Biopharmaceutics and Clinical Pharmacokinetics

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Introduction to Pharmacokinetics

Advancements in biopharmaceutics have come about largely through the development and application of pharmacokinetics. Pharmacokinetics is the study and characterization of the time course of drug absorption, distribution, metabolism, and excretion, and the relationship of these processes to the intensity and time course of therapeutic and toxicologic effects of drugs. Pharmacokinetics is used in the clinical setting to enhance the safe and effective therapeutic management of the individual patient. This application has been termed *clinical pharmacokinetics*.

DISTRIBUTION AND ELIMINATION

The transfer of a drug from its absorption site to the blood, and the various steps involved in the distribution and elimination of the drug in the body, are shown in schematic form in Figure 1–1. In the blood, the drug distributes rapidly between the plasma and erythrocytes (red blood cells). Rapid distribution of drug also occurs between the plasma proteins (usually albumin but sometimes α_1-acid glycoproteins and occasionally globulin) and plasma water. Since most drugs are relatively small molecules they readily cross the blood capillaries and reach the extracellular fluids of almost every organ in the body. Most drugs are also sufficiently lipid soluble to cross cell membranes and distribute in the intracellular fluids of various tissues. Throughout the body there is a distribution of drug between body water and proteins or other macromolecules that are dispersed in the body fluids or are components of the cells.

The body can be envisioned as a collection of separate compartments, each containing some fraction of the administered dose. The transfer of drug from one compartment to another is associated with a rate constant (k). The magnitude of the rate constant determines how fast the transfer occurs.

The transfer of drug from blood to extravascular fluids (i.e., extracellular and intracellular water) and tissues is called *distribution*. Drug distribution is usually a rapid and reversible process. Fairly quickly after intravenous (iv) injection, drug in the plasma exists in a distribution equilibrium with drug in the erythrocytes, in other body fluids, and in tissues. As a consequence of this dynamic equilibrium, changes in the concentration of drug in the plasma are indicative of changes in drug level in other tissues including sites of pharmacologic effect (bioreceptors).

The transfer of drug from the blood to the urine or other excretory compartments (i.e., bile, saliva, and milk), and the enzymatic or biochemical transformation *(metabolism)* of drug in the tissues or plasma to metabolic products, are usually irreversible processes. The net result of these irreversible steps, depicted in Figure 1–1, is called *drug elimination*. Elimination processes are responsible for the physical or biochemical removal of drug from the body.

The moment a drug reaches the bloodstream, it is subject to both distribution and elimination. The rate constants associated with distribution, however, are usually much larger than those related to drug elimination. Accordingly, drug distribution throughout the body is usually complete while most of the dose is still in the body. In fact, some drugs attain distribution equilibrium before virtually any of the dose is eliminated. In such cases, the body appears to have the characteristics of a single compartment.

This simplification, however, may not be applied to all drugs. For most drugs, concentrations in plasma measured shortly after iv injection reveal a

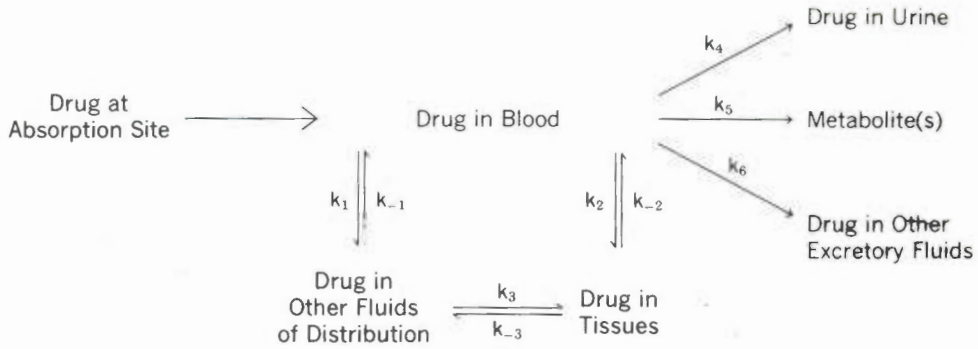


Fig. 1–1. Schematic representation of drug absorption, distribution, and elimination.

distinct distributive phase. This means that a measurable fraction of the dose is eliminated before attainment of distribution equilibrium. These drugs impart the characteristics of a multicompartment system upon the body. No more than two compartments are usually needed to describe the time course of drug in the plasma. These are often called the rapidly equilibrating or central compartment and the slowly equilibrating or peripheral compartment.

PHYSICAL SIGNIFICANCE OF DRUG CONCENTRATION IN PLASMA

Blood samples taken shortly after intravenous administration of equal doses of two drugs may show large differences in drug concentration despite the fact that essentially the same amount of each drug is in the body. This occurs because the degree of distribution and binding is a function of the physical and chemical properties of a drug and may differ considerably from one compound to another.

At distribution equilibrium, drug concentrations in different parts of the body are rarely equal. There may be some sites such as the central nervous system or fat that are poorly accessible to the drug. There may be other tissues that have a great affinity for the drug and bind it avidly. Drug concentrations at these sites may be much less than or much greater than those in the plasma.

Despite these complexities, once a drug attains distribution equilibrium its concentration in the plasma reflects distribution factors and the simple relationship between amount of drug in the body (A) and drug concentration in the plasma (C) shown in Equation 1–1 applies:

$$A = VC \qquad (1–1)$$

The proportionality constant relating amount and concentration is called the apparent volume of distribution (V). In most situations, V is independent of drug concentration. Doubling the amount of drug in the body (e.g., by doubling the iv dose) usually results in a doubling of drug concentration in plasma. This is called *dose proportionality;* it is often used as an indicator of *linear pharmacokinetics*.

The apparent volume of distribution is usually a characteristic of the drug rather than of the biologic system, although certain disease states and other factors may bring about changes in V. The magnitude of V rarely corresponds to plasma volume, extracellular volume, or the volume of total body water; it may vary from a few liters to several hundred liters in a 70-kg man. V is usually not an anatomic volume but is a reflection of drug distribution and a measure of the degree of drug binding.

Acid drugs, such as sulfisoxazole, tolbutamide, or warfarin, are often preferentially bound to plasma proteins rather than extravascular sites. Although these drugs distribute throughout body water, they have small volumes of distribution ranging from about 10 to 15 L in man. A given dose will result in relatively high initial drug concentrations in plasma.

On the other hand, many basic drugs including amphetamine, meperidine, and propranolol are more extensively bound to extravascular sites than to plasma proteins. The apparent volumes of distribution of these drugs are large, ranging from 4 to 8 times the volume of total body water (i.e., 180 to 320 L in a 70-kg man). The frequently small doses and large distribution volumes of these drugs often make their quantitative detection in plasma difficult.

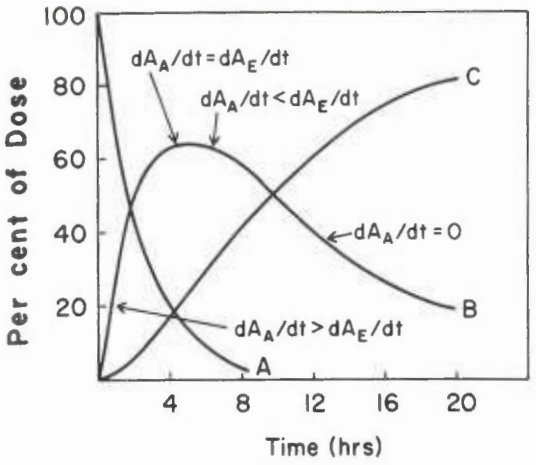


Fig. 1–2. Time course of drug disappearance from the absorption site (curve A) and appearance of eliminated drug in all forms (curve C). The net result is curve B, which depicts the time course of drug in the body.

PHARMACOKINETIC CONSIDERATIONS OF DRUG CONCENTRATIONS IN PLASMA

The plasma contains measurable quantities of many endogenous chemicals. In healthy individuals these biochemicals are present in concentrations that are reasonably constant, and it is appropriate to speak of creatinine or bilirubin levels in the plasma. Drug levels or concentrations in the plasma are rarely level. One usually finds different concentrations of drug in the plasma at different times after administration. These changes reflect the dynamics of drug absorption, distribution, and elimination (Fig. 1–2).

Intravenous Administration

Absorption need not be considered when a drug is given by rapid iv injection. As soon as the drug is administered it undergoes distribution and is subject to one or more elimination pathways. The amount of drug in the body and the drug concentration in plasma decrease continuously after injection. At the same time, there is continuous formation of metabolites and continuous excretion of drug and metabolites. Eliminated products accumulate while drug levels in the body decline.

Most drugs distribute rapidly so that shortly after iv injection, distribution equilibrium is reached. Drug elimination at distribution equilibrium is usually described by *first-order kinetics*. This means that the rate of the process is proportional to the amount or concentration of substrate (drug) in the system. As drug concentration falls, the elimination rate falls in parallel. The proportionality constant relating rate and amount or concentration is called a rate constant. Accordingly, the elimination rate is written as follows:

$$-\frac{dA}{dt} = \frac{dA_E}{dt} = kA \qquad (1–2)$$

where A is the amount of drug in the body at time t, A_E is the amount of drug eliminated from the body (i.e., the sum of the amounts of metabolites that have been formed and the amount of drug excreted) at time t, and k is the first-order elimination rate constant.

The elimination rate constant is the sum of individual rate constants associated with the loss of parent drug. For example, the overall elimination rate constant (k) in the model depicted in Figure 1–1 is given by

$$k = k_4 + k_5 + k_6 \qquad (1–3)$$

Dimensional analysis of Equation 1–2 indicates that the units of k are reciprocal time (i.e., day^{-1}, hr^{-1}, or min^{-1}).

Since there is a relationship between the amount of drug in the body and the drug concentration in the plasma (Eq. 1–1), we may rewrite Equation 1–2 as

$$-\frac{d(VC)}{dt} = -V\frac{dC}{dt} = k(VC)$$

or

$$-\frac{dC}{dt} = kC \qquad (1–4)$$

Integrating this expression between the limits t = 0 and t = t yields

$$\log C = \log C_o - \frac{kt}{2.303} \qquad (1–5)$$

Equation 1–5 indicates that a plot of log C versus t will be linear once distribution equilibrium is reached. The term C_o is the intercept on the log concentration axis, on extrapolation of the linear segment to t = 0.

Figure 1–3 shows the average concentration of a semisynthetic penicillin in the plasma as a function of time after an intravenous injection of a 2-g dose. The concentration values are plotted on a log scale; the corresponding times are plotted on a linear scale. The semilogarithmic coordinates make it convenient to plot first-order kinetic data for they

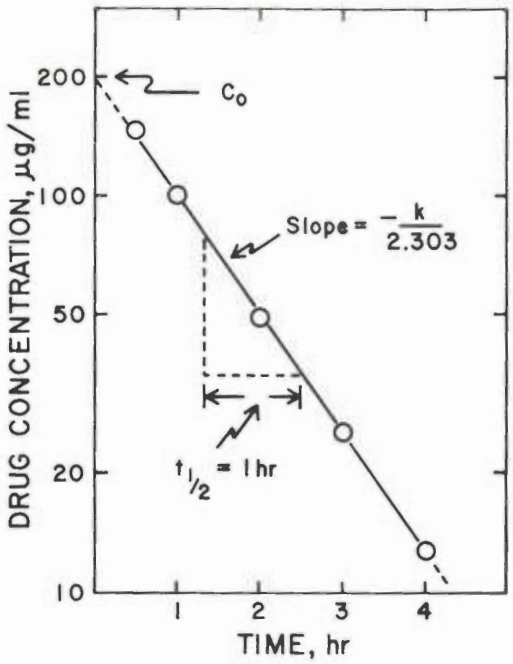


Fig. 1–3. Semilogarithmic plot of penicillin concentrations in plasma after a 2-g intravenous dose. Concentrations decline in a first-order manner with a half-life of 1 hr.

avoid the necessity of converting values of C to log C.

According to Equation 1–5, the linear portion of the semilogarithmic plot of C versus t has a slope corresponding to $-k/2.303$ and an intercept, on the y-axis (i.e., at $t = 0$), corresponding to C_o. If a drug were to distribute almost immediately after injection, C_o would be a function of the dose and the apparent volume of distribution. Therefore, we would be able to calculate V as follows:

$$V = \frac{\text{iv dose}}{C_o} \qquad (1–6)$$

For the data shown in Figure 1–3 we can determine that $C_o = 200$ mg/ml and that $V = 10$ L.

This approach, however, is seldom useful; Equation 1–6 usually gives a poor estimate of V, always larger and sometimes substantially larger than the true volume of distribution. Equation 1–6 assumes that drug distribution is immediate, whereas most drugs require a finite time to distribute throughout the body space. Other methods to calculate V will be described subsequently.

