4 General Principles of Drug Distribution

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4.1 INTRODUCTION

Drug distribution describes the process by which a drug moves between the blood and various tissues (e.g., muscle and fat) or organs (e.g., brain). It is an integral part of ADME (absorption, distribution, metabolism, and excretion), which encompasses the complete fate of a drug when it enters the body and further background is provided in Section 4.2. Many factors can influence the ability of a drug to distribute in the body, including physiochemical and physiological factors, which are discussed in Sections 4.3 and 4.4, respectively. Uptake and efflux transporters also have an important role in drug tissue distribution as discussed in Section 4.5. Methods to investigate distribution continue to evolve and are employed *in vivo*, in both animal and human studies and in more sophisticated *in vitro* methods. Dosing of subjects with radiolabeled drugs is common and can be coupled with analytical techniques such as microdialysis, magnetic

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resonance imaging (MRI), and positron emission tomography (PET). Classical and current *in vitro* and *in vivo* methods to investigate drug distribution are reviewed in Sections 4.6 and 4.7. Different models employed in the calculation of drug distribution from data are also outlined in Section 4.8. Finally, examples of the clinical implications of drug distribution are presented in Section 4.9.

4.2 BACKGROUND OF DRUG DISTRIBUTION

Drug distribution is an important part of a larger pharmacokinetic picture known as *absorption, distribution, metabolism, and excretion* (ADME), which has become integral in understanding and explaining drug effects. ADME is the interplay between absorption, distribution, metabolism, and elimination. Drug distribution describes the process by which a drug moves between the blood and various tissues (e.g., muscle and fat) or organs (e.g., the brain). Many factors can influence the ability of a drug to distribute in the body, including physiochemical and physiological factors and the contribution of transporters. When a drug is administered intravenously, or absorbed after oral dosing, it will rapidly circulate through the body in the bloodstream. A complete circulation of the blood typically takes about 1 min, and after this time, a drug will begin to distribute into tissues [1]. Factors that can affect the ability of the drug to distribute into tissues include water and fat solubility. A drug that is more water soluble will tend to stay in the blood or interstitial spaces, whereas a fat-soluble drug may concentrate in fatty tissues. Drugs can also be limited in their ability to penetrate into tissues depending on their ability to cross barriers, such as the blood–brain barrier (BBB) or placental barrier. Drug binding to plasma proteins can also affect its ability to distribute. Bound drug is not able to distribute into tissues; only the fraction unbound (free) moves into tissues and determines the pharmacodynamics or efficacy of the drug.

4.3 MULTIPLE PHYSIOCHEMICAL FACTORS INFLUENCE DRUG DISTRIBUTION

Cell membranes form the barriers between all the compartments of the body. There are four main ways in which small molecules and drugs can cross membranes and gain access to the different body compartments. First, they can diffuse directly through the lipid, they can diffuse through aqueous pores formed by special proteins in the membrane, they can combine with a transmembrane carrier protein, and, finally, they can transverse the membrane by pinocytosis. Of these four, diffusion through the lipid and transport via carrier-mediated mechanisms are the most important in terms of the disposition of drugs, including both paracellular and transcellular distribution. The aqueous pores in membranes are too small (ca. 0.4 nm) for most drugs to pass through, and pinocytosis is generally more important for the transfer of macromolecules (such as insulin) but not for small molecules. Significant advances in identifying and understanding transporters for xenobiotics have been made in recent years, and it is estimated that at least 5% of all human genes are transporter related [2]. This indicates the importance of transport across barriers in normal physiological processes. Transfer via carrier-mediated mechanisms is dealt with in more detail in Section 4.5, and this

discussion will therefore concentrate on factors that influence the passive diffusion of drug molecules through the lipid membrane.

4.3.1 Lipophilicity, pH, pK_a, and Ionization

The ability of drugs to cross membranes depends on their solubility in the membrane (determined by the partition coefficient of the drug between the membrane lipid phase and the aqueous phase) and the diffusion coefficient. There is therefore a close relationship between the lipid solubility of a drug and its ability to permeate a cell membrane. As a result, lipid solubility is a major determinant of the pharmacokinetics of a drug, and, for example, its absorption from the intestine, ability to transverse the BBB, and rate of renal elimination can to a large extent be predicted from its lipid solubility.

Many drugs are either weak bases or acids and can exist as both ionized and nonionized forms, and as such their lipid solubility depends to a large extent on the pH of the environment. The dissociation constant, pK_a, can be determined by the Henderson–Hasselbalch equation, and this relationship can be used to predict the extent of ionization of weak acids and bases in different pH environments. For a weak base, the relationship is

$$pK_a = pH + \log \frac{[\text{ionized form}]}{[\text{nonionized form}]}$$

and for a weak acid,

$$pK_a = pH + \log \frac{[\text{nonionized form}]}{[\text{ionized form}]}$$

For both weak acids and bases, the ionized form is not generally lipid soluble, and cannot cross membranes, unless by a specific transporter mechanism. On the other hand, the lipid solubility of the nonionized form depends on the structure of the drug, and many nonionized drugs will permeate rapidly. However, there are exceptions to this and among them are polycationic aminoglycoside antibiotics, which as a group are not well absorbed orally and do not penetrate into the BBB or into most other body fluid compartments. Elimination of these drugs is almost exclusively by glomerular filtration, and 50–60% of a therapeutic dose is excreted unchanged by patients within 24 h [3].

Drugs have a wide range of pK_a values, and the values found among a selection of common drugs are depicted in Table 4.1. Where a pH difference exists between body compartments, then the extent of ionization of a drug would influence its ability to distribute within the body, with the ratio of ionized to nonionized drug being determined by the pK_a of the compound and the pH of the compartment fluid. An example of this is shown in Fig. 4.1, which illustrates the distribution of a weak acid, aspirin (pK_a 3.5), and a weak base, erythromycin (pK_a 8.8), between the stomach contents (pH 2), plasma (pH 7.4), and urine (at pH 6, which is the normal situation, and at alkaline pH 8). In this model, it is assumed that the nonionized drug will cross membranes and reach equal concentrations in each compartment, whereas the ionized drug will not cross at all. As a result, the total drug concentration in each compartment will be different, and an acidic drug will be trapped as an ion in the compartment with the highest pH. Theoretically, large concentration gradients can be built between compartments if there is a large difference in pH. However, in practical terms, this extreme situation does not

TABLE 4.1 pK_a Values for Some Acidic and Basic Drugs

Drug	pK_a value
Bases	
Chloroquine	10.8
Amphetamine	9.9
Atropine	9.9
Ephedrine	9.6
Desmethylimipramine	9.5
Propranolol	9.5
Chlorpromazine	9.3
Erythromycin	8.8
Dopamine	8.4
Morphine	7.9
Codeine	5.8
Diazepam	3.3
Acids	
Levodopa	2.3
Penicillins	2.7
Probenacid	3.4
Aspirin	3.5
Flufenamic acid	3.9
Warfarin	5.0
Phenobarbitone	7.4
Phenytoin	8.3

Bases are arranged in decreasing strength, acids are arranged in decreasing strength, with the strongest acids at the top, and the strongest bases at the top.

happen because fluids in body compartments are not static, and the constant flux of fluids between compartments reduces the concentration gradients. It is also of course unrealistic to assume that ionized drug is totally nonpermeant, and a small percentage will diffuse across membranes.

It is important to realize that for gastrointestinal (GI) absorption, a major overriding contributing factor is the enormous absorptive area of the villi of the small intestine compared with the smaller absorptive area of the stomach. For this reason, partition between different pH environments is not a major determinant of the site of absorption of drugs from the GI tract. Nevertheless, weak acids and bases are well absorbed from the intestine, while strong acids and bases are not, as discussed by studies such as that published in 1957 by Schanker and coworkers, which shows the absorption of a range of drugs across the rat [4] and human [5] stomach as a function of their pK_a values. They showed that strong acids, with pK_a of <2, and strong bases, with p$K_a > 10$, are not well absorbed. The usefulness of the low oral bioavailability of a strong base is well demonstrated by the case of curare, a mixture of alkaloids found in various South American plants, used as an arrow poison by South American Indians. The effective constituent is tubocurare, a quaternary-ammonium-containing compound, and the low

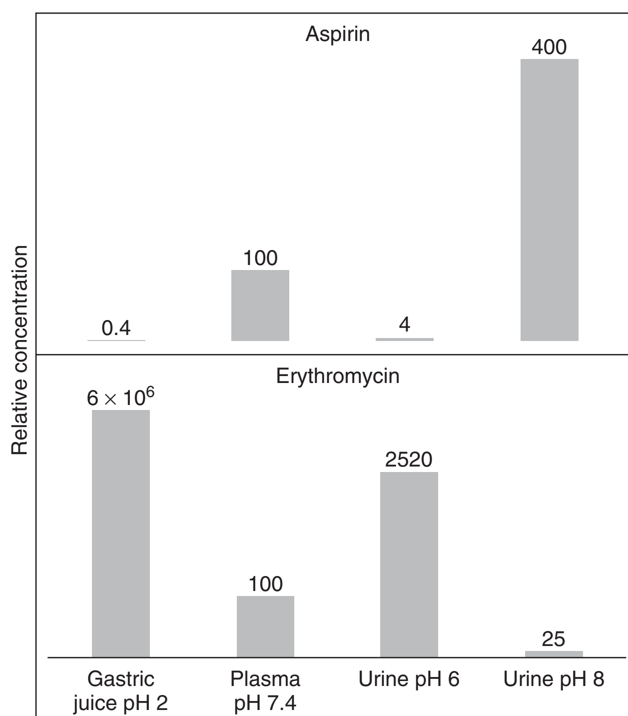

Figure 4.1 Theoretical partition of a weak acid (aspirin) and a weak base (erythromycin) between gastric juice, plasma, and urine (at the normal situation of pH 6 and at alkaline pH of 8). The numbers represent the relative partition compared with plasma, which has been taken as 100%, and illustrate the large differences in total drug concentrations that could result from the different fractions of ionized species present as a function of environment pH.

oral absorption of tubocurare allowed it to be used safely in the hunting of animals for food. It was the earliest nondepolarizing neuromuscular blocking agent identified. Tubocurare is not used clinically now in medicine being superceded by synthetic non-depolarizing blocking agents such as pancuronium, atracurium, and vecuronium, all of which are quaternary ammonium compounds with varying durations of action and low oral bioavailability [6].

The pH of the environment and the pK_a does, however, have important consequences for urinary excretion and for the distribution of drugs into the brain. If the urine becomes acidic then the excretion of weak acids is decreased, and that of weak bases is increased, and if urine becomes basic, this has the opposite effects. This means of enhancing the excretion of drugs by altering the pH of the urine has been used extensively to increase drug elimination in cases of overdose, particularly with aspirin and phenobarbitone, where alkalinization of the urine is an established clinical protocol to promote excretion of the weakly acidic drugs. This is discussed further in Section 4.9, with examples provided from the literature. In addition to altering urine pH, administration of sodium bicarbonate increases plasma pH and will increase the movement of weak acids from the central nervous system (CNS) into the plasma. In

overdose, administration of sodium bicarbonate would thus both increase the elimination of phenobarbitone and decrease its CNS toxicity. Conversely, the administration of a carbonic anhydrase inhibitor, such as acetazolamide, decreases plasma pH and causes weakly acidic drugs to become concentrated in the CNS, thus increasing their neurotoxicity.

4.3.2 Molecular Size

The rate of diffusion of a substance depends on its molecular size, the diffusion coefficient for small molecules being inversely proportional to the square root of the molecular weight. Although large molecules diffuse more slowly than small ones, because most drugs have molecular weights between 200 and 1000 kD, the effect of molecular weight does not have a major effect on their pharmacokinetics.

In terms of renal elimination, molecular weight is not an important factor as the glomerular capillaries allow molecules below a weight of about 20,000 kD to diffuse into the filtrate. Plasma albumin, with a molecular weight of about 68,000 kD, is virtually completely excluded, but most drugs with the exception of macromolecules cross the glomerulus freely. Molecular weight is, however, a major determinant controlling whether a chemical is excreted into bile. As a general rule, low molecular weight substances (<325 kD) are poorly excreted into bile [7]. Biliary excretion is regulated by the presence of the xenobiotic transporters present on the canalicular membrane of hepatocytes and has consequences for the biological half-life and toxicity of compounds. The role of transporters in drug distribution has been further discussed in Section 4.5.

