by the rate constant K_{el}. For this procedure to work, K_a must be greater than K_{el} so that at later time points e^{-Kat} approaches zero. If $K_a < K_{el}$, the same C-T profile will result; however, now the terminal slope will be K_a as it is the rate-limiting process! One just "*flip-flopped*" K_a for K_{el}. In fact, recalling the discussion in Chapter 2 on slow-release dosing formulations, we termed the resulting effect on disposition of drug in the body an example of the *flip-flop* phenomenon, the origin of which is this relation. When an extravascular route of administration is used, one can never be certain that the C-T profile is not dependent upon a slow, and thus rate-limiting, absorptive process secondary to a formulation factor. If a depot or slow-release formulation is

administered such that $K_a < K_{el}$, the terminal slope will reflect the rate of absorption rather than the rate of elimination. T1/2 may be overestimated as it will now reflect $0.693/K_a$ rather than $0.693/K_{el}$. Complete absorption also cannot be assured (e.g., F = 1); thus one never truly knows the size of the absorbed dose. Accurate estimates of Cl and Vd, reflecting the true pharmacokinetic disposition of a drug, are required as input to determine these relations. These are best calculated after a complete intravenous injection. Finally, as discussed above for intravenous administration, urine analysis may also be used to estimate absorption parameters.

As we leave this section, it is a good time to underscore the relationship of some ADME processes as they translate to pharmacokinetic parameters; namely absorption, clearance, and volume of distribution, and further relate to the main determinants of a dosage regimen – namely, F and T1/2. This can be appreciated as



F was determined by Equation 2.4 as the ratios of AUC_{oral}/AUC_{iv}. The intravenous D is a function of Cl and AUC from Equation 3.16. Half-life was a function of Vd and Cl in Equation 3.18. This scheme shows how F is a function of absorption and clearance processes (AME) while half-life is a function of clearance and distribution (DME).

Why are these relationships important? One of the main clinical applications of pharmacokinetic principles is to construct dosage regimens, the approach that is fully presented below. Diseases that change any of these primary pharmacokinetic parameters would be expected to change the plasma concentrations achieved after dosing, and thus drug effect. For example, renal disease that reduced GFR, might reduce Cl_B for drugs primarily eliminated by the kidney. Similarly, liver disease might alter disposition of drugs cleared by the liver. In contrast, diseases that resulted in severe elimination and fluid accumulation could alter Vd. Both of these scenarios would increase T1/2 and alter plasma concentrations. The relationship between concentration and effect is extensively discussed in the next chapter on pharmacodynamics.

Two-Compartment Models

Many drugs are not described by a simple one-compartment model since the plasma concentration time profile is not a straight line. This reflects the biological reality that for many drugs, the body is not a single homogeneous compartment, but instead is composed of regions that are defined by having different *rates* of drug distribution. Such a situation is reflected in the two-compartment model depicted in Figure 3.10. The drug initially is distributed in the central compartment and by definition is eliminated from this compartment. The difference comes because now the drug also distributes into other body regions at a rate that is different from that of the central compartment.

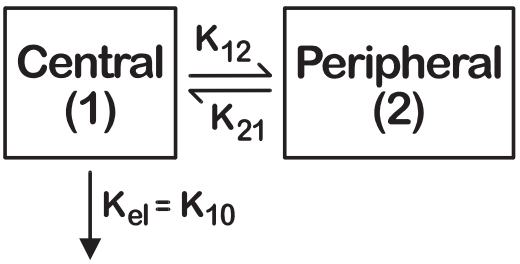


Figure 3.10 Generalized open two-compartment pharmacokinetic model after intravenous administration with elimination (K_{el}) from the central compartment. K_{12} and K_{21} represent intercompartmental micro-rate constants.

As presented in Chapter 2, there are many factors that determine the rate and extent of drug distribution into a tissue (e.g., blood flow, tissue mass, blood/tissue partition coefficient, etc.). When the composite rates of these flow and diffusion processes are significantly different than K_{el}, then the C-T profile will reflect this by assuming a biexponential nature. For many drugs, the central compartment may consist of blood plasma and the extracellular fluid of highly perfused organs such as the heart, lung, kidneys, and liver. Distribution to the remainder of the body occurs more slowly, which provides the physiological basis for a two-compartment model. Such a peripheral compartment is defined by a distribution rate constant (K_{12}) out of the central compartment and a redistribution rate constant (K_{21}) from the peripheral back into the central compartment. As discussed in the distribution chapter, depots or sinks may also occur. This is a pharmacokinetic concept where the distribution rate constants are significantly slower than K_{el} and thus become the rate-limiting factor defining the terminal slope of a biexponential C-T profile, a situation analogous to flip-flop in absorption studies.

We will begin the discussion of multicompartmental models with the principles of analyzing a two-compartment model after intravenous administration (Figure 3.11). This is the most common scenario encountered in comparative medicine and the principles easily translate to more complicated models. The fundamental principle involved is that the observed serum concentration time profile is actually the result of two separate pharmacokinetic processes that can be described by two separate exponential terms, commonly written as:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \tag{3.25}$$

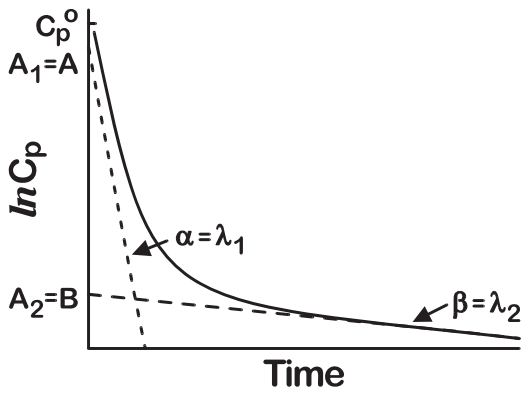


Figure 3.11 Semilogarithmic plasma concentration versus time profile of a drug described by a two-compartment open model. Parameters are defined in the text.