Although it is possible to calculate the elimination rate constant from the slope of the line, it is much easier to determine k by making use of the following relationship:

$$k = 0.693/t_{1/2} \qquad (1–7)$$

where $t_{1/2}$ is the half-life of the drug (i.e., the time required to reduce the concentration by 50%). This parameter is determined directly from the plot (see Fig. 1–3). In a first-order process, the half-life is independent of the dose or initial plasma concentration. One hour is required to observe a 50% decrease of any plasma concentration of the semisynthetic penicillin, once distribution equilibrium is attained. It follows that the elimination rate constant of this drug is equal to $0.693/t_{1/2}$ or 0.693 hr^{-1}. Knowledge of the half-life or elimination rate constant of a drug is useful because it provides a quantitative index of the persistence of drug in the body. For a drug that distributes very rapidly after iv injection and is eliminated by first-order kinetics, one-half the dose will be eliminated in one half-life after administration; three-quarters of the dose will be eliminated after two half-lives. Only after four half-lives will the amount of drug in the body be reduced to less than one-tenth the dose. For this reason, the half-life of a drug can often be related to the duration of clinical effect and the frequency of dosing.

Short-Term Constant Rate Intravenous Infusion

Few drugs should be given as a rapid intravenous injection (bolus) because of the potential toxicity that may result. Many drugs that require intravenous administration, including theophylline, procainamide, gentamicin, and many other antibiotics, are given as short-term constant rate infusions over 5 to 60 min, or longer. The following scheme describes this situation:

$$\text{Drug in reservoir} \xrightarrow{\text{Constant rate}} \text{Drug in body} \xrightarrow{k} \text{Eliminated drug}$$

The rate of change of the amount of drug in the body (A) during infusion is given by

$$dA/dt = k_o - kA \qquad (1–8)$$

where k_o is the infusion rate expressed in amount per unit time (e.g., mg/min), kA is the elimination rate, and k is the first-order elimination rate constant. This relationship assumes that the drug reaches distribution equilibrium quickly. Integrating Equation 1–8 from $t = 0$ to $t = t$ yields

$$A = k_o[1 - \exp(-kt)]/k \qquad (1–9)$$

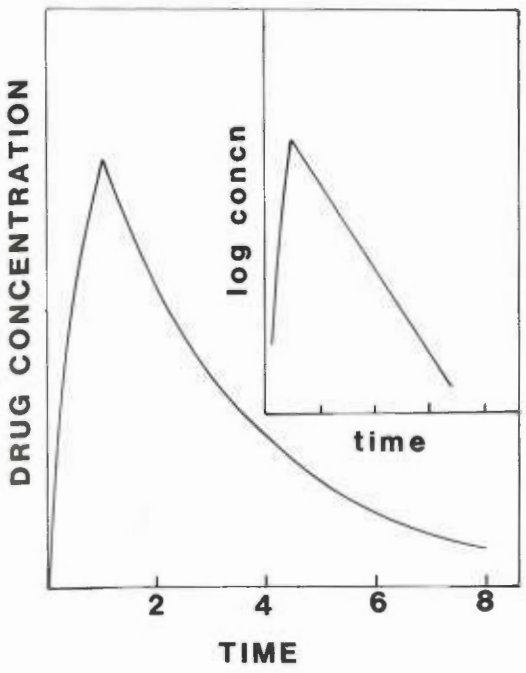


Fig. 1–4. Drug concentration in plasma during and after a 1-hr constant rate intravenous infusion. The inset shows the same data, plotted on semilogarithmic coordinates.

or

$$C = k_o[1 - \exp(-kt)]/kV \qquad (1–10)$$

According to Equation 1–10, drug concentration in plasma increases during infusion. When the entire dose has been infused at time T, drug concentration reaches a maximum given by

$$C_{max} = k_o[1 - \exp(-kT)]/kV \qquad (1–11)$$

and thereafter declines. The declining drug concentration is described by

$$C = C_{max} \exp(-kt') \qquad (1–12)$$

or

$$\log C = \log C_{max} - (kt'/2.303) \qquad (1–13)$$

where $t' = t - T$. Equations 1–12 and 1–13 apply when distribution equilibrium is essentially reached by the end of the infusion. A semilogarithmic plot of C (post-infusion drug concentration in plasma) versus t′ yields a straight line, from which the half-life and elimination rate constant can be estimated. The entire drug concentration-time profile during and after a short-term infusion is shown in Figure 1–4.

Equation 1–11 may be arranged to calculate V, since all other terms are known. This estimate may be less than accurate but it is always better than that provided by Equation 1–6.

The maximum or peak drug concentration in plasma is always lower after intravenous infusion than after bolus injection of the same dose. The more slowly a fixed dose of a drug is infused, the lower the value of C_{max}. Consider a rapidly distributed drug with a half-life of 3 hr. A given dose administered as an iv bolus results in an initial plasma level of 100 units. The same dose, infused over 3 hr ($T = t_{1/2}$) gives a C_{max} value of 50 units ($C_{max}/2$); infused over 6 hr ($T = 2t_{1/2}$), it gives a concentration of 25 units ($C_{max}/4$). Also, since C_{max} is a linear function of k_o, doubling the infusion rate and infusing over the same period of time (i.e., doubling the dose) doubles the maximum concentration.

Extravascular Administration

A more complex drug concentration-time profile is observed after oral, intramuscular, or other extravascular routes of administration because absorption from these sites is not instantaneous, nor does it occur at a constant rate. As shown in Figure 1–2, the rate of change of the amount of drug in the body (dA/dt) is a function of both the absorption rate (dA_A/dt) and the elimination rate (dA_E/dt); that is,

$$\frac{dA}{dt} = \frac{dA_A}{dt} - \frac{dA_E}{dt} \qquad (1–14)$$

or

$$\frac{dC}{dt} = \frac{1}{V}\left[\frac{dA_A}{dt} - \frac{dA_E}{dt}\right] \qquad (1–15)$$

where V is the apparent volume of distribution. When the absorption rate is greater than the elimination rate (i.e., $dA_A/dt > dA_E/dt$), the amount of drug in the body and the drug concentration in the plasma increase with time. Conversely, when the amount of drug remaining at the absorption site is sufficiently small so that the elimination rate exceeds the absorption rate (i.e., $dA_E/dt > dA_A/dt$), the amount of drug in the body and the drug concentration in the plasma decrease with time. The maximum or peak concentration after drug administration occurs at the moment the absorption rate equals the elimination rate (i.e., $dA_A/dt = dA_E/dt$). The faster a drug is absorbed, the higher is the maximum concentration in plasma after a given

dose, and the shorter is the time after administration when the peak is observed.

First Order In—First Order Out

Many drugs appear to be absorbed in a first-order fashion and the following scheme often applies:

$$\text{Drug at absorption site} \xrightarrow{k_a} \text{Drug in body} \xrightarrow{k} \text{Eliminated drug}$$

Under these conditions

$$dA/dt = k_a A_A - kA \qquad (1\text{–}16)$$

where k_a is the apparent first-order absorption rate constant, k is the first-order elimination rate constant, A is the amount of drug in the body, and A_A is the amount of drug at the absorption site. Integrating Equation 1–16 from t = 0 to t = t and converting amounts to concentrations results in the complicated equation shown below:

$$C = k_a FD[\exp(-kt) - \exp(-k_a t)]/V(k_a - k) \qquad (1\text{–}17)$$

where F is the fraction of the administered dose (D) that is absorbed and reaches the bloodstream, V is the apparent volume of distribution, and C is the drug concentration in plasma any time after administration. Equation 1–17 is often used to describe drug concentrations in plasma after extravascular administration.

The absorption rate constant of a drug is frequently larger than its elimination rate constant. In this case, at some time after administration, the absorption rate term in Equation 1–15 approaches zero, indicating that there is no more drug available for absorption, and Equation 1–17 simplifies to

$$C = k_a FD[\exp(-kt)]/V(k_a - k) \qquad (1\text{–}18)$$

or

$$C = C_o^* \exp(-kt) \qquad (1\text{–}19)$$

and

$$\log C = \log C_o^* - \frac{kt}{2.303} \qquad (1\text{–}20)$$

Equation 1–18 assumes that distribution equilibrium is essentially reached by the end of the absorption phase.

When absorption is complete, the rate of change of the amount of drug in the body equals the elimination rate, and Equation 1–15 reduces to Equation 1–4. The portion of a drug concentration in the plasma versus time curve, commencing at the time absorption has ceased, is called the postabsorptive phase. During this phase, the decline in drug concentration with time follows first-order-kinetics. A semilogarithmic plot of drug concentration in the plasma versus time after oral or other extravascular routes of administration usually shows a linear portion that corresponds to the postabsorptive phase. A typical plot is shown in Figure 1–5; the slope of the line is equal to $-k/2.303$.

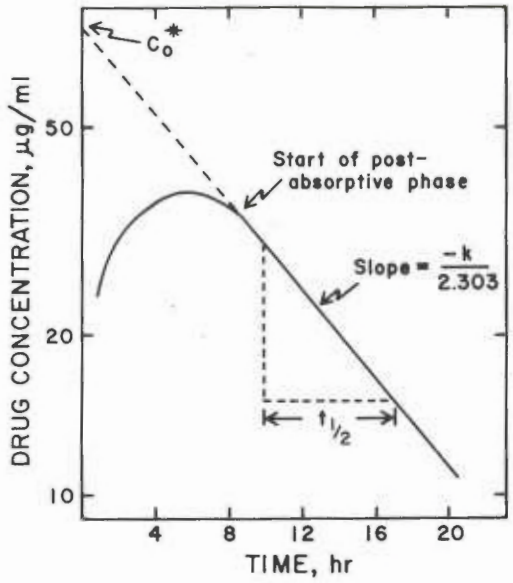
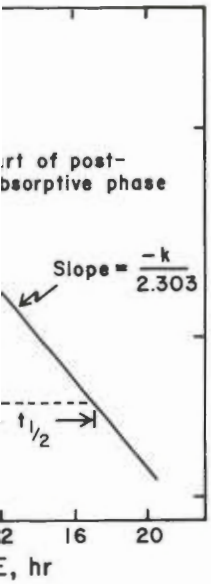


Fig. 1–5. Typical semilogarithmic plot of drug concentration in plasma following oral or intramuscular administration of a slowly absorbed form of the drug.

The intercept of the extrapolated line (C_o^*) is a complex function of absorption and elimination rate constants, as well as the dose or amount absorbed and the apparent volume of distribution. It is incorrect to assume that the intercept approximates the ratio of dose to volume of distribution unless the drug is very rapidly and completely absorbed, and displays one-compartment characteristics (i.e., distributes immediately). This rarely occurs.

Occasionally, the absorption of a drug is slower than its elimination, a situation that may be found with drugs that are rapidly metabolized or excreted and with drugs that are slowly absorbed because of poor solubility or administration in a slowly releasing dosage form. When this occurs, a semilogarithmic plot of drug concentration versus time



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(see Fig. 1–5) after oral administration cannot be used to estimate k or half-life because the slope is related to the absorption rate constant rather than the elimination rate constant. The drug must be administered in a more rapidly absorbed form or given intravenously.

Patient-To-Patient Variability

The time course of drug in the plasma after administration of a fixed dose may show considerable intersubject variability. The variability after intravenous administration is due to differences between patients in distribution and elimination of the drug. These differences may be related to disease or concomitant drug therapy or they may be genetic in origin. Variability is greater after intramuscular administration because, in addition to differences in distribution and elimination, absorption may be variable. Differences in absorption rate after intramuscular injection have been related to the site of injection and the drug formulation. Still greater variability may be found after oral administration. The absorption rate of a drug from the gastrointestinal tract varies with the rate of gastric emptying, the time of administration with respect to meals, the physical and chemical characteristics of the drug, and the dosage form, among other factors. Similarly, the amount of an oral dose of a drug that is absorbed depends on biologic, drug, and dosage form considerations. Many commonly used drugs are less than completely available to the bloodstream after oral administration because of incomplete absorption or presystemic metabolism.

Absorption Rate and Drug Effects

The influence of absorption on the drug concentration-time profile is shown in Figure 1–6. Administration of an equal dose in three different dosage forms results in different time courses of drug in the plasma. The faster the drug is absorbed, the greater is the peak concentration and the shorter is the time required after administration to achieve peak drug levels.

Many drugs have no demonstrable pharmacologic effect or do not elicit a desired degree of pharmacologic response unless a minimum concentration is reached at the site of action. Since a distribution equilibrium exists between blood and tissues, there must be a minimum therapeutic drug concentration in the plasma that corresponds to, though may not equal, the minimum effective concentration (MEC) at the site of pharmacologic effect. Thus, the absorption rate of a drug after a single dose may affect the clinical response. For example, it is evident from Figure 1–6 that the more rapid the absorption rate, the faster is the onset of response. The drug is absorbed so slowly from dosage form C that the minimum effective level is never attained. No effect is observed after a single dose, but effects may be seen after multiple doses.