4.3.3 Drug Delivery Systems

Particle size has a major effect on GI absorption, and this has been a factor in the standardization of formulations of pharmaceutical agents such as digoxin. For example, Lindenbaum *et al*. [8] demonstrated the marked variability in plasma concentrations seen in patients given four different tablet preparations of digoxin, and observations such as these contributed to the standardization of formulations. Digoxin has a low therapeutic index (maximum nontoxic dose/minimum effective dose), and control of circulating concentrations is crucial; problems with bioavailability contribute to unexpected clinical results [9], and capsule preparations proved to be more consistent than tablets [10]. The aim of clinical pharmacy is to deliver a drug for an appropriate time, within the therapeutic window, to a specific target, outside the systemic toxic concentration range. Conventional routes of administration, such as oral or injection, cannot always achieve this satisfactorily. Controlled release systems match the rate of drug delivery to its elimination, and thereby the concentration remains within the therapeutic range for most of a 24-h period. Today, such controlled release can be provided by a wide range of processes such as encapsulation, transdermal patches, bioadhesive systems, osmotic micropumps, microimplants, drug-eluting medical devices, and nanoparticles [11,12]. Polymers are employed to delay the dissolution of drugs and control their flux from the delivery system. This is usually achieved using a polymeric coating or matrix that degrades at a slower rate than the drug diffuses and releases. Copolymers of various ratios of lactic acid to glycolic acid [poly(lactide-*co*-glycolide)] are commonly used, the ratio controlling the rate of degradation [13]. The

development of delivery systems for small molecules, proteins, and DNA has taken an enormous step forward with advances in nanotechnology, and these are now making a significant contribution to the global pharmaceutical market. It is known that nanoparticles are taken up by cells more effectively than larger sized microparticles [14]. Nanodelivery systems can be designed with different compositions and biological properties, such as nanoparticles, dendrimers, nanocages, micelles, molecular conjugates, and liposomes, and these have been extensively investigated for drug and gene delivery systems [15]. The effect of particle size on the distribution of nanocarriers within the body has been established, and following intravenous administration, small particles (<30 nm) are eliminated by renal excretion [16], particles of 30–150 nm are taken up into the bone marrow, heart, kidney, and stomach [17,18]. Larger particles (>200 nm) are sequestered by the mononuclear phagocytic system and located mainly to the reticular endothelial system in the liver [19]. In addition, recently, the role of shape in the biodistribution of nanoparticles has been suggested [20]. In this context, Devarajan and coworkers [21] have described the effect of nanoparticle shape on *in vivo* distribution in rabbits and dogs. They have shown that irregularly shaped polymeric lipid nanoparticles evade macrophages and localize in the spleen opening up particle shape as a new possibility in controlling the disposition of nanoparticulate drug delivery systems.

4.4 PHYSIOLOGICAL FACTORS AFFECTING DRUG DISTRIBUTION

Drug distribution begins on absorption of a drug into the bloodstream. The various regions of the systemic circulation are perfused with blood through innumerable branching pathways, which are effectively arranged in parallel (Fig. 4.2) [1]. Distribution of drug occurs through the blood via the circulatory system. Blood is continually flowing from the heart to all areas of the body through arteries, to capillaries through organs and skin, reconverging into veins that return to the heart. The blood then travels to the lung for reoxygenation via the pulmonary circulation before returning to the heart to complete the process over and over again. Uninterrupted movement of blood is required for maintaining the supply of oxygen from the lungs and nutrients from the gut, as well as for the distribution of hormones, chemicals, including xenobiotics, water, and heat, and the delivery of waste for excretion [1]. The first stage of drug distribution is dependent on the cardiac output and regional blood flow to the various organs. Drug distribution is rapid to highly perfused organs, where the rate of blood flow is higher and these organs receive most of the drug, while distribution to adipose tissues, which are not so well perfused, is generally slower and drug concentrations are markedly less. Drug distribution is a reversible process in equilibrium so drug can move between organs and the circulation. Distribution of drug from systemic circulation to tissues is dependent on the interplay of the physiochemical properties of the drug such as lipid solubility, ionization, and molecular size (Section 4.3) and physiological factors such as binding to plasma proteins, rate of blood flow, susceptibility to passively diffuse, or to undergo active transport. It can be affected by local ionic and cellular conditions and special barriers such as biological membranes like the BBB and placental barrier. The drug exists in two forms in blood, the free form, which is active and available for biotransformation and excretion, or the bound form, which is usually pharmacologically inactive. The main physiological compartments, where drugs

are distributed, are the plasma (5% of body weight), interstitial fluid (16% of body weight), intracellular fluid (37% of body weight), fat (20% of body weight), specific organs (liver/kidney), as well as muscle, bone, and the CNS [22]. Additional factors can influence the ability of a drug to distribute, including the propensity of the drug to bind to plasma proteins. In the plasma, drugs generally bind to albumin although drugs can also bind to globulins (α, β, γ), α-1-acid glycoprotein (AGP) or lipoproteins. Since protein binding can have a large impact on drug distribution properties, Section 4.4.4 is devoted to covering this topic in more detail. The lipid solubility of the drug can also affect its distribution (Section 4.3.1). Additionally, the disease state itself can play an important role in determining the extent of distribution since disease-induced changes to many of the physiological parameters can markedly influence drug distribution. Many of these physiological factors are explained in further detail in subsequent sections.

4.4.1 Distribution Between Body Water

Drug distribution and dilution can occur between various organ compartments of water within the body and this information is sometimes useful for comparing the distribution of drugs with the volumes of the body water compartments by calculating a volume of distribution or V_d. The volume of distribution is a proportionality constant that relates the amount of drug in the body [dose (mg)] to the plasma concentration. $C_p = C_{p0}e^{-kt}$, where C_{p0} equals the initial plasma concentration and is a function of the distribution of the drug in the body:

$$V_d(\text{volume of distribution}) = \frac{\text{Dose (mg)}}{\frac{[\text{Plasma}]\text{mg}}{\text{mL}}}$$

V_d values can describe where drug is and do not necessarily correspond to real, physiological volumes. The V_d can reflect the ability of the drug to cross cell membranes (polarity), bind to plasma proteins (limits distribution), or bind to tissue proteins (increases distribution).

The amount of body water can be as much as 75% of the total body weight with the average being about 60% or around 44 L. This body water is distributed between four main compartments, the intracellular fluid (61%), interstitial fluid (27%), blood plasma (7%), and blood cell (5%) [22]. The V_d calculated for individual drugs can provide information on the extent of distribution between body water and tissues. Distribution between body water compartments is dependent on their volume and the barriers present, such as lipid membranes, binding to plasma proteins, and pH gradients that are encountered. Following entry into the body, regardless of route of administration, a drug has the propensity to be distributed into any one of these distinct body water compartments or to be sequestered within a cellular site. When a drug is administered directly to the bloodstream, it enters the plasma compartment. When a drug is absorbed, it passes through cell linings of the absorbing organ (skin, lung, or GI tract) into the fluids surrounding cells of the organ known as the *interstitial fluid*. The interstitial and intracellular fluids are in equilibrium with water and electrolytes, which move slowly into and out of cells in contrast to fast-moving blood. Drug can leave interstitial fluid by entering local tissue cells, entering blood capillaries (systemic circulation) or entering

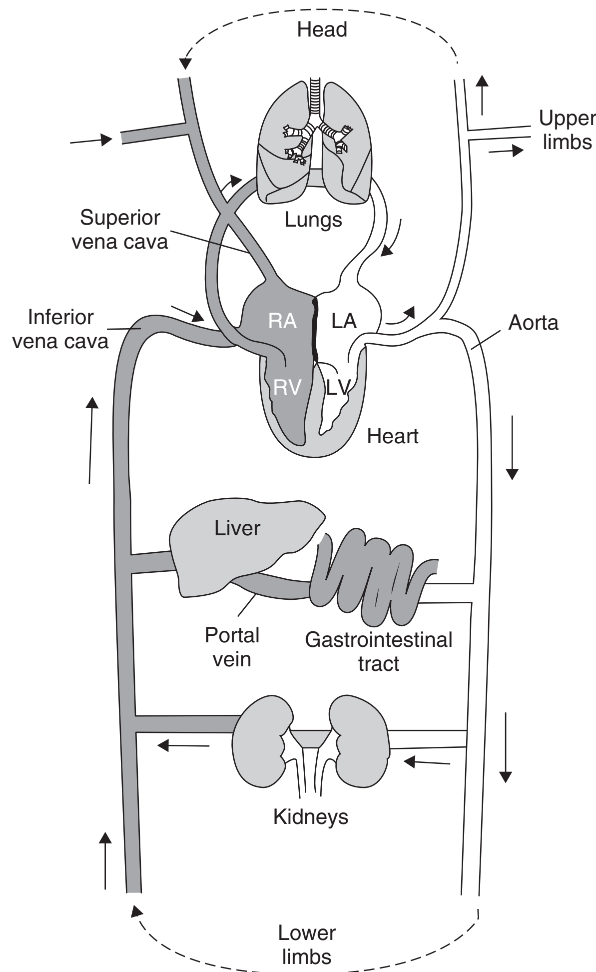

Figure 4.2 Simplified schematic of the circulatory system showing direction of flow from the inferior vena cava and superior vena cava to the right atrium. From the right atrium, the blood will enter the right ventricle where it is pumped into the lungs oxygenated and returned to the left atrium where it is pumped by the left ventricle to the rest of the body and returned to the right atrium through the IVC and SVC as depicted above.

the lymphatic system. Drug can also enter transcellular fluids by passing through an epithelium. Examples of transcellular fluids are cerebrospinal fluid (CSF), aqueous humor of the eye, contents of renal tubules, urine in the bladder, glandular contents, synovial fluid in joints, pleural fluids, peritoneal fluids, bile, saliva, and GI secretions. Figure 4.3 shows a schematic of drug distribution after various routes of administration through to elimination. While these compartments of water (intracellular fluid, interstitial fluid, blood plasma, and blood cell/transcellular fluid) are important in describing the distribution of drugs, compounds rarely associate solely within them, but most often distribute among several compartments, often binding to cellular components as described in Section 4.4.8.

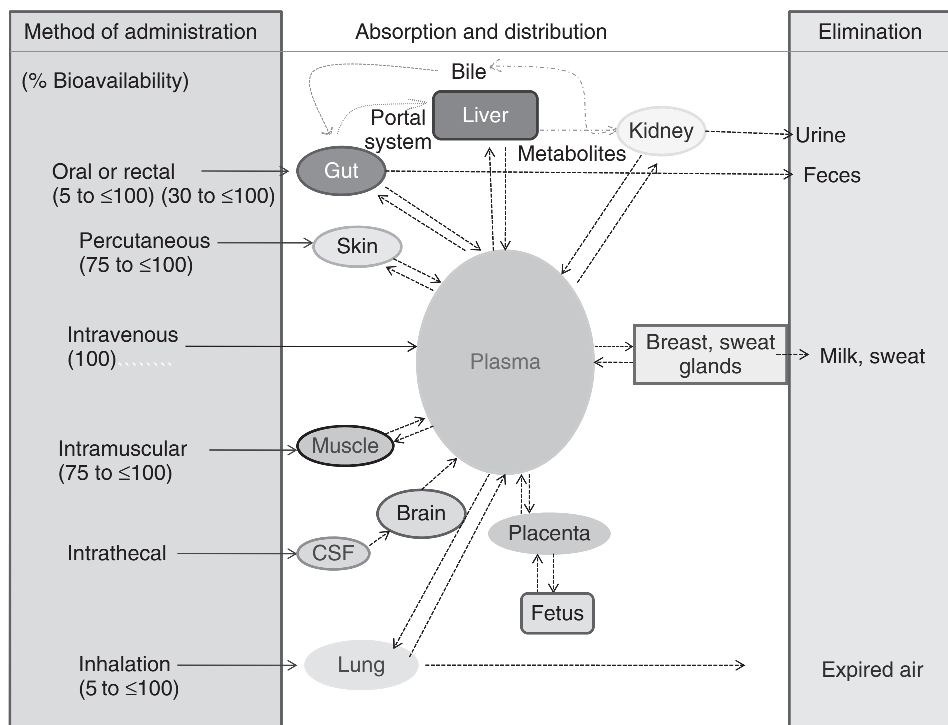

Figure 4.3 Pictorial representation of the disposition of a drug on administration. The theoretical bioavailability of the drug is highlighted below the route of administration on the left. Once the drug has been administered, it is absorbed and potentially distributed into various compartments of the body as detailed above. The methods for elimination will be dependent on a host of factors, including extent of distribution to eliminating organs such as the liver and kidneys. (See color insert.)

4.4.2 Drug Distribution Patterns

On entering the vascular system, a drug is distributed throughout body fluids, as described in Section 4.4.1, or into various tissues. The pattern of distribution that the drug exhibits reflects both the physiochemical nature of the drug and its ability to penetrate different membranes. The V_d can provide useful information on the type of distribution pattern displayed by a drug, and four different distribution patterns are recognized. Drugs exhibiting pattern 1 remain largely within the vascular system. This would encompass drugs such as dextran with a V_d in the region of 3–5 L, which approximates the volume of plasma [23]. Drugs that are distributed throughout the body water and not limited to the vascular system exhibit pattern 2 and demonstrate a V_d of around 30–50 L. Xenobiotics that exhibit this pattern are generally low molecular weight and water soluble. Examples of pattern 2 drugs would be ethanol (molecular weight = 46.07 g/mol), atenolol (molecular weight = 266 g/mol), and some sulfonamides (molecular weights typically <250 g/mol). Pattern 3 distribution evokes very large V_d values because of tissue partitioning, which may or may not be related to the site of action. Highly lipid-soluble compounds that can concentrate into fat tissue also tend to have higher V_d values. Most drugs follow a nonuniform distribution

pattern, also referred to as *pattern 4*, which is a combination of patterns 1–3 [23]. These drugs display different lipid/water solubility and varying abilities to pass through membranes. In some cases, this may result in higher concentrations within sites with higher rates of blood flow such as the kidney and liver, which can be reflective of the amount of drug being excreted. These drugs can have a V_d value within a wide range.

4.4.3 Rate of Blood Flow

The rate of distribution of a drug to organs is dependent on the blood flow rate to these regions. Highly perfused organs such as the heart, liver, lungs, kidneys, and brain reach equilibrium more rapidly because the rate of blood flow is higher. Areas where blood flow is lower such as skin, bone, and fat have a slower rate of equilibration. The rate of blood flow can also differ between rest and exercise and is dependent on physiological cues.