Note the similarity of this biexponential equation to that presented for absorption in Equation 3.24. In this case we have terms with slopes (α and β) and corresponding intercepts (*A* and *B*). The C-T profile on semilogarithmic plots is depicted in Figure 3.11. By definition, $\alpha \gg \beta$ and thus β is the terminal slope. If $\alpha \approx \beta$, the slopes of the two lines would be equal and we would be back to the single line of Figure 3.5 and a one-compartment model!

When dealing with multicompartmental models, it becomes necessary to introduce new nomenclature to denote the intercept terms and slopes of the C-T profile because, as will be shown shortly, the observed slopes are no longer synonymous with the elimination and distribution micro-rate constants as they were when we were analyzing absorption plots for K_{el} and K_a. When these models were constructed, the defining differential rate equations could be written in terms of the mass of drug in the central compartment (X). In the two-compartment model of Figure 3.10, this equation now must describe drug movement in terms of the mass of drug in compartment one and two. The solution to these differential equations are the slopes of the biexponential C-T profile giving α and β. Multicompartmental models have their own syntax: the slopes of the C-T profile are named using the Greek alphabet, starting with the most rapid rate α for distribution followed by β for elimination. The intercept terms are denoted using the Roman alphabet as A related to α and B to β.

A preferred nomenclature carries less phenomenological context and uses the Greek letter λ_n, with n = 1, 2, 3, . . . progressing from the most rapid to the slowest rate process. The corresponding intercept terms are denoted as A_n. This nomenclature describes any multicompartmental model without implying a physiological basis to the underlying mechanism responsible for the different rates observed. The biexponential equation for a two-compartment model may now be written as

$$Cp = A_1 e^{-\lambda_1 t} + A_2 e^{-\lambda_2 t} \tag{3.26}$$

The actual rate constants describing flux between compartments are now termed micro-rate constants and denoted by k_{xy}, where compound moves from x → y. When the origin or destination of a compound is outside of the body, x or y is denoted as 0, respectively. K_a thus becomes k_{01} and K_{el} becomes k_{10}. With a two-compartment model, three Vds may be calculated: the volume of the central compartment V_c or V_1, the peripheral compartment V_p or V_2, and the total volume of distribution in the body V_t or $V_1 + V_2$. As will be seen below, the actual Vd calculated from the data is dependent upon the method used; however, the only estimate of V_t which can be broken into its component central and peripheral volumes is the volume of distribution at steady state, Vd_{ss}.

Now that we have the appropriate nomenclature, it is instructive to derive the differential rate equations for λ_n and A_n based on the microconstants which define them. For a two-compartment model after intravenous injection of dose D with elimination occurring from the central compartment, the following differential equation describes the rate of drug disposition:

$$dC_1/dt = -(k_{12} + k_{10})\,C_1 + (k_{21})\,C_2 \tag{3.27}$$

Processes that remove compound from the central compartment (k_{10} and k_{12}) are grouped together and have a negative rate since they result in a descending C-T profile. The only process that adds chemical to the central compartment (k_{21}) – that is, redistribution from the peripheral compartment – is assigned a positive rate and results in an ascending C-T profile. The rate of this process is driven by the concentration of compound in the peripheral compartment. Note the similarity of this equation to the differential equation for absorption in a one-compartment model (Equation 3.23). In this model, the only process that added drug to the central compartment was k_a, which therefore was assigned a positive sign, while the only process removing drug was $-K_{el}$. Similarly, as stressed throughout this text, the driving mass for this passive absorption process was the fraction of administered dose (F X) available for absorption. The power and essence of pharmacokinetic analysis is that the physiological processes driving drug disposition can be quantitated by using differential equations describing drug flux into and out of observable compartments, with most models structured to reflect the central compartment, which is monitored via blood sampling as the primary point of reference. Solution of the differential Equation 3.27 by integration yields Equations 3.25 or 3.26 describing the biexponential C-T profile characteristic of a two-compartment open model.

The observed slopes λ_1 and λ_2 and intercepts A_1 and A_2 are related to the microconstants as

$$k_{21} = (A_1\lambda_2 + A_2\lambda_1)/(A_1 + A_2) \quad (3.28)$$

$$k_{el} = \lambda_1\lambda_2/k_{21} \quad (3.29)$$

$$k_{12} = \lambda_1 + \lambda_2 - k_{21} - k_{10} \quad (3.30)$$

Similarly, each of the slopes now has a corresponding T1/2 calculated as

$$T1/2\lambda_1 = 0.693/\lambda_1 \quad \{\text{Distribution}\} \quad (3.31)$$

$$T1/2\lambda_2 = 0.693/\lambda_2 \quad \{\text{Elimination}\} \quad (3.32)$$

The slope of the terminal phase of the C-T profile reflects the elimination T1/2 and is the primary parameter used to calculate dosage regimens. Note that since $\gamma_1 \gg \gamma_2$, $T1/2_{\gamma 1} \ll T1/2_{\gamma 2}$ and at later time points (recall the five T1/2 rule), distribution will be complete and the biexponential Equation 3.26 collapses to the monoexponential equation $Cp = A_2e^{-\lambda 2t}$. This equation is similar in form to the one-compartment Equation 3.13 except the intercept is now A_2 and not Cp_0 and the slope is $-\gamma_2$ and not K_{el}. This property of "disappearing" exponentials with large γs at later time points provides the basis for analyzing polyexponential C-T profiles using the curve "stripping" approach (technically called the method of residuals) discussed earlier.