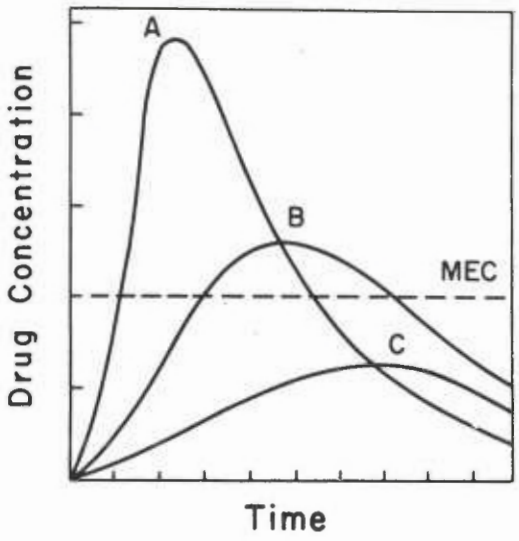


Fig. 1–6. The effects of absorption rate on drug concentration-time profile. The same amount of drug was given orally with each dosage form. The drug is absorbed most rapidly from dosage form A. Drug absorption after administration of dosage form C is slow and possibly incomplete. The dotted line represents the minimum effective concentration (MEC) required to elicit a pharmacologic effect.

The intensity of many pharmacologic effects is a function of the drug concentration in the plasma. The data in Figure 1–6 suggest that administration of dosage form A may evoke a more intense pharmacologic response than that observed after administration of dosage form B since A produces a higher concentration of drug. When dosage form C is considered, it is clear that an active drug may be made to appear inactive by administering it in a form that results in slow or incomplete absorption.

BIOAVAILABILITY

The bioavailability of a drug is defined as its rate and extent of absorption. Rapid and complete absorption is usually desirable for drugs used on an acute or "as needed" basis for pain, allergic re-

sponse, insomnia, or other conditions. As suggested in Figure 1–6, the more rapid the absorption, the shorter is the onset and the greater is the intensity of pharmacologic response. The efficacy of a single dose of a drug is a function of both the rate and extent of absorption. In such cases, there is no assurance of the bioequivalence of two dosage forms of the same drug simply because the amount of drug absorbed from each is equivalent; the absorption rate of drug from each drug product must also be comparable. Rapid absorption may also reduce the frequency and severity of gastrointestinal distress observed after oral administration of certain drugs, including aspirin and tetracycline, by reducing the contact time in the gastrointestinal tract.

Usually, a useful estimate of the relative absorption rate of a drug from different drug products or under different conditions (e.g., with food or without food) can be made by comparing the magnitude and time of occurrence of peak drug concentrations in the plasma after a single dose.

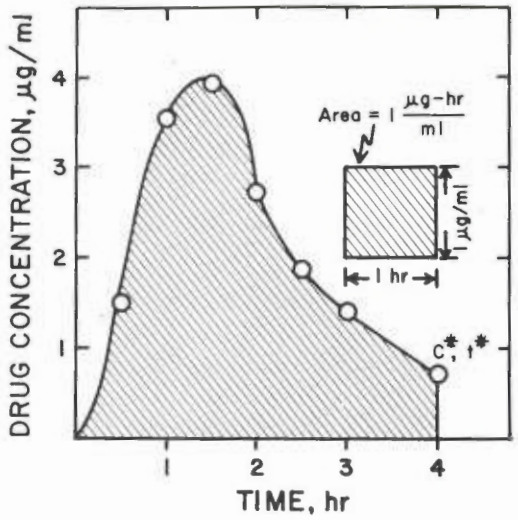


Fig. 1–7. Typical rectilinear plot of drug concentration in the plasma following an oral dose. The area under the concentration-time plot from t = 0 to t = 4 hrs is denoted by shading.

Estimating the Extent of Absorption

The extent of absorption or relative extent of absorption of a drug from a product can be estimated by comparing the total area under the drug concentration in plasma versus time curve (AUC), or the total amount of unchanged drug excreted in the urine after administration of the product to that found after administration of a standard. The standard may be an intravenous injection, an orally administered aqueous or water-miscible solution of the drug, or even another drug product accepted as a standard. When an iv dose is used as the standard and the test product is given orally (or via some other extravascular route), we determine absolute bioavailability. If, following equal doses of the test product and the iv standard, the AUC values are the same, we conclude that the drug in the test product is completely absorbed and not subject to presystemic metabolism.

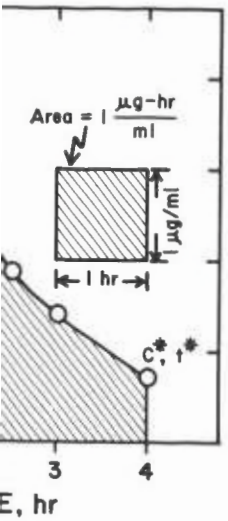
Frequently, however, the standard is an oral solution or an established product. If, following equal doses of the test product and standard, the AUC values are the same, we conclude that the test product is 100% bioavailable, *relative* to the standard; we need use the word relative because we do not know *a priori* that the standard is completely absorbed or completely available. When two products produce the same peak concentration of drug in plasma and the same AUC, the products are *bioequivalent*.

The area under a drug concentration in the plasma versus time curve has the units of concentration-time (e.g., μg − hr/ml), and can be estimated by several methods. One method is to use a planimeter, an instrument for mechanically measuring the area of plane figures. Another procedure, known as the "cut and weigh method," is to cut out the area under the entire curve on rectilinear graph paper and to weigh it on an analytical balance. The weight thus obtained is converted to the proper units by dividing it by the weight of a unit area of the same paper (Fig. 1–7). The most common method of estimating area under curves is by means of the trapezoidal rule, which is described in Appendix I.

Sometimes, single dose bioavailability studies are not carried out long enough to allow drug concentrations to fall to negligible levels. We cannot determine directly the total AUC, only the partial AUC. In this case, a widely used method is to determine the AUC from t = 0 to the last sampling time (t*), by means of the trapezoidal rule, and to estimate the missing area by means of the equation

$$\text{Area from } t^* \text{ to } \infty = C^*/k \qquad (1\text{–}21)$$

where C* is the drug concentration at t = t*, and k is the apparent first-order elimination rate con-



t of drug concentration in
. The area under the con-
o t = 4 hrs is denoted by

the products are *bio-*

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s the units of concen-
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One method is to use
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$$_{o} = C^*/k \qquad (1–21)$$

tration at t = t*, and
elimination rate con-

stant. This area must be added to the area calculated from time zero to t* to obtain the total area under the curve.

The total area under the drug level-time curve for drugs eliminated by first-order kinetics is given by

$$AUC = \frac{\text{Amount of drug reaching the bloodstream}}{k \cdot V} \qquad (1–22)$$

It follows that the bioavailability (F) of a drug from a drug product may be determined from the expression

$$F = \frac{(AUC)_{\text{Drug product}}}{(AUC)_{\text{Standard}}} \qquad (1–23)$$

when equal doses are administered. If different doses of the product and standard are given, the area estimates should be scaled appropriately to permit comparison under conditions of equivalent doses, assuming AUC is proportional to dose.

The amount of drug excreted unchanged in the urine (A_u) after administration is given by

$$A_u = F \cdot \text{Dose} \cdot (k_u/k) \qquad (1–24)$$

where k_u is the urinary excretion rate constant and k is the overall elimination rate constant. It follows that the fraction of the dose absorbed from a drug product relative to that absorbed from a standard may be calculated from the expression

$$F = \frac{(A_u)_{\text{Drug product}}}{(A_u)_{\text{Standard}}} \qquad (1–25)$$

The usefulness of Equation 1–25 depends on how much of the drug is eliminated by urinary excretion, the sensitivity of the assay for drug in urine, and the variability in urinary output of the drug. Many drugs are extensively metabolized and little, if any, appears unchanged in the urine. In such cases, bioavailability is estimated from plasma concentration data.

CONTINUOUS DRUG ADMINISTRATION

Most drugs are administered in a constant dose given at regular intervals for prolonged periods of time. For some of these drugs a therapeutic plasma concentration range has been identified. By prescribing a drug in an appropriate dosing regimen, the physician hopes to elicit a prompt and adequate clinical response. This is often predicated upon the prompt attainment of adequate drug concentration in the plasma.

Constant Rate Infusion

It is convenient to consider first the simpler case of continuous administration of a drug by intravenous infusion; this method of drug administration results in a plasma concentration-time profile that is similar in many ways to that found on intermittent repetitive dosing. Figure 1–8 illustrates the time course of drug concentration in plasma during and after infusion at a constant rate. At the outset, drug concentration increases gradually but at a diminishing rate. If infusion is continued, drug concentration eventually reaches a plateau or steady state. A steady state is reached because the amount of drug in the body reaches a level where the elimination rate, given by kA, is equal to the infusion rate (k_o). Whenever input rate equals output rate, dA/dt = 0, dC/dt = 0, and steady state exists.

By considering Equation 1–10, which describes drug concentration in plasma during constant rate infusion, at times that are sufficiently large so that exp(−kt) approaches zero, drug concentration at steady state (C_{ss}) is given by

$$C_{ss} = k_o/kV \qquad (1–26)$$

Since attainment of steady state often represents the stabilization of a patient on a given course of therapy, it is of interest to know how long it takes to reach steady state. For drugs with pharmacokinetic characteristics that can be described by a one-compartment model (i.e., drugs that distribute rapidly) we have a relatively simple relationship between attainment of steady state and the half-life of the drug. One half the steady-state concentration is reached within a period of time equal to the half-life of the drug. Following a period of infusion equal to four times the half-life, the plasma concentration is within 10% of the eventual steady-state concentration.

If the time to reach steady-state represents an unacceptable delay, one may wish to use an iv bolus loading dose or a series of iv bolus minidoses before starting the infusion. The loading dose is estimated from the ratio of infusion rate (k_o) to elimination rate constant (k). This approach works well for most drugs given intravenously.

If one knows the drug level (C_{ss}) needed to produce a satisfactory response, Equation 1–26 can be used to calculate the infusion rate (k_o) needed to reach the desired level. Under these conditions, $k_o = C_{ss} \cdot k \cdot V$ and loading dose = $C_{ss} \cdot V$.

2

Compartmental and Noncompartmental Pharmacokinetics

The basic principles outlined in Chapter 1 are useful for many drugs but they do not apply to all drugs. When a drug distributes relatively slowly, the relationships that have been described do not strictly apply; rigorous pharmacokinetic analysis is much more complicated. The purpose of this chapter is to describe the difficulties encountered with drugs that impart multicompartmental characteristics to the body, and to introduce methods that permit noncompartmental pharmacokinetic analysis of drugs, irrespective of their distribution characteristics.

MULTICOMPARTMENTAL CHARACTERISTICS

On intravenous bolus administration, many drugs distribute sufficiently slowly so that a significant fraction of the dose is eliminated before distribution equilibrium is achieved. When this occurs, a semilogarithmic plot of drug concentration in plasma versus time looks like the curve shown in Figure 2–1. The data cannot be described by a single exponential expression (i.e., a single compartment). At the outset drug concentrations decline rapidly; ultimately, a linear relationship between log concentration and time is observed. The entire curve can usually be described by a mathematical expression that contains either two or three exponential terms [e.g., $C = A \exp(-\alpha t) + B \exp(-\beta t)$].

The mathematical models that apply to this situation are shown in Figure 2–2. In the simpler of the two models (the two-compartment model), the drug is assumed to distribute instantaneously into a space called the central compartment; the apparent volume of this space is usually larger than blood volume. The drug is simultaneously but more slowly distributed into a second space (the peripheral or tissue compartment) and eliminated. The three-compartment model assumes that there are two distinct spaces to which the drug distributes from the central compartment at measurably different rates. In either model, after administration, the apparent volume of the drug increases and the rate constant associated with the rate of decline of drug concentrations in plasma decreases until distribution equilibrium is achieved.

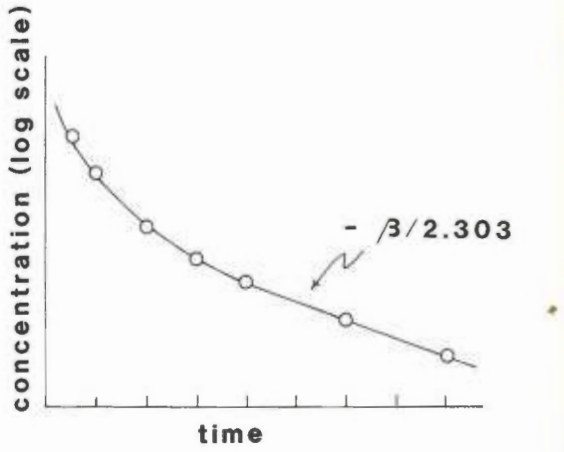

Fig. 2–1. Semilogarithmic plot of plasma concentration versus time after intravenous bolus administration of a drug with multicompartment pharmacokinetic characteristics. The slope of the terminal linear segment of the curve is indicated.

