5

Distribution

Drug concentration in blood or plasma depends on the amount of the drug present in the body as well as how extensively it is distributed. The latter can be assessed from its volume of distribution.

5.1. DEFINITION

5.1.1. Proportionality Factor

The apparent volume of distribution (V) of a drug can be viewed simply as a proportionality factor between the total amount of drug present in the entire body and the drug concentration in the reference body fluid, usually the plasma, at any given time:

$$V(t) = A(t)/C_p(t) \tag{5.1}$$

A(t) is the amount of drug in the body at time t after administration, $C_p(t)$ is the drug concentration in the plasma at time t, and V(t) is the apparent volume of distribution with respect to the drug concentration in the plasma at time t.

There are several different volume terms depending on the reference fluids, where drug concentrations are measured, including blood, plasma, and plasma water, although the apparent volume of distribution based on the *plasma* drug concentration is the one most frequently determined. Relationships among different volume terms based on drug concentrations in different reference fluids are shown below:

$$V_b(t) \cdot C_b(t) = V(t) \cdot C_p(t) = V_u(t) \cdot C_u(t) \quad [= A(t)] \tag{5.2}$$

where $C_b(t)$, $C_p(t)$, and $C_u(t)$ are the drug concentration in blood, plasma, and plasma water, respectively, and $V_b(t)$, V(t), and $V_u(t)$ are the apparent volumes of distribution with respect to blood, plasma, and unbound drug concentrations, respectively. All the volume of distribution terms discussed hereafter will be the estimated volume of distribution based on the *plasma* drug concentrations, unless otherwise indicated.

Table 5.1. General Relationships among the Volume of Distribution at Steady State (V_{ss}), the Extent of Protein Binding, and the Distribution Characteristics of Drugs[a]

V_{ss}[b] (liter/kg)	Drug	General trend in protein binding	Distribution characteristics in the body
>1	Basic	More binding in tissue than in plasma	A drug may be concentrated in particular tissues in the body[c]
0.4–1	—	Similar binding between plasma and tissue	A drug distributes uniformly throughout the body
<0.4 (close to extracellular volume)	Acidic	More binding in plasma than in tissue	Drug molecules are confined mainly in the plasma pool with limited distribution to the tissues

[a]Data taken from Nau, 1986.
[b]Volume of distribution at steady state estimated based on drug concentration in plasma.
[c]The most common mechanisms for concentrating drug in tissues are binding to tissue macromolecules or partitioning into lipids within tissues. Another factor, which can lead to V_{ss} of the drug being apparently greater than total body volume, is reversible metabolism, ie., metabolite(s) produced from drug converts back to the parent drug.

UNITS: Usually liters or liters/kg when normalized to kg body weight.

5.1.2. Pharmacokinetic Implications of the Volume of Distribution

5.1.2.1. Proportionality Factor between the Amount of a Drug in the Body and the Drug Concentrations

The amount of drug in the body at any given time can be estimated by multiplying V(t) by the plasma drug concentration at time t.

5.1.2.2. Extent of Distribution of Drug into Tissues

The volume of distribution is a direct measure of the extent of the distribution of a drug. Although it does not represent a real physiological volume, the volume of distribution at steady state (V_{ss}) can be used to assess the extent of distribution of a drug from the plasma into the tissues. Table 5.1 summarizes the general considerations concerning the extent of protein binding and distribution into tissues based on V_{ss}.

5.1.2.3. Determinants of Half-Life

The terminal half-life of a drug, which is indicative of the duration of drug exposure during the terminal phase of a plasma drug concentration–time profile, is affected by both the *volume of distribution at the terminal phase* (V_β), not V_{ss}, and the *systemic clearance* (Cl_s):

$$t_{1/2} = \frac{0.693 \cdot V_\beta}{Cl_s} \tag{5.3}$$

5.1.3. Summary of Characteristics of the Volume of Distribution

1. V(t) is an imaginary volume correlating the amount of a drug present in the body to the concentrations of the drug measured in the reference body fluid, usually plasma, at any given time t.
2. V(t) can vary depending on the reference fluids in which the drug concentrations are measured. In general, the volume of distribution is referred to the *plasma* drug concentrations, unless otherwise indicated.
3. As an independent parameter, V(t) is indicative of the extent of space or volume into which the drug is distributed, referred to drug concentration, not the rate of distribution. How fast the distribution occurs can be estimated by distributional clearance (Cl_d).

If a drug is confined to the plasma pool, its V(t) approaches the actual volume of plasma in the body, and may not change much with time. However, if drug molecules gradually diffuse from plasma into other tissues, V(t) of the drug changes with time upon administration and can be greater than the total plasma volume.

5.2. DIFFERENT VOLUME TERMS

When a plasma concentration–time profile after intravenous injection of the drug exhibits a biexponential decline and the body can be viewed as a two-compartment model (the central and the peripheral compartments, Fig. 5.1) there are three different volumes of distribution that can be considered: the apparent volume of distribution of the central compartment (V_c), the apparent volume of distribution at steady state (V_{ss}), and the apparent volume of distribution at pseudodistribution equilibrium (V_β).

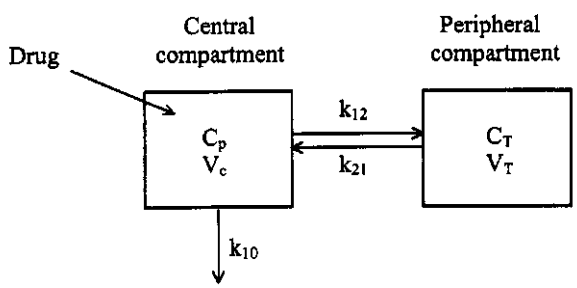

Figure 5.1. Two-compartment model of the body for drug disposition. Drug is administered and eliminated from the central compartment and distributes between the central and the peripheral compartments. C_p is the plasma drug concentration and C_T is a hypothetical average drug concentration in the peripheral compartment; k_{10} is the elimination rate constant, and k_{12} and k_{21} are the distribution rate constants; V_c and V_T are the apparent volumes of the central and the peripheral compartments, respectively.

5.2.1. Apparent Volume of Distribution of the Central Compartment

The apparent volume of distribution of the central compartment (V_c) is a proportionality factor between the amount of drug in the central compartment and the drug concentrations in the plasma. The central compartment may represent the plasma and highly perfused tissues and organs such as the liver, kidney, and spleen with which an instantaneous equilibrium of the drug in the plasma occurs.

Lower limit of V_c: After intravenous injection, drug molecules instantaneously disperse within the plasma pool and further distribute into blood cells and/or other tissues at a slower rate. Thus, V_c cannot be smaller than the actual volume of plasma in the body. The plasma volume of a human being (70 kg body weight) is approximately 3 liters (0.04 liter/kg) (Benet and Zia-Amirhosseini, 1995).

5.2.2 . Volume of Distribution at Steady State

Two different approaches for estimating the volume of distribution at steady state (V_{ss}) will be discussed. V_{ss} based on a two-compartment system for the body will be described according to pharmacokinetic relationships with other volume terms. In addition, a physiologically more relevant description of V_{ss} will be discussed based on the extent of plasma and tissue protein binding and actual plasma and tissue volumes.

5.2.2.1. V_{ss} *Based on a Two-Compartment System*

By definition, V_{ss} is a ratio between the amount of drug in the body and its concentration in plasma *at steady state.* A steady state implies a condition in which the rate of change in the amount of drug [A(t)] in the body is zero, i.e., $dA(t)/dt = 0$, and can be achieved after continuous infusion of a drug when the rate of infusion equals the rate of elimination. V_{ss} can be also estimated after a single intravenous dosing. After a single intravenous bolus injection of a drug for which the disposition profile can be adequately described by a two-compartment system, the volume of distribution of that drug becomes V_{ss} at one time point, when its distribution between the central and the peripheral compartments reaches equilibrium. At this time point (ie., distributional equilibrium), the rates of drug distribution from the central compartment into the peripheral compartment and vice versa become equal (Fig. 5.2). The pharmacokinetic expression of V_{ss} at this time point based on a two-compartment model is as follows:

(5.4) $$V_{ss} = V_c \cdot (1 + k_{12}/k_{21})$$

where k_{12} and k_{21} are the distribution rate constants from the central to the peripheral compartments and vice versa, respectively. V_c, k_{12}, and k_{21} can be calculated from the disposition parameters (A, B, α and β with curve-fitting of a biexponential equation [$C_p(t) = Ae^{-\alpha t} + Be^{-\beta t}$] to the plasma drug concentration–time profile (see Chapter 2).