It is often difficult to accurately estimate distribution parameters when γ_1 is very rapid since early blood samples must be collected, sometimes before blood has completely circulated. In a large animal such as a horse or cow, this requires a few minutes and thus very early samples (e.g., <5 minutes) will not have sufficient time for this mixing to occur. Secondly, small errors in sample timing result in a large % error (1 minute off for a 5-minute sample; error is 20%) and thus the data obtained at very early time points is often extremely variable. In contrast, 5 minutes off of a 6-hour sample is only a 1% error making estimates of terminal slopes much less variable.

Volumes of Distribution

There are now three volumes of distribution to contend with: V_c or V_1, V_p or V_2, and $V_t = (V_1 + V_2)$. These are again calculated by a knowledge of intercepts and administered dose (assuming intravenous administration). The relevant intercept is C_p^0, which is now simply $A_1 + A_2$:

$$V_1 = D/C_p^0 = D/(A_1 + A_2) \quad (3.33)$$

$$Vd_{ss} = V_1[(k_{12} + k_{21})/k_{21}] \quad (3.34)$$

$$V_2 = Vd_{SS} - V_1 \quad (3.35)$$

$$Vd\ (B) = D/B = D/A_2 \quad (3.36)$$

$$Vd_{area} = D/[(AUC)(\lambda_2)] = D/[(AUC)(\beta)] \quad (3.37)$$

$$= Vd_\beta = (k_{10}V_1)/\lambda_2 \quad (3.38)$$

The relation between these estimates are

$$Vd\ (B) > Vd_{area} > Vd_{SS} > V_C \quad (3.39)$$

The easiest to discard is *Vd(B)*, the apparent volume of distribution by extrapolation, since it is often used when a complete analysis of the curve is avoided and only the terminal slope and its intercept A_2 is determined. As discussed above, this estimate completely ignores V_1. Similarly, V_c is defined as only the central compartment volume. It is the volume from which clearance is determined and is used in some infusion calculations.

The volume of distribution at steady-state, Vd_{ss}, is the most "robust" estimate since it is mathematically and physiologically independent of any elimination process or constant. It is the preferred Vd estimate for interspecies extrapolations and the study of the effects of altered physiology on Vd since it is independent of elimination. Theoretically, Vd_{ss} describes the Vd at only a single time point when the rate of elimination equals that of distribution. The point at which this occurs is the inflection point or bend in the C-T profile that occurs because the more rapid tissue distribution phase has now peaked. This is best appreciated in Figure 3.12 when the concentrations in the central and tissue compartments are plotted.

Vd_{area} is often used when clinical dosage regimens are constructed because it reflects the area during the elimination phase of the curve which predominates in any dosage regimen (see Figure 3.12). This is absolutely equivalent to Vd_β, the so-called volume of distribution at pseudodistribution equilibrium. If the rate of elimination is very prolonged (slow), as seen in severe renal disease, the terminal slope of the concentration-time profile may

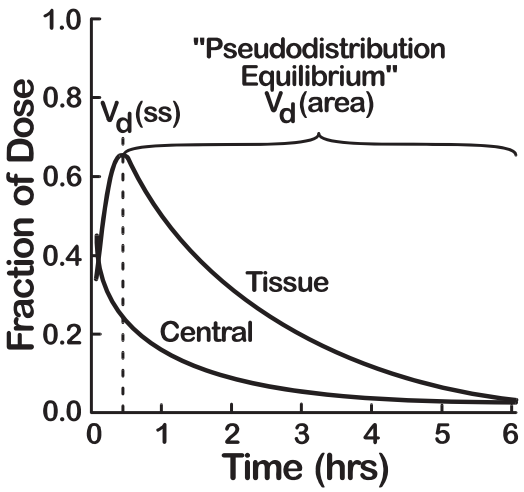


Figure 3.12 Relationship between Vd_{ss} and Vd_{area} for a drug described by a two-compartment model. Note that Vd_{ss} is only descriptive of the volume of distribution at the peak of the tissue compartment concentration versus time profile, while Vd_{area} describes the volume throughout the terminal elimination phase.

approach zero (plateaus; T1/2 becomes very long), which effectively "stretches out" the curve's inflection due to a plateau in the peripheral tissue compartment. Under this scenario, Vd_{area} becomes equal in value to Vd_{ss}.

Physiologically, a way to conceptualize Vd is to compare the individual compartment volumes based on plasma versus tissue binding, as:

$$Vd = V_{plasma} + V_{tissue}(fu_{plasma}/fu_{tissue}) \quad (3.40)$$

This relationship nicely shows the effect that both plasma and tissue protein binding can have on volume of distribution. Note that V_{plasma} and V_{tissue} do not directly correspond to V_1 and V_2, respectively, as the latter are determined by relative rates since both volumes actually include plasma and tissue.