4.4.3.1 Rate of Blood Flow to Organs and Blood Perfusion Rate. Systemic flow is the culmination of flow through many parallel circuits supplying different organs and tissues. Since systemic and pulmonary circulation is one continuous circuit, the flow through the lungs is entirely dependent on the flow through the systemic circulation. Because they are integrated, both the systemic and pulmonary flow must each be equivalent to the volume pumped by each ventricle, which is the cardiac output. Cardiac output is the product of the stroke volume and the number of heartbeats per minute. In a typical 70-kg male volunteer at rest, the average stroke volume of the left ventricle is 0.08 L, while the average heart rate is 69 beats/min; therefore, at rest, cardiac output is about 5.5 L/min [1]. However, due to biological barriers, capillary permeability, and other physiological requirements, the quantity of blood flowing through each organ or region supplied by the systemic circulation is regulated. Table 4.2 outlines the cardiac output to various organs when a healthy, 70-kg man is resting. Cardiac output can increase with exercise or during emotional or physical stress, which can include illness, trauma, or disease. In contrast, if there is a decrease in stroke volume from dehydration or blood loss, there will be a decrease in cardiac output. Therefore, it should be considered that there are ranges to the outputs described in Table 4.2.

TABLE 4.2 Blood Perfusion Rates for Selected Organs

Organ	Perfusion Rate (mL/min/mL of Tissue)	Percentage of Cardiac Output
Skin	0.05	5
Bone	0.02	5
Muscle	0.03	15
Fat	0.01	2
Brain	0.55	15
Heart	0.7	4
Liver	0.75	20
Kidneys	4.5	24

Source: Extracted from Ref. 22.

4.4.4 Plasma Protein Binding

Plasma comprises ~55% of the total blood volume and is composed of water containing trace amounts of salts, minerals, and nutrients. Plasma contains a variety of proteins such as immunoglobulin and fibrinogen (for clotting); however, one of its main constituents is albumin, which comprises ~60% of the total plasma protein [24].

Among serum proteins, human serum albumin (HSA) and AGP play important roles in protein binding of many drugs, which is important in determining drug distribution throughout the body [25]. Albumin is basic, and therefore preferentially binds acidic and neutral compounds, while basic drugs can bind to AGP, which is acidic. Since albumin is a good carrier for acidic drugs, many drugs will circulate through the bloodstream bound to it. While drugs can bind to other plasma proteins, such nonspecific binding occurs to a much lesser extent. Binding of drugs to albumin, which enables circulation throughout the body, can also limit distribution to the surrounding tissues, since only the unbound portion is free to enter tissues from the bloodstream [26]. Since protein binding may retain and thereby determine the amount of drug within the central blood compartment, a higher degree of protein binding will produce a lower V_d. It has been shown that some medical conditions that alter the levels or binding properties of plasma proteins can affect the degree of plasma protein binding of drugs, and thus lead to drug interactions [27–29]. For instance, disease and stress can induce conformational changes in plasma or tissue proteins through synthesis of endogenous substances that can interfere with binding. Conjunctive tissue diseases, such as aging, prolonged bleeding, and starvation, are characterized by reduced plasma proteins, while heart infarction or liver morbidities can increase AGP levels, which can change binding profiles of basic drugs and affect distribution or pharmacokinetic and pharmacological parameters [27,28,30]. During pregnancy, albumin levels have been shown to decrease, thereby resulting in notable increases in unbound fractions for diazepam, valproic acid, phenytoin, phenobarbitone, salicylic acid, pethidine, lignocaine, dexamethasone, sulfafurazole, and propranolol [31,32]. A summary of pathological and physiological conditions that can affect protein binding through modulation of HSA, AGP, or lipoprotein levels can be found in Table 4.3 [33]. It is important to note that changes in the unbound drug fraction do not always result in proportional changes in clearance or distribution volume. Important contributors to the extent of concentration change will also be the extent of binding, type of clearance, the size of the distribution volume, administration route, and concomitant changes in intrinsic (cellular) clearance function caused, for example, by induction or inhibition of hepatic enzymes [28].

4.4.5 Red Blood Cell Partitioning

Although not as routinely discussed as plasma protein binding, red blood cell (RBC) partitioning is an important factor for determining V_d and clearance. Establishing the kinetic properties of drug binding to RBC enables appropriate selection of a biological fluid (whole blood, plasma, or serum) for analyzing drug concentration. This ensures more physiologically relevant PK parameters and improves the analytical sensitivity for drugs with K_b/p (whole blood-to-plasma ratio) or K_e/p (drug affinity to binding sites in erythrocytes relative to those in plasma) >2.0. Chiral characteristics, lipophilicity, and molecular size are all important determinants of RBC partitioning. In some cases, partitioning can occur by passive diffusion (organic, anionic drugs, and nonelectrolytes), through aqueous channels by small hydrophilic compounds (<150 D), or, in

the case of liphophilic compounds, through dissolving into the lipid bilayer membrane. Binding of drug to RBC can occur to hemoglobin and [34] membrane or proteins within the cytosol such as carbonic anhydrase. Additionally, RBCs have the ability to metabolize some drugs, which can add another consideration to the disposition of drugs and the predictions of PK parameters. The rate and extent of RBC binding can

TABLE 4.3 Effect of Physiologic and Pathological Conditions on Plasma Serum Protein Levels

Physiologic and Pathological Condition	Protein					
	Albumin		AGP		Lipoprotein	
	Decrease	Increase	Decrease	Increase	Decrease	Increase
Age fetal			×			
Age geriatric	×			×		
Age neonate	×					
Bacterial pneumonia	×					
Benign tumor		×				
Burns	×					
Celiac disease				×		
Cirrhosis of liver	×					
Crohn's disease				×		
Cystic fibrosis	×					
Diabetes						×
Exercise		×				
GI disease	×					
Histoplasmosis	×					
Hyperthyroidism					×	
Hypothyroidism		×				×
Injury				×	×	
Liver abscesses	×					
Liver disease					×	×
Malignant neoplasm	×					
Multiple myeloma	×					
Myocardial infarction				×		
Nephrotic syndrome	×		×			×
Neurological disease		×				
Neurosis		×				
Pancreatitis	×					
Paranoia		×				
Pregnancy	×					
Psychosis		×				
Renal failure	×			×		
Rheumatoid arthritis				×		
Schizophrenia		×				
Severe malnutrition	×					
Stress				×		
Surgery	×			×		
Trauma	×			×	×	
Use of oral contraceptives			×			

Source: Extracted from Ref. 33.

be determined by *in vitro* methodologies where drug is added to suspended RBC in plasma or water containing plasma. Or by *ex vivo* methods where drug is administered to humans, samples are collected and RBC and plasma are separated through centrifugation after which levels of drug are measured in both biological fluids. In some cases, such as with neuroleptic drugs (butaperazine, haloperidol, and thioridazine), the RBC concentration is better correlated with therapeutic effect or dose than plasma concentrations and in the case of digoxin toxicity, RBC concentration better distinguishes between toxic and nontoxic drug levels than plasma concentrations [34].

4.4.6 Perfusion and Diffusion

The distribution of the free fraction of drug can be limited by tissue perfusion rate or by diffusion of the drug across barrier membranes. Only small molecules that are not bound to albumin can undergo diffusion because, when bound to albumin, the molecular weight of the complex is so high (>68,000 kD) that it limits diffusion [35]. However, this protein binding is not a static process and can be described with an on–off rate equation. Passive diffusion occurs when drug moves from an area of high concentration to an area of low concentration following the equation of Fick's law of diffusion [22]:

$$\text{Rate of drug diffusion} = \frac{-DKA(C_{\mathrm{p}} - C_{\mathrm{t}})}{h}$$

In this equation, C_{p} is the concentration of drug in plasma, C_{t} is the concentration of drug in tissue, A is the surface area of the membrane, K is the lipid–water partition coefficient, D is the diffusion constant and is inversely proportional to the weight of the drug, while h is the thickness of the membrane. The negative sign denotes the net transfer of drug from inside the capillary lumen into tissues and extracellular spaces. In this manner, the diffusion is both spontaneous and temperature dependent. In the case of nonpolar, highly lipid-soluble drugs there can be a perfusion rate limitation. Being described as perfusion rate-limited assumes that on entry into blood circulation, the drug is free to distribute completely and instantly across membranes without diffusion barriers. In this case, it is limited only by the rate of blood flow to the organ. If drug distribution is limited by perfusion, the distribution equilibrium may take longer to achieve when perfusion is low and the partition coefficient is high. If the permeability coefficient is high, then the concentration of drug at the tissue must be high before equilibrium takes place. In the case of diffusion rate-limited distribution, as would most likely occur with polar drugs, the rate of distribution into tissue is a function of the permeability coefficient of the drug, the concentration gradient, the distance that the drug travels, the surface area for diffusion, the temperature, and the molecular weight of the drug since smaller lighter molecules will diffuse faster than larger heavier ones. Capillary permeability can also have a profound effect on drug distribution.

4.4.7 Capillary Permeability

The lining of endothelial cells that make up the blood vessels, the lymphatic vessels, and the inside of the heart can affect capillary permeability to drugs depending on how tight the junction between the cells is. The capillary endothelium has discontinuous junctions, which can make the capillary walls quite permeable [36]. For instance,

lipid-soluble drugs can pass through vascular endothelium rapidly, whereas water-soluble drugs will penetrate more slowly, and the rate is dependent on physiochemical properties such as molecular size. Additional physiochemical properties that can affect the rate of transfer across the capillary membrane are whether the drug is ionized and this depends on the pK_a of the drug and the pH of the blood as discussed in Section 4.3.1. Membrane permeability tends to restrict the transfer and distribution of drugs once they are delivered to the tissue. The other major factors that determine the rate of drug distribution is blood perfusion and the expression of transporters.

Two deviations to the typical capillary structure can result in variation from normal drug tissue permeability.

1. The renal and hepatic capillaries in the membrane of the endothelial cells, known as *fenestrations*, are larger, thereby increasing permeability that enables more extensive distribution of many drugs out of the capillary beds of the kidney and liver. Since both these organs serve as sites of elimination, this would serve to reduce the plasma concentration by acting as a mechanism of clearance (refer to Section 4.8).
2. An opposite phenomenon exists within the brain where capillaries seem to have relatively impermeable walls compared with normal vascular endothelium. This greatly restricts the permeability of molecules from blood to brain tissue, despite the cardiac output being relatively high.

The brain capillaries and the renal and hepatic capillaries are two important deviations from the normal capillary wall and both have a profound effect on drug distribution [36]. Capillaries within the brain have tight junctions and have endothelial cells that are so close together that they form the BBB. This limits the distribution of drugs into the brain, and generally only lipid-soluble drugs, or drugs that resemble endogenous substances and are actively transported, can enter the brain. In general, polar or ionized drugs cannot distribute in the CNS. In contrast, the capillary walls in the kidney and liver are very permeable since there are large gaps in the junctions between the endothelial cells and fenestrations through the cell cytoplasm allowing movement of drugs and plasma proteins. This enables rapid and extensive distribution of drugs and proteins out of the capillary bed, which is well suited for these organs since they play such an important role in drug elimination. Since the distribution of a drug to tissues can be dependent on the permeability of the capillaries at the site of distribution, Table 4.4 describes the various pore sizes of some of these barrier membranes. These

TABLE 4.4 Capillary Permeability

Barrier Membrane	Pore Diameter (Å)
Intestinal epithelium	4
Capillary endothelium	40–80
Muscle capillaries	60
Glomerular capillaries	75–100
Glomerular endothelium	1000
Liver capillaries	1000

barriers to distribution are described in more detail in Section 4.4.9. Other capillary beds with tight junctions that can limit distribution include those found in the testicles, eyes, and synovia [36]. Disease states such as inflammation, liver disease, and diabetes can affect capillary barriers and therefore disable or enable entry of drugs and thus change the normal distribution pattern observed in the healthy state [36].

4.4.8 Tissue Storage

As mentioned in Section 4.4.1, some drugs may accumulate in certain types of tissues and act as a reservoir, slowly releasing the drug into the bloodstream, thus keeping the blood level of the drug from decreasing rapidly and prolonging its effect. These drugs generally exhibit pattern 3 distribution, as described in Section 4.4.2, and tend to have very large V_d values. For example, long-term administration of the antimalarial agent quinacrine results in accumulation in the liver [37] and gentamicin binding to tissue results in accumulation in the kidney and vestibular system [38]. This phenomenon can be attributed to binding of drug to cellular constituents such as proteins, phospholipids, or nuclear proteins, as described above. An additional mechanism of accumulation within tissues can be attributed to active transport (e.g., liver enrichment of statins), which is covered in more detail in Section 4.5. Fat can also serve as a reservoir for many lipid-soluble drugs and since the blood flow to adipose tissue is only 2% of cardiac output, it serves as a rather stable reservoir. Drugs that accumulate in fatty tissues can leave the tissue so slowly that the drug can still be present in the bloodstream for days after dosing has ceased. An example of highly lipid-soluble xenobiotics that extensively distribute into fat are polychlorinated biphenyls and dicophane. Other examples of drugs stored in fat would be the lipid-soluble barbiturate thiopental, remifentanil, and some other β-blockers [39,40].

Bone can also serve as a site of accumulation for drugs such as tetracycline antibiotics, other divalent metal-ion chelating agents, and heavy metals [41]. Drugs and other toxic agents such as lead or radium can accumulate into the bone crystal surface and incorporate into the crystal lattice where effects can persist long after exposure [42]. This type of accumulation and exposure can have therapeutic advantages for treatments aimed at osteoporosis. Bound drug in the bloodstream can also serve as a reservoir since the drug will be released as the unbound levels decrease. As an example, digitoxin and phenytoin can be sequestered by plasma proteins, which then act as a reservoir for drug [43].