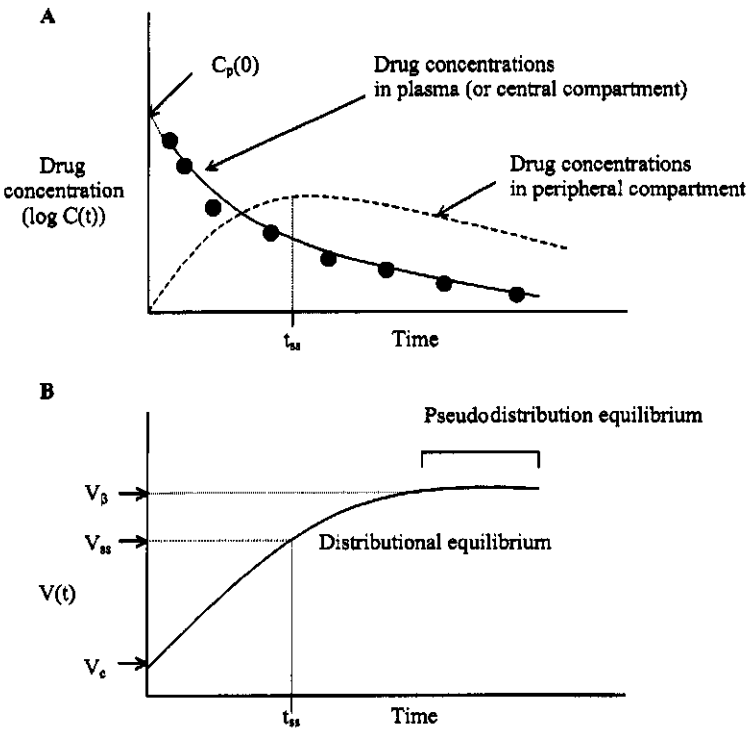

Figure 5.2. Semilogarithmic plots of measured drug concentrations (●) in plasma (central compartment, —) and estimated drug concentrations in the peripheral compartment (----) *vs.* time (A), and the corresponding changes in volume of distribution (B) with time, when the drug disposition profile can be best described with a two-compartment model. Only at time t_{SS} when V(t) becomes V_{SS}, is distributional equilibrium between the central (plasma) and the peripheral compartments attained.

5.2.2.2. V_{ss} *Based on Physiological Parameters*

Among several different volume of distribution terms, V_{ss} is especially important in that it has a certain physiological relevance that reflects the extent of drug distribution from the plasma pool into tissues and organs in the body. At equilibrium between the plasma and the tissues, the extent of distribution of the drug depends on its binding to plasma proteins, blood cells, and tissue components, and the actual total volumes of plasma and tissues:

(5.5)
$$V_{ss} \cdot C_{p,ss} = V_p \cdot C_{p,ss} + V_t \cdot C_{t,ss}$$

Amount of drug in the body at steady state	*Amount of drug in plasma at steady state*	*Amount of drug in tissues at steady state*

where $C_{p,ss}$ and $C_{1,ss}$ are a plasma drug concentration and an average concentration

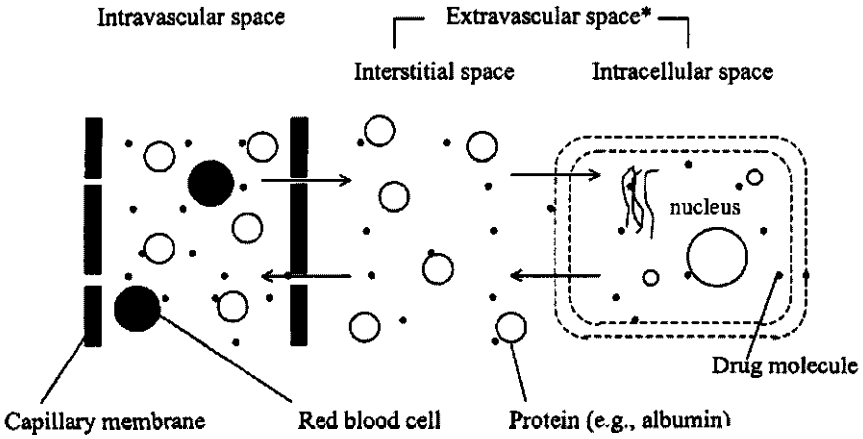

Figure 5.3. Schematic description of drug distribution. Note that only drug molecules (•) not bound to proteins (O) can transport from intravascular space into the rest of the total body water in extravascular and intracellular spaces. *Approximately 55–60% of the total extracellular (ie., intravascular and interstitial) albumin and 40% of total $\propto_1$ -acid glycoprotein are located in interstitial space.

of the drug in the tissues at steady state, and V_p and V_t are, respectively, the actual volume of the plasma and the extravascular space plus the erythrocyte volume into which the drug distributes. Therefore,

$$V_{ss} = V_p + \frac{C_{t,ss}}{C_{p,ss}} \cdot V_t \tag{5.6}$$

Drug molecules bound to a plasma protein such as albumin are not able to penetrate membrane lipid bilayers owing to the large sizes and charges of proteins. It is, therefore, assumed that only drug molecules not bound to (or free from) plasma proteins are capable of transporting between intravascular, extravascular, and intracellular spaces for distribution (free-drug hypothesis). Figures 5.3 illustrates unbound drug molecules transporting across different spaces in the body. Consequently, unbound drug concentrations in plasma and within tissues should be equal at steady state, i.e.,

Unbound drug concentration in plasma ↓ $f_u \cdot C_{p,ss}$ = *Unbound drug concentration in extravascular space and red blood cells* ↓ $f_{u,t} \cdot C_{t,ss}$

and, hence,

$$C_{t,ss} = f_u \cdot C_{p,ss}/f_{u,t} \tag{5.7}$$

where f_u and $f_{u,t}$ are the ratios between unbound- and total-drug concentrations in plasma and tissues (extravascular space), respectively. Incorporating Eq. (5.7) into

Eq. (5.6) yields

$$V_{ss} = V_p + (f_u/f_{u,t}) \cdot V_t \quad (5.8)$$

As is clear from Eq. (5.8), the degree of protein binding in both the plasma and tissues can significantly affect V_{ss}. For instance, a drug with extensive binding to plasma proteins (small f_u) generally exhibits a small V_{ss} (Φ ie and Tozer, 1979). When the drug has a high affinity for tissue components (small $f_{u,t}$), V_{ss} can be much greater than the actual physiological volume of the body. Based on limited data, it has been suggested that tissue binding appears to be similar across species, whereas plasma protein binding can vary significantly and tends to be more extensive in larger animals. Unlike plasma protein binding, tissue binding of a drug cannot be readily measured, and it is thus difficult to assess the effects of changes in the latter on V_{ss}. Table 5.2 lists the physiological volumes of different body fluids.

5.2.2.3. Factors Affecting V_{ss}

Whenever physiological factors governing the volume of distribution are discussed, V_{ss} with respect to plasma drug concentrations should be considered. Basically four different factors affect the extent of V_{ss}, as was indicated in Eq. (5.8). (1) the size of entire plasma pool (V_P); (2)the actual body tissue volume (V_t); (3) the ratio between unbound- and total-drug concentrations in the plasma (f_u); (4) the ratio between unbound- and total-drug concentrations in the tissues ($f_{u,t}$). The magnitude of V_{ss} is affected not only by the physiological volume of reference fluids and tissues into which the drug distributes, but also by its physicochemical properties such as hydrophilicity and lipophilicity, which govern protein binding affinity.

5.2.3. Volume of Distribution at Pseudodistribution Equilibrium

The volume of distribution at pseudodistribution equilibrium (V_β) is simply a proportionality constant that relates the total amount of a drug present in the body

Table 5.2. The Physiological Volumes of Different Body Fluids and Tissues in an Average Adult (Body Weight 70 kg)[α]

	Volume	
Fluid	liters	liters/kg
Total body water	42	0.60
Plasma	3–3.3	0.04–0.05
Blood	5.5	0.08
Extravascular fluid	39	0.56
Interstitial fluid	11–12	0.16–0.17
Body solids and fat	20	0.28

[α]Data taken from Balant and Gex-Fabry (1990), Benet (1995), and Φie and Tozer (1979).

to the plasma drug concentrations during the pseudodistribution equilibrium phase (so-called, β- , postdistribution, elimination, or terminal phase). Once the pseudo-distribution equilibrium phase is attained, V_β multiplied by C_P (t) during the phase provides a correct estimate of the amount of drug in the body at any time t thereafter.

5.3. ESTIMATING THE VOLUME OF DISTRIBUTION

The volume of distribution values can be estimated only after *intravenous bolus injection.* Equations (5.9), (5.10), and (5.11) for the measurement of different volume terms (V_c, V_{ss}, and V_β) should not be applied to data obtained after administration of a drug via other routes, even if complete bioavailability can be assumed. If a plasma drug concentration–time profile follows a monoexponential decline after intravenous injection, i.e., the body behaves like a single compartment, the three different volume terms described above become the same, and volume of distribution [V(t)] is independent of time. On the other hand, V(t) of a drug after intravenous administration changes from V_c, V_{ss} to V_β immediately after injection toward the terminal phase (Fig. 5.2), when the disposition of the drug in the body is better described with a two-compartment model, ie., a biphasic profile of log C_p(t) *vs.* time.

5.3.1. Apparent Volume of the Central Compartment

The apparent volume of the central compartment (V_c) can be estimated by dividing the intravenous dose (D_{iv}) by an estimated drug concentration in plasma at time 0, assuming that the drug injected into the systemic circulation instantaneously distributes into tissues and organs represented by the central compartment, but does not yet transport into tissues and organs represented by the peripheral compartment:

(5.9)
$$\boxed{V_c = D_{iv}/C_p(0)}$$

C_p(0) is an estimated concentration at time 0 determined by backextrapolating the first two drug concentrations after intravenous injection (Fig. 5.2A), and V_c is sometimes called the initial dilution volume ($V_{extrapo}$).

5.3.2. Volume of Distribution at Steady State

The simplest way to estimate V_{ss} of a drug is to use moment analysis on the plasma drug concentration profiles after intravenous bolus injection:

(5.10)
$$\boxed{V_{ss} = \frac{AUMC_{0-\infty,iv}}{AUC_{0-\infty,iv}} \cdot \frac{D_{iv}}{AUC_{0-\infty,iv}} = MRT_{iv} \cdot Cl_s}$$

$AUC_{0-\infty}$,iv and $AUMC_{0-\infty}$,iv are, respectively, the area under the plasma drug concentration *vs* time curve and the area under the first moment curve (plasma drug concentration × time *vs.* time curve) from time zero to infinity following intravenous bolus injection. Cl_s and MRT_{iv} are the systemic plasma clearance and mean residence time of a drug after intravenous injection.