The kinetics of the situation might be better understood by considering the mathematical relationships that apply. For the two-compartment model,

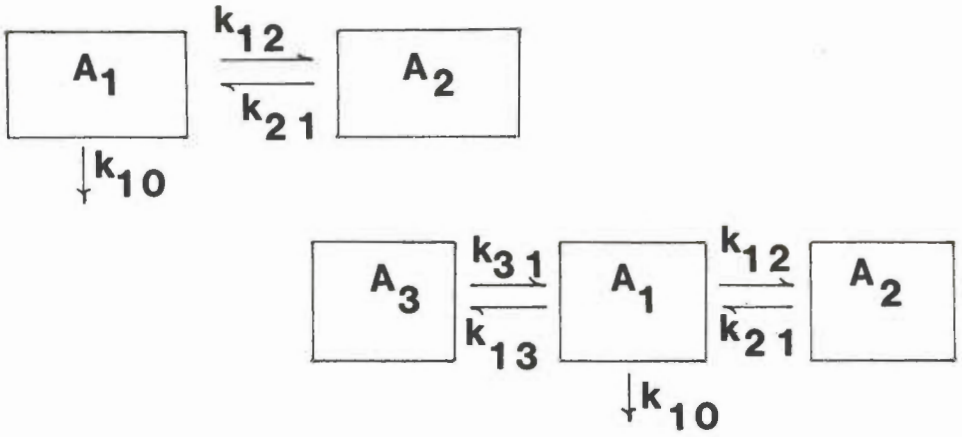

Fig. 2–2. Examples of a two- and three-compartment pharmacokinetic model. A_1 denotes the central compartment in each model and A_2 and A_3 are peripheral compartments. Immediately after an iv bolus injection, the central compartment contains an amount of drug equal to the dose. The general case for extravascular administration assumes that drug is transferred from the absorption site to the central compartment.

$$\text{Rate of loss of drug from central compartment} = \text{Rate of distribution} + \text{Rate of elimination} - \text{Rate of redistribution} \quad (2\text{–}1)$$

where

$$\text{Rate of loss of drug from central compartment} = -dA_1/dt \quad (2\text{–}2)$$

$$\text{Rate of distribution} = k_{12}A_1 \quad (2\text{–}3)$$

$$\text{Rate of elimination} = k_{10}A_1 \quad (2\text{–}4)$$

$$\text{Rate of redistribution} = \quad (2\text{–}5)$$

where A_1 and A_2 represent the amounts of drug in the central and peripheral compartments, respectively (see Fig. 2–2).

Immediately after administration, $-dA_1/dt$ is at a maximum equal to the product of $(k_{12} + k_{10})$ and dose; since there is no drug in the tissue compartment, there is no redistribution. As drug levels (A_1) in the central compartment decline because of distribution and elimination, there is a corresponding fall in $-dA_1/dt$, but as drug levels build up in the tissue compartment and the rate of redistribution becomes significant, there is a braking effect on the rate of decline of A_1.

At distribution equilibrium a fixed relationship exists between A_1 and A_2 such that

$$A_2 = \mathcal{Z}A_1 \quad (2\text{–}6)$$

where $\mathcal{Z}$ is a complex constant incorporating both distribution and elimination parameters. Under these conditions

$$-dA_1/dt = k_{12}A_1 + k_{10}A_1 - k_{21}\mathcal{Z}A_1 \quad (2\text{–}7)$$

or

$$-dA_1/dt = (k_{12} + k_{10} - k_{21}\mathcal{Z})A_1 \quad (2\text{–}8)$$

Expressing Equation 2–8 in terms of drug concentrations rather than amounts yields

$$-dC/dt = (k_{12} + k_{10} - k_{21}\mathcal{Z})C = \beta C \quad (2\text{–}9)$$

where $\beta = k_{12} + k_{10} - k_{21}\mathcal{Z}$. Equation 2–9 is a typical first-order rate expression. Thus, irrespective of the complexity of the model, drug concentrations in the plasma decline in a first-order manner once distribution equilibrium is achieved. The rate constant describing this first-order portion of the curve is usually termed β.

Data Analysis at Distribution Equilibrium

Integration of Equation 2–9 indicates that the log-linear region of the curve shown in Figure 2–1 will have a slope equal to $(-\beta/2.303)$. Therefore, for drugs that require multicompartmental description, a terminal half-life may be defined as

$$t_{1/2} = 0.693/\beta \qquad (2–10)$$

It is important to remember that this half-life reflects the persistence of only a fraction of the dose; the balance of the dose is eliminated more rapidly. It is also important to note that, irrespective of the model, the half-life of a drug always reflects both distribution and elimination. This is evident when Equation 2–9 is considered.

The mathematical relationships that apply when distribution equilibrium is reached also make it possible to calculate an apparent volume of distribution. This apparent volume, usually termed V_β, is given by

$$V_\beta = \frac{\text{iv dose}}{(\text{AUC})\beta} \qquad (2–11)$$

where AUC denotes the total area under the drug concentration-time profile and β is the terminal first-order elimination rate constant. V_β is a proportionality constant relating the amount of drug in the body to drug concentration in the plasma during the terminal (log-linear) phase of drug elimination (i.e., at distribution equilibrium).

An analogous expression that can be applied to drugs that distribute rapidly is

$$V = \frac{\text{iv dose}}{(\text{AUC})k} \qquad (2–12)$$

where k is the first-order elimination rate constant.

Equations 2–11 and 2–12 can usually be applied to data obtained after intramuscular administration of a drug; in this case, the term "iv dose" is replaced by "im dose." These equations should not ordinarily be applied to data obtained after oral administration. If they are, the term "iv dose" must be replaced by "amount absorbed" or, more precisely, by "amount of drug actually reaching the bloodstream."

Equation 2–12 is a mathematically rigorous and widely applied equation for the estimation of apparent volume of drugs that distribute rapidly once they reach the bloodstream. Equation 2–11 is a useful approximation of the volume of distribution of most drugs that require a multicompartmental description. However, V_β has several inherent problems not the least of which is that it reflects elimination as well as distribution. In all cases, V_β will overestimate the volume of distribution of a drug; in most cases, the overestimate is small and of little consequence, but it can be unacceptably large for drugs with pronounced multicompartmental characteristics. The dependence of V_β on drug elimination also means that changes in drug elimination may cause a change in V_β even though the perturbation has no effect on distribution per se.[1]

Sometimes it is also useful to calculate the apparent volume of the central compartment (V_1). This is usually done by curve-fitting the concentration-time data after iv bolus injection, by means of a computer-based nonlinear regression program, to an equation of the form

$$C = A\exp(-\alpha t) + B\exp(-\beta t) \qquad (2–13)$$

where $\alpha > \beta$. The iv dose divided by the sum of the coefficients is equal to the volume of the central compartment, i.e.

$$V_1 = \text{iv dose}/(A+B) \qquad (2–14)$$

V_1 is always smaller than the total volume of distribution (V). For this reason, high drug concentrations (i.e. dose/V_1) may occur immediately after a rapid iv injection. These levels fall quickly but could be dangerous. Good sense dictates that iv injections be given relatively slowly.

In the previous chapter, it was noted that the peak concentration of a drug is always smaller after iv infusion than after iv bolus. The difference in concentration for drugs that distribute immediately is a function of the infusion time and half-life of the drug. Strictly speaking, a drug must be infused over at least one half-life to see a 50% change in peak concentration. In practice, much shorter infusion times are almost always helpful because most drugs display a distributive phase and multicompartment characteristics on iv administration.

The initial rapid fall in drug levels after iv bolus injection, the distribution-elimination phase, is sometimes characterized by a half-life, the so-called alpha half-life (i.e., $0.693/\alpha$). The alpha half-life is usually much smaller than the beta half-life (i.e., $0.693/\beta$). Under these conditions, the difference in peak concentration after an iv bolus and an iv infusion is a function of the alpha half-life.

Consider a drug that shows two-compartment

characteristics after iv administration. Assume that the iv dose is 1 g, V_1 = 10 L, α half-life = 15 min, and β half-life = 6 hr. After an iv bolus, the initial drug concentration is 100 mg/L. In contrast, the peak concentration of the drug is only about 25 mg/L when it is infused over 30 minutes.

Other Problems with Multicompartmental Analysis

The number of exponentials and, therefore, the number of compartments required to describe the decline of drug concentration after intravenous bolus injection is not well defined, but depends on both the frequency and timing of blood samples. More frequent sampling right after administration tends to yield data that must be described by equations containing more exponential terms than would be required by less frequent sampling. Thus, the compartmental model required to describe the pharmacokinetics of a drug depends, in part, on the experimental design. In turn, estimates of half-life are dependent on the model selected.

Various statistical considerations are useful in minimizing the problems associated with model selection, but they do not overcome them. Studies with a single drug in a group of patients may result in some patients requiring a two-compartment model to describe the pharmacokinetics of the drug, whereas others require a three-compartment model. We frequently find that drugs requiring multicompartmental analysis after intravenous administration can be described by a one-compartment model after oral administration. Since pharmacokinetic analysis based on compartmental models can lead to unreconcilable difficulties, more and more investigators and clinicians who use pharmacokinetics are turning to noncompartmental approaches that can be applied to all drugs.

NONCOMPARTMENTAL METHODS

Noncompartmental methods for calculating absorption, distribution, and elimination parameters are based on the theory of statistical moments.[2,3] The zero moment of a drug concentration in plasma versus time curve is the total area under the curve from time zero to infinity (AUC), which has been described in Chapter 1. Estimates of AUC are not only useful for calculating bioavailability, but can also be used for calculating drug clearance, which is equal to the ratio of the intravenous dose to AUC.

The first moment of a plasma concentration-time profile is the total area under the curve resulting from a plot of the product of drug concentration and time versus time. Table 2–1 shows concentration data obtained after constant rate intravenous infusion of a drug. Also listed are the values of C · t. These values are plotted versus time in Figure 2–3. The area under the C · t versus t plot from t = 0 to the last sampling time, t*, can be calculated by means of the trapezoidal rule (see Appendix I). Provided that blood samples have been collected for a sufficiently long period of time so that the last sample may be considered in the post-absorptive and, where applicable, postdistributive

Table 2–1. Drug Concentration and Drug Concentration-Time Data, During and After a 1-hr Constant Rate Intravenous Infusion

Time (hr)	Concentration (μg/ml)	Concentration-Time (μg/ml)(hr)
0.5	3.2	1.6
1.0	5.9	5.9
2.0	4.2	8.4
3.0	3.0	9.0
4.0	2.1	8.4
5.0	1.5	7.5
6.0	1.1	6.6
8.0	0.5	4.0

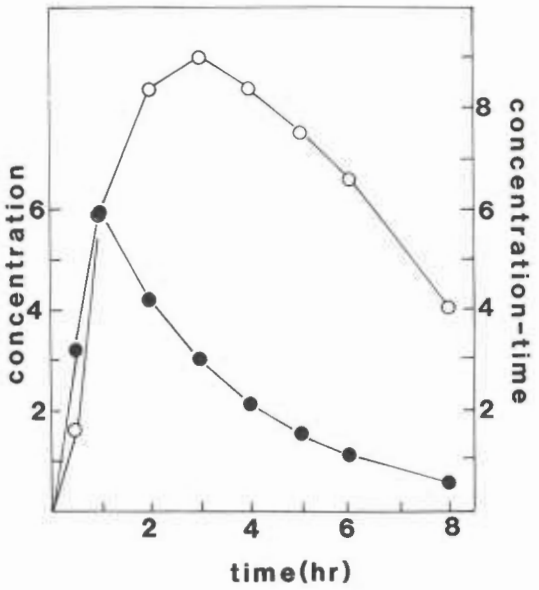

Fig. 2–3. Plots of drug concentration (μg/ml) (●) and drug concentration-time (μg-hr/ml) (○) versus time, during and after a 1-hr constant rate intravenous infusion. The area under the drug concentration versus time plot to infinity is AUC; the area under the drug concentration-time versus time plot to infinity is AUMC.

phase of the curve, the area from t* to ∞ may be estimated from the following equation:[4]

$$\int_{t^*}^{\infty} t \cdot C = \frac{t^* C^*}{\beta} + \frac{C^*}{\beta^2} \qquad (2\text{–}15)$$

where the integral term on the left-hand side of the equation is the partial area under the curve, C* is drug concentration at the last sampling time, t*, and β is the terminal first-order elimination rate constant. This area is then added to the area from t = 0 to t = t*, determined by the trapezoidal rule, to estimate the total area. The total area under the C · t versus t plot is termed the AUMC or area under the first moment curve.