4.4.9 Physiological Barriers to Drug Distribution

Epithelial and endothelial cells act as internal barriers, while external barriers include keratinized epithelium (skin), ciliated epithelium (lung), or epithelium with microvilli such as is found in the intestine. These types of cells are connected by zonulae occludens, which is a tight junction that creates an unbroken phospholipid bilayer [44]. There are also internal or blood–tissue barriers that are made up of endothelial cells joined via zonulae occludens [45]. In these cases, drug permeation occurs mostly in the capillary beds, for example, endocrine glands in the gut have fenestrations on endothelial cells, which allow for passage of small molecules, whereas the liver has large fenestrations (~100 nm), thereby allowing drugs to exchange freely between the blood and interstitium [45]. Cardiac muscle has high endo- and transcytotic activity,

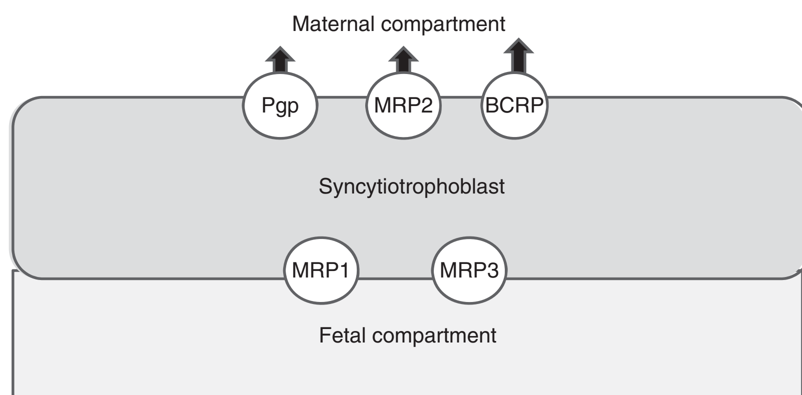

Figure 4.4 A depiction of xenobiotic transporter expression at the placental barrier where the expression of efflux transporters serves to protect the fetal compartment from exposure to substances that are substrates for the expressed transporters. *Source*: Extracted from Ref. 81. (See color insert.)

which enables drug transport via vesicles, whereas the CNS has endothelia that lack pores and possess only a little transcytotic activity, therefore limiting diffusion to small lipophilic drugs that can undergo transcellular passive diffusion [46]. This provides a barrier that is very restrictive and limits the types of drugs that can permeate.

4.4.9.1 Maternal:Fetal Barrier. The composition of the placental barrier changes over the course of pregnancy, but it consists of the chorion frondosum (fetal tissue) and deciduas basalis (maternal tissue), which are divided into cotyledons separating the maternal and fetal circulation [47]. It is composed of a single layer of polarized epithelial cells known as the trophoblast, which contains the syncytiotrophoblast, and the fetal capillary endothelia (refer to Fig. 4.4). Drug flux can occur across the apical (toward maternal) and basolateral (toward fetal) membranes of the syncytiotrophoblast [47]. The placenta is very well perfused and therefore not as impenetrable as often thought [48]; while most lipophilic drugs can readily cross by diffusion, there are a large number of efflux transporters present, similar to those expressed in the brain, allowing export of drugs out of the placenta. Uptake transporters are also present [49], which facilitate the transport of more hydrophilic nutrients. Although these mechanisms are covered in more detail in Section 4.5, it is important to note that the presence of efflux transporters can limit intracellular concentrations and hence exposure to the fetus, despite the ability of lipophilic drugs to readily diffuse. The physiochemical properties of the drug and whether it is a substrate for transport processes have a large impact on the actual fetal exposure. Physiological and physiochemical factors previously covered such as lipid solubility, extent of plasma binding, and degree of ionization of weak acids and bases are also important general determinants in drug transfer across the placenta.

4.4.9.2 Mammary Gland. The mammary gland represents an important barrier that limits drug exposure to a suckling infant. The functional unit for milk production and the milk–blood barrier is a single layer of epithelial cells in the alveoli, which are extensively proliferating during lactation [50]. The mammary gland is highly perfused

with a large surface area, and therefore distribution of drug to milk is generally similar to that of blood with very little lag time. However, there is high expression of transporters that can serve to limit the extent of exposure to drug. Additional factors that may influence drug distribution include the slightly lower pH of milk (7.2) compared with blood, thereby allowing ion trapping of weak bases [51]. There can also be a degree of binding to milk proteins and, since the milk fat content is high, lipophilic drugs may have large milk to serum concentration ratios due to partitioning into milk [52]. There is also evidence of transporter expression on the mammary epithelium, mostly for endogenous substrate transfer. While there is more extensive discussion of transporters in Section 4.5, it is worth noting that some members of the organic anion-transporting polypeptide (OATP) and multidrug resistance protein (MRP) families have been detected, while ABCB1 (MDR1) and ABCG2 (breast cancer resistance protein (BCRP)) have also been shown to play a role in drug accumulation in secreted milk [53,54].

4.4.9.3 Blood–Brain Barrier. The BBB serves to protect the brain from the contents of the CSF and blood, and it comprises a layer of endothelial cells sealed by tight junctions supported in terms of nutrition and communication by astrocytes [55]. Projections from astrocytes form a communication link between the capillary endothelium and the neurons of the brain, and lipids within the astrocyte cell walls and the tight junctions between the adjacent endothelial cells limit the passage of water-soluble molecules at the BBB. This is not totally impenetrable but slows the rate at which drugs can cross into brain tissue, while still allowing essential nutrients such as oxygen to pass through [56]. The expression of transporters at the apical membrane of the epithelial cells can also prevent distribution of drugs across the barrier and contribute to drug resistance phenotypes [57,58]. As discussed in Section 4.4.3, the rate of blood flow to the brain is quite high, since the percentage of the cardiac output distributed to the brain is around 15%. This enables some highly lipid-soluble drugs, such as certain anesthetics, and small liphophilic compounds, such as barbiturates and benzodiazepines, to diffuse rapidly into the CNS. The role of efflux transporters on the apical membrane can effectively serve to actively exclude a large variety of lipophilic drugs from the brain capillary endothelial cells, thereby limiting the distribution of these types of drugs. This can be beneficial in reducing adverse neurotoxic effects and may also reduce the treatment options for neurological disorders. Therefore, there may be therapeutic advantages to transporter modulation as a mechanism for distribution of novel therapeutics. In contrast to the BBB, the choroid plexus contains fenestrated endothelium, which allows for drug distribution across the choroid plexus [59,60]. The choroid plexus is a continuous layer of epithelial cells that function to control CSF homeostasis. CSF flows from the ventricles across the surfaces of the brain and spinal cord into the venous blood sinuses [61]. The CSF–blood barrier (Fig. 4.5) is composed of choroidal epithelial cells joined together by tight junctions at their apical surface. In this manner, the barrier prevents all but the most lipid-soluble drugs from entering the CSF [57]. This can result in profound differences in concentration of drug between the aqueous CSF and the CNS tissue [62–64]. Similar to the other barriers discussed, there is a large expression of various uptake and efflux transporters, including SLC22A8 (Oat3), SLC16A1 (MCT-1), SLCO1A2 (OATP1A2), ABCB1 [P-glycoprotein (Pgp)], ABCC4 (MRP4), ABCC1 (MRP1), and ABCG2 (BCRP), which profoundly affect drug distribution into the CNS and these are discussed in further detail in the following section [65].

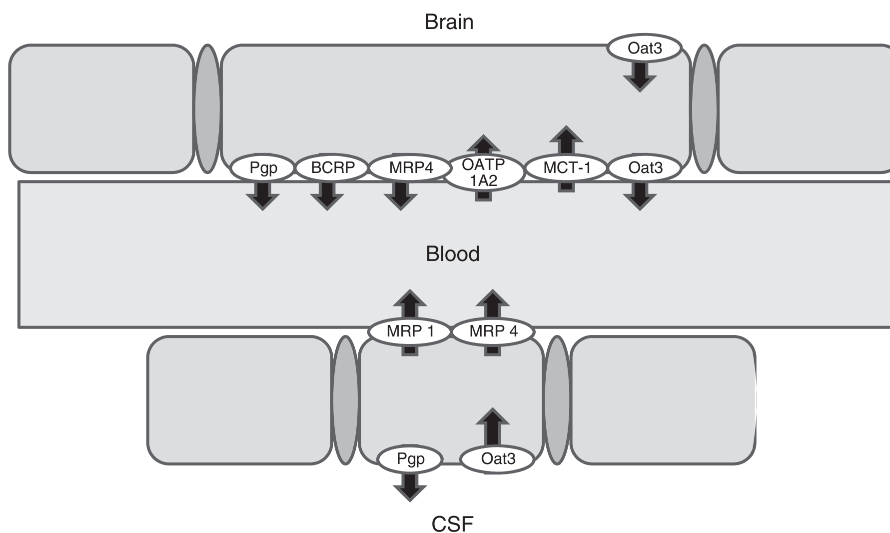

Figure 4.5 A depiction of xenobiotic transporter expression at the blood–brain barrier (BBB) and choroid plexus, cerebral spinal fluid (CSF)–blood barrier. As evidenced in detail above, the expression of efflux transporters is pronounced along the apical membrane of the BBB, thus limiting the exposure of substrates for these transporters into the brain. *Source*: Extracted from Ref. 64. (See color insert.)

4.5 THE ROLE OF TRANSPORTERS IN DRUG DISTRIBUTION

It has become increasingly recognized that most drugs are distributed across cellular membranes with the assistance of transporter proteins. These transporters have been identified in every living cell in varying amounts and are distributed differentially throughout tissues and barrier membranes [66–70]. It is thought that transporters may play an important role not only in distributing drug molecules to their effector site but also in distribution to almost every organ and tissue in the body [66]. This has been shown to contribute to both pharmacological and toxicological effects and to nonlinear pharmacokinetics.

4.5.1 Transporter Subtypes Involved in Distribution

Since the discovery of Pgp as an important contributor to efflux drug transport across membranes and its underlying role in multidrug resistance (MDR) [71], it has become more commonly accepted that transporters can have a large impact in drug disposition. The identification of various transporters has resulted in classification systems based on the method and direction of transport. Transporters that have been identified as requiring the direct binding and hydrolysis of ATP have been grouped into a superfamily of ABC (ATP-binding cassette) transporters. So far, there are at least 49 members of the ABC superfamily identified in humans and these are grouped into seven families and contain therapeutically important and well-studied transporters such as ABCB1, ABCC1, ABCC2, and ABCG2 [72]. Another important large family of transporters is the SLC (solute carrier) transporters of which there are as many as 50–55 gene families

and at least 362 members [73,74]. Included in this class are all the major classes of uptake transporters, including the OATPs and organic cation transporter (OCTs), which have both been implicated in drug–drug interactions [68]. Unlike the ABC transporters, the SLC transporters do not directly utilize an energy source [66]. Together the ABC and SLC transporters are increasingly being implicated in regulation of the passage of endogenous and exogenous chemicals across cellular and tissue membranes, thereby playing a pivotal role in drug distribution and subsequent disposition, efficacy, and toxicity [66]. An additional consideration in understanding the differential distribution of drugs through body compartments within a human population is the occurrence of single-nucleotide polymorphisms within the transporter molecules [75–77]. Since transporters clearly play an important role in drug distribution having an understanding of whether a drug of interest is a substrate/inhibitor of specific transporters can provide a more lucid interpretation of V_d and result in more predictive physiologically based pharmacokinetic (PBPK) modeling (refer Section 4.8.4). This field is rapidly evolving and expanding, therefore while a vast number of transporters have been identified and their respective tissue distribution has been described, it is understood that not all these transporters may result in clinically relevant drug–drug interactions. In 2007, an international transporter consortium was established, and in 2010, a review article was published recognizing the importance of transporters in drug disposition and making recommendations regarding the conduct of transporter studies and data analysis [78].

4.5.1.1 ABC Transporters. ABC transporters make up the largest class of membrane-bound proteins in biology and function by enabling active transfer of molecules across biological membranes, through binding and hydrolysis of ATP [66]. As stated earlier, there are currently at least 49 members within seven families (ABCA–ABCG), which are identified by the presence of a characteristic amino acid motif within the nucleotide binding domains (LSGGQ) [79]. A number of ABC transporters have been shown to be expressed at barrier membranes and are therefore considered important to drug distribution. Table 4.5 summarizes the expression of the ABC transporter mRNA in multiple tissue types in descending order of expression, while Table 4.6 summarizes the gene identification and the alias of the transporters that are involved in xenobiotic transport.