5.3.3. Volume of Distribution at Pseudodistribution Equilibrium

The volume of distribution at pseudodistribution equilibrium (V_β) of a drug can be estimated by dividing Cl_s by the slope of the terminal phase (β) after intravenous administration (see Chapter 2):

(5.11)
$$V_\beta = \frac{Cl_s}{\beta}$$

5.3.4. Differences among V_c, V_{ss}, and V_β

V(t) of a drug with a two-compartmental disposition profile following intravenous injection increases from V_c immediately after injection, to V_{ss} when distributional equilibrium between the plasma and the peripheral compartment is achieved, and eventually to V_β when pseudodistribution equilibrium is attained (Gibaldi *et al.,* 1969). The trend of these volume changes with time is illustrated in Fig. 5.2.

Immediately after bolus injection of a drug, most of the drug molecules will remain momentarily in the plasma pool and highly perfused organs before distributing into other tissues. The drug dose divided by plasma concentration [$C_p(O)$] estimated at time zero yields the apparent volume of distribution corresponding to the central compartment, i.e., V_c, representing the volume of distribution term during this early phase.

Drug molecules will further distribute into the more slowly equilibrating tissues and/or organs (ie., the peripheral compartment). During this initial stage of distribution, the rate of drug distribution from the plasma into those tissues is faster than that from the tissues to the plasma, simply because not much drug has yet been accumulated in the tissues. More drug molecules will be accumulated in the tissues with time, and thus the rate of drug distribution from the tissues to the plasma increases. At a certain time point (t_{ss}), the rate of drug distribution from the plasma into the tissues becomes equal to the rate of drug distribution back from the tissues to the plasma, i.e., the rate of change of the amount of the drug in the tissues becomes zero. At t_{ss}, an average drug concentration in the tissues (the peripheral compartment) reaches its highest point. The apparent volume of distribution of the drug at t_{ss} becomes V_{ss}.

After V_{ss} is attained, V(t) continues to increase as time goes on until the pseudodistribution equilibrium (terminal phase) between the plasma drug concentrations and those in all the tissues is achieved. At the pseudodistribution equilibrium stage, the ratio of the amounts of a drug between the plasma (central compartment) and the tissues (peripheral compartment) remains constant. The apparent volume of distribution of a drug during this phase is called V_β.

It is noteworthy that V_{ss} multiplied by $C_p(t)$ equals the amount of a drug remaining in the body only at one time point, t_{ss}, after intravenous bolus injection. Before or after t_{ss}, respectively, the amount of the drug in the body estimated by multiplying V_{ss} by $C_p(t)$ is either an over- or an underestimate of the true value.

5.3.5. Relationships among V_c, V_{ss}, V_β, Cl_s and Cl_d

When a log $C_p(t)$ *vs.* time plot exhibits biphasic behavior, i.e., the slope of the plot during the initial distribution phase is much steeper than that of the terminal phase, V_β can be approximated with respect to V_c, V_{ss}, and Cl_s and distributional clearance (Cl_d) as follows (Jusko and Gibaldi, 1972; Kwon, 1996):

(5.12) $$V_\beta = V_{ss} + V_{ss} - V_c). \ (Cl_s/Cl_d)$$

REFERENCES

Benet L. Z. and Zia-Amirhosseini P., Basic principles of pharmacokinetics, *Toxicol. Pathol.* **23** 115–123, 1995.

Balant L. P. and Gex-Fabry M., Physiological pharmacokinetic modelling, *Xenobiotica* **20:** 1241–1257, 1990.

Gibaldi M. *et al.,* Relationship between drug concentration in plasma or serum and amount of drug in the body, *J. Pharm. Sci.* **58:** 193–197, 1969.

Jusko W. J. and Gibaldi M., Effects of change in elimination on various parameters of the two-compartment open model, *J. Pharm. Sci.* **61:** 1270-1273, 1972.

Kwon Y., Volume of distribution at pseudo-distribution equilibrium: relationship between physiologically based pharmacokinetic parameters and terminal half-life of drug, *Pharm. Sci.* **2:** 387–388, 1996.

Nau H., Species differences in pharmacokinetics and drug teratogenesis, *Environ. Health Perspect.* **70** 113–129, 1986.

Ше S. and Tozer T. N., Effect of altered plasma protein binding on apparent volume of distribution, *J. Pharm. Sci.* **68:** 1203–1205, 1979.

6

Clearance

Clearance is a measure of the ability of the body or an organ to eliminate a drug from the blood circulation (Gibaldi, 1986; Tozer 1981; Wilkinson, 1987). Systemic (or total body) clearance is a measure of the ability of the entire body to eliminate the drug, whereas organ clearance such as hepatic or renal clearance is a measure of the ability of a particular organ to eliminate the drug.

6.1. DEFINITION

6.1.1. Proportionality Factor

The most general definition of clearance is a proportionality factor between the rate of elimination of a drug from the entire body (systemic clearance) or an organ (organ clearance), and its concentration at the site of measurement, i.e., reference body fluid such as blood or plasma. For instance, when drug concentrations are measured in the plasma, systemic *plasma* clearance (Cl_s) can be defined as

$$\text{Systemic plasma clearance} = \frac{\text{Elimination rate of drug from the entire body}}{\text{Drug concentration in plasma}}$$

(6.1)

$$\boxed{Cl_s = \frac{-dA(t)/dt}{C_p(t)}}$$

A(t) is the amount of drug in the body and $C_p(t)$ is the plasma drug concentration at time t.

6.1.2. Apparent Volume of Reference Fluid Cleared of a Drug per Unit Time

A physiologically more meaningful definition of systemic clearance is the *apparent* volume of reference fluid such as plasma (or blood) cleared of drug per unit time. It is important to note that the clearance terms depend on where the drug concentrations are measured. The estimated clearance based on drug concentrations in blood, plasma, and plasma water (plasma without proteins) are referred to as

blood clearance (Cl_b), plasma clearance (Cl_P), and clearance based on the unbound drug concentration (Cl_u), respectively. Systemic *blood* or *unbound-drug* clearance can be obtained by replacing $C_P(t)$ in Eq. (6.1) with the drug concentration in the blood [$C_b(t)$] or unbound drug concentration [Cu(t)], respectively.

NOTE: PHARMACOKINETIC IMPLICATIONS OF CLEARANCE. Clearance is not an indicator of how much of a drug is being eliminated per unit time, but rather *how much apparent volume of reference fluid is cleared of a drug per unit time.* The amount of a drug eliminated from the body over a certain period of time depends on both the extent of clearance and the concentrations of the drug in the reference fluid. For a better understanding, let us assume that there is a tank filled with water that is being well circulated and continuously filtered by a pump (Fig. 6.1). If a drop of blue dye is added to the tank, then dye molecules will be instantaneously and evenly distributed in the water by the continuous circulation caused by the pump. As the water passes through the filter, which can remove the dye molecules, its color will gradually fade. The tank and the water can be viewed as a whole body and a reference fluid, respectively. The pump can be viewed as the heart and the filter can be considered the elimination mechanisms of the body such as metabolism or renal elimination. A drop of blue dye is an intravenous dose of drug and thus blue dye molecules can be viewed as drug molecules in the body. The changes in the intensity of the color of the water with time can be viewed as a concentration *vs.* time profile of a drug in a reference fluid in the body after intravenous bolus injection.

The efficiency of the filter in removing dye molecules from the water depends on how much water passes through the filter and how efficiently the filter can remove the dye molecules from the water. The volume of the water completely cleared of the dye molecules per unit time is equivalent to the clearance of the drug in the body. For instance, the amount of dye removed from the water (the amount of drug eliminated from the body) per unit time can be calculated by multiplying the volume of water cleared of the dye per unit time (systemic clearance) by the concentration of

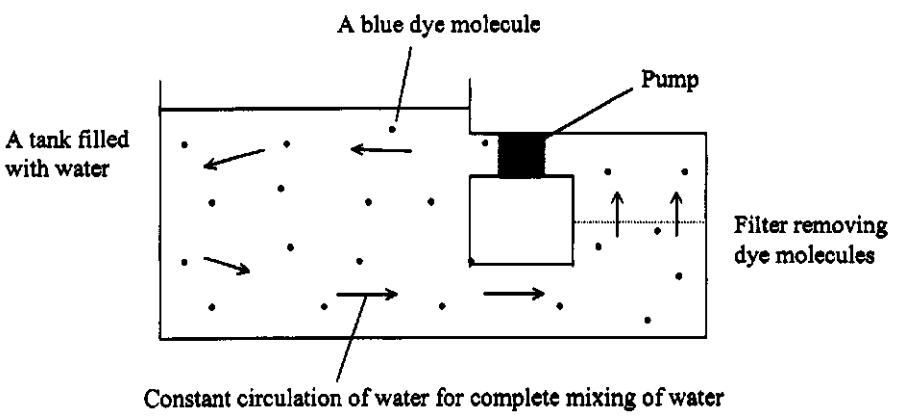

Figure 6.1. A tank filled with water being continuously circulated by a punip and filtered.