Clearance

Knowing V_1, one can easily calculate the systemic clearance since Cl_B occurs from the central compartment and is essentially the same as a one-compartment model.

$$Cl = K_{10}V_1 \quad (3.41)$$

Alternatively, Cl_B may be calculated using the model-independent intravenous infusion Equation 3.21 presented earlier. The only difference is that with the more complex distribution kinetics present in a multicompartmental model, the time to reach C^{ss} may be significantly longer. Finally, Cl_B may also be determined using Equation 3.16 based on AUC. In a two-compartment model, AUC may be calculated using slopes and intercepts by the relation

$$AUC = (A_1/\lambda_1) + (A_2/\lambda_2) \quad (3.42)$$

which can be generalized for a multicompartmental model to

$$AUC = \sum A_i/\lambda_i \quad (3.43)$$

Using Vd_{ss} and Cl_B, Equation 3.18 can again be used to calculate the overall T1/2 of drug in the body. This T1/2 reflects both distribution and elimination processes and is very useful as input into an interspecies allometric analysis. This is not equivalent to the terminal elimination half-life, T1/2 (λ_2), and must be calculated from the Cl_B and Vd_{ss} parameters.

Absorption in a Two-Compartment Model

When an extravascular dose is administered as input into a two-compartment model (Figure 3.13), the differential equation defining this model is

$$V_1 dC_1/dt = -(k_{12} + k_{10})C_1V_1 + k_{21}C_2V_2 + k_{01}X \quad (3.44)$$

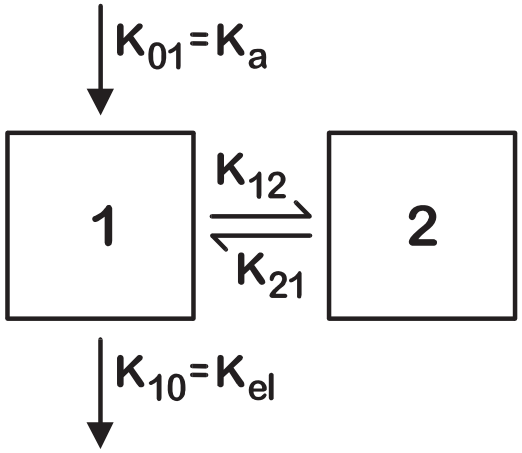


Figure 3.13 Generalized open two-compartment pharmacokinetic model with first-order absorption (K_{01}) into and elimination (K_{el}) from the central compartment. K_{12} and K_{21} represent intercompartmental constants reflecting distribution.

The movement of drug in the central compartment is now driven by three different concentrations: C_1, C_2 as well as the fraction of the administered dose D that is available for absorption (X). There are a number of approaches to solve this model. An example of the equation describing such a plasma profile would be

$$Cp = k_{01}D/V_1\left[A'_1e^{-\lambda_1 t} + A'_2e^{-\lambda_2 t} - A'_3e^{-k_{01}t}\right] \quad (3.45)$$

In this case, the intercepts (A_n') are different than those obtained from an intravenous study (A_n) and significantly more complex since the "driving" concentrations in compartments one and two are now dependent upon the fraction absorbed in a fashion analogous to the terms of Equation 3.24 seen for absorption in a one-compartment model. However, in reality, it is difficult to separate k_{01} from λ_1 since the two are of a similar order of magnitude, coupled with the earlier discussed concern that early time points are often prone to large errors. Depending on the ratio of rate constants, the C-T profile may even appear monoexponential! The final complication is that absorption flip-flop may also occur making selection of k_{01} and λs very difficult. The only method to reliably address all of these problems is to conduct an independent intravenous bolus study using a two-compartment model and independently estimate λ_1 and λ_2 to arrive at an estimate of the absorbed dose. These equations are now easily analyzed using modern computer software.

Data Analysis and its Limitations

Clearly, as pharmacokinetic models become more complex, one must question the wisdom of pursuing such analyses. In reality, there are mathematical limitations to

the complexity of the model able to be fit to an experimental data set which is based on the "information density," that is, how many data points are analyzed relative to how many parameters need to be calculated. This is similar to the statistical concept of "degrees of freedom." In practice, there are better approaches to model complex absorption using noncompartmental strategies of residence times and linear system deconvolution analysis, which are discussed in advanced texts. The final consideration with two-compartmental models, and one that is even more serious for multicompartmental models, is the actual structure of the model studied. Up until now, we have *assumed* that input into (absorption) and output from (elimination) the model are via the central compartment (model A), and furthermore, all samples are taken from this compartment and expressed as differential equations based on dC_1/dt. However, other possible structures exist for the basic two-compartment model. For example, drug may be infused into a tissue bed, or drug could distribute to the organ before metabolism and thus elimination in that organ. Many of these latter type of problems occur when the rate of distribution is actually slower than elimination making the initial exponential term reflect elimination. Very lipophilic chlorinated hydrocarbon chemicals may initially distribute extensively throughout the body and then slowly (periods of months) redistribute to the blood where metabolism would then occur. The redistribution rate constant would be the rate-limiting process. All would generate C-T profiles described by the sum of exponential very similar to those discussed above. However, the equations which *link* these fitted parameters to the underlying micro-rate constants would be very different.