4.5.1.2 SLC Transporters. SLC transporters represent a basic and abundant mechanism by which endogenous and exogenous molecules are distributed across membranes [66]. SLC transporters do not directly access an energy source and they are able to function as uniporters (facilitated diffusion of substrates across membranes), symporters (transport of two compounds in the same direction at the same time), and antiporters (transport of two compounds in opposite directions at the same time) [66,68]. While the majority of the SLC transporters serve to facilitate transfer of highly specific endogenous substrates, a number of them can contribute to MDR and limit accumulation by transporting drugs into and out of cells [68]. As an example, multiple statin drugs are substrates for OATPs that are highly expressed in the basolateral membrane of hepatocytes and this contributes to their ability to accumulate into the liver [75]. Certain SLC transporters can function bidirectionally depending on substrate concentration gradients so they can play the role of efflux transporter. Many OCTs can function in this manner [68]. Often times the extent and direction of drug movement into organs, including the intestine and liver, is determined by the unique interplay between apically and

TABLE 4.5 ABC Transporter Expression in Various Tissues[a]

Tissue	Expression of the 10 Most Abundant Human ABC Transporters in Various Tissues Ranked from Highest to Lowest									
Adrenal gland	ABCA1	ABCC3	ABCB1	ABCD1	ABCA8	ABCG1	ABCF2	ABCF1	ABCA6	ABCD3
Bladder	Not known									
Bone marrow	ABCB10	ABCF1	ABCD3	ABCF2	ABCC4	ABCE1	ABCB2	ABCC1	ABCC10	ABCB8
Brain	ABCA2	ABCA5	ABCF2	ABCD2	ABCF1	ABCD3	ABCE1	ABCC5	ABCB9	ABCB8
Colon	ABCD3	ABCC3	ABCB2	ABCC7	ABCA1	ABCF2	ABCA5	ABCE1	ABCD1	ABCB10
Heart	ABCA8	ABCF2	ABCC9	ABCD3	ABCF1	ABCE1	ABCA9	ABCA5	ABCB1	ABCD1
Kidney	ABCD3	ABCC4	ABCF2	ABCF1	ABCB1	ABCC2	ABCE1	ABCA2	ABCC3	ABCB8
Liver	ABCA6	ABCG8	ABCB11	ABCB4	ABCG5	ABCA1	ABCD3	ABCC2	ABCC9	ABCF1
Lung	ABCA6	ABCA1	ABCB2	ABCF1	ABCC4	ABCA8	ABCF2	ABCD3	ABCG1	ABCE1
Mammary gland	Not known									
Ovary	Not known									
Pancreas	ABCC7	ACF1	ABCA5	ABCD3	ABCE1	ABCF2	ABCC3	ABCD1	ABCC1	ABCC10
Peripheral leukocytes	ABCB2	ABCF1	ABCD1	ABCF2	ABCE1	ABCB3	ABCB10	ABCA2	ABCC1	ABCA1
Placenta	ABCD1	ABCF1	ABCA1	ABCB8	ABCF2	ABCG2	ABCG1	ABCB2	ABCD3	ABCC10
Prostate	ABCC4	ABCF1	ABCD3	ABCF2	ABCA5	ABCE1	ABCB2	ABCC1	ABCA1	ABCB10
Retina	Not known									
Salivary gland	ABCF1	ABCF2	ABCD3	ABCA6	ABCE1	ABCC7	ABCD1	ABCB2	ABCB10	ABCG1
Skeletal muscle	ABCF1	ABCF2	ABCA5	ABCD1	ABCD3	ABCC1	ABCC9	ABCA6	ABCB10	ABCA1
Small intestine	ABCD1	ABCD3	ABCF1	ABCG5	ABCB2	ABCF2	ABCG8	ABCB10	ABCC3	ABCG2
Smooth muscle	Not known									
Spinal cord	ABCA2	ABCD3	ABCA8	ANCF1	ABCA6	ABCA1	ABCE1	ABCA5	ABCB2	ABCB8
Spleen	ABCB2	ABCF1	ABCF2	ABCG1	ABCA1	ABCD1	ABCE1	ABCC1	ABCD3	ABCB10
Stomach	ABCD3	ABCF1	ABCC3	ABCF2	ABCE1	ABCB2	ABCA6	ABCA5	ABCC1	ABCA8
Testis	ABCF1	ABCF2	ABCD1	ABCB9	ABCE1	ABCC1	ABCD3	ABCA5	ABCA8	ABCC12
Thymus	ABCF1	ACA1	ABCB2	ABCD3	ABCF2	ABCC1	ABCG1	ABCD1	ABCA7	ABCC10
Thyroid gland	ABCF1	ABCA2	ABCF2	ABCA6	ABCD3	ABCA5	ABCE1	ABCA8	ABCC4	ABCA1
Trachea	ABCF1	ABCD3	ABCB2	ABCC1	ABCA1	ABCG1	ABCA5	ABCA8	ABCC4	ABCE1
Uterus	ABCF1	ABCF2	ABCA6	ABCD1	ABCD3	ABCA1	ABCG2	ABCB2	ABCE1	ABCB10

[a] Ref. 66.

TABLE 4.6 ABC Transporter Alternative Names and Distribution[a]

Transporter Gene	Alternative Name	Distribution
ABCA1	ABC1	Ubiquitous
ABCA2	ABC2	Brain
ABCA3	ABC3, ABCC	Lung
ABCA4	ABCR	Rod photoreceptors
ABCA5	—	Muscle, heart, testes
ABCA6	—	Liver
ABCA7	—	Spleen, thymus
ABCA8	—	Ovary
ABCA9	—	Heart
ABCA10	—	Muscle, heart, testes
ABCA11	—	Stomach
ABCA12	—	Low in all tissues
ABCB1	MDR1, PGP	Adrenal, kidney, brain
ABCB2	TAP1	Ubiquitous, ER
ABCB3	TAP2	Ubiquitous, ER
ABCB4	PGP3, MDR3	Liver
ABCB5	—	Ubiquitous
ABCB6	MTABC3	Mitochondria
ABCB7	ABC7	Mitochondria
ABCB8	MABC1	Mitochondria
ABCB9	—	Heart, brain
ABCB10	MTABC2	Mitochondria
ABCB11	SPGP, BSEP	Liver
ABCC1	MRP1	Ubiquitous
ABCC2	MRP2	Liver
ABCC3	MRP3	Lung, intestine, liver
ABCC4	MRP4	Prostate
ABCC5	MRP5	Ubiquitous
ABCC6	MRP6	Kidney, liver
ABCC7	CFTR	Exocrine tissues
ABCC8	SUR	Pancreas
ABCC9	SUR2	Heart, muscle
ABCC10	MRP7	Low in all tissues
ABCC11	MRP8	Low in all tissues
ABCC12	MRP9	Low in all tissues
ABCD1	ALD	Peroxisomes
ABCD2	ALD1,ALDR	Peroxisomes
ABCD3	PMP70, PXMP1	Peroxisomes
ABCD4	PMP69, P7R	Peroxisomes
ABCE1	OABP	Ovary, testes, spleen
ABCF1	ABC50	Ubiquitous
ABCF2	—	Ubiquitous
ABCF3	—	Ubiquitous
ABCG1	ABC8	Ubiquitous
ABCG2	BCRP	Placenta, intestine
ABCG4	White2	Liver
ABCG5	Sterolin 1	Liver, intestine
ABCG8	Sterolin 2	Liver, intestine

[a] Ref. 80.

basolaterally expressed transporters. In this manner, transporters that result in vectorial drug transfer into the systemic circulation are referenced as *absorptive transporters*, whether they are efflux or uptake transporters. Transporters involved in the excretion of substrates from circulation are known as *secretory transporters*. Expression of these transporters on the canalicular membrane of hepatocytes further effects the disposition of drugs since substrates can be effectively eliminated from distribution through hepatic phase I and II biotransformation and subsequent efflux into bile. Table 4.7 summarizes the expression of the SLC transporter mRNA in multiple tissue types in descending order of expression, while Table 4.8 summarizes the gene identification and the alias of the transporters that are involved in xenobiotic transport.

4.5.2 Transporter Expression at Barrier Membranes

The expression of multiple ABC and SLC transporters in the apical and basolateral sides of epithelial and endothelial cells lining pharmacological barriers indicates their importance in protection and detoxification [70]. ABCB1 (PGP) is highly expressed in most barrier tissues, such as the BBB, testis, placenta, and endothelial cells of human cardiac vasculature. Additional transporters of importance include the ABCC family, which consists of the MRPs and ABCG2 (BCRP) and are also expressed in many of the epithelia and endothelia that constitute important barrier membranes. Transporter expression at some important barriers to distribution is shown in Figs. 4.4 and 4.5, which describe transporter location at the BBB and CSF–blood barrier and at the placental barrier, respectively. A number of transporters are also expressed at the blood–testis barrier where tight junctions between sertoli cells allow polarization and barrier formation, which protect the germ cells. These transporters include Pgp, GLUT1, 2, and 3, urea transporter, and potentially OCT1, 3, OCTN1, and 2. The latter are thought to contribute to accumulation of propranolol and imipramine in the testis [81], which may result in adverse effects in this tissue. Additionally, the role of transporters on the apparent V_d should be considered, since transporter-mediated uptake into hepatocytes can affect the extent of clearance and therefore the apparent V_d. An example of this would be cerivastatin, which is highly distributed to the liver both through uptake processes and cellular binding. This has been shown as a mechanism for drug–drug interaction between cerivastatin and CsA, since CsA acts as an inhibitor of cerivastatin uptake.

4.6 *IN VITRO* METHODOLOGIES FOR DETERMINING DRUG DISTRIBUTION

Some of the different parameters of drug distribution can be determined using *in vitro* methodologies. Well-established methods for evaluating plasma protein binding and determining the role of transporters as well as more recently reported methods are discussed in the following sections. A more detailed description of the transporters involved in drug distribution is given in Section 4.5.

4.6.1 Plasma Protein Binding

Plasma protein binding studies play an important role in the process of candidate drug selection and development. The amount of drug that binds to protein is dependent on

TABLE 4.7 SLC Transporter Expression in Various Tissues[a]

Tissue	Expression of the 10 Most Abundant Human SLC Transporters in Various Tissues Ranked from Highest to Lowest									
Adrenal gland	SLC16A9	SLC40A1	SLC25A3	SLC25A37	SLC39A9	SLC24A6	SLC3A2	SLC30A1	SLC6A6	SLC25A44
Bladder	SLC25A6	SLC25A23	SLC25A3	SLC40A1	SLC25A5	SLC25A24	SLC8A1	SLC25A44	SLC25A37	SLC39A9
Bone marrow	SLC25A37	SLC4A1	SLC40A1	SLC16A3	SLC25A6	SLC25A39	SLC2A3	SLC25A3	SLC25A5	SLC30A6
Brain	SLC22A17	SLC25A23	SLC39A10	SLC25A6	SLC17A7	SLC25A39	SLC1A2	SLC25A44	SLC25A5	SLC12A5
Colon	SLC25A6	SLC25A5	SLC25A3	SLC39A5	SLC25A23	SLC39A9	SLC25A24	SLC16A3	SLC25A44	SLC30A6
Heart	SLC25A37	SLC8A1	SLC16A7	SLC40A1	SLC25A30	SLC25A23	SLC25A11	SLC25A6	SLC25A37	SLC25A5
Kidney	SLC39A5	SLC25A6	SLC25A5	SLC5A12	SLC7A7	SLC12A1	SLC27A5	SLC34A1	SLC3A2	SLC25A3
Liver	SLC22A17	SLC40A1	SLC2A2	SLC25A5	SLC13A5	SLC39A5	SLC38A3	SLC22A7	SLC25A37	SLC39A9
Lung	SLC40A1	SLC34A2	SLC6A4	SLC25A6	SLC2A3	SLC39A8	SLC16A3	SLC25A37	SLC25A24	SLC25A3
Mammary gland	SLC25A37	SLC25A37	SLC25A6	SLC40A1	SLC25A39	SLC25A44	SLC37A3	SLC12A2	SLC20A2	SLC16A3
Ovary	SLC25A6	SLC40A1	SLC30A6	SLC25A37	SLC16A9	SLC25A39	SLC25A5	SLC25A23	SLC25A24	SLC24A6
Pancreas	SLC25A6	SLC25A5	SLC39A5	SLC40A1	SLC25A3	SLC39A9	SLC39A8	SLC30A6	SLC30A1	SLC39A6
Peripheral leukocytes	SLC25A37	SLC16A3	SLC25A6	SLC2A3	SLC25A3	SLC25A44	SLC25A5	SLC40A1	SLC30A6	SLC7A7
Placenta	SLC2A1	SLC40A1	SLC25A6	SLC3A2	SLC39A9	SLC7A2	SLC30A6	SLC39A6	SLC25A5	SLC39A8
Prostate	SLC30A4	SLC39A10	SLC39A6	SLC25A6	SLC25A37	SLC39A9	SLC40A1	SLC14A1	SLC25A37	SLC25A23
Retina	SLC16A3	SLC25A6	SLC25A3	SLC23A2	SLC24A44	SLC25A5	SLC30A6	SLC16A14	SLC40A1	SLC25A23
Salivary gland	SLC25A6	SLC39A9	SLC25A5	SLC13A5	SLC25A3	SLC31A1	SLC5A5	SLC9A1	SLC25A23	SLC25A37
Skeletal muscle	SLC16A3	SLC25A37	SLC25A11	SLC25A4	SLC40A1	SLC25A23	SLC25A30	SLC19A2	SLC25A9	SLC25A6
Small intestine	SLC5A1	SLC25A6	SLC40A1	SLC25A5	SLC25A3	SLC39A5	SLC2A5	SLC39A9	SLC25A24	SLC13A2
Smooth muscle	SLC25A6	SLC25A23	SLC25A3	SLC8A1	SLC40A1	SLC25A5	SLC30A6	SLC12A2	SLC25A24	SLC25A37
Spinal cord	SLC22A17	SLC25A6	SLC39A10	SLC14A1	SLC16A9	SLC25A23	SLC39A9	SLC25A3	SLC39A6	SLC25A44
Spleen	SLC25A6	SLC16A9	SLC39A10	SLC25A5	SLC16A3	SLC25A39	SLC25A37	SLC30A6	SLC39A9	SLC40A1
Stomach	SLC25A6	SLC25A5	SLC25A3	SLC40A1	SLC25A37	SLC39A9	SLC16A3	SLC30A6	SLC25A23	SLC39A7
Testis	SLC25A37	SLC30A6	SLC16A3	SLC30A4	SLC30A1	SLC25A33	SLC40A1	SLC2A5	SLC16A9	SLC25A6
Thymus	SLC25A6	SLC25A5	SLC40A1	SLC25A37	SLC25A4	SLC25A8	SLC30A6	SLC39A9	SLC25A11	SLC16A3
Thyroid gland	SLC25A6	SLC5A3	SLC26A7	SLC5A5	SLC25A23	SLC25A44	SLC25A3	SLC30A6	SLC25A37	SLC40A1
Trachea	SLC25A6	SLC12A2	SLC16A3	SLC25A37	SLC40A1	SLC25A3	SLC25A5	SLC39A9	SLC16A9	SLC25A44
Uterus	SLC25A6	SLC40A1	SLC25A3	SLC25A5	SLC16A9	SLC39A9	SLC25A37	SLC25A23	SLC37A3	SLC30A6

[a] Ref. 66.