Table 6.1. Important Pharmacokinetic Implications of Clearance

Parameters	Relationship with clearance
Amount of a drug eliminated per unit time	Systemic clearance × concentration of a drug in reference fluid at time t
Amount of a drug eliminated	Systemic clearance × $AUC_{t_1-t_2}$ from time t_1 to t_2
Terminal half-life of a drug	Proportional to the apparent volume of reference fluid divided by the systemic clearance
Steady state drug concentration after continuous infusion	Infusion rate divided by systemic clearance

dye in the water (drug concentration in a reference fluid). Also, how fast the dye molecules disappear from the water (the terminal half-life of the drug) is governed by both the volume of the water completely cleared of the dye per unit time (clearance) and the total volume of the water containing the dye molecules in the tank (the apparent volume of distribution of the reference fluid). The important pharmacokinetic implications of clearance are summarized in Table 6.1.

UNITS: The unit of clearance is the same as flow rate (ie., volume/time). For instance, ml/min or ml/min · kg (i.e., ml/min/kg) when normalized to body weight (kg).

6.2. SYSTEMIC (PLASMA) CLEARANCE

6.2.1. Estimation

In general, the systemic plasma clearance (Cl_s) of a drug can be estimated from the plasma drug concentration–time profile after intravenous bolus injection. Systemic clearance can also be measured following administration via routes other than intravenous injection as long as bioavailability is known and the total absence of administration-route-dependent differences in kinetics can be assumed. The integral of the numerator and denominator of Eq. (6.1) with respect to time yields

$$Cl_s = \frac{-\int_0^\infty [dA(t)/dt]\,dt}{\int_0^\infty C_p(t)\,dt} = \frac{A(0) - A(\infty)}{AUC_{0-\infty,iv}} \tag{6.2}$$

A(0) and A(∞) are the total amount of drug present in the body after intravenous bolus injection at time zero and infinity, respectively. A(0) is, therefore, equal to the intravenous dose (D_{iv}), and A(∞) is zero because at infinite time there should be no drug left in the body. $AUC_{0-\infty,\,iv}$ is the area under the plasma drug concentration *vs.* time curve from time zero to infinity after intravenous injection.

The systemic plasma clearance of the drug can then be estimated by dividing D_{iv} by $AUC_{0-\infty\ ,iv}$:

(6.3) $$\boxed{\text{Systemic plasma clearance} = D_{iv}/AUC_{0-\infty,iv}}$$

For example, if $AUC_{0-\infty\ ,iv}$ of a drug in the plasma is 5 μg. hr/ml after intravenous administration of 10 mg/kg body weight in rats, systemic plasma clearance of this drug in rats would be 33.3 ml/min/kg as shown below:

$$\text{Systemic plasma clearance} = \frac{\overset{\downarrow}{10\ \text{mg/kg}}}{\underset{\uparrow}{(5\ \mu\text{g}\cdot\text{hr})/\text{ml}}} = \frac{(10\cdot 1000\ \mu\text{g})/\text{kg}}{(5\ \mu\text{g}\cdot 60\ \text{min})/\text{ml}}$$

D_{iv} above the left fraction; $AUC_{0-\infty,iv}$ below the middle fraction

$$= 33.3\ \text{ml/min/kg}$$

In most cases, drug concentrations are measured in *plasma* rather than in *blood* because sample preparation and drug analysis in plasma are simpler than in blood, and thus systemic clearance implies systemic *plasma* clearance, unless otherwise indicated.

6.2.2. Relationship between Systemic Clearance and the Volume of Distribution

The clearance and the volume of distribution at steady state are somewhat related to each other in that the extent of plasma protein binding of a drug can affect both parameters (see Chapter 7). In most other cases, however, the clearance and the volume of distribution can be assumed to be independent of one another.

6.2.3. Relationship between Systemic Clearance and the Terminal Half-Life

Terminal half-life ($t_{1/2}$) of a drug after intravenous injection is a function of both the systemic clearance and the volume of distribution at pseudodistribution equilibrium (V_β).

(6.4) $$\boxed{t_{1/2} = \frac{0.693\cdot V_\beta}{Cl_s}}$$

6.2.4. Amount of Drug Eliminated from the Body

The amount of a drug eliminated from the body up to time t after administration can be estimated by multiplying Cl_s by AUC from time 0 to t (AUC_{0-t}):

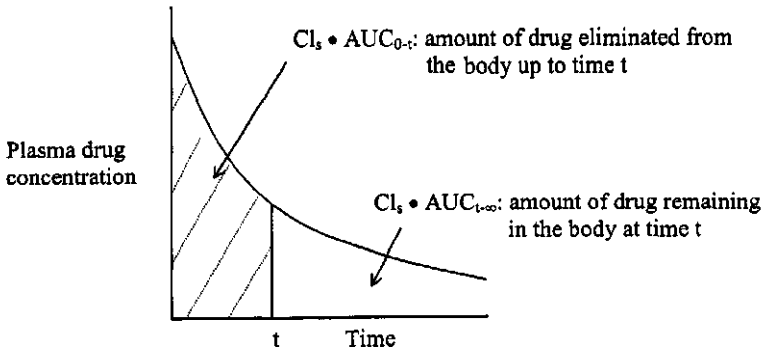

Figure 6.2. Plasma concentration–time curve of a hypothetical drug after intravenous administration on a linear scale. If the area up to time t (AUC_{0-t}) is 30% of the total AUC (ie., $AUC_{0-\infty}$), then the indications are that 30% of the intravenous dose has been eliminated by time t. The remaining 70% of $AUC_{0-\infty}$ accounting for AUC from time t to ∞ represents the fraction of the dose remaining in the body at time t.

(6.5)
$$\text{Amount of drug eliminated from the body up to time t after administration} = Cl_s \cdot AUC_{0-t}$$

Equation (6.5) holds true at any time after drug administration, regardless of the route of administration. Obviously, the amount of drug remaining in the body at time t can be calculated by subtracting Cl_s AUC_{0-t} from the total amount of drug administered into the systemic circulation. Figure 6.2 illustrates the relationship between the amount of drug eliminated from the body up to time t and the amount of drug remaining in the body at time t after intravenous bolus injection.

6.3. ORGAN CLEARANCE

Organ clearance reflects the ability of the eliminating organ to remove a drug from the *blood.* A pharmacokinetically more meaningful interpretation of organ clearance would be the real physiological volume of blood cleared of drug per unit time. This is because organ clearance is estimated based on the drug concentration in the *blood,* not in the *plasma,* and therefore, when organ clearance is referred to, it generally implies *blood clearance.* It is important to note that whenever physiological factors governing the clearance of drug are considered, blood clearance by the organ has to be used.

Like systemic clearance, organ clearance can also be viewed as a proportionality constant relating the elimination rate of a drug from blood by the organ to the drug concentration in blood perfusing through the organ. This concept can be best illustrated by considering elimination processes of a drug in a single organ using isolated organ perfusion under a steady state condition (Fig 6.3).

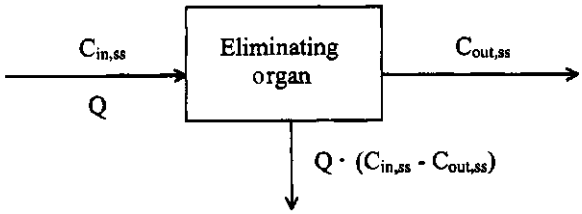

Figure 6.3. Schematic representation of organ perfusion under steady state conditions: $C_{in,ss}$: drug concentration in blood entering the organ at steady state; $C_{out,ss}$: drug concentration in blood leaving the organ at steady state; and Q: blood flow rate.

Based on mass balance at steady state, the rate of elimination of a drug by the organ is equal to the difference between the input and output rates of the drug through the organ:

$$\text{Rate of elimination at steady state} = \text{Input rate at steady state} - \text{Output rate at steady state} = Q \cdot C_{in,ss} - Q \cdot C_{out,ss}$$

$C_{in,ss}$ and $C_{out,ss}$ are, respectively, the drug concentrations in the blood entering and leaving the organ at steady state, and Q is the blood flow rate. According to the definition of clearance, organ clearance can be also described as a proportionality constant between the rate of elimination of the drug by the organ and the drug concentration in the blood, i.e., inlet blood drug concentration, at steady state:

$$\text{Organ clearance} = \frac{\text{Rate of drug elimination by the organ at steady state}}{\text{Inlet drug concentration in blood at steady state}}$$

(6.6) $$\boxed{\text{Organ clearance} = \frac{Q \cdot (C_{in,ss} - C_{out,ss})}{C_{in,ss}}}$$

(6.7) $$\text{Organ clearance} = Q \cdot E$$

(6.8) $$E = \frac{C_{in,ss} - C_{out,ss}}{C_{in,ss}}$$

As in Eq. (6.7), organ clearance is often expressed as the blood flow rate multiplied by the extraction ratio [Eq. (6.8)]. The extraction ratio (E) represents the fraction of the amount (or concentration) of drug entering the organ that is extracted by the organ during perfusion. E is dimensionless and ranges between 0 and 1 (sometimes expressed as a percent). E = 0 means that the organ does not remove drug at all during perfusion, whereas E = 1 indicates the complete elimination of a drug from the blood by the organ during perfusion. In other words, E reflects the organ's efficiency in removing drug from the blood stream.