The ratio of AUMC to AUC for any drug is a measure of its mean residence time (MRT).[5,6] MRT calculated after intravenous administration is the statistical moment analogy to drug half-life; it provides a quantitative estimate of the persistence of a drug in the body. Like half-life, MRT is a function of both distribution and elimination.

Comparison of MRT values after intravenous bolus administration with the MRT after some other route of administration provides information regarding the mean absorption time.[7] Similar comparisons can be made between two dosage forms given orally to obtain relative absorption data.

One of the most useful properties of statistical moments is that they permit the estimation of a volume of distribution that is independent of drug elimination.[4,6] Using these methods, the volume of distribution of a drug is given by the product of the intravenous bolus dose and the ratio of AUMC to AUC squared.

Drug Clearance

Clearance is a function of both the intrinsic ability of certain organs, such as the kidneys and liver, to excrete or metabolize a drug and the blood flow rate to these organs. This concept is best illustrated by considering elimination in a single organ as depicted schematically in Figure 2–4. Under these conditions, the venous concentration of drug (C_V) will always be less than the arterial concentration (C_A) because some of the drug is eliminated or extracted during the passage of the blood through the organ. The rate at which drug enters the organ is equal to the product of blood flow (Q) and arterial concentration. The rate at which drug leaves the organ is equal to the product of blood flow and venous concentration. The difference between the

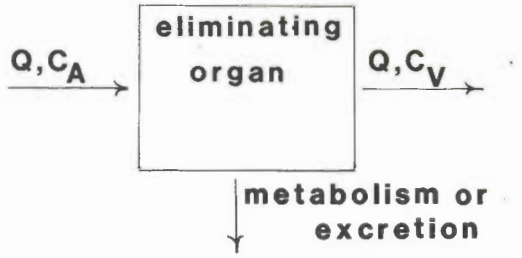

Fig. 2–4. Schematic representation of drug elimination by a single organ. Blood flows through the organ at a rate equal to Q. Drug concentration entering the organ is C_A; drug concentration leaving the organ is C_V; C_V is less than C_A.

input rate and the output rate is the rate of elimination of drug by the organ;

$$\text{Elimination rate} = Q(C_A - C_V) \qquad (2\text{–}16)$$

The ratio of the elimination rate to the drug input rate (QC_A) is termed the extraction ratio (ER) and is given by

$$ER = (C_A - C_V)/C_A \qquad (2\text{–}17)$$

The extraction ratio of a drug ranges from 0 to 1 depending on how well the organ eliminates or extracts the drug from the blood flowing through it. If the organ does not eliminate the drug, then $C_V = C_A$ and ER = 0; if the organ avidly extracts the drug so that $C_V \cong 0$, then ER = 1.

By definition, the organ clearance (Cl) of a drug represents the volume of blood cleared per unit time. It may be viewed as a proportionality constant relating the elimination rate of a drug to the drug concentration in the blood, as expressed in the following equation:

$$Cl = \text{Elimination rate}/C_A \qquad (2\text{–}18)$$

It follows from Equation 2–16 that

$$Cl = Q(C_A - C_V)/C_A \qquad (2\text{–}19)$$

or, according to Equation 2–17

$$Cl = Q(ER) \qquad (2\text{–}20)$$

Thus, clearance is equal to the product of blood flow and extraction ratio. Since elimination rate is expressed in units of amount per unit time, and concentration is expressed in units of amount per unit volume, it follows that clearance has units of volume per unit time (e.g., ml/min or L/hr), the same as flow rate. If drug elimination is a first-order process, then clearance is independent of drug concentration.

These equations, which have been developed for a single organ, can be extended to the elimination of a drug from the body. The total body clearance of a drug from the blood is equal to the ratio of the overall elimination rate of the drug to the drug concentration in blood, where the overall elimination rate is the sum of the elimination processes occurring in all organs.

By means of integral calculus, it can be shown that the ratio of the overall elimination rate of a drug to its concentration in the blood is equal to the ratio of the amount of drug ultimately eliminated to the total area under the drug concentration-time curve. Since, after intravenous administration, the amount eliminated is equal to the dose, clearance can be expressed as

$$Cl = \text{dose}/(AUC) \qquad (2\text{–}21)$$

Equation 2–21 provides the basis for the routine estimation of the total body clearance of a drug after a single dose. To estimate clearance, drug is ordinarily given intravenously, but Equation 2–21 usually applies as well to intramuscular administration. Clearance cannot be estimated after oral administration unless it can be assumed that the total dose reaches the bloodstream. Application of Equation 2–21 to data obtained after oral administration when bioavailability is incomplete results in an overestimate of clearance.

Clearance can also be estimated at steady state after prolonged constant rate intravenous infusion. Under these conditions

$$Cl = k_o/C_{ss} \qquad (2\text{–}22)$$

where k_o is the infusion rate and C_{ss} is the drug concentration at steady state.

It is sometimes useful to keep in mind that clearance can also be expressed as the product of V_β and β. For drugs that distribute rapidly and can be described by a single compartment, $Cl = Vk$.

Apparent Volume of Distribution

The most useful volume term in pharmacokinetics is the apparent volume of distribution at steady state or V_{ss}. It represents the proportionality constant relating the amount of drug in the body at steady state after prolonged constant rate intravenous infusion or repetitive administration to the drug concentration or average drug concentration at that time. V_{ss} is independent of drug elimination and reflects solely the anatomic space occupied by a drug and the relative degree of drug binding in the blood and extravascular space.

Estimation of V_{ss} does not require data obtained at steady state; this distribution parameter can be calculated after a single dose of a drug by means of the following equation:[4,6]

$$V_{ss} = \text{iv dose}(AUMC)/(AUC)^2 \qquad (2\text{–}23)$$

where AUMC is the total area under the first moment curve.

Although Equation 2–23 applies only to intravenous bolus administration, the relationship can be modified easily to accommodate the different ways drugs are administered. If a drug is given by a short-term constant rate intravenous infusion,[8] then

$$V_{ss} = \frac{\text{infused dose}(AUMC)}{(AUC)^2} - \frac{\text{infused dose}(T)}{2(AUC)} \qquad (2\text{–}24)$$

where T is the duration of infusion. Since the infused dose is equal to k_oT, we can also express Equation 2–24 as

$$V_{ss} = \frac{k_oT(AUMC)}{(AUC)^2} - \frac{k_oT^2}{2(AUC)} \qquad (2\text{–}25)$$

Relationship of Half-Life, Clearance, and Volume of Distribution

Earlier, we noted that clearance is equal to the product of V_β and β. This relationship does not imply, however, that clearance is dependent on volume of distribution and half-life. Both clearance and distribution volume are independent parameters, although both may be affected by a change in plasma protein binding. Half-life is a dependent parameter. For a multicompartment model, $t_{1/2} = 0.693\ V_\beta/Cl$.

This relationship shows that the larger is the distribution volume, the longer is the half-life. Independently, the larger is the clearance of a drug, the smaller is the half-life. An increase in half-life should not be interpreted as a decrease in drug elimination; it may merely reflect an increase in distribution volume. Changes in elimination are represented by changes in clearance.

Mean Residence Time

The mean residence time (MRT) of a drug after administration of a single dose is given by

$$MRT = (AUMC)/(AUC) \qquad (2\text{–}26)$$

The MRT of a drug after intravenous bolus administration provides a useful estimate of the persistence time in the body and in this sense is related to half-life. When applied to drugs that distribute rapidly it can be shown that

$$MRT_{iv} = 1/k \qquad (2\text{–}27)$$

where k is the first-order elimination rate constant. The half-life of a drug is equal to 0.693/k. Half-life tells us the time required to eliminate 50% of the dose; MRT_{iv} tells us the time required to eliminate 63.2% of the dose.

The MRT of a drug that distributes slowly and requires multicompartment characterization is a complex function of the model rate constants for distribution and elimination. However, in noncompartmental terms, the following relationship is useful:

$$MRT_{iv} = 1/\bar{k} \qquad (2\text{–}28)$$

where $\bar{k}$ is a rate constant equal to the ratio of clearance to V_{ss}. For drugs with multicompartment characteristics, $\bar{k} > \beta$. For drugs that distribute almost immediately, $\bar{k} = k$. In many cases, the ratio of 0.693 to $\bar{k}$ serves as the effective half-life of a drug.

Irrespective of the distribution characteristics of a drug, MRT represents the time required for 63.2% of an intravenous bolus dose to be eliminated. As such, it may be possible to determine MRT from urinary excretion data alone by determining the time required to excrete 63.2% of that amount which is ultimately excreted as unchanged drug.

Mean residence time is a function of how we give the drug. The MRT values for noninstantaneous administrations will always be greater than the MRT following intravenous bolus administration. However, the MRT_{iv} can be estimated following other modes of drug administration. For example, following a constant rate intravenous infusion

$$MRT_{iv} = MRT_{inf} - (T/2) \qquad (2\text{–}29)$$

where T is the duration of the infusion. MRT_{inf} is calculated according to Equation 2–26.

DRUG ABSORPTION

Noncompartmental methods for estimating the extent of absorption of a drug after oral or other extravascular routes of administration have been described in Chapter 1. Essentially, these methods require a comparison of areas under the curve. The fraction of an oral dose that actually reaches the bloodstream can be estimated from the ratio of AUC after oral administration to AUC after intravenous administration of equivalent doses of the drug. The extent of absorption of drug in a test dosage form relative to its absorption from a standard dosage form, such as an aqueous solution, can be estimated from the ratio of AUC after the test dose to AUC after the standard.

Noncompartmental methods for estimating the rate of absorption of a drug after extravascular administration are based on differences in MRT after different modes of administration. In general,[7]

$$MAT = MRT_{ni} - MRT_{iv} \qquad (2\text{–}30)$$

where MAT is the mean absorption time, MRT_{ni} is the mean residence time after administration of the drug in a noninstantaneous manner, such as orally, intramuscularly, or by iv infusion and MRT_{iv} is the mean residence time after intravenous bolus administration.

When absorption is a first-order process

$$MAT = (1/k_a) \qquad (2\text{–}31)$$

where k_a is the first-order absorption rate constant. Under these conditions, $k_a = 1/MAT$, and the absorption half-life is given by 0.693 (MAT). When absorption or input is a zero-order process

$$MAT = (T/2) \qquad (2\text{–}32)$$

where T is the time over which absorption or input takes place.

Moment analysis and the concept of MRT may also be useful for comparing the absorption characteristics of a drug from different formulations. This application is considered in Chapter 8.

A limitation of moment theory is seen when the difference between MRT_{ni} and MRT_{iv} is small. In this case, it may be difficult to estimate MAT with adequate accuracy.

A useful application of moment theory, to evaluate the pharmacokinetics of furosemide after iv and oral administration, has been reported.[9] The mean MRT after an iv dose of the loop diuretic to eight healthy subjects was less than 1 hr, suggesting an effective half-life of about 40 min. Absorption after oral administration, however, was slow and incomplete. Bioavailability was only about half the dose. The difference in MRT after oral and iv administration (MAT) was 84 min. The mean absorption time for furosemide was significantly

larger than the MRT_{iv}, suggesting absorption rate-limited elimination of the drug.

Predicting Steady-State Concentrations

When a drug is given continuously or intermittently for a sufficient period of time it accumulates and eventually reaches a steady state with respect to drug concentration in the blood (see Figs. 1–8 and 1–9). Drug concentration at steady state is solely a function of the effective rate of dosing and the total body clearance of the drug in the patient, both of which are noncompartmental parameters.

The steady-state concentration (C_{ss}) following constant rate intravenous infusion may be determined by rearranging Equation 2–22 which yields

$$C_{ss} = k_o/Cl \qquad (2–33)$$

where k_o is the infusion rate and Cl is the clearance of the drug.

A similar equation can be written to describe the average drug concentration at steady state ($\overline{C}$) following repetitive intermittent administration of a fixed dose (D) given at fixed intervals (τ) (see Fig. 1–9). Under these conditions,

$$\overline{C} = F(DR)/Cl \qquad (2–34)$$

where F is the fraction of the administered dose that actually reaches the bloodstream and DR is the average dosing rate; if a drug is given in a dose of 400 mg every 8 hr, then DR = 50 mg/hr.

If a drug is given at irregular intervals during the day (e.g., 3 times a day or after meals and at bedtime rather than every 8 hr or every 6 hr), one can use Equation 2–34 to calculate the average drug concentration over the day by setting DR equal to (total daily dose)/24 hr.