TABLE 4.8 SLC Transporter Alternative Names and Distribution[a]

Transporter Gene	Alternative Name	Distribution
SLC22A6	OAT1	—
SLC22A7	OAT2	—
SLC22A8	OAT3	—
SLC22A9	OAT4	—
SLC21A2	Hpgt	—
SLC21A3	OATP-A	—
SLC21A9	OATP-B	—
SLC21A8	OATP8	—
SLC21A6	OATP-C	—
SLC21A11	OATP-D	—
SLC2112	OATP-E	—
SLC21A14	OATP-F	—
SLC22A1	OCT1	Small intestine, liver, kidney
SLC22A2	OCT2	Small intestine, kidney
SLC22A3	OCT3	Small intestine, liver, kidney
SLC22A16	OCT6	Testis, monocytes, lymphocytes, hematopoetic progenitor cells
SLC22A4	OCTN1	Brain, lymph nodes, testis, monocytes, CD4+-lymphocytes
SLC22A5	OCTN2	Brain, monocytes, CD4+-lymphocytes
SLC28A1	CNT1	—
SLC28A2	CNT2	—
SLC28A3	CNT3	—
SLC29A1	ENT1	—
SLC29A2	ENT2	—
SLC29A3	ENT3	—
SLC23A1	NCBT1	—
SLC23A2	NCBT2	—
SLC15A1	PEPT1	—
SLC15A2	PEPT2	—
SLC2A5	GLUT5	Small intestine
SLC2A1	GLUT1	—
SLC5A1	Sodium/gluc cotransporter	—
SLC25A6	ADP-ATP translocase	—
SLC25A5	ADP-ATP translocase 2	—
SLC25A3	Phosphate carrier protein	—
SLC25A37	Mitoferrin-1	—
SLC39A5	Zip5, zinc transporter	—
SLC39A9	Zip9	—
SLC19A9	MOT9, monoxcarboxylate transporter	—
SLC40A1	Ferroportin-iron	—
SLC14A1	Urea	—
SLC16A3	MOT4	—

[a]Ref. 53 and 66.

the concentration of protein, the concentration of free drug, and the drug's affinity for the binding sites of the protein molecule. Plasma protein binding studies allow the determination of the amount of unbound (free) drug concentrations in the blood. A low fraction unbound (fu) value (e.g., fu < 0.1) indicates that a compound is highly bound to plasma proteins. Plasma protein binding data should be considered with other *in vitro* data to determine the potential of a drug for efficacy in a clinical context. Low protein binding may be more favorable than high protein binding. However, factors such as solubility of the drug and nonspecific binding need to be considered when interpreting data.

These are basic concepts that are important to the understanding of the overall topic of drug distribution.

There are numerous *in vitro* methods for the determination of protein binding, including equilibrium dialysis, dynamic dialysis, ultrafiltration, ultracentrifugation, exclusion chromatography, and circular dichroism [82]. The three most widely used *in vitro* protein binding techniques in pharmaceutical research are those which allow high throughput, namely chromatographic separation, 96-well ultrafiltration, and 96-well equilibrium dialysis [83]. These are discussed further herein. The method of chromatographic separation, for example, using a HSA-immobilized column, separates multiple test compounds on the same column. This allows relative ranking by calculation of percent binding for each compound. This method is easy to set up and use and is relatively inexpensive. With the 96-well ultrafiltration method (an automated and rapid method for analyzing multiple compounds), percent binding is calculated. However, nonspecific binding of the analyte to the plastic housing or ultrafiltration membrane surface can affect the quality of the data. Nevertheless, it is frequently used in industry. The 96-well equilibrium dialysis method is the "gold standard" means of protein binding analysis [83]. Protein binding studies are traditionally performed with ^{14}C-labeled compounds. With the equilibrium dialysis method, the procedure is performed in a 96-well system. There is a membrane in each of the 96 wells with buffer solution added to one side of the membrane and an equal volume of plasma containing the test compound added to the other side of the membrane. The plate is incubated at 37°C with rotation for up to 24 h to allow the incubations to reach equilibrium. The long equilibration times sometimes required could be an issue if the analyte is not stable in plasma under these incubation conditions. Samples are removed from either side of the membrane for analysis by LC/MS/MS. The equation to calculate free fraction is as follows:

$$\text{fu} = \frac{C_{\text{buffer}}}{C_{\text{plasma}}}$$

where C_{buffer} is the unbound compound concentration in buffer after dialysis, and C_{plasma} is the postdialysis plasma concentration [83]. This equation assumes that error added as a result of volume shift is negligible since potential error in the 96-well format is minimized.

The percentages of drug unbound (free) and bound to protein are calculated as follows [83]:

$$\%\text{Free} = \text{fu} \times 100\%\text{bound} = (1 - \text{fu}) \times 100$$

Recovery of test compound at the end of the study should theoretically be 100%. Any deviation from 100% may indicate binding of the compound to the dialysis equipment

or it could also indicate a solubility issue with the compound:

$$\text{Percentage recovery} = \frac{(\text{Buffer compartment [After dialysis]} + \text{Plasma compartment concentration [After dialysis]})}{(\text{Initial solution concentration [Buffer]} + \text{Initial solution concentration [Plasma]})} \times 100$$

Both plasma protein and RBC binding studies are conducted as a routine part of the ADME process. There are many representative examples of routine protein binding studies demonstrating both high and low binding compounds. However, one interesting example is the plasma protein binding studies conducted with radiolabeled dihydroartemisinin in rat and human plasma [84] Dihydroartemisinin exhibits high binding capacity with both rat and human plasma proteins (76–82%). The concentration of total radioactivity in the plasma fraction is <25% of that in whole blood with total radioactivity distributed in RBCs about three- to fourfold higher than that in plasma, suggesting that the antimalarial potency of dihydroartemisinin (DHA) in treatment of blood stage malaria may relate to the high RBC binding.

4.6.2 Transporter Studies

Transporters and methods to investigate the role of transporters in the body has been discussed more extensively in the chapter titled ***Solute Carrier (SLC) Family Transporters***. The objectives and strategies for conducting these types of studies will be related to their impact on drug development. The current methods for investigating protein binding, V_d, and other parameters are described in brief in the following sections.

4.6.2.1 Membrane-Based Assay Systems. There are several established *in vitro* methods to evaluate the role of efflux transporters in the distribution of a drug. The most simple of these methods is the use of "inside out" membranes and vesicles such as those from baculovirus-transfected Sf9 insect cells, which have been engineered to overexpress the efflux ABC transporters specifically. Substrates of the transporter are taken up into the vesicles in an ATP-dependent manner. The two main assays are the vesicular transport assay and the ATPase assay. The vesicular transport assay is a direct measure of transporter activity. Following incubation, vesicles can be separated from the incubation solution by rapid filtration through filters or nitrocellulose membranes. The test compound, trapped inside the vesicles, is retained on the filter. The amount of the transported molecule is determined by quantitative measurement with high performance liquid chromatography (HPLC), LC/MS/MS, radioactivity, or fluorescence measurement. This method is not suitable for lipophilic or adsorptive drugs; however, it can be used for "nonsticky" transporters regardless of basal ATPase activity. As an alternative assay method, ABC transporter membranes that are dependent on hydrolysis of ATP can be used with an ATPase assay to determine possible substrate or inhibitor properties of test compounds. The ATPase assay is an indirect measure of transporter activity. ATP hydrolysis yields inorganic phosphate, which can be measured by a simple colorimetric reaction. The amount of inorganic phosphate is directly proportional to the activity of the transporter. This assay system is simple to use and can be used in a high throughput manner. It is suitable for lipophilic drugs but not appropriate for use with transporters of high basal ATP activity such as MRP4, MRP8, bile salt export

pump (BSEP), and BCRP. This assay requires less transporter protein than the vesicle transport assay; however, high concentrations of compound are required.

The advantages of using membranes and vesicles are the simplicity of the system and the focus on a single transporter. When testing hydrophobic compounds, there can be a high degree of background binding of the compound to cell membranes in vesicle or cellular systems. However, these assays when used with other *in vitro* methods help the investigator to understand, more thoroughly, the relevant PK properties of new drugs.

4.6.2.2 Cell-Based Assay Systems. Cell-based transporter assays can determine if a test compound is a substrate or inhibitor of an individual transporter. Whole cell models have an advantage over membrane and vesicle assays as they can be utilized to study both uptake and efflux of test compounds. These systems can thus be used in studies to determine transport mechanisms. Bidirectional transport, namely apical-to-basolateral and basolateral-to-apical flux, can be measured to determine the transport of a test compound across the small intestine (Caco-2 cell line) or BBB. Caco-2 cells endogenously express numerous uptake transporters, which can make identifying uptake transporter substrates in these cells difficult. As an alternative approach, cell lines such as the epithelial cell line, Madin-Darby canine kidney (MDCK), can be specifically transfected to express recombinant uptake, efflux, or both transporters. For example, MDCK cells transfected with the MDR1 gene (MDCK-MDR1) express the efflux transporter, human Pgp. The MDCK-MDR1 cell line has superior transepithelial electrical resistance (TEER) values compared with Caco-2 and wild-type MDCK cells. Higher TEER creates a very tight cell system that allows for increased sensitivity in identifying Pgp substrates. Therefore, a transporter effect observed in the MDCK-MDR1 cell system may not necessarily be observed in the Caco-2 cell system. The lower TEER values of the Caco-2 cells create a more “leaky” system, which may be more representative of the human small intestine. Use of transfected cell lines such as MDCK-MDR1 allows focus on a single transporter and could help identify drug permeability issues early on in drug development.

Cells isolated from intact tissue (primary cells), such as hepatocytes isolated from liver tissue, can also be used to evaluate ADME properties of a compound, including the role of transporters in distribution. Primary cells are more difficult to maintain in culture than cell lines. In the case of hepatocytes, the sandwich culture method was developed to help maintain more *in vivo*-like properties such as cell polarity and shape and bile canaliculi reformation. One disadvantage of the cell culture systems is the static nature of the culture without “flow” properties. The different primary hepatocyte cell models such as suspension, monolayer, and sandwich culture formats are discussed in more detail in the chapter titled ***Solute Carrier (SLC) Family Transporters***.

4.6.3 *In Vitro* Methods to Study the Brain Tissue Distribution

In vitro models of the brain have been developed for estimating unbound drug concentrations in the brain interstitial and intracellular fluids [85]. The method uses brain slices from rat and guinea pig for uptake studies and brain homogenate for binding studies. Agreement between *in vivo* and *in vitro* methods for estimating the unbound brain volume of distribution ($V_{u,brain}$) has been established by comparing *in vitro* data with microdialysis data *in vivo*. Microdialysis is discussed in more detail in Section 4.7.2.1.

The brain slice method is suitable for measuring tissue distribution of hydrophilic through to very lipophilic compounds in a high throughput manner [86] and measures the partitioning of drug between buffer and brain slice to yield, $V_{u,brain}$. The unbound drug concentration in the brain interstitial fluid, the most relevant measure of central drug exposure, can then be calculated from the measured total amount of drug in brain tissue using $V_{u,brain}$ as a conversion factor. Friden and colleagues have optimized their method and combined the use of brain slice and brain homogenate studies to define the intracellular to extracellular partitioning of unbound drug in the brain tissue [86,87]. Models to assess drug distribution in the BBB have been reviewed by Alavijeh *et al*. [82]. With respect to *in vitro* cell models of drug distribution in the brain, MDCK cells are considered a good predictor of BBB permeability as they have a high transmembrane resistance causing tight junction between the cells, as exists in the BBB. Assessment of passive diffusion across the BBB and Pgp transport can also be studied with MDCK cells. The combination of the two parameters allows BBB permeation and active efflux out of the BBB to be modeled. However, the endothelial cells such as Caco-2 express a greater range of transporter molecules, which makes them much better suited for studies investigating different transporters within the BBB. *In vitro* methods assessing transport function in the liver and kidneys is discussed in the chapter titled ***Solute Carrier (SLC) Family Transporters***.

4.7 *IN VIVO* METHODOLOGIES FOR DETERMINING DRUG DISTRIBUTION

Drug distribution can be difficult to determine *in vivo*, particularly in man where whole tissues or organs cannot be analyzed directly. Traditional methods, in addition to modern methods such as imaging and analysis, are discussed in the following sections. Many of these current techniques have been used in both animal and human studies. Most current tissue distribution studies consist of a combination of both traditional and modern methods.

4.7.1 *In Vivo* Studies in Preclinical Animals and *Ex Vivo* Organs

Drug distribution studies have been performed in preclinical species by collection and analysis of organs and techniques such as whole body autoradiography (WBA). Generally, a study would involve single oral or intravenous doses of increasing concentrations of radiolabeled drug being given to a preclinical species such as rat. Blood samples would be taken at time intervals from animals for pharmacokinetic analyses. For tissue distribution analysis, animals are euthanized at each time point and tissue and organ samples taken. Aqueous homogenates are prepared from most tissues and radioactivity measured in each. Feces and urine are collected from animals housed in metabolism cages and analyzed. Furthermore, blood, fat, the contents of the stomach, and contents of different sections of the intestine are also collected and analyzed. For example, radiolabeled dihydroartemisinin, a powerful antimalarial drug has been studied in rats [84] to determine pharmacokinetics, tissue distribution, and mass balance. Tissue distribution study methods are described in detail in the study by Xie *et al*. [84]. WBA is a drug distribution process using radiolabeled drug in live animals such as rat. It is a sensitive and quantitative method. The main advantage of WBA is that it

allows collection of maps of the spatial and temporal distribution of a drug in individual tissues and throughout the body. However, it is not possible to distinguish whether the radioactivity detected is from the drug or from its metabolites. Furthermore, it is limited in the identification of the distribution of more than one compound administered simultaneously. It is an invasive technique and analysis is postmortem, therefore a large number of animals are required.