Let us assume that a rat liver is perfused with blood containing 10 μg/ml of a drug at 12 ml/min via the portal vein, and that the blood concentration of the drug in the hepatic vein at steady state is 2 μg/ml. The hepatic clearance of this drug is, then, 9.6 ml/min, and the extraction ratio of the liver for the drug is 0.8, as described below:

$$\text{Hepatic clearance} = \frac{\overset{\text{Flow rate (ml/min)}}{12} \times (\overset{C_{in,ss}\ (\mu g/ml)}{10} - \overset{C_{out,ss}\ (\mu g/ml)}{2})}{\underset{C_{in,ss}\ (\mu g/ml)}{10}} = 12 \times \underset{E}{0.8} = 9.6 \text{ ml/min}$$

NOTE: EXTRACTION RATIO. In general, when E is greater than 0.7, between 0.3 and 0.7, or smaller than 0.3, organ clearance is considered to be high, moderate, or low, respectively. Availability of a drug after it passes through the eliminating organ can be expressed as 1 – E, which is the fraction of the amount of drug that entered the organ and was not cleared or the fraction of the volume of blood containing the drug that passed through the organ without being cleared.

Upper limit of organ clearance: As indicated in Eq. (6.6), maximum organ clearance, which can be achieved with complete removal of the drug by the organ during single-pass perfusion, i.e., $C_{out,ss} = 0$, cannot be greater than the blood flow rate perfusing through the organ.

6.3.1. Hepatic Clearance

The liver is the most important organ in the elimination of a drug from the body. It is highly perfused and under normal conditions receives approximately 75% of its blood supply from the portal vein and 25% from the hepatic artery. The highly branched capillary system and fenestrated endothelium enable direct contact between the blood components and all cell types within the organ, including, e.g., hepatocytes, Kupfer cells, and fat storage cells. Hepatocytes, the principal cell type in the liver, contain various metabolizing enzymes such as cytochrome P450 and uridine diphosphate glucuronyl transferase (UDPGT) and are well equipped with active transporters for efficient uptake of drug and excretion into the bile. In general, hepatic drug clearance implies clearance via both metabolism and biliary excretion.

Although Eq. (6.6) elucidates the concept of organ clearance rather intuitively, it has a limited applicability in estimating organ clearance because of the specific experimental conditions required, i.e., *in situ* organ perfusion at steady state, or in characterizing physiological factors governing organ clearance *in vivo.* Several pharmacokinetic models have been developed to enable estimates of organ clearance without the need for organ perfusion studies or an understanding of the physiological factors that govern organ clearance. The most well-known hepatic clearance models include "well-stirred (or venous equilibrium)," "parallel-tube (or sinusoidal perfusion)," and "dispersion" models. Pharmacokinetic differences among the models are due mainly to the differences in assumptions made on the *anatomical structure of the liver* and the *extent of blood mixing within the liver.*

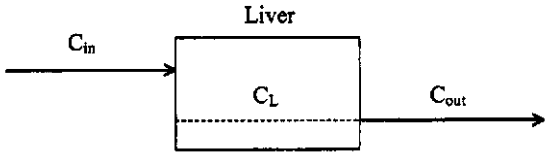

Figure 6.4. The well-stirred model: C_{in}: drug concentration in portal vein blood or the systemic circulation; C_L : drug concentration within the liver; and C_{out}: drug concentration in hepatic vein blood (ie., emergent venous blood).

6.3.1.1. Well-Stirred (Venous Equilibrium) Model

The well-stirred model, which is the most popular one for hepatic clearance (Fig. 6.4), assumes that the entire liver, i.e., both the liver tissues including hepatocytes and the blood in the sinusoid, is well mixed, so that drug molecules are distributed instantaneously and homogeneously within the liver upon entering. As a result, the drug concentration within the liver is assumed to be equal throughout the organ (Pang and Rowland, 1977). In other words, the well-stirred model views the liver as a single compartment *(anatomy of the liver)* with a complete mixing of blood *(extent of blood mixing).* Important assumptions for the well-stirred model for hepatic clearance include: (a) only unbound drug in blood is subject to elimination (metabolism and/or biliary excretion), (b) no membrane transport barrier, (c) no concentration gradient of the drug within the liver, (d) the concentration of the drug within the liver is equal to that in emergent venous blood, and (e) linear kinetics.

Hepatic clearance (Cl_h) based on the well-stirred model is described as follows:

(6.9)
$$Cl_h = \frac{Q_h \cdot f_{u,b} \cdot Cl_{i,h}}{Q_h + f_{u,b} \cdot Cl_{i,h}}$$

$Cl_h = Q \cdot E$; therefore

(6.10)
$$E = \frac{f_{u,b} \cdot Cl_{i,h}}{Q_h + f_{u,b} \cdot Cl_{i,h}}$$

$Cl_{i,h}$ is the intrinsic hepatic clearance, Q_h is the hepatic *blood* flow rate, and $f_{u,b}$ is the ratio between unbound and total (both bound and unbound) drug concentrations in *blood,* not plasma. Since in most studies, the fraction of a drug unbound in plasma, f_u, is more frequently measured than $f_{u,b}$, it is useful to note the relationship between $f_{u,b}$ and f_u:

(6.11)
$$f_{u,b} = \frac{f_u \cdot C_p}{C_b}$$

since $f_{u,b} \cdot C_b = f_u \cdot C_p$, where C_b and C_p are the drug concentrations in blood and plasma, respectively. Equation (6.9) is one of the most important equations in

pharmacokinetics with various applications to understanding fundamental concepts of hepatic clearance.

6.3.1.2. Parallel-Tube (Sinusoidal Perfusion) Model

The parallel-tube model (Fig. 6.5) views the liver as a group of identical tubes *(anatomy of the liver)* arranged in parallel, with metabolizing enzymes and biliary excretion functions evenly distributed around the tubes with a bulk flow of blood *(extent of blood mixing)* passing through them (Pang and Rowland, 1977). The parallel-tube model produces a concentration gradient of a drug within the liver along the blood flow path from the periportal (portal vein) to the perivenous (hepatic vein) regions. Important assumptions for the parallel-tube model are: (a) only the drug not bound to blood components is subject to elimination (metabolism and/or biliary excretion), (b) no membrane transport barrier, (c) a concentration gradient of the drug within the liver ranged between inlet and outlet drug concentrations, and (d) linear kinetics.

Hepatic clearance based on the parallel-tube model is

$$Cl_h = Q_h \cdot (1 - e^{-f_{u,b} \cdot Cl_{i,h}/Q_h}) \tag{6.12}$$

The average drug concentration ($C_{L,avg}$) within the liver is

$$C_{L,avg} = \frac{C_{in} - C_{out}}{\ln(C_{in}/C_{out})} \tag{6.13}$$

The differences between the well-stirred and the parallel-tube models are summarized in Table 6.2.

6.3.1.3. Dispersion Model

The well-stirred and the parallel-tube models represent the two extreme cases of the anatomical features of the liver and hepatic blood flow patterns. The dispersion model views the liver somewhere between these two extremes. It considers the liver to be a meshed organ *(anatomy of the liver)* with internal blood dispersion, the degree of which can be reflected by the so-called "dispersion number (D_N, *extent of blood mixing)"* (Roberts and Rowland, 1985, 1986).

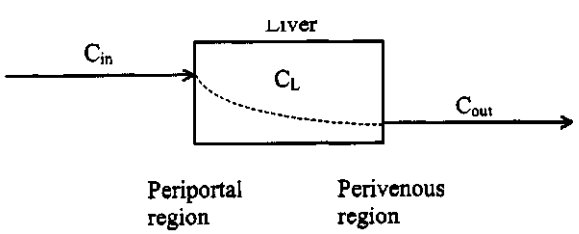

Figure 6.5. The parallel-tube model (see Fig. 6.4 for abbreviations).

Table 6.2. **Differences between the Well-Stirred and the Parallel-Tube Models of Hepatic Clearance**

	Well-stirred model	Parallel tube model
Liver anatomy	A single homogeneous compartment	A group of identical tubes
Blood flow	Complete mixing	Bulk flow
Drug concentration in the liver	Constant and equal to that of emergent venous blood	$(C_{in}-C_{out})/[]ln(C_{in}/C_{out})]$, decreases from periportal to perivenous regions
Hepatic clearance	$(Q_h \cdot f_{u,b} \cdot Cl_{i,h})/(Q_h + f_{u,b} \cdot Cl_{i,h})$	$Q_h \cdot (1 - e^{-f_{u,b} \cdot Cl_{i,h}/Q_h})$
Extraction ratio[a]	$f_{u,b} \cdot Cl_{i,h}/(Q_h + f_{u,b} \cdot Cl_{i,h})$	$1 - e^{-f_{u,b} \cdot Cl_{i,h}/Q_h}$

[a]The difference in hepatic clearance of the same drug between the models is not significant when its clearance is low (E<0.3). When the clearance of a drug is high (E>0.7), hepatic clearance estimated based on the parallel-tube model tends to be slightly higher than that using the well-stirred model. Thus, the selection of the models becomes important only for drugs showing extensive clearance.

Hepatic clearance based on the dispersion model is expressed as

$$Cl_h = Q_h \cdot (1 - F_h) \tag{6.14}$$

and

$$F_h = \frac{4a}{(1 + a)^2 \cdot \exp[(a - 1)/2D_N] - (1 - a)^2 \cdot \exp[-(a + 1)/2D_N]}$$

where, $a = (1 + 4R_N \cdot D_N)^{1/2}$ and $R_N = f_u \cdot Cl_{i,h}/Q \cdot D_N$ ranges between 0 and 1, and the well-stirred and the parallel-tube models are the two extreme cases of the dispersion model with $D_N = 1$ (complete dispersion of blood) and 0 (no dispersion of blood) within the liver, respectively. It is likely that a D_N value somewhere between these two extremes would be more reasonable for describing the degree of blood mixing inside the liver. It appears that the estimates of D_N depend heavily on the experimental conditions and substrates studied.