Multicompartmental Models

The final level of compartmental model complexity to be dealt with in this chapter is the three-compartment model depicted in Figure 3.14 that generates the C-T profile in Figure 3.15. These data were obtained following intravenous gentamicin administration to dogs. In this case, gentamicin distributes into two different compartments from the central compartment, one with

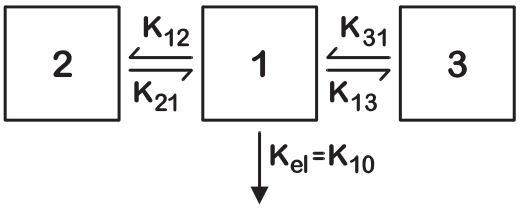


Figure 3.14 Three-compartment pharmacokinetic model after intravenous administration. Parameters are defined in the text.

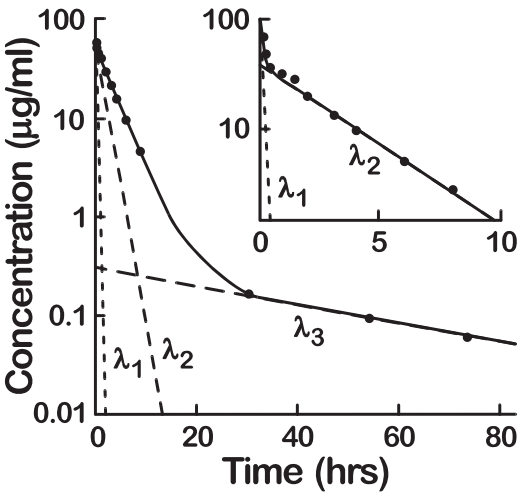


Figure 3.15 Semilogarithmic plot of plasma concentration versus time for intravenous gentamicin in the dog. Disposition is described by a three-compartment model when samples are collected over 80 hours and a two-compartment model when samples are collected for only 10 hours (insert).

rates faster (k_{12}/k_{21}) and the other with rates slower (k_{13}/k_{31}) than k_{10}. The slopes of the C-T profile for λ_1 primarily reflects the contribution of rapid distribution while λ_3, the terminal slope, primarily reflects the contribution of slower distribution into the so-called deep compartment. This model is applicable to many three-compartment drugs encountered in veterinary medicine (e.g., aminoglycosides, tetracyclines, persistent chlorinated hydrocarbon pesticides). Drug elimination from the central compartment is primarily reflected in λ_2 or β and through general usage is termed the β *elimination phase*.

These types of models are generally employed when experiments are conducted over long time frames and C-T profiles monitored to low concentrations. If the data are truncated at earlier times as shown in the insert of Figure 3.15, a normal two-compartment model is adequate to describe the data. However, if the goal of a study were to describe the tissue residue depletion profile of a drug in a food-producing animal, the tissue C_3-T profile would be of interest since it is the tissue where legal tolerances are established. This makes such complicated models useful in food animal veterinary medicine.

Models consisting of more than three compartments have been used when the data are of sufficient quality (sensitive analytical method, sufficient samples) to warrant such an analysis. The polyexponential equation describing n-compartment models (i = 1,2,3— n) is

$$C_p = \sum A_i\, e^{-\lambda_i t} \tag{3.46}$$

The differential equations needed to link these slopes and intercepts to the micro-rate constants are exceedingly

complex and will not be discussed further. These complex models are only presented to give an appreciation of the types of models encountered when tissue residue predictions are encountered.

In respect to prediction of tissue residues, when a tissue sample is taken, one is not measuring just concentrations in that tissue since the vascular and extracellular fluid components of that tissue are actually part of the central compartment. Similar arguments can be made for other components. When one is looking at deep compartment disposition, this may be satisfactory since release from these depots are rate-limiting, making this tissue component larger than any other phase that has already reached equilibrium. Equations are available to fractionate a tissue mass into vascular, extracellular, and cellular components based again on Vd estimates. Alternatively, tissue cages or microdialysis probes may be inserted into the tissue mass and extracellular kinetics modeled. These data are often presented in veterinary studies dealing with antimicrobial distribution to infected tissues.

Compartmental modeling concepts and techniques have defined the discipline of pharmacokinetics and continue to be extremely useful tools. One- and two-compartment analyses form the basis for most models used in human as well as veterinary and comparative medicine. These two models also serve as the foundation upon which many of the other techniques now to be briefly discussed are based. Modern computers have facilitated the analysis of these data to the point that the user no longer has to derive all of the relevant differential equations. Comprehensive software packages are available to effortlessly perform these calculations, even if the data does not support the model analyzed! Concerns such as these have led many clinical pharmacologists in both human and veterinary medicine to move away from complex multicompartmental models and adopt so-called *model-independent* approaches when their goal is to predict dosage regimens for clinical applications.

Noncompartmental Models

Over the last two decades, there has been generalized adoption of noncompartmental methods in veterinary and comparative pharmacokinetics. Noncompartmental models were first developed and applied to radiation decay analysis and remain dominant in the physical and biological science literature for general applications. Since their first application to problems in pharmacokinetics by Yamaoka in 1979, noncompartmental methods have grown steadily in use. This approach is for the most part actually an application of well-developed statistical moment theory, a full discussion of which is beyond the scope of this text. The approach involves primarily calculation of the Slopes, Heights, Areas and Moments (SHAM) of plasma concentration time curves. Statistical moment theory describes drug behavior based on the mean or average time an administered drug molecule spends in a kinetically homogeneous space, a concept identical to that of a compartment. The difference again is that no specific inferences are being made about the structure of these spaces.