A still simpler method for estimating average drug concentration at steady state than that suggested by Equation 2–34 is also available. As may be seen in Figure 1–9, $\overline{C}$ is a concentration intermediate between the maximum and minimum drug concentrations at steady state. Specifically,

$$\overline{C} = AUC_{ss}/\tau \qquad (2–35)$$

where AUC_{ss} is the area under the curve from t = 0 to t = τ during a dosing interval at steady state. In other words, $\overline{C}$ is the height of a rectangle of width τ that has an area ($\overline{C} \times \tau$) equal to the area under the curve during a dosing interval at steady state. Steady-state bioavailability studies comparing AUC_{ss} for test product and reference standard are widely used for evaluating sustained-release dosage forms. By definition, AUC_{ss} is equal to AUC, the total area under the curve from t = 0 to t = ∞ after a single dose. Under these conditions

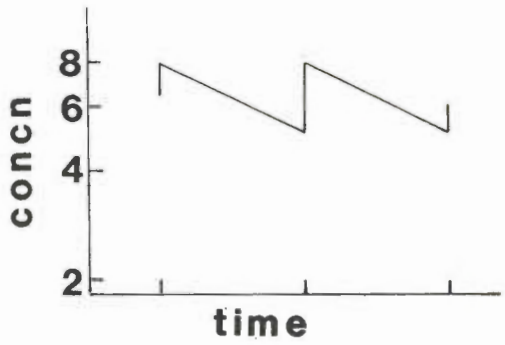

Fig. 2–5. Steady-state concentrations after repetitive administration of a rapidly distributing drug with a 12-hr half-life given every 8 hr. The ratio of C_{max} to C_{min} is 1.6.

$$\overline{C} = AUC/\tau \qquad (2–36)$$

By merely knowing the AUC of a drug after a single dose administered in the same way that will be used for repetitive dosing, we can predict the average drug concentration at steady state.

Although $\overline{C}$ is a useful parameter and easy to calculate, we must remember that it tells us nothing about the time course of drug concentrations during a dosing interval. This limitation is of little consequence for drugs with long half-lives that distribute rapidly and are dosed relatively frequently (i.e., $\tau < t_{1/2}$). In this case, the steady-state ratio of C_{max} to C_{min} will be less than 2 and the drug concentration profile at steady state will be relatively flat (Fig. 2–5). On the other hand, large fluctuations may be seen with drugs having relatively short half-lives that are given less frequently than every half-life (Fig. 2–6) and with drugs that distribute slowly and display multicompartment characteristics (Fig. 2–7). In these cases, the steady-state ratio of C_{max} to C_{min} will exceed 2. For certain drugs, the attainment of an acceptable value of $\overline{C}$, well within the therapeutic concentration range, may belie the fact that C_{max} is too high and adverse effects may result or that C_{min} is too low and for some time during the dosing interval the patient may not be receiving the optimal benefit of the drug. Noncompartmental methods are generally not useful for describing the time course of drug in the blood. It is probably best to handle such considerations with the concept of half-life and the application of compartmental analysis. Questions

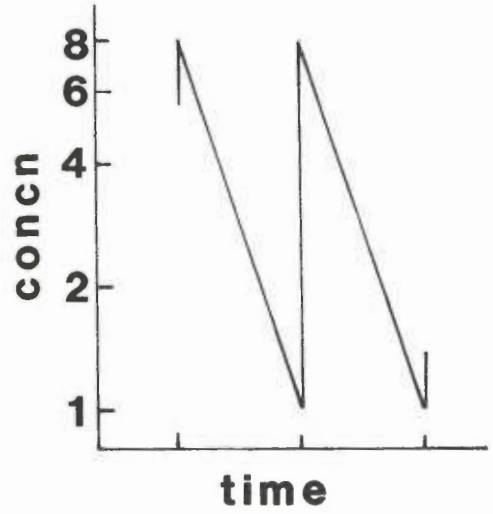

Fig. 2–6. Steady-state concentrations after repetitive administration of a rapidly distributing drug with a 2-hr half-life given every 6 hr. The ratio of C_{max} to C_{min} is 8.

regarding drug accumulation and loading dose may also be better answered by applying compartment theory, as described in Chapter 1. A noncompartmental alternative based on the principle of superposition is described in Appendix II.

Predicting the Time to Steady State

The time required to reach steady state on continuous constant rate intravenous infusion of a drug that distributes rapidly is a function of the half-life of the drug. After a period of infusion equal to 4 half-lives, the drug concentration in blood or plasma will be within 90% of the steady-state concentration; after a period equal to 7 half-lives, drug concentration is within 99% of the steady-state level. The same drug given as repetitive intravenous boluses of fixed doses at fixed intervals will show similar characteristics; after a period of dosing equal to 4 half-lives, the average drug concentration will be within 90% of the average steady-state concentration.

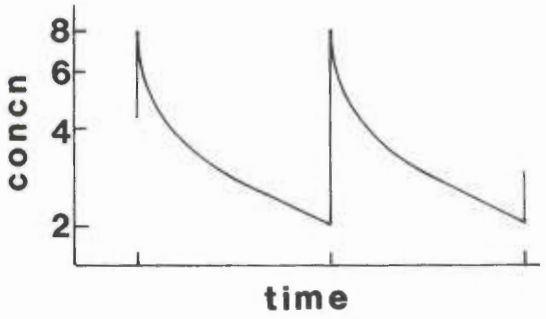

Fig. 2–7. Steady-state concentrations after repetitive administration of a slowly distributing drug with a 12-hr half-life given every 12 hr. The ratio of C_{max} to C_{min} is 4.

In practice, the time after the start of dosing to attain a certain fraction (e.g., 90%) of the steady-state concentration is not only a function of half-life, but also of the way we give the drug and of the distribution characteristics of the drug. Repetitive extravascular or noninstantaneous administration of a drug requires a longer period to attain steady state than we would predict from its half-life. On the other hand, repetitive administration of a drug that distributes slowly and shows multicompartment characteristics requires a shorter period to reach steady state in the plasma than we would predict from its terminal half-life. Exact equations to solve for the time after starting dosing at which a certain percentage of steady state is reached for different drugs under different conditions of use are both complex and difficult to solve.

Moment analysis provides a unique solution to this problem. Chiou has shown that by means of AUC analysis one can calculate the time to steady state for any drug after a single dose given in the same way that will be used for repetitive dosing.[10] In essence, the time required after giving the dose for the partial area under the curve (AUC_0^t) to be equal to a certain fraction of the total area under the curve (AUC) is the same as the time required to reach the same fraction of steady state on repetitive dosing of the drug.[11] This idea is expressed in the following equation:

$$f_{ss} = AUC_0^t/AUC \qquad (2–37)$$

where f_{ss} is the fraction of the steady-state concentration reached at time t on repetitive dosing and the area terms refer to a single dose.

When using Equation 2–37, one does not explicitly solve for time. Rather, one selects a time after giving the dose and carries out an area analysis to calculate f_{ss}. The time required to reach a desired f_{ss} (e.g., 90%) is estimated by trial and error. Usually two trials followed by interpolation should be sufficient to provide a useful estimate of the required time.

Parameters Based on Free Drug Concentration

The noncompartmental methods described in this chapter are based on total drug concentrations in blood or plasma. Most drugs are bound to some extent to plasma proteins and formed elements in

the blood. Therefore, we can speak of a free drug concentration and a total drug concentration (free plus bound) in blood or plasma.

The usual analytic methods determine total drug concentration in plasma (C). Total drug concentration in blood (C_b) can be estimated by the following equation:

$$C_b = C_{rbc} \cdot HCT + C(1 - HCT) \quad (2–38)$$

where C_{rbc} is drug concentration in the red blood cell and HCT is hematocrit.

The ratio of free (C_f) to total drug concentration in blood or plasma is termed the free fraction (f). Free fraction is usually determined in plasma (f_p) by means of equilibrium dialysis or ultrafiltration. Free fraction in blood is calculated by the following equation:

$$f_b = f_p C/C_b \quad (2–39)$$

The plasma or blood binding of most drugs given in usual doses is independent of drug concentration. Therefore, by determining total drug concentration and by determining free fraction at a given concentration, we can calculate free drug concentration.

In theory, free rather than total drug concentration in blood or plasma is more closely related to pharmacologic effects. There is some experimental and clinical data to support this idea. In the absence of inter- or intrasubject differences in binding, a given total drug concentration always reflects the same free drug concentration. However, some patients bind a drug much more or much less effectively than average because of disease-related factors. During a course of therapy, there may be a change in binding because of concomitant drug therapy. Therefore, an undesirably low or high total drug concentration may not reflect a corresponding low or high free drug concentration.

Total drug concentration at steady state is a function of clearance (see Eq. 2–34). The clearance of drugs with a low hepatic or renal extraction ratio depends on binding as well as the efficiency of the eliminating organs. The clearance of total drug may increase or decrease simply because of a change in binding. In this case, there will be a change in the steady-state concentration of total drug but not of free drug. Since free drug concentration at steady state is unchanged, an unusually high or low total drug concentration may not require a change in dosing rate.

Under these conditions, it may be desirable to determine the clearance of free drug (Cl_f) as well as the clearance of total drug. Free drug clearance from plasma is given by the following equation:

$$Cl_f = Cl/f_p \quad (2–40)$$

CONCLUSIONS

The noncompartmental methods described in this chapter permit a comprehensive pharmacokinetic analysis without resort to curve-fitting, computers, or tedious mathematical equations. Although these methods cannot be applied to all pharmacokinetic problems, they are useful for most problems and are particularly useful for the clinical application of pharmacokinetics. In the following pages, you will find many of these relationships used to answer important clinical questions.

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8

Bioavailability

The time course of a drug in the body depends on how the drug is given. Blood levels are likely to be different after a single oral dose compared with the same dose given by rapid intravenous injection. There are two reasons for this difference: one is related to the completeness of absorption and the other to the rate of absorption of the drug. These two characteristics of drug absorption are called the *bioavailability* of the drug.

In most cases, we are particularly concerned with the fraction of the oral dose that actually reaches the bloodstream, because this amount is the *effective dose* of a drug. In some cases, notably those involving drugs used as a single dose for acute purposes, such as sedation or pain, we are also concerned with the rate of absorption of the drug.

Many drugs are not completely available after oral administration. Some drugs have low permeability and are slowly absorbed even when given in solution; examples include cromolyn, neomycin, and riboflavin. Since the residence of a drug at absorption sites in the gastrointestinal tract is limited by motility, there may be insufficient time for complete absorption. The availability of these compounds may be increased by administering them with food or with drugs that decrease motility, or by developing more lipid-soluble prodrugs.

Other drugs are so poorly water soluble that dissolution may be incomplete during the period of time available for absorption; some examples are phenytoin, griseofulvin, and isotretinoin. The availability of these drugs may be increased, in some cases dramatically, by dosage form changes, such as particle size reduction, or by means of water-soluble prodrugs.

A large number of drugs demonstrate incomplete bioavailability because of chemical degradation in the stomach (e.g., penicillin G), preabsorptive metabolism by enzymes in the proximal small intestine (e.g., aspirin) or bacteria in the distal small intestine and colon (e.g., digoxin), or presystemic metabolism in the gut wall (e.g., isoproterenol) or liver (e.g., propranolol) during absorption. A drug subject to presystemic metabolism may be completely absorbed but incompletely available, because part of the dose is metabolized to other products during the drug's passage from the gut lumen to the systemic circulation.

The availability of drugs subject to acid hydrolysis in the stomach may be improved by the use of enteric-coated dosage forms. Few strategies are available to improve the availability of drugs subject to preabsorptive or presystemic metabolism.

ESTIMATING THE BIOAVAILABILITY OF A DRUG

The fraction or percent of an administered dose that actually reaches the systemic circulation is called the *absolute* or *systemic bioavailability* of a drug. Systemic bioavailability is determined from blood level or urinary excretion data after oral administration, with reference to similar data after intravenous administration.

The total area under the drug level in blood or plasma versus time curve (AUC), after a single dose, reflects the amount of drug reaching the bloodstream. For most drugs, if we double the amount injected intravenously, we double the AUC. It follows that if we compare the AUC after oral administration with that obtained after intravenous administration, we can determine the fraction (F) of the oral dose available to the systemic circulation. In other words,

$$F = (AUC)_{oral}/(AUC)_{iv} \qquad (8\text{–}1)$$

If only 60% of an oral dose reaches the bloodstream, then F = 0.60; if the entire dose is available, then F = 1.0

As noted in Chapter 1, the usual bioavailability study is terminated before drug concentrations in blood return to negligible levels. The AUC beyond the last concentration data point (C* at t*) is estimated from the equation:

$$\text{Area from } t^* \text{ to } \infty = C^*/k \qquad (8\text{–}2)$$

where k is the first-order elimination rate constant. This partial area is added to the area from t = 0 to t = t*, calculated by means of the trapezoidal rule (see Appendix I), to determine AUC.

We sometimes recognize, from preliminary data, that the intravenous dose must be smaller than the oral dose to achieve comparable blood levels. In this case, for purposes of safety, different oral and intravenous doses are used for estimating systemic availability. Under these conditions,

$$F = (AUC)_{oral}D_{iv}/(AUC)_{iv}D_{oral} \qquad (8\text{–}3)$$

where D refers to the dose.