NOTE: INTRINSIC HEPATIC CLEARANCE $Cl_{i,h}$ reflects the inherent ability of the liver to eliminate the drug not bound in blood components (to be exact, drug molecules not bound to tissue components within the hepatocytes and is goverened solely by

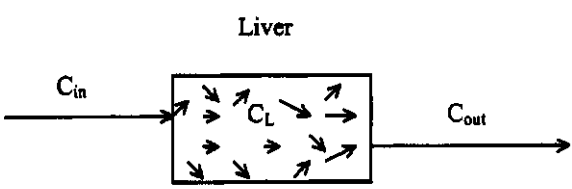

Figure 6.6. The dispersion model (see Fig. 6.4 for abbreviations).

the activities of metabolizing enzymes and/or biliary excretion (both active and passive transport of a drug into the bile). In theory, it is the same as the hepatic clearance of a drug when there are no limitations in drug delivery to the liver (sufficient and fast blood flow), protein binding (no protein binding), and other factors such as cofactor availability when needed. Physiological factors governing $Cl_{i,h}$ are as follows:

(6.15)
$$Cl_{i,h} = \frac{V_{max}}{K_m + C_{L,u}}$$

$V_{max,}$ is the maximum rate of metabolizing enzyme(s) and/or biliary excretion, K_m is the apparent Michaelis–Menten constant of metabolizing enzyme(s) and/or biliary excretion activity, and $C_{L,u}$ is the concentration of unbound drug within the hepatocytes available for metabolizing enzyme(s) and/or biliary excretion. If $C_{L,u}$ is much smaller than $K_m(C_{L,U} + 0.1 \cdot K_m)$, $C_{li,h}$ becomes simply a ratio between V_{max} and K_m. At low $C_{L,u}$, therefore, $C_{1i,h}$ is a concentration-independent constant:

(6.16)
$$Cl_{i,h} = V_{max}/K_m$$

(a) Factors Affecting Hepatic Clearance. According to Eq. (6.9), there are basically three different physiological factors governing hepatic clearance: (1) *hepatic blood flow rate,* indicating how fast the drug can be delivered to the liver, (2) *fraction of a drug not bound to blood components,* reflecting what portion of a drug in the blood is available for clearance, (3) *intrinsic hepatic clearance,* representing the intrinsic ability of the liver to remove a drug from the blood by metabolism and/or biliary excretion, when there are no restrictions in drug delivery (blood flow), protein binding, and cofactor availability.

(b) Applications of Hepatic Clearance Models. The most important value of hepatic clearance models is probably their ability to elucidate how the three different factors described above that affect hepatic clearance, i.e., blood flow, protein binding, and intrinsic clearance, are related each other. In some models, the effects of the degree of blood dispersion inside the liver on clearance can also be taken into account.

(i) Estimation of intrinsic hepatic clearance. $Cl_{i,h}$ of a drug can be estimated according to Eq. (6.9), if Cl_h, Q_h, and $f_{u,b}$ are known. Cl_h can be estimated either by measuring the extraction ratio from *in situ* perfusion studies under steady state conditions [Eq. (6.6)] or by determining the *in vivo* systemic clearance. If there is experimental evidence suggesting that the systemic clearance of a drug is solely via hepatic elimination, systemic blood clearance of the drug determined based on blood drug concentrations can be considered to equal Cl_h. For Q_h, published values (Table 6.3) are usually used; $f_{u,b}$ can be measured experimentally *in vitro* (see Chapter 7).

Table 6.3. Hepatic Blood Flow Rate of Laboratory Animals and Humans[a]

Species	Body weight (kg)	Hepatic blood flow rate (ml/min/kg)
Mouse	0.02	90
Rat	0.25	47.2, 81
Monkey	5	43.6
Dog	10	30.9
Human	70	20.7

[a]Data taken from Davies and Morris (1993) and Houston (1994).

(ii) Estimating hepatic clearance from in vitro experiments. It is possible to predict Cl_h if $Cl_{i,h}$ can be estimated from experimentally measured V_{max} and K_m values of metabolizing enzymes *in vitro* (see Chapter 12).

(c) Limits of Hepatic Clearance. C_{lh} of a drug cannot be greater than Q_h, which is the upper limit of Cl_h. Let us think about the following two extreme cases of Cl_h based on the well-stirred model [Eq. 6.9)].

IF $f_{u,b}$ $Cl_{i,h}$ IS MUCH GREATER THAN Q_h (HIGH CLEARANCE):

Cl_h approaches Q_h:

$$Cl_h = \frac{Q_h \cdot f_{u,b} \cdot Cl_{i,h}}{Q_h + f_{u,b} \cdot Cl_{i,h}} \approx \frac{Q_h \cdot \cancel{f_{u,b}} \cdot \cancel{Cl_{i,h}}}{\cancel{f_{u,b}} \cdot \cancel{Cl_{i,h}}} = Q_h$$

When Cl_h is greater than 70% of Q_h (in other words, $E \geq 0.7$), it is generally considered high extraction or high hepatic clearance for the drug. In this case, changes in $f_{u,b}$ and/or $C_{li,h}$ do not affect Cl_h to any significant extent, whereas alterations in Q_h can have substantial effects.

IF $f_{u,b} \cdot Cl_{i,h}$ IS MUCH SMALLER THAN Q_h (LOW CLEARANCE):

Cl_h becomes limited by $f_{u,b} \cdot Cl_{i,h}$:

$$Cl_h = \frac{Q \cdot f_{u,b} \cdot Cl_{i,h}}{Q + f_{u,b} \cdot Cl_{i,h}} \approx \frac{\cancel{Q} \cdot f_{u,b} \cdot Cl_{i,h}}{\cancel{Q}} = f_{u,b} \cdot Cl_{i,h}$$

When Cl_h of a drug is lower than 30% of Q_h ($E \leq 0.3$), it is generally considered low extraction or low hepatic clearance. In this case, Cl_h of a drug is not much affected by changes in Q_h, whereas alterations in $f_{u,b}$ and/or $C_{li,h}$ can have significant effects.

6.3.2. Biliary Clearance

Once a drug molecule gets into the hepatocytes, it can be subject to both metabolism and biliary excretion. Excretion of a drug into the bile occurs through

canalicular membranes surrounding the bile canaliculi of the hepatocytes. Hepatic clearance (Cl_h) of a drug is the sum of its clearances via both metabolism (Cl_m) and biliary excretion (Cl_{bl}):

$$Cl_h = Cl_m + Cl_{bl} \tag{6.17}$$

By definition, Cl_{bl} of a drug is a proportionality constant relating the rate of biliary excretion of unchanged drug to drug concentration in the blood:

$$Cl_{bl} = \frac{\text{(Bile flow rate)} \cdot \text{(Drug concentration in bile)}}{\text{Drug concentration in blood}} \tag{6.18}$$

As the bile flow is relatively slow, approximately 0.06 and 0.008 ml/min/kg in rats and humans, respectively, Cl_{bl} would be small, unless the drug concentration in the bile is substantially higher than that in the blood.

Enterohepatic circulation: A drug excreted in the bile passes into the intestine. Some or most of the secreted drug may be reabsorbed from the intestine, undergoing enterohepatic circulation (EHC) and the rest of it is excreted in the feces. This may be repeated many times until the drug is ultimately eliminated from the body by other elimination processes such as metabolism or renal or fecal excretion. In this way, EHC may increase the persistence of a drug in the body. If some of the drug excreted in the bile is subject to EHC, the biliary excretion process is no longer solely an elimination process, but rather contains components of both elimination and distribution processes.

6.3.3. Renal Clearance

The renal clearance (Cl_r) of a drug consists of four different processes — glomerular filtration (Cl_f), active secretion (Cl_{rs}), passive reabsorption (F_r), and renal metabolism($C1_{rm}$):

$$Cl_r = (Cl_f + Cl_{rs}) \cdot (1 - F_r) + Cl_{rm} \tag{6.19}$$

$$Cl_f = f_{u,b} \cdot GFR \tag{6.20}$$

$$Cl_{rs} = \frac{Q_r \cdot f_{u,b} \cdot Cl_{i,s}}{Q_r + f_{u,b} \cdot Cl_{i,s}} \tag{6.21}$$

Cl_f, Cl_{rs}, and Cl_{rm} are clearances representing glomerular filtration, active secretion, and renal metabolism. $Cl_{i,s}$ and Q_r are intrinsic renal tubular secretion clearance by active transporter(s) and renal blood flow rate, respectively. F_r is the fraction of the drug reabsorbed into the blood from the urine after excretion. GFR is the glomerular filtration rate, at which plasma water is filtered through the glomerulus, e.g., approximately 125 ml/min in a 70-kg man.