In pharmacokinetic studies conducted in preclinical animals or in humans, the most common procedure is where a mixture of known amounts of labeled and nonlabeled drug are administered in experiments to evaluate the mass balance and tissue distribution of the drug. The total dose used in the radiolabel studies is usually a pharmacologically relevant dose. This dose can be determined from results of early efficacy studies. If a specific dose has not yet been established, a total dose of radioactivity could be 100 μCi, or less, providing that exposure of a specific tissue to the radiolabeled drug does not exceed radiation limits to avoid local radiation damage [87]. The most commonly used radioisotope is ^{14}C. It is highly stable and therefore no radiolytic or chemical decomposition of the compound occurs. Disadvantages of using radiolabeled compounds include the obvious potential health risk of radioactivity exposure to humans, the expense to synthesize and purify the radiolabeled compound, the time required for this process, and the further expense of correct handling and disposal of radioactive material.

There are also *ex vivo* and other *in vivo* models to study drug transport and V_d. *In vivo* models such as gene-knockout and transporter-deficient animal models are now used in drug distribution studies. Transporter knockout models can be used to establish the extent of the importance of the pathway removed by the knockout process to the overall clearance of a drug. In these knockout models, compensatory mechanisms may come into play by upregulation of other pathways involving alternative transporters and metabolizing enzymes, and this will complicate interpretation of results. Factors such as sex, species, strain, housing conditions, and diet can affect the outcome of these studies and also need to be considered when interpreting results and in cross-species comparisons [78].

There is particular importance in studying transporters in drug distribution not only due to the potential inhibition of a transporter by a comedicant but also because of the genetic polymorphisms of transporters in the human population. Recently, matrix-assisted laser desorption ionization (MALDI) imaging mass spectrometry coupled with high definition histology has been used to follow the *in vivo* transport of unlabeled drugs within specific organ and tissue compartments [88]. The authors tracked and quantified the distribution of an inhaled reference compound, tiotropium, a bronchodilator used in the treatment of chronic obstructive pulmonary disease (COPD), within the lungs of dosed rats. Drug ion distribution patterns compared between adjacent tissue sections, showing tiotropium parent MS ions and fragmented daughter MS/MS ions, were dispersed into the lung and pleura, in a concentration gradient. For comparison, levels of drug measured in the lung compartments by a chemical extraction method were similar. Change in volume of distribution for any reason, such as a disease process, aging or genetic polymorphisms in transporter genes, for example, can lead to a significant change in drug efficacy or likelihood of toxicity, as well as a change in its half-life independent of a change in clearance. Comparing studies reported in the literature, Grover and Benet [89] concluded that the liver is a larger contributor to distribution volume than the kidneys, taking into consideration both uptake and efflux transporters.

4.7.2 Studies in Man

Classical methods for pharmacokinetic measurements were the major option to determine tissue distribution of a test compound in patients. In these studies, plasma levels of parent drug and metabolites are commonly determined following intravenous administration of a drug by infusion over time. For example, Fogli *et al*. [90] administered paclitaxel followed by gemcitabine to patients with non-small-cell lung cancer and were able to determine volume of distribution as well as other pharmacokinetic parameters to optimize their treatment regimens in phase I trials. In another interesting ADME study, the adipose tissue and plasma distribution of various antiretrovirals in HIV-1-infected patients was investigated [91]. Plasma samples were collected in a routine manner. Adipocyte samples were collected, as a by-product of lipostructure plastic surgery in patients suffering from lipodystrophy. Protease inhibitor (PI) and nonnucleoside reverse transcriptase inhibitor (NNRTI) concentrations in plasma and adipocyte lysates were determined by HPLC assays coupled with either ultraviolet photodiode array or spectrofluorimetric detection. The concentrations of these compounds in adipocyte lysates indicated that in patients with effective nadir plasma concentrations of PI and NNRTI, PIs may diffuse into fat tissue. The authors concluded that NNRTIs have a high affinity for fat tissue and may accumulate there.

4.7.2.1 Microdialysis. The technique of *in vivo* microdialysis has become one of the major tools to sample endogenous and exogenous substances in extracellular spaces [92]. Microdialysis is a semi-invasive technique based on the passive diffusion of substances across a small semihollow fiber permeable membrane (a probe), connected to an inlet and outlet tubing with a small diameter to allow the interior of the probe to be perfused with a suitable carrier solution [93]. Although developed for use with intracerebral measurements, it is now used to measure drug disposition in many different tissues, organs, and biological fluids. One advantage is that the samples taken are generally stable due to the absence of enzymes in the sample. Therefore, samples can be analyzed after the procedure. Samples are commonly separated using liquid chromatography or capillary electrophoresis to isolate the drug and/or drug and its metabolites from endogenous compounds. Detection is usually by fluorescence, electrochemical, or mass spectrometry [93]. This method allows continuous monitoring of drug or metabolite concentrations over significant time periods. Extracellular concentration gradients caused by transport of the drug or perfusion differences can also be evaluated. Compared with other techniques, microdialysis does not cause fluid loss or pressure in the tissue being measured since the samples are replaced by fresh fluid. Cells, protein, and debris are excluded from the dialysate by the semipermeable membrane. This helps prevent enzyme degradation of the sample and maintains clean samples that can be analyzed without the need for a clean-up step.

The microdialysis technique was reviewed by Zhou and Gallo [92] and they discussed many examples where microdialysis has been used to measure tumor-specific drug concentrations compared with plasma drug concentrations, as an indicator of tumor response to chemotherapy. Microdialysis has also been used to measure the unbound drug concentration in biological fluids. This permits direct measurement of plasma protein binding *in vivo* [94]. Disadvantages include local damage to the tissue, circulation, and membrane barrier integrity. It has lower spatial and temporal resolution compared with some other techniques. If low flow rates are used in the experiment,

this technique may not be suitable for use with lipophilic compounds as they may stick to the probe.

4.7.2.2 Positron Emission Tomography. PET is a noninvasive technique that involves the patient being injected with a biologically active, radiolabeled drug (radiotracer) that localizes at targeted sites within the body. Radiation emitted from the site or sites of accumulation is detected by a bank of detectors to produce a three-dimensional image of the radiotracer of drug distribution. A positron-emitting radionuclide is used as the label in PET [95]. PET methods are very sensitive and allow determination of absolute radioactivity concentrations within tissues [96]. Rosso and coworkers [97] characterized the distribution of temozolomide, an important alkylating agent used in the treatment of brain tumors. These studies predicted normal brain and brain tumor temozolomide concentration profiles for different temozolomide dosing regimens allowing optimization of treatment schedules.

4.7.2.3 Magnetic Resonance Techniques. Two other imaging methods to measure drug disposition *in vivo* noninvasively are MRI and magnetic resonance spectroscopy (MRS).

MRI can be thought of most simply as the measuring and mapping of the magnetic properties of an object. Instead of measuring the magnetism point to point in the object, MRI can create an image that depends on the rates of magnetization for each point [95]. MRS produces spectra that depict individual changes in the concentrations of parent drug and metabolite for a sensitive volume. However, there is no simple means with MRS to measure absolute concentrations [96]. Compared with PET, MRS imaging has low spatial resolution and average drug concentrations are usually obtained from large regions of interest (e.g., whole brain).

Human blood-oxygenation-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) is based on the fact that changes in the oxygen level in the blood can affect the fraction of hemoglobin in the deoxygenated state. This can be used to create image contrast [98], and the technique has been used to investigate how anxiolytics such as benzodiazepines affect amygdala functioning in healthy volunteers [99]. This technique is relatively novel and its range of applications is still being established.

4.7.2.4 Modeling. PBPK models are also used to predict ADME, including drug distribution. Some of these are discussed in detail in the study by Theil *et al*. [100] and mentioned elsewhere in this chapter. Mathematical models to reflect different aspects of drug distribution are described in the literature. Examples are models for plasma protein binding and how it affects rates of uptake of drugs to the brain [101] and models to predict the distribution of drugs delivered intracranially to the brain [111].

4.8 CALCULATING DISTRIBUTION

The relationship between the concentrations of a drug found in the different parts of the body with time after administration of a dose is known as *pharmacokinetics*, and an understanding of pharmacokinetics is crucial to enable both optimal and safe use of drugs in patients and new product development. Pharmacokinetic data are obtained by

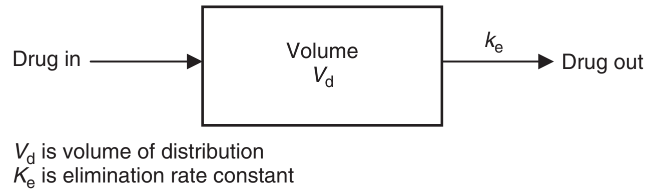

Figure 4.6 One-compartment model with a well-perfused central compartment.

measuring the concentration of drug in various tissues and body fluids over a period of time and fitting them to a mathematical model of the body.

4.8.1 One-Compartment Model

Although the movement of drug between the compartments of the body is a complex dynamic process, a simple analysis can be made by assuming that all the body compartments are in rapid equilibrium with a central compartment, which is usually interpreted as the blood, and that the concentration of a drug is constant throughout this compartment (Fig. 4.6). In this simple model, the body is considered to be a single well-stirred compartment, and it is assumed that if a drug is injected (e.g., intravenous injection) into this compartment, it will instantaneously distribute throughout it. Thus, the concentration of drug at zero time (C_0) can be calculated, and if C_0 is known, then V_d, the volume of distribution, can be calculated:

$$C_0 = \frac{D}{V_d}$$

where V_d is the volume of distribution and D the dose.

V_d does not represent the volume of an anatomical compartment, but links the total amount of drug in the body to its concentration in blood plasma. As described in Section 4.4.1, total body water is divided into the plasma water, the interstitial water, the blood cell/transcellular fluid, and the intracellular water, and the way in which a drug distributes between these will affect its plasma concentration. If the drug only distributes into plasma water (about 3 L in man), then its plasma concentration will be higher than if it distributed into all the extracellular water (about 14 L) or indeed total body water (about 40 L).

Although the V_d is a mathematical parameter, without absolute physiological meaning, it gives important information about a compound. A very high V_d value indicates that the drug is localized or sequestered in a storage site such as fat or bone. On the other hand, if the V_d is low, then it indicates that the drug is retained in the plasma (an example of various drugs and their corresponding V_d can be found in Table 4.9).

Clearance of the drug from the central compartment takes place at a rate determined by the elimination rate constant, K_{el}, which represents removal of drug by all processes from the biological system. For many drugs, a plot of log drug concentration versus time yields a straight line and indicates first-order kinetics where the rate of elimination is proportional to the drug concentration (Fig. 4.7). This means that the time taken for the plasma concentration of drug to halve will always be the same regardless of where on the curve the original value is taken. This time is referred to as the *half-life* of

TABLE 4.9 Values of Volume of Distribution for Selected Drugs

Drug	V_{ss} (L)
Humira (mAb)	5
Warfarin	8
Gentamicin	15
Phenytoin	50
Cyclosporine	300
Digoxin	400
Desipramine	3000
Quinacrine	40,000

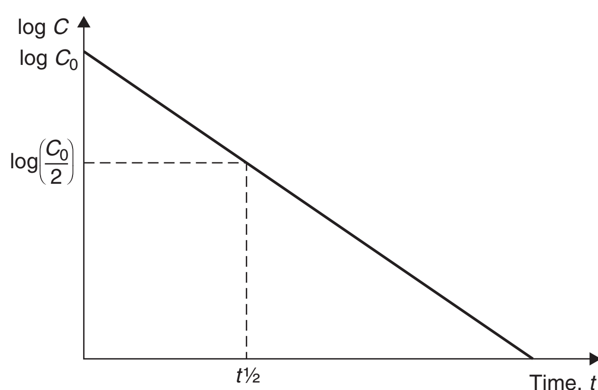

Figure 4.7 Theoretical log plasma drug concentration versus time curve for a one-compartment model.

the drug ($t_{1/2}$). The elimination rate constant is inversely related to the half-life of the drug:

$$K_{el} = \frac{0.693}{t_{1/2}}$$

The faster the clearance of the drug, the faster one half of the concentration of the drug will be cleared and the shorter will be the half-life. If the pathways of elimination of a drug are saturated (e.g., a cofactor may become rate limiting), then the elimination rate becomes independent of drug concentration, and the drug is being cleared as fast as possible. This is seen with the metabolism of acetylsalicylic acid [102] where the conjugation pathways are saturable and with ethanol metabolism where the cofactor, nicotinamide adenine dinucleotide (NAD), is the rate-limiting factor.

4.8.2 Two-Compartment Model

The simple single-compartment model assumes that the rates of absorption, metabolism, and excretion are all directly proportional to the concentration of drug in the compartment from which the transfer is occurring. However, the different organs of the body,

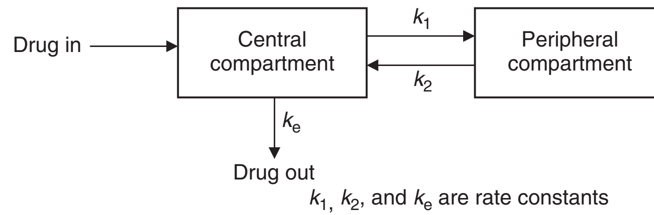

Figure 4.8 The two-compartment model with a central and peripheral compartment.