For some drugs, urinary excretion data can also be used to estimate availability. After intravenous administration of a drug, a fraction of the dose is excreted unchanged in the urine; the rest of the dose is subject to nonrenal elimination. In some cases, this fraction is so small as to represent a negligible amount or an amount too small to measure with precision. Under these conditions, urinary excretion data will not be useful. On the other hand, there are drugs for which evaluation of urinary excretion data is the method of choice for estimating availability. The thiazide class of diuretics is an example.[1]

For most drugs, the same fraction of the dose is excreted in the urine regardless of the size of the intravenous dose. Accordingly, by comparing the total amount of drug excreted unchanged (A_u) after a single oral and intravenous dose of a drug, we can determine the fraction (F) of the oral dose available to the bloodstream. In other words,

$$F = (A_u)_{oral}/(A_u)_{iv} \qquad (8\text{–}4)$$

When different oral and intravenous doses are used, the following equation applies:

$$F = (A_u)_{oral}D_{iv}/(A_u)_{iv}D_{oral} \qquad (8\text{–}5)$$

Absolute bioavailability has been determined for comparatively few drugs. The principal reason for this lack of information is that most drugs are not approved for intravenous use. Intramuscular administration may be an alternative absolute standard, particularly for soluble drugs, but again, relatively few drugs are approved for intramuscular administration. Because of this, the bioavailability of a drug is usually determined against a relative standard, one that does not assure complete availability.

A commonly used relative standard is an aqueous oral solution of the drug. Blood levels or urinary excretion data are compared after a single dose of the drug administered as the test product or the oral solution. To determine the availability of the drug from the test dosage form *relative* to that from the standard dosage form (F_{rel}), the following equations apply:

$$F_{rel} = (AUC)_{test}/(AUC)_{standard} \qquad (8\text{–}6)$$

and

$$F_{rel} = (A_u)_{test}/(A_u)_{standard} \qquad (8\text{–}7)$$

It can be debated that the maximum availability of a drug from an oral dosage form can never exceed that found from an aqueous oral solution. This is probably true in most instances; however, it may not be true for drugs that are poorly soluble in acid and precipitate in the form of coarse crystals in the stomach on swallowing the aqueous solution, or for drugs that are subject to acid hydrolysis and for which the test dosage form provides protection not afforded by the solution. In these cases, F_{rel} may exceed unity.

Some drugs defy formulation as aqueous solutions and one must resort to other relative standards; these include nonaqueous oral solutions, oral suspensions, or other solid oral dosage forms.

The physicochemical basis for using a nonaqueous solution of a drug as a bioavailability standard has been considered by Serajuddin et al.,[2] who studied the absorption of an investigational drug coded REV 5901. The drug existed in both solid and metastable liquid forms, had a pK_a of 3.7, and low water solubility (0.002 mg/ml at 37°). Appreciable solubility was observed only at pH values of 2 or less. Dissolution rate at pH >3 was practically zero.

REV 5901 was quite soluble in several nonaqueous solvents approved for oral use. The bioavailability of some of these nonaqueous solutions as well as an aqueous suspension was compared. Bioavailability was 76% after administration of a solution in polysorbate 80 (Tween 80), 61% when

given as a solution in peanut oil, and 35% as an aqueous suspension, relative to an oral solution of polyethylene glycol 400 (PEG 400), which provided the highest blood levels.

The investigators observed that on dilution of the water-miscible organic solutions (PEG 400 and Tween 80) with aqueous media, the drug immediately formed saturated solutions and the excess drug separated as emulsified oily globules. The dispersability of the globules improved when surfactants were present in the aqueous media. The average globule size was 1.6 μM, compared with a particle size of 5 to 10 μM when the drug was suspended in water. Therefore, a considerably larger surface area was available when the drug was ingested as a solution in Tween or PEG 400, rather than as an aqueous suspension.

Although the investigational drug was practically insoluble at the pH of the small intestine, its solubility was increased dramatically when bile salts and lecithin were added to the aqueous media. Serajuddin et al. concluded that the large surface area of the drug separating from organic solutions would facilitate dissolution in the presence of biological surfactants and increase bioavailability.

The innovator's dosage form, regardless of its availability, is often used as a relative standard, because presumably its efficacy is established. When a relative standard, other than an aqueous oral solution, is used, it is not uncommon to find that $F_{rel} > 1.0$.

Figure 8–1 shows blood levels of the antihypertensive drug prazosin after oral administration of 5 mg by capsule or hydroalcoholic solution.[3] The mean AUC for the test capsule was 174 ng/hr per ml whereas that for the solution was 199.0 ng/hr per ml. According to Equation 8–6, the relative availability of prazosin from the capsule is 0.87 or 87%.

A relative availability of 1.0 does not imply complete availability; we can only conclude from this information that the availability of drug from the test dosage form is equal to that from the standard. Propoxyphene gives almost the same blood levels after oral administration of commercial capsules or aqueous solution,[4] but the systemic availability of the drug after either dosage form is only about 20% because of presystemic metabolism.[5]

Most bioavailability studies are carried out by giving a single dose of drug to ambulatory, healthy subjects, after an overnight fast. There is concern that, in some instances, this kind of study does not

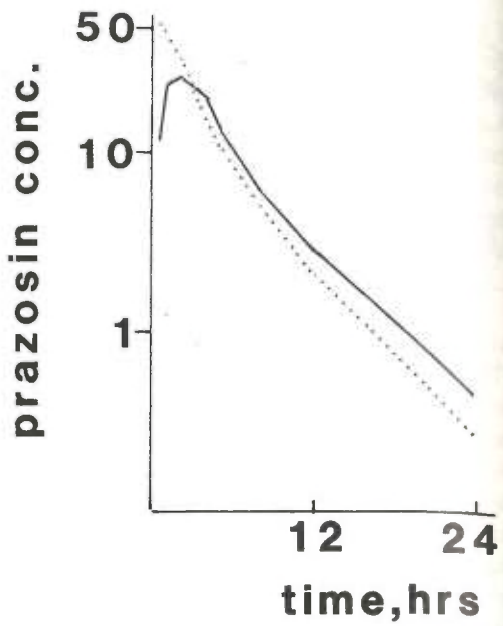

Fig. 8–1. Semilogarithmic plot of prazosin concentrations in plasma (ng/ml) following a 5-mg oral dose by capsule (—) or solution (···). (Data from Hobbs, D.C., Twomey, T.M., and Palmer, R.F.[3])

reflect the general use of the drug and may provide misleading information. This concern is particularly evident for the evaluation of prolonged-release dosage forms. We have learned enough about drug absorption to recognize that, in some cases, food, activity (sleeping vs awake), and disease may have differential effects on drug availability from oral dosage forms. Two dosage forms that differ in their release rates of drug may show equivalent AUC values in normal subjects but different values in a population with above average gastrointestinal motility. Differences between fed and fasted populations may also occur.

Oral administration of two 0.25 mg digoxin tablets and two 0.2 mg digoxin capsules containing a water-miscible solution of the drug yields similar values for AUC, indicating bioequivalence. The area under the curve following the tablets is 103% relative to the capsules. When either dosage form is given with propantheline, an anticholinergic that slows stomach emptying and decreases gastrointestinal motility, there is an increase in AUC but the change is larger for the tablets than for the capsules—24% versus 13%. Consequently, under conditions of hypomotility, digoxin AUC after administration of the tablets is 113% relative to the capsules.[6]

The oral absorption of digoxin in tablet form has been reported to be reduced after cancer chemotherapy and radiation therapy. Bjornsson et al.[7] studied possible differences in the effect of high-dose cancer chemotherapy on the relative bioavailability of digoxin given in tablet form and in solution-in-capsule form. Each subject received a single oral dose of either 0.5 mg tablets or 0.4 mg capsules before and after chemotherapy.

Before chemotherapy, the AUC following the tablets was 104% relative to the capsules. Chemotherapy reduced the average AUC after tablet administration by nearly 50%, compared with a reduction of only 15% with the capsules. Consequently, after chemotherapy, digoxin AUC following the tablets was only 74% relative to the capsules.

These concerns have led to increasing interest in steady-state studies for the evaluation of relative availability. When a constant dose of a drug is given at constant dosing intervals (e.g., 150 mg every 12 hr), the AUC during a single dosing interval at steady state (AUC_{ss}) is equal to the total AUC after a single dose (AUC). It follows from Equation 8–6 that:

$$F_{rel} = (AUC_{ss})_{test}/(AUC_{ss})_{standard} \qquad (8–8)$$

We can also show that:

$$F_{rel} = (A_{u,ss})_{test}/(A_{u,ss})_{standard} \qquad (8–9)$$

where $A_{u,ss}$ is the amount of drug excreted unchanged in the urine during a single dosing interval at steady state. Since the average drug concentration in blood or plasma at steady state, $\overline{C}_{ss}$, is equal to the ratio of AUC_{ss} to the dosing interval, τ, it follows that:

$$F_{rel} = (\overline{C}_{ss})_{test}/(\overline{C}_{ss})_{standard} \qquad (8–10)$$

By obtaining blood levels or urinary excretion data at steady state for a relatively short period of time (one dosing interval), we can determine the relative availability of a drug. Moreover, this assessment takes into account the general conditions of use of the drug, particularly when patients rather than healthy subjects are studied.

Dickerson and co-workers[8] determined the steady-state levels of pseudoephedrine after multiple dosing of two prolonged-release capsules given every 12 hr; one capsule (A) contained 120-mg pseudoephedrine and the other (B) contained 150 mg of the drug. The mean steady-state concentrations, $\overline{C}_{ss}$, were 447 ng/ml for capsule A and 510 ng/ml for capsule B. Adjusting these data for the difference in dose (120 mg vs 150 mg), we can calculate that the bioavailability of pseudoephedrine from capsule A relative to capsule B is 110%. Therefore, the dosage forms are nearly bioequivalent.

An advantage of steady state over single dose evaluation of availability is evident in the results of studies with the anticonvulsant drug carbamazepine.[9] Figure 8–2 shows serum concentrations of carbamazepine after single 200-mg doses of two different commercial tablets. It is difficult to determine from these data whether the higher serum levels resulting from product A are the result of greater availability of carbamazepine or merely faster absorption. Steady-state concentrations, shown in Figure 8–3, resulting from multiple dosing of each product at equal daily doses in each patient, clearly indicate that the products are bioequivalent.

Bioavailability studies are typically of a cross-over design; each person in a panel of subjects receives each treatment. This design avoids the problem of intersubject variability in drug elimination, which could obscure comparisons of AUC or A_u; all dosage forms are compared in each individual. The cross-over design, however, does not account for intrasubject variability (i.e., variability in drug elimination in the same subject from one administration to another). Drugs that show a high degree of intrasubject variability require large panels of subjects to differentiate dosage forms or to conclude that dosage forms are bioequivalent with an adequate degree of certainty.

When two products are given to the same individual on separate occasions and result in different AUC values, the dissimilarity may either be due to different bioavailability characteristics or to variability in drug clearance from one occasion to the other. In a two-period crossover study, we may incorrectly interpret the variation in clearance as reflecting a difference in bioavailability. Therefore, we would like to correct for the variability in clearance to improve our evaluation of bioavailability.