6.3.3.1. Glomerular Filtration

In the Bowman capsule (glomerulus), drug molecules not bound to blood components are physically filtered through the glomerular capillary because of the renal artery blood pressure (Fig. 6.7). For instance, molecules smaller than 15 Å in

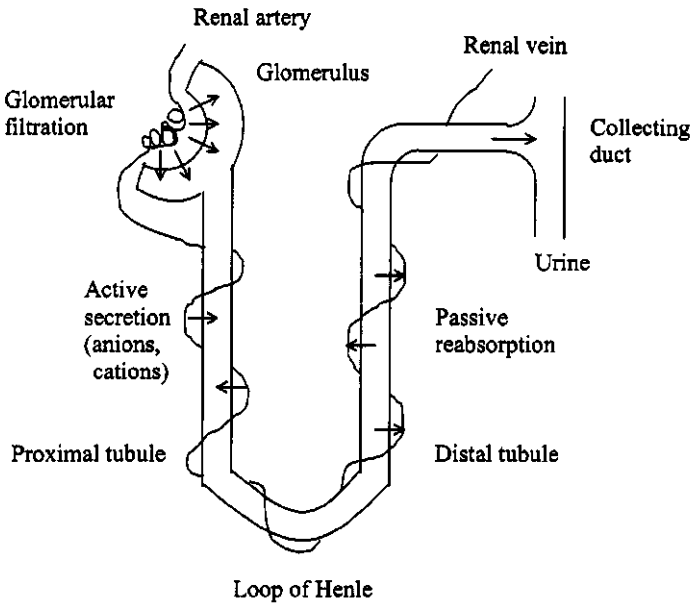

Figure 6.7. A schematic description of renal clearance processes.

diameter can readily pass through the glomeruli with approximately 125 ml of plasma/min in a healthy adult [glomerular filtration rate (GFR)], which is less than 20% of the total renal blood flow rate of 650 to 750 ml/min. This physical filtration process is represented by the Cl_f of a drug. As only unbound drug in the blood can be filtered, Cl_f is a function of both $f_{u,b}$ and GFR [Eq. (6.20)]. Creatinine (endogenous substance) or inulin can be used to assess GFR because of their negligible protein binding, tubular secretion, and reabsorption.

6.3.3.2. Active Secretion

Active secretion of a drug occurs mainly at the proximal tubule by transporters located in the tubular membranes. It has been reported that there are several distinct transporters for various cationic or anionic substrates (Giacomini, 1997). Equation (6.21), which describes Cl_{rs} is similar in principle to the well-stirred hepatic clearance model. The difference is that in the kidney, active secretion clearance is mediated by membrane transporters, whereas hepatic clearance is governed by both metabolizing enzyme and biliary excretion activities. Cl_{rs} is affected by Q_r, $f_{u,b}$, and $Cl_{i,s}$. $Cl_{i,s}$ can be described as T_{max}/K_m, where T_{max} is the maximum capacity of transporter(s) and K_m is the apparent Michaelis–Menten constant under linear conditions.

6.3.3.3. Reabsorption

In general, reabsorption of drug molecules into the renal venous blood occurs mainly at the distal tubule. Owing to reabsorption of the drug from the urine before

it is excreted into the bladder, renal clearance has to be corrected for the fraction of drug (F_r) excreted in urine that is reabsorbed before release into the bladder. The extent of F_r depends on the lipophilicity and ionizability of a drug. As a rule of thumb, reabsorption can be nearly complete for a drug with log $D > 0$. Because this process is considered to be passive and diffusional, urinary pH can also play an important role for the reabsorption of weak acidic or basic drugs, as drug molecules un-ionized at urinary pH are more readily reabsorbed than their ionized counterparts. On an average, urinary pH is close to 6.3. Diet, drugs, and various disease states can alter urine pH. Under forced acidification and alkalinization, urinary pH can vary from approximately 4.4 to 8.2.

6.3.3.4. Renal Metabolism

Although metabolism in the kidney is a minor elimination pathway for most compounds, renal metabolism, especially glucuronidation and amino acid conjugation, appears to play an important role in renal clearance of several drugs, such as zidovudine (Lohr *et al.,* 1998).

NOTE: ESTIMATING RENAL CLEARANCE. Equations (6.19)–6.21) are useful for understanding physiological mechanisms of renal drug elimination; however, they have limited practical value in estimating Cl_r, owing to experimental difficulties in measuring $Cl_{i,s}$ and F_r *in vivo.* The Cl_r of a drug can be determined experimentally *in vivo* without organ perfusion simply by measuring drug concentrations in blood and urine simultaneously. By definition, Cl_r is a proportionality ratio between renal elimination rate of a drug and its concentration in blood.

$$Cl_r = \frac{(\text{Urine flow rate}) \cdot (\text{Drug concentration in urine})}{\text{Drug concentration in blood}} \tag{6.22}$$

One drawback of this approach is that it needs measurement of the *in vivo* urine flow rate, which is difficult to measure accurately. Alternatively, Cl_r can be estimated by dividing the amount of unchanged drug excreted into the urine over an extended period of time (usually over 24 hr after drug administration in small laboratory animals) by AUC_{0-t} in blood, regardless of the route of administration [Eq. (6.23)]. The amount of unchanged drug excreted into the urine can be obtained by the urinary drug concentration multiplied by the volume of urine collected, and, in general, $AUC_{0-t} > 90\%\ AUC_{0-\infty}$ is desirable for a reliable estimate of Cl_r:

$$Cl_r = \frac{\text{Amount of unchanged drug excreted in the urine up to time t}}{AUC_{0-t}} \tag{6.23}$$

The difference between systemic clearance (Cl_s) and Cl_r, both experimentally measured, is called nonrenal clearance (Cl_{nr}), which can be assumed to be equal to Cl_h when the liver is the major clearance organ:

$$Cl_s = Cl_r + Cl_{nr} \tag{6.24}$$

Creatinine clearance and "intact nephron hypothesis": Renal clearance of creatinine, an endogenous end product of muscle metabolism with a narrow range of constant concentrations in the blood, is widely recognized as a reliable indicator of kidney impairment. Plasma protein binding of creatinine is negligible, and its tubular active secretion and reabsorption are also considered minimal. Owing to these characteristics of creatinine, its renal clearance is close to the actual GFR, which ranges from 100 to 125 ml/min in man. It has been observed that creatinine clearance correlates well with the entire kidney function; e.g., creatinine clearance lower than 50 ml/min is indicative of moderate to severe renal failure. These empirical observations suggest that renal impairments do not selectively affect any particular kidney function or cell type, but rather affect the entire nephron. This relationship between creatinine clearance and the functions of the entire nephron is known as "the intact nephron hypothesis."

6.4. RELATIONSHIP BETWEEN SYSTEMIC BLOOD AND ORGAN CLEARANCES

The systemic blood clearance (Cl_b) can equal the sum of the organ clearances by each of the eliminating organs when the following conditions are met: (1) Elimination of the drug from the body occurs solely via the eliminating organs (in a case where the drug is unstable in the blood, systemic blood clearance might be greater than the sum of organ clearances). (2) The eliminating organs except the liver and intestine are anatomically arranged independently of each other:

$$Cl_b = Cl_r + Cl_{h,app} + Cl_{others} \tag{6.25}$$

where Cl_r is the renal clearance, $Cl_{h,app}$ is the (apparent) hepatic clearance, and Cl_{others} is the sum of clearances via other organs, including, e.g., the lung, brain, and muscle (but not the intestine).

For instance, the liver and the kidney neither share a blood vessel nor are they connected to each other, so that the extent of drug clearance by one of them does not affect that by the other. The intestine and the liver are, however, directly connected to each other by a single vein, ie., the portal vein, which makes most of the blood perfusing through the intestine, except blood from the lower part of rectum, flow into the liver as well. Because of this anatomical arrangement of the organs, the extent of apparent hepatic clearance of a drug *in vivo* is also affected by intestinal elimination. In general, it is assumed that the systemic intestinal metabolism, i.e., the elimination of a drug in the systemic circulation by the intestine, is negligible for most compounds owing to the limited diffusibility of compounds across the basement membranes between the splanchnic blood and the enterocytes. When drug molecules in blood are not subject to intestinal metabolism, $Cl_{h,app}$ in Eq. (6.25) becomes the true hepatic clearance of the drug. For those compounds subject to both systemic intestinal and hepatic clearances *in vivo,* $Cl_{h,app}$ becomes the apparent clearance, reflecting both intestinal and hepatic elimination [Eq. (6.26)]. This is because the liver is connected to the intestine via a single vein, which makes both

organs act as one eliminating organ in a kinetic sense (Kwon, 1997):

$$Cl_{h,app} = Cl_g + (1 - E_g) \cdot Cl_h \tag{6.26}$$

where Cl_g is intestinal clearance, E_g is the intestinal extraction ratio of the drug, and Cl_h is hepatic clearance.

6.5. APPARENT CLEARANCE FOLLOWING ORAL DOSING

Apparent clearance following oral dosing (Cl_{po}) of a drug is simply the ratio between an oral dose (D_{po}) and AUC from time zero to infinity after oral administration ($AUC_{po,0-\infty}$), and is sometimes referred to as "oral clearance":

$$Cl_{po} = D_{po}/AUC_{po,0-\infty} \tag{6.27}$$

The Cl_s of a drug is D_{iv}/AUC_{iv}, and if D_{po} and D_{iv} are the same, then

$$Cl_{po} = \frac{AUC_{iv,0-\infty} \cdot Cl_s}{AUC_{po,0-\infty}} = \frac{Cl_s}{F} \tag{6.28}$$

where F is the oral bioavailability. Cl_{po} does not have any particular pharmacokinetic implications other than as the ratio between the systemic clearance and the oral bioavailability, except when the following conditions are met: (a) complete absorption of a drug after oral administration, (b) only hepatic clearance for drug elimination, (c) linear kinetics, and (d) blood clearance.