Rather than being based on diffusion, these models are based on *probability density functions* that define drug disposition in terms of the probability of the drug being in a specific location. Instead of determining rates in terms of rate constants or half-lives, they describe processes in terms of statistical moments; the most useful is the mean residence time (MRT; τ). These are based on plasma concentration data and are determined by calculating areas under concentration versus time curves. MRT is calculated as

$$MRT = \frac{\int_0^\infty t\,C\,(t)\,dt}{\int_0^\infty C\,(t)\,dt} = \frac{AUMC}{AUC} \tag{3.47}$$

The denominator of this equation is the AUC we have discussed earlier in terms of calculating clearance and bioavailability. The numerator is known as the area under the [first] moment curve (AUMC), which is the CT-T profile. AUC and AUMC are depicted in Figure 3.16.

The MRT could be thought of as the statistical moment analogy to the half-life (T1/2), and it is inversely related to the first-order elimination rate of a one-compartment open model:

$$MRT_{TV} = 1/K_{el} \tag{3.48}$$

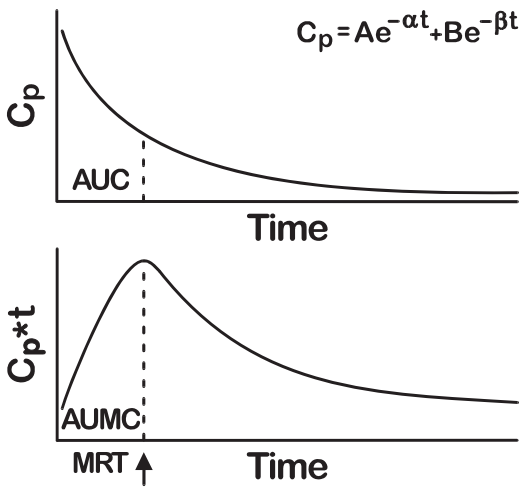


Figure 3.16 Plasma concentration versus time (C-T) and its first-moment (CT-T) plots demonstrating AUC, AUMC, and MRT.

Rearranging demonstrates that $K_{el} = 1 / MRT_{iv}$. Recalling Equation 3.18 where $T1/2 = 0.693/K_{el}$, substitution gives us

$$T1/2 = 0.693\ (MRT) \tag{3.49}$$

The MRT thus becomes an excellent parameter to describe the length of drug persistence in the body, much as the half-life is used in many compartmental pharmacokinetic models. The T1/2 used in this context is the elimination T1/2 in the body, and not that calculated from the terminal exponential phase for multicompartmental models. If the dose of drug is administered by intravenous infusion, the MRT_{iv} may be calculated as

$$MRT_{iv} = MRT_{infusion} - (Infusion\ Time)/2 \tag{3.50}$$

where $MRT_{infusion}$ is simply calculated from the observed data using Equation 3.47.

The primary task to solve model–independent or noncompartmental models is the direct estimation of the moments from data. This essentially is determining the relevant AUCs and moments from the C-T profile. When the C-T profile is described by a polyexponential equation of the form $f(t) = A_i e^{-\lambda it}$, Equation 3.43 ($AUC = \Sigma A_i / \lambda_i$) can be generally used to determine AUC. The AUMC may then be calculated as

$$AUMC = \sum A_i/\lambda_i^2 \tag{3.51}$$

The simplest and most commonly used method for estimating area under any curve is the trapezoidal rule. This technique is important since it is the primary method used to assess bioavailability by regulatory agencies. The approach is again illustrated in Figure 3.17.

$$AUC = \sum_{n=1}^{N} \frac{C_n + C_{n+1}}{2}(t_{n+1} - t_n) \tag{3.52}$$

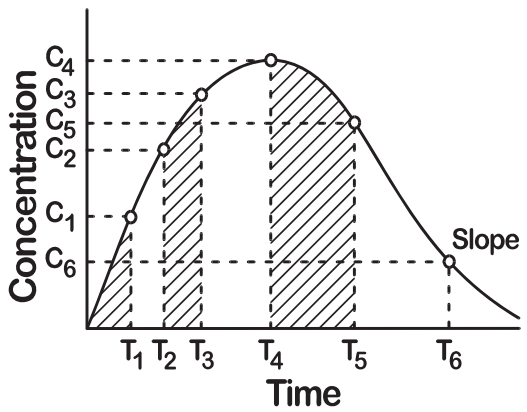


Figure 3.17 Breakdown of a plasma concentration versus time curve into trapezoids used to calculate the area under the curve. The terminal area from T_6 to T_∞ is calculated from extrapolating the terminal slope.

The summation is over N trapezoids, formed by N+1 data points. This algorithm is quick and, if enough data points are available, relatively accurate. It is also a simple algorithm to implement on a computer. The area under each pair of connected points describes a trapezoid (except when one of the points has zero value, in which case one of the legs of the trapezoid has zero length, making a triangle). The area under the entire curve is then the sum of the areas of the individual trapezoids, which can easily be calculated. The area under the final triangle is estimated by the AUC and must be estimated to infinite time. Generally, this portion of the AUC should be less than 20% of the total. Many methods have been proposed for this, the most common being $AUC_{T\to\infty} = C_T / \lambda_n$, where λ_n is the terminal slope of the C-T profile. The estimation of the first moment for calculation of MRT is the summed trapezoids plotted on a CT-T graph. The attraction of statistical moment analysis to pharmacokinetics is the use of trapezoids to determine the relevant areas. No assumptions but the underlying mechanisms of drug disposition are made, and computer curve fitting is not required.