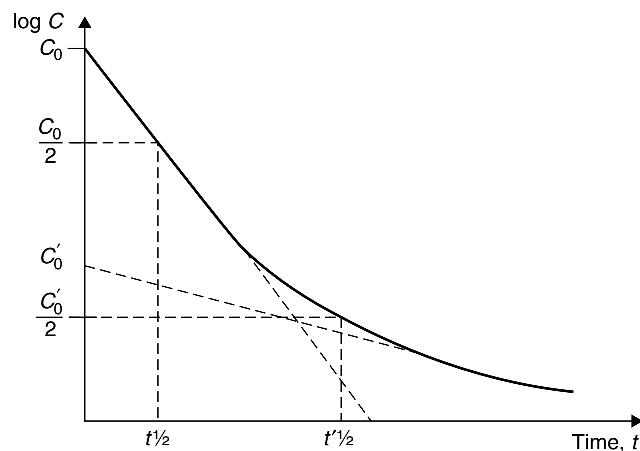

Figure 4.9 Theoretical log plasma drug concentration versus time curve for the two-compartment model.

for example, the brain, body fat, and muscle tissue, are very different in terms of their blood supply, permeability of their capillaries to drugs, and the partition coefficient for drugs, and all these differences affect the time course of drug distribution. A two-compartment model, which introduces a separate peripheral compartment to represent the tissues, in contact with the central blood compartment, resembles the real body situation more closely (Fig. 4.8). In this case, the plot of log drug concentration (in the central compartment, i.e., in the blood) shows two distinct slopes (Fig. 4.9). The first steeper slope represents the distribution of the drug from the blood to the tissues, which results in a rapid fall in the blood concentration, and the second slower phase is the elimination of the drug from the central compartment. The half-life for the slow phase provides an estimate of the K_{el}. If a drug is rapidly metabolized, then the two phases are not well separated.

The mathematical analysis of a two-compartment model is more complex than a one-compartment model, and in practical terms, drugs are not usually given intravenously. Where a drug is administered by a route other than intravenously, the plasma concentration profile will be a composite of absorption in addition to distribution and elimination. For a one-compartment model, the absorption rate constant (K_a) and the elimination rate constant K_{el} can be calculated by extrapolation of the appropriate part of the log dose curve (Fig. 4.10), but in the two-compartment model, absorption, distribution,

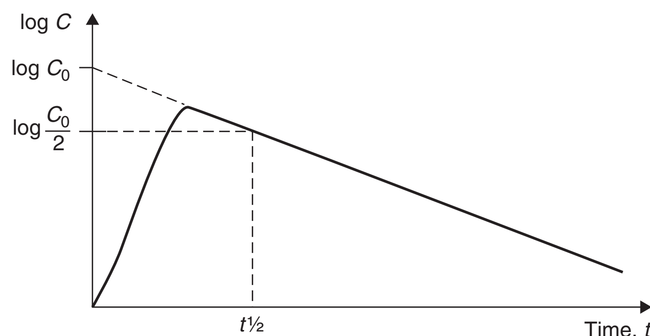

Figure 4.10 Theoretical log plasma drug concentration versus time curve for a one-compartment model with an initial absorption phase.

and elimination are occurring simultaneously and the curve is difficult to analyze satisfactorily. Pharmacokinetic data from orally administered drugs are also difficult to analyze. The administered dose of the drug may not be the same as the dose which is absorbed and available systemically. This is due partly to incomplete absorption from the GI tract and to first-pass metabolism mainly by the liver. The extent of first-pass metabolism by the liver can be extensive. For example, approximately 70% of the drug propranolol is metabolized before it reaches the systemic circulation when given orally [103]. The dose of a drug actually absorbed can be quantified by measuring the area under the plasma concentration versus time curve (AUC), which is a measure of the total body load of a drug. The oral bioavailability can be compared by

$$\text{Bioavailability} = \frac{\text{AUC}_{\text{oral}}}{\text{AUC}_{\text{iv}}}$$

4.8.3 Hepatic Drug Clearance

The main means of removing a drug from the central compartment is by hepatic drug metabolism. The ability of the liver to eliminate a drug is related to the hepatic blood flow and the intrinsic clearance of the liver. When intrinsic clearance is much greater than hepatic blood flow, the liver metabolizes and extracts all the drug from the blood presented to it, and the higher the blood flow through the liver, the more drug will be extracted from it. On the other hand, if the intrinsic clearance is much less than blood flow, then the hepatic clearance is dependent on the rate of metabolism. These two extremes are called *flow-limited* and *metabolism-limited extraction*, respectively. Examples of the former are propranolol, and lignocaine, and of the latter antipyrine. The ability of the liver to clear a drug from the blood is dependent not only on the metabolic activity of the organ but also on factors that influence the hepatic blood flow, such as cardiac output and redistribution of blood during exercise.

4.8.4 Tissue Distribution Prediction Models for PBPK

PBPK modeling attempts to predict ADME parameters using mathematical modeling of *in vitro* and preclinical derived data. There are some main assumptions to generic PBPK models related to compound distribution. These include the following:

- Distribution is mainly a passive process with no significant active transport involvement.
- Distribution into tissues is rate limited primarily by site-specific blood flow.
- Each tissue is viewed as a well-stirred compartment.
- Permeability across the membrane does not hinder or limit the ability of compounds to distribute into tissues.

Several mathematical models to predict distribution of drugs into various organs and tissues have been proposed. Input data generally required for these models include both parameters related to the compound and to the system. Compound-related input parameters are log P, pK_a, and blood plasma partitioning, while system-related parameters are the volume of lipids, phospholipids, and water in plasma and tissues, and the pH. The models aim to estimate the partition coefficients (K_p). Currently, it is common practice to use a number of the proposed models for predicting distribution parameters and compare them across known data sets to determine which model is most suitable. Five commonly used distribution models are described in Table 4.10. A major limitation with PBPK modeling predictions is lack of accounting for the presence of active uptake components, which can lead to a lack of correlation with *in vivo* data derived from preclinical species. Efforts to improve prediction models by incorporating transport parameters are on-going and are described in the literature [104,105].

4.9 CLINICAL IMPLICATIONS OF ALTERED DRUG DISPOSITION

Understanding the role of disposition in the pharmacokinetics of a drug is essential both for drug development and also for clinicians. The latter group must understand how the dosage of products is ascertained so that they can use the drug optimally in all groups of patients, appreciate that subgroups of patients may be at risk during therapy, and understand any limitations. This is particularly important for drugs with a low therapeutic index. An example of a group at risk is patients with deficient renal function. It is crucial to carefully monitor their response to drugs excreted predominantly by the kidney, for example, digoxin and aminoglycoside antibiotics, to avoid adverse effects.

4.9.1 Absorption

Absorption plays an important role in a clinical context and this can be illustrated, for example, in that the low oral bioavailability of many drugs limits their route of administration. For example, heparin is not absorbed from the GI track because of its size and charge, and it is therefore administered by injection, intravenously in emergency situations, and also routinely by patients at home subcutaneously [112]. On the other hand, treatment of *Clostridium difficile* infection resident in the gut by vancomycin has taken advantage of its poor GI absorption, which gives rise to high local concentrations in the colon [113].

4.9.2 Renal Elimination

Treatment of poisoning or drug overdose is often concentrated on minimizing absorption and also on increasing the elimination of the toxin. In many cases, the latter may

TABLE 4.10 Commonly Used Models for Predicting Distribution Parameters

Prediction Model (Reference)	Input Data	Output Data	Important Assumptions	Accurately Predicts	Mispredictions/ Additional Information
Poulin and Theil [106]	log P, fup, pK_a (compound)	Partition coefficients Pt:p based on volumes of lipid, phospholipids water, and plasma proteins of each tissue	Lipid subcompartment mainly phospholipids, passive diffusion. Equation 4.1: homogeneous distribution; Equation 4.2: nonhomogeneous distribution	Acids, bases, and neutral compounds	Zwitterions and some acids and bases. Improvements to method have been published
Berezhkovskiy [107]	log P, fup, pK_a	Same as Poulin model	Same as Poulin and Theil	Modified Poulin model	Additionally considers the exchange of drugs between compartments and peripheral drug elimination
Rodgers *et al*. [108]	log P, fup, pK_a, BP	Same as Poulin model	Passive distribution and nonsaturating mechanisms. Electrostatic interactions between ionized compounds (bases) and anionic phospholipids (based on B:P). Interactions with intracellular neutral phospholipids and neutral lipids (based on olive oil–water partition). Consideration of interactions with intracellular neutral phospholipids and neutral lipids	Moderate to strong bases and improved prediction for zwitterions	Peripheral elimination, binding to tissue constituents other than phospholipids and when there is active transport

Willman *et al.*, PK-Sim [109]	log P, fup, pK_a, estimated RBC, membrane affinity	Same as Poulin model	Well-stirred system	Moderate to strong bases	*In silico* measured membrane affinity is used as a marker of lipophilicity
Schmitt [110]	log P, fup, pK_a, membrane affinity	Same as Poulin model but includes neutral lipids, neutral and acidic phospholipids, and proteins of each tissue	Lipid subcomparment consists of neutral lipids, neutral phospholipids, and acidic phospholipids	Class-independent model	Recommends use of membrane affinity to describe binding to phospholipids, if unknown can apply log P as an approximation. Accounts for electrostatic interactions between charged molecules at physiological pH with acidic phospholipids. Considers the difference in pH of tissues and plasma

be achieved by altering the pH of the urine. For example, acute aspirin or phenobarbitone poisoning is treated by alkalinization of the urine. To achieve this, treatment with intravenous bicarbonate infusion is used and this may raise the urine pH between 7.5 and 8.1. As a result, the plasma half-life of aspirin has been shown to decrease from 19.4 h in control patients to 5.0 h in the treated group providing an effective means of removing the toxin [114]. Similarly, the renal clearance of phenobarbitone can be increased from 0.8 to 5.1 mL/h/kg in patients treated with sodium bicarbonate [115]. Although, theoretically, similar advantages may be gained from acidification of the urine for accelerating the clearance of basic drugs such as amphetamine or phencyclidine, there are significant risks associated with this procedure, such as acute renal failure and acid–base/electrolyte disturbances. Acidification of urine is therefore not recommended as a therapeutic method for treatment of overdose.

4.9.3 Transporter-Mediated Distribution

The distribution of many drugs is regulated by their uptake and efflux into body compartments and organs regulated by their affinity for various transporter systems as discussed in Section 4.5. Interference with transporter systems was used to clinical advantage during the World War II when penicillin was still in short supply. Doctors maximized its efficacy by coadministration of probenacid, which competitively inhibits its secretion from the renal tubules [116]. These transporters also of course transport endogenous ligands, for example, bilirubin, and indeed Campbell and coworkers [117] have suggested that the unconjugated hyperbilirubinemia induced during indinavir, rifamycin SV, and cyclosporine A therapy may be partly caused by competition and resultant inhibition of OATP1B1-mediated bilirubin uptake.

Coadministration of drugs is commonplace in pharmacy, and adverse drug–drug interactions can result in changes in both pharmacokinetics and pharmacodynamics. These interactions can occur during GI absorption, by interference with plasma protein binding, or with carrier-mediated transport (including hepatic or renal uptake and biliary or urinary excretion) or by metabolism. The latter mechanism is well established and has received a great deal of attention (reviewed by Campbell *et al*. [117] and discussed in detail elsewhere in this volume); however, the hepatic clearance of many poorly metabolized drugs has been found to be determined by hepatic uptake. Competition between different classes of drugs for the uptake transporter mechanisms is recognized as a mechanism responsible for several clinically significant drug–drug interactions. One of the classes of drugs involved in these interactions is the cholesterol lowering 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, commonly referred to as the *statins*. The major target site of action for these drugs is the liver and they are taken up into hepatocytes by the organic anion transporter polypeptide OATP family, as mentioned previously in Section 4.5.1.2. Coadministration of various drugs has been reported to increase the plasma concentrations of statins [118] and this can occasionally lead to severe side effects such as myopathy and rhabdomyolysis [119,120]. Cyclosporin A interacts with the statins that are not appreciably metabolized in the liver, and the interaction with cerivastatin [121] and pitavastatin [122] has been demonstrated to occur because the cyclosporine A inhibits OATP1B1-mediated uptake. Hirano and coworkers [122] have predicted the degree of inhibition of OATP1B1 in humans *in vivo* by relating the blood unbound fraction of the inhibitor, the concentration of the inhibitor present at the inlet to the liver, and the *in vitro* K_i value for

inhibition of transport by OATP1B1 (measured in cultured HEK293 cells) for several compounds. As a result of their calculations, they have suggested that cyclosporine A, rifampicin, rifamycin SV, clarithromycin, and indinavar would interact with pitavastatin in a clinical situation to increase the plasma concentration, affect its cholesterol lowering activity, and more importantly cause side effects. Caution should also be advocated with inhibitors of OAT-mediated transport such as cephalosporins [122]. The occurrence of drug–drug interactions through competition and inhibition of transporter mechanisms has been extensively reviewed by Shitara *et al*. [123], and they constitute a major contribution to clinical adverse drug reactions, especially when the incidence of polypharmacy is considered.

4.10 SUMMARY

All the parameters affecting distribution, that is, physicochemical and physiological factors, and the action of metabolism and transporters, act together to determine the volume of distribution of drugs, and an understanding of these processes leads to better decision making about drug development and therapeutic use by pharmaceutical scientists and clinicians. To improve this decision-making process, the way forward should be to develop better models for future use concentrating on *in vitro* methods that allow accurate extrapolation to the *in vivo* patient situation. Strategies should be in place to encourage development in the areas of safety evaluation of candidate pharmaceuticals and minimize attrition in the drug discovery process.

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