Under the above conditions, F becomes equal to the drug availability (1 – E) of hepatic clearance, and thus Cl_{po} becomes the same to $f_{u,b} \cdot Cl_{i,h}$ by substituting 1 – E for F. This relationship according to the well-stirred model for hepatic clearance is as follows:

$$Cl_s = \frac{Q \cdot f_{u,b} \cdot Cl_{i,h}}{Q + f_{u,b} \cdot Cl_{i,h}} \quad \text{and} \quad 1 - E = \frac{Q}{Q + f_{u,b} \cdot Cl_{i,h}}$$

Therefore,

$$Cl_{po} = Cl_s/F = f_{u,b} \cdot Cl_{i,h} \tag{6.29}$$

6.6. DISTRIBUTIONAL CLEARANCE

One of the least explored parameters in pharmacokinetics may be distributional clearance (Cl_d). Cl_d reflects the ability of the body to distribute drug molecules from the plasma into the organs or tissues and vice versa (Jusko, 1986), and is a

function of drug permeability (P) across the membrane and the surface area of the membrane(S):

(6.30) $$\boxed{Cl_d(cm^3/sec) = P(cm/sec) \cdot S(cm^2)}$$

Cl_d has the same unit of flow rate, usually cm^3/sec, as any other clearance term.

6.7. BLOOD *VS.* PLASMA CLEARANCES

Estimates of clearance can vary depending on the reference body fluids, such as blood or plasma, for which drug concentrations are measured. For instance, the systemic clearance of a drug, which is referred to as drug concentration in *blood,* is "systemic *blood* clearance," whereas if drug concentrations in *plasma* are used, the clearance becomes "systemic *plasma* clearance."

6.7.1. Blood Clearance

Blood clearance can be viewed as the *actual* volume of blood cleared of a drug per unit time from the entire blood pool in the body (systemic blood clearance) or from the blood pool passing through the eliminating organs (organ blood clearance). The systemic blood clearance is the sum of organ blood clearances.

6.7.2. Plasma Clearance

Plasma clearance does not represent the actual volume of plasma cleared of a drug. It is rather the *apparent* volume of plasma cleared per unit time, reflecting simply the ratio between the rate of drug elimination from the entire body (or the organs) and the drug concentrations in the plasma. Plasma clearance is more widely used than blood clearance, however, because sample preparation and analysis are easier in plasma than in blood. If one wishes to estimate the extraction ratio via the eliminating organ, the plasma clearance value has to be converted to the blood clearance value based on the concentration ratios between the plasma and the blood.

6.7.3. Relationship between Blood and Plasma Clearances

For most pharmacokinetic applications, it does not matter much whether clearance measurements are based on blood or plasma drug concentrations. However, this does become important when clearance estimates are compared directly with an organ blood flow rate to obtain the extraction ratio of the organ clearance. In this case, blood clearance must be used because the organ clearance relates to organ *blood* flow rate, not *plasma* flow rate, and the extraction ratio is measured based on differences in drug concentrations in *blood,* not plasma, between entering and leaving the organ. A direct comparison of a plasma clearance value with the blood flow rate of the eliminating organ to assess the organ's drug extraction ratio is, therefore, incorrect, unless both the plasma and the blood drug concentrations are

the same. In general, blood clearance is considered a more appropriate measure of organ function than plasma clearance whenever physiological implications of clearance are considered. The relationships between blood clearance (Cl_b) and plasma clearance (Cl_p) can be obtained from the definition of clearance, which is the ratio between the rate of drug elimination (dA/dt) and drug concentrations in the reference fluid, blood (C_b), or plasma (C_p):

$$Cl_b = \frac{dA/dt}{C_b(t)} \quad \text{and} \quad Cl_p = \frac{dA/dt}{C_p(t)}$$

Therefore,

(6.31) $$\boxed{Cl_b \cdot C_b = Cl_p \cdot C_p}$$

6.7.4. Relationship between Blood and Plasma Concentrations

Relationships between blood and plasma drug concentrations and unbound drug concentration are illustrated in Fig. 6.8 and can be described by the following equation:

(6.32) $$\boxed{C_b = Hct \cdot C_r + (1 - Hct) \cdot C_p}$$

where C_b, C_p, and C_r are the drug concentrations in the blood, plasma, and red blood cells, respectively, and Hct is the hematocrit (a ratio of the volume of erythrocytes to the volume of blood, which is usually 0.4–0.5).

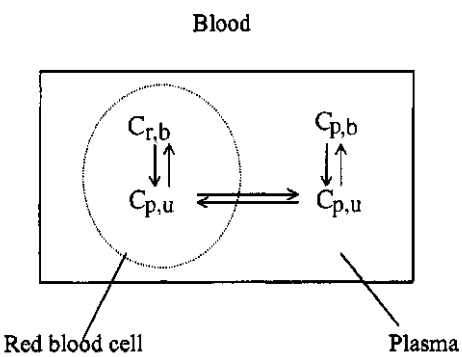

Figure 6.8. Diagram showing drug concentrations in different blood components. The unbound drug concentration in plasma is the same as that inside the red blood cells, if drug transport across the red blood cell membranes occurs via passive dffusion: $C_{p,b}$: concentration of drug bound to plasma proteins; $C_{p,u}$: concentration of drug not bound to plasma proteins; and $C_{r,b}$: concentration of drug bound to red blood cell components.

If drug binding is more extensive to red cells than to plasma proteins, $C_b/C_p > 1$, and if there is stronger binding to plasma proteins, $C_b/C_p < 1$. Plasma clearance is often found to be greater than the blood flow rate of the eliminating organ(s) such as the liver, which is the upper limit of the organ clearance. This may indicate that the drug partitions more to red blood cells than to plasma, and drug molecules bound to red blood cells are also available for extraction by the organ. C_b/C_p ratios of most drugs are between 0.8–1.2. It is, therefore, reasonable to assume that Cl_p of drugs is generally similar to Cl_b, and the extent of binding to plasma proteins or red blood cells for most drugs within the therapeutic window is independent of drug concentration.

6.7.5. Clearance Based on Unbound Drug Concentration in Plasma

Occasionally, it is observed that the plasma clearance increases with the drug concentration. This apparent concentration-dependent clearance may be simply due to nonlinear protein binding. In this case, clearance measurements based on unbound drug concentration in plasma (Cl_u) should remain unchanged, regardless of the drug concentration. The relationship between Cl_p and Cl_u is

(6.33) $$\boxed{Cl_p \cdot C_p = Cl_u \cdot C_u}$$

Since C_u equals $f_u \cdot C_p$ and f_u is a ratio between the unbound and total drug concentrations in plasma, Cl_p equals $Cl_u \cdot f_u$. It is important to note that Cl_u does not have any particular physiological meaning and should be viewed simply as a proportionality constant between the elimination rate of a drug from the body and the unbound drug concentrations in plasma.

6.7.6. Relationship among Blood, Plasma, and Unbound Drug Clearances

From Eqs. (6.31) and (6.33) (Tozer, 1981), the following relationship can be established:

(6.34) $$\boxed{Cl_b \cdot C_b = Cl_p \cdot C_p = Cl_u \cdot C_u}$$

For example, Cl_p of 33.3 ml/min/kg does not mean that 33.3 ml of plasma is cleared of drug per min per kg of body weight. Rather, it shows that 33.3 ml of *apparent* volume of plasma, which is equal to Cl_b, ie., the *actual* volume of blood cleared of drug per minute in the entire body per kg body weight, multiplied by C_b/C_b, a ratio of drug concentrations between the blood and the plasma.

REFERENCES

Davies B. and Morris T., Physiological parameters in laboratory animals and humans, *Pharm. Res.* **10:** 1093–1095, 1993.

Giacomini K. M., Membrane transporters in drug disposition, *J. Pharmacokinet. Biopharm.* **25:** 731–-741, 1997.

Gibaldi M., The basic concept: clearance, *J. Clin. Pharmacol.* **26:** 330–331, 1986.

Houston J. B., Utility of *in vitro* drug metabolism data in predicting *in vivo* metabolic clearance, *Biochem. Pharmacol.* **47:** 1469–1479, 1994.

Jusko W. J., Guidelines for collection and analysis of pharmacokinetic data, in W. E. Evans, J. J. Schentag, and W. J. Jusko (eds.), *Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring,* 2nd ed., Applied Therapeutics, Washington, 1986, pp. 19–37, 1986.

Kwon Y., Effects of diffusional barriers on the extent of presystemic and systemic intestinal elimination of drugs, *Arch. Pharm. Res.* **20:** 24-28, 1997.

Lohr J. W. *et* al., Renal drug metabolism, *Pharmacol. Rev.* **50:** 107–141, 1998.

Pang K. S. and Rowland M., Hepatic clearance of drugs: I. Theoretical considerations of a "well-stirred" model and a "parallel tube" model. Influence of hepatic blood flow rate, plasma and blood cell binding, and the hepatocellular enzymatic activity on hepatic drug clearance, *J. Pharmacokinet. Biopharm.* **5:** 625–654, 1977.

Roberts M. S. and Rowland M., Hepatic elimination-dispersion model, *J. Pharm. Sci.* **74:** 585–587, 1985.

Roberts M. S. and Rowland M., A dispersion model of hepatic elimination: 1. Formulation of the model and bolus consideration, *J. Pharmacokinet. Biopharm.* **14** 227–260, 1986.

Tozer T. N., Concepts basic to pharmacokinetics, *Pharmacol. Ther.* **12:** 109–131, 1981.

Wilkinson G. R., Clearance approaches in pharmacology, *Pharmacol. Rev.* **39:** 1–47, 1987.