To complete this brief introduction to this topic that is the basis of many commercial software packages, one other residence time having general application in clinical pharmacology is the mean absorption time (MAT). Other residence times may be calculated but are not used in determining dosage regimens in clinical medicine. MAT is technically the mean arrival time into the systemic circulation of bioavailable absorbed molecules. MAT is the statistical moment theory equivalent of estimating K_a. MAT is a computationally straightforward method to characterize the rate of drug absorption in bioavailability studies. The simplicity of this approach is that transit times are additive. MAT is the mean time for drug molecules to remain unabsorbed. MAT is simply the difference in MRT following intravenous injection (MRT_{IV}) and another noninstantaneous administration (MRT_{Route}):

$$MAT = MRT_{Route} - MRT_{IV} \tag{3.53}$$

Assuming absorption is described by a first-order process with an apparent rate constant of k_a, then

$$MAT = k_a^{-1} \tag{3.54}$$

making the absorption half-life

$$T1/2_{[abs]} = \ln 2 \cdot MAT \tag{3.55}$$

On the other hand, when absorption is assumed to be a zero-order process (e.g., constant rate), then

$$MAT = T/2 \tag{3.56}$$

where T is the duration of the absorption. Note the similarity to the infusion Equation 3.50 above. In reality, a

constant rate infusion is a zero-order absorption whose MAT is just one-half the length of the infusion.

The reader should recall that the determination of systemic availability expressed in Equation 2.4 [$F = (AUC_{Route})(Dose_{IV})/(AUC_{IV})(Dose_{Route})$] is a noncompartmental analysis. An AUC should be determined by the trapezoidal methods presented in this chapter. If the point of an analysis is to determine bioequivalence between two formulations, one is actually calculating relative systemic availability, a concept equivalent to determining whether two formulations are clinically interchangeable. In addition, the metrics time to peak concentration (T^{max}) and peak concentration (C^{max}) are often compared. Comparison of MATs would also shed light on the equivalence of two formulations. The reader should consult the references in the section "Selected Readings," later in this chapter for further approaches and the Food and Drug Administration website (www.fda.gov/CVM) for current bioequivalence guidelines for veterinary products.

The determination of Cl_B using statistical moment theory is easily obtained using the previously defined Equation 3.16 where $Cl_B = D/AUC$. Using the trapezoidal methods to estimate AUC makes this a robust estimate of clearance. The volume of distribution at steady-state (Vd_{ss}), according to statistical moment theory, is simply the product of MRT and CL:

$$Vd_{SS} = Cl_B\ MRT \tag{3.57}$$

This, incidentally, affords the expression for half-life as a function of clearance, by solving Equation 3.57 for MRT:

$$T_{1/2} = \frac{\ln 2 \cdot Vd_{SS}}{Cl_B} \tag{3.58}$$

which is the same as that presented in Equation 3.18. Substitution of the respective expressions for MRT (Equation 3.47) and CL (Equation 3.16) into Equation 3.57 yields

$$Vd_{SS} = \frac{D_{i.v.} \cdot AUMC}{AUC^2} \tag{3.59}$$

Another volume parameter also calculated using statistical moments sometimes encountered for dosage regimens is Vd_{area}:

$$Vd_{area} = \frac{D_{i.v.}}{k_{el} \cdot AUC} \tag{3.60}$$

Statistical moment methods provides a powerful tool for calculating many of the common pharmacokinetic parameters that are routinely encountered in veterinary medicine. This includes the concept of bioequivalance discussed above, as well as generating parameters that are used to construct dosage regimens and assess the effect of disease on drug effects. As can now be even more fully appreciated, Cl_B and Vd_{ss} are truly independent parameters that quantitate distribution and excretion using computationally robust techniques based on minimal model-specific assumptions. We have presented this approach since it is the primary method by which pharmacokinetic parameters are now determined in veterinary medicine. Table 3.2 is a compilation of equations useful to calculate these parameters from an analysis of a C-T profile.

Table 3.2 Noncompartmental equations for calculating common pharmacokinetic parameters

$Cl_B = Dose / AUC$
$Cl_D = V_c\ \lambda_1 - Cl_B$
$Vd_{ss} = (Dose \times AUMC) / AUC^2$
$V_c = Dose / Cp_0$
$MRT_{iv} = AUMC/AUC = V_d(ss) / Cl_B$
$MAT = MRT_{route} - MRT_{iv}$
$T1/2 = 0.693\ MRT = 0.693\ Cl_B / Vd_{ss}$
$T1/2\ (\lambda) = 0.693 / \lambda$
$F = (AUC_{route})\ (Dose_{iv}) / (AUC_{iv})\ (Dose_{route})$
$AUC = \Sigma\ A_i / \lambda_i$
$AUMC = \Sigma\ A_i / (\lambda_1)^2$

Note that AUC and AUMC could be calculated using trapezoidal analysis of areas rather than fitting curves to obtain estimates of A_i and λ_i.

Nonlinear Models

Most pharmacokinetic models incorporate the common assumption that drug elimination from the body is a first-order process, and the rate constant for elimination is assumed to be a true constant, independent of drug concentration. In such cases, the amount of drug cleared from the body per unit time is directly dose or concentration dependent, the percentage of body drug load that is cleared per unit time is constant, and the drug has a single constant elimination half-life. Fortunately, first-order elimination (at least apparent first-order elimination) is typical in drug studies. First-order linear systems application greatly simplifies dosage design, bioavailability assessment, dose–response relationships, prediction of drug distribution and disposition, and virtually all quantitative aspects of pharmacokinetic simulation.

However, drugs most often are *not* eliminated from the body by mechanisms that are truly first-order by nature. Actual first-order elimination across all concentrations applies only to compounds that are eliminated exclusively by mechanisms not involving enzymatic or active transport processes (i.e., processes involving energy). As presented in Chapter 2, they are primarily driven by diffusion and obey Fick's Law. The subset of drugs not requiring a transfer of energy in their elimination is restricted to those that are cleared from the body by urinary and biliary excretion and, among those, only drugs that enter the renal tubules by glomerular filtration or

passive tubular diffusion. All other important elimination processes require some form of energy-consumptive metabolic activity or transport mechanism. What is the impact of this on pharmacokinetic parameters?

The reason energy-involved processes are not strictly first-order is that they are generally *saturable,* or more specifically are *capacity-limited*. At clinical dosages, the majority of drugs do not reach saturation concentrations at the reaction sites, and follow first-order linear kinetics. Recalling for first-order processes, a constant percentage of remaining drug is cleared per unit time, and the drug has a discrete, concentration-independent elimination rate constant (K) and thus half-life. For drugs eliminated by zero-order kinetics or saturated pathways, however, a constant quantity of drug is eliminated per unit of time, and this quantity is drug concentration independent and the drug does not have a constant, characteristic elimination half-life. The potential impact of saturable, leading to zero-order (versus first-order) elimination, can be profound, and its effects include altered drug concentration profiles, scope and duration of drug activity, and distribution and disposition among tissues. Saturable hepatic metabolism may markedly affect drug absorption due to reduced clearance (lower hepatic extraction) and altered first-pass activity after oral administration. Nonlinearity is associated with a nonconstant T1/2 at different doses or when a plot of dose versus AUC is not linear, indicating that Cl is reduced as dose increases.

The primary technique used to model saturable metabolic process employs the Michaelis–Menten rate law. This can be expressed as

$$dC/dt = -(V_{\max})(C)/(K_m + C) \tag{3.61}$$

where V_{max} is the maximum velocity (rate) of the reaction, and K_m is the Michaelis constant that relates concentration to effect. There are two notable simplifying conditions of the Michaelis–Menten equation. If $K_m \gg C$, then Equation 3.61 reduces to

$$dC/dt = -(V_{\max})(C)/(K_m) \tag{3.62}$$

This is equivalent to first-order elimination after IV administration in a one-compartment model where $dC/dt = -K_{el}C$. Thus, assuming elimination by a single biotransformation process, the first-order elimination rate constant K_{el} becomes V_{max}/K_m. If however, $K_m \gg C$, saturation is occurring and then Equation 3.62 collapses to

$$dC/dt = -V_{\max} = -K_0 \tag{3.63}$$

The rate in this case is independent of drug concentration (i.e., a constant), which describes a zero-order process, and the rate of drug elimination is now equal to $-K_0$.

Often, drugs are found to be eliminated by both first-order and nonlinear processes in parallel. In such cases, Equation 3.62 must be expanded to include the strictly first-order elimination processes:

$$\frac{dC}{dt} = -\frac{V_{\max}C}{K_m + C} - k'_{el}C \tag{3.64}$$

What is the impact of this on clinical veterinary pharmacology? The precise calculation of V_{max} and K_m is not done in clinical practice. However, values of Cl_B and T1/2 are assumed to be constant. When saturation occurs, Cl_B decreases and T1/2 increases, resulting in dosage regimens that accumulate with potential adverse effects. Recalling discussions in Chapter 2 on metabolism and saturation, the same phenomenon can also occur if enzyme inhibition occurs. In contrast, enzyme induction would increase clearance and reduce T1/2 resulting in decreased and potentially ineffective plasma concentrations.

Summary of Modeling Approaches

With the advent of modern computers, advanced software packages and sensitive and high-throughput analytical techniques, more comparative pharmacokinetic data are becoming available, and such data are being included in package inserts of approved products. The focus of this chapter is to develop basic pharmacokinetic concepts that serve as the foundation of dosage regimen construction in clinical practice. Obviously, many of the more complex models were only presented in order to illustrate these basic concepts. It is crucial that the practitioner know assumptions and limitations involved in a specific clinical case. However, there are additional pharmacokinetic approaches that are used in comparative medicine which in some cases complement the basic approaches above, and in others offer significant advantages. Two of these will be briefly introduced. Standard pharmacokinetic textbooks should be consulted for more detail.

Population Pharmacokinetics

All of the models discussed to this point have been focused on predicting drug concentrations in the individual animal. However, in many cases populations are of interest. For example in dogs, it would be ideal to know the basic pharmacokinetic parameters for a drug in the population at large that would apply to all breeds, ages, and gender. More important would be knowledge of which subpopulation had significantly different parameters. This is normally achieved by collecting a large number of plasma samples in individual animals and averaging resulting pharmacokinetic data from small (4–6 animals) studies. Recently, techniques have been developed that allow one to conduct studies in large numbers of individuals with less individual sampling. The approach