Some investigators have suggested that if half-lives are different between two treatments, this might reflect a difference in clearance. The equation for this correction is as follows:

$$F = (AUC)_{test}(t_{1/2})_{standard}/(AUC)_{standard}(t_{1/2})_{test} \qquad (8–10a)$$

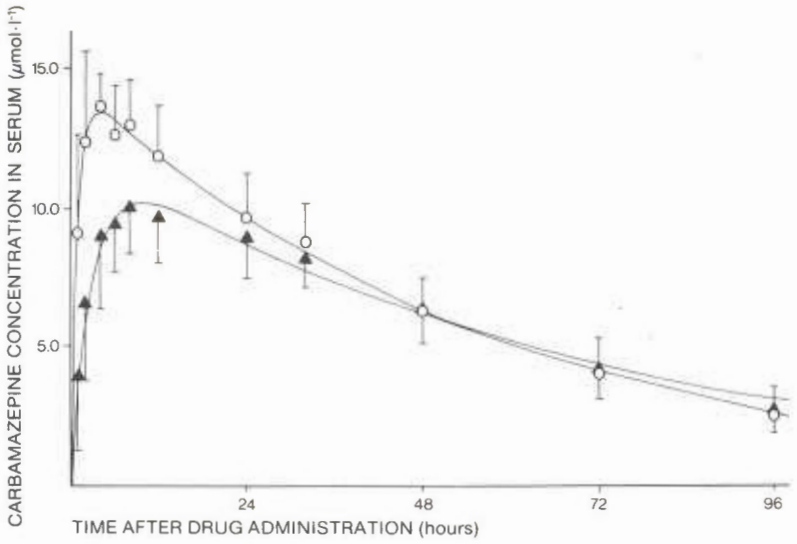

Fig. 8–2. Carbamazepine concentrations in serum after single 200-mg oral doses in 2 different tablet products. (Data from Anttila, M., et al.[9])

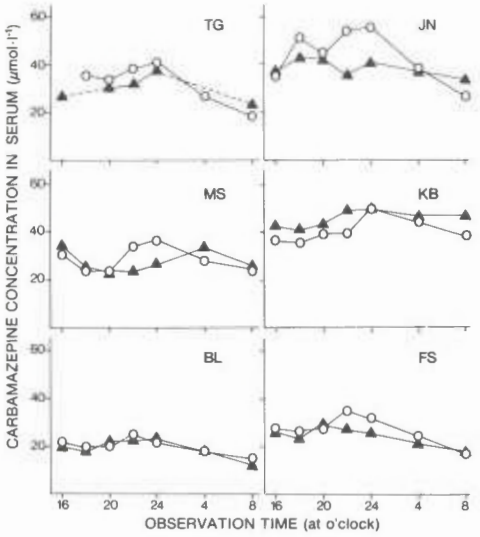

Fig. 8–3. Carbamazepine concentrations in serum at steady state in different subjects after repetitive oral dosing with 2 different tablet products. (Data from Anttila, M., et al.[9])

If half-life estimates are randomly distributed for test and reference treatments, then half-life correction is warranted, if the variance of the corrected bioavailability (F) value is less than for uncorrected values. In those situations where half-life estimates are not randomly distributed across treatments (i.e., the half-life for one treatment is consistently larger than for another), then prolonged absorption of the drug rather than variation in clearance may be causing the apparent half-life change. In this circumstance, half-life correction is not appropriate.

A more rigorous correction can be applied by administering simultaneously the oral dosage form and an intravenous solution of labeled drug. In this manner, clearance can be calculated independently for each leg of the study. Alternatively, an oral solution containing labeled drug can be given at the same time as the test dosage form. Interest in reducing the effect of intrasubject variability on bioavailability studies by correcting for differences in clearance has been stimulated by increased availability of stable isotopes (e.g., drug molecules containing ^{2}H or ^{13}C atoms), which are considered safer than radioactive isotopes, and the advances in gas chromatography-mass spectrometry (GC-MS).[10]

One report describing the use of stable isotopes was concerned with the bioavailability of maprotiline, a tetracyclic antidepressant.[11] Six subjects were given simultaneous single 50-mg oral doses of tablets containing maprotiline HCl and an aqueous solution containing trideuterated maprotiline HCl. The mean AUC values for the solution and tablet had coefficients of variation (CVs) of about 65%, whereas the mean value for relative bioavailability (AUC_{tab}/AUC_{soln}) had a CV of only 5%.

More recently, Shinohara et al.[12] used stable isotopes to determine the bioavailability of methyl-

testosterone (MT) tablets in 8 subjects. The study was carried out in a crossover manner in order to compare the stable isotope method with the conventional crossover method. Each subject was given a 10-mg MT tablet with a reference solution containing 10 mg trideuterated methyltestosterone (MT3D) on one occasion, and a solution containing 10 mg MT with the MT3D reference solution on another. Serum samples were analyzed for MT and MT3D by GC-MS.

When the tablet and reference solution were given at the same time, the peak concentration of MT3D (reference solution) was almost twice as great as that for MT (tablet), but the average AUC values were nearly identical. Mean relative bioavailability for the tablet was 101%. The mean AUCs for the reference solution and tablet had CVs of 42% and 45%, respectively. The mean relative bioavailability had a coefficient of variation of only 18%.

Relative bioavailability was also determined from AUC values for MT after administration of tablet and solution on separate occasions. The mean was 97%, similar to the results in the stable-isotope study, but the coefficient of variation was 38%, more than twice that observed in the isotope study. The investigators concluded that the assumption of a constant clearance in individual subjects on different occasions may be a poor one, certainly for methyltestosterone, and probably for most drugs.

Shinohara et al. also made theoretical calculations to estimate the number of subjects required to detect (with a probability of 0.8) a difference of 20% between the tablet and solution. They estimated that 40 subjects were required for a conventional crossover study, whereas only 12 subjects would be needed for the stable-isotope method.

In 1979, investigators from the FDA and other laboratories reported a new approach to comparative bioavailability testing.[13] They proposed the usual crossover design but added that each formulation would be taken with a solution containing a stable isotope of the drug. They used this approach to compare the bioavailability of two brands of imipramine tablets.

A solution containing 25 mg dideuterated imipramine (IMP2D) was taken each time an imipramine (IMP) tablet was administered. Blood samples were collected after drug administration and plasma was analyzed for IMP and IMP2D. Crossover studies were run 1 week apart.

The data were analyzed in the conventional way by comparing the AUC resulting from each tablet, as well as in a new way by comparing relative parameters. The AUC for IMP from tablet *A* relative to the AUC for IMP2D from the reference solution given at the same time was compared with the corresponding values for tablet *B* relative to its reference solution.

Although both methods of comparison suggested that the two imipramine tablets were bioequivalent, statistical power differed remarkably. This is readily seen when the data set is used to calculate the number of subjects needed to detect (with a probability of 0.8) a difference in AUC of 20% between the two tablets. The conventional crossover study was found to require 20 subjects, whereas the relative crossover study (using a stable isotope as an internal standard) would require only 4 subjects.

ESTIMATING THE ABSORPTION RATE OF A DRUG

Rigorous methods are available to evaluate the kinetics of drug absorption after administration of a test dosage form, but these methods require concentration-time data after rapid intravenous injection of the drug in the same individual.[14] Unfortunately, an intravenous reference curve is not available for most drugs.

At this time there are no completely satisfactory methods to evaluate absorption kinetics solely from data obtained after oral administration. Despite the limited methodology, there is keen interest in some quarters for comparative absorption rate data. Regulatory agencies often ask for a quantitative evaluation of absorption kinetics as part of the pharmacokinetic characterization of new drugs; this is considered particularly important for those drugs where rapid absorption is needed for clinical response and for drugs in prolonged-release dosage forms.

The pharmaceutical industry has an additional interest in the evaluation of absorption rate, to establish in vivo-in vitro correlations. Quantitative correlations between gastrointestinal absorption and in vitro dissolution rates may permit rapid screening of new dosage forms and serve as a quality control tool to quickly assess the potential effects of small changes in processing or composition

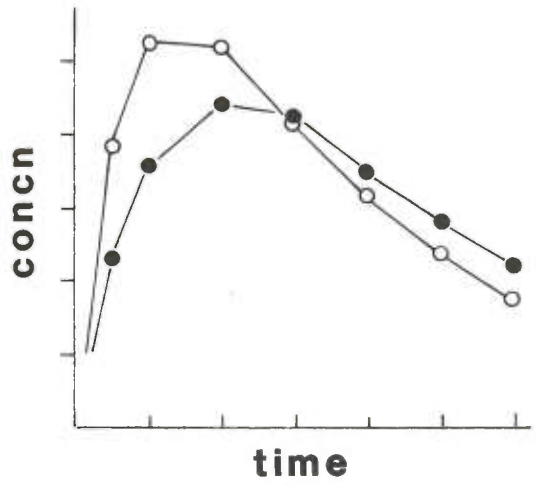

Fig. 8–4. Effect of absorption rate on the time course of drug in the plasma after a single oral dose. The faster the absorption, the higher is the peak concentration and the shorter is the time to peak.

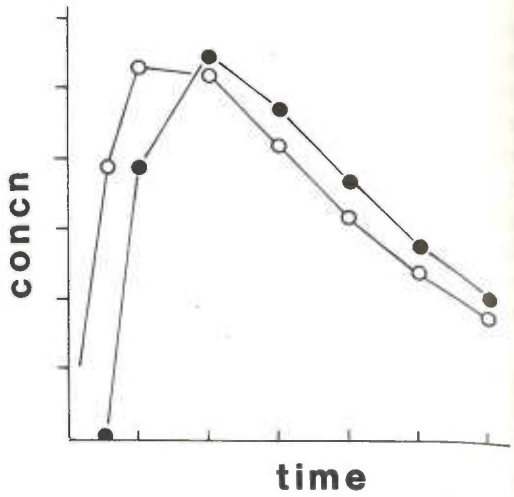

Fig. 8–5. Effect of a delay in gastric emptying or drug release from the dosage form on the time course of drug in the plasma after a single oral dose. The peak concentrations after each dose are similar but there is a difference in the time to peak.

or of product age on the bioavailability of drug from the dosage form.

For clinical purposes, most investigators find it sufficient to compare peak concentrations of drug in blood or plasma and the time required to reach the peak after a single dose of the drug in different dosage forms. The faster the absorption of a drug, the larger is the peak concentration, and the shorter is the time to peak (Fig. 8–4). Sometimes, one may find two dosage forms that release drug at about the same rate but differ in their dependence on gastric emptying or in the time for onset of drug release. The latter may be observed when a film-coated tablet is compared with an uncoated tablet. When this occurs, the peak concentrations will be about the same, but the time to peak will differ, because of the difference in lag time before absorption begins (Fig. 8–5).

Precise definition of the time to peak is often difficult because of limited opportunities to take blood samples. Ronfeld and Benet have shown that, with normal biological and experimental variability, it may be impossible to differentiate, on the basis of peak times, two dosage forms that differ in their release rates of drug by a factor of two.[15] Accordingly, this method for comparing absorption rates may be insufficiently sensitive for some needs. Furthermore, estimates of relative times to peak or peak concentrations are of little use in the evaluation of prolonged release dosage forms, which may produce no well defined peak concentration.

The statistical moments theory offers an attractive alternative for the evaluation of absorption data. As noted in Chapter 2, the difference between the mean residence time (MRT) after administration of a test dosage form (MRT_{test}) and the MRT after rapid intravenous injection (MRT_{iv}) is the mean absorption time (MAT):

$$MAT = MRT_{test} - MRT_{iv} \qquad (8–11)$$

If absorption is first-order, then:

$$MAT = 1/k_a \qquad (8–12)$$

where k_a is the first-order absorption rate constant.

Even in the absence of intravenous data, MAT is useful. For example, the relative ranking of MRT values following several dosage forms mirrors the relative ranking of the dosage forms with respect to drug release and absorption.

Riegelman and Collier proposed that the difference in MRT after a test oral dosage form and an aqueous solution, (MRT_{soln}) is equivalent to the mean dissolution time (MDT) or mean release rate of drug from the dosage form in the gastrointestinal tract:[16]

$$MDT = MRT_{test} - MRT_{soln} \qquad (8–13)$$

This approach has the potential to be a useful tool in the biopharmaceutic evaluation of dosage forms.

The absorption of furosemide has been studied by means of moment analysis.[17] The mean residence time after an intravenous bolus of furosemide, MRT_{iv}, was 51 min. After oral administration of a furosemide tablet to fasting subjects MRT was 135 min. The difference (MAT) is 84 min. The mean absorption time for oral furosemide was significantly greater than MRT_{iv}, indicating absorption rate-limited elimination kinetics.

The mean absorption time for furosemide tablets given immediately after a meal was 144 min, considerably longer than the mean value calculated when the tablets were given to fasting subjects. The difference in MAT values for the tablet given to fasted and fed subjects, 60 min in this case, is a representation of the delay in absorption resulting from the meal. It might be looked upon as the mean increase in gastric emptying time.

When an oral solution of furosemide was given after a meal, MAT was 109 min. The difference between MAT for the tablet and solution given after a meal was 35 min, representing the mean postprandial dissolution time for furosemide tablets.

PREABSORPTIVE HYDROLYSIS AND METABOLISM

The principal sites of chemical or biochemical (metabolic) conversion of a drug in the gut lumen are the stomach (acid), small intestine (esterases and other enzymes), and distal small intestine and colon (gut bacteria). These conversions can take place in parallel with or precede drug absorption and result in reduced availability.

Some drugs are not chemically stable at the low pH of the stomach; examples include penicillin G, methicillin, erythromycin, and digoxin. After oral administration, they are subject to acid hydrolysis in the stomach to form inactive products; less than 100% of the administered dose is available for absorption. This problem can usually be predicted from in vitro chemical stability studies.

The availability of drugs subject to acid hydrolysis in the stomach is a function of the rate of dissolution and the residence time of the drug in the stomach. Minimizing the dissolution of the drug in the stomach leads to increased availability. Factors that promote gastric emptying or increase gastric pH also result in improved bioavailability.

The importance of enzymatic hydrolysis in the fluids of the small intestine in determining the availability of drugs is unknown. Esterases are certainly ubiquitous in the body and could, in principle, degrade drugs like aspirin or ester prodrugs like pivampicillin or chloramphenicol palmitate before or in competition with the absorption process. In general, however, the gut wall is likely to be a more important site for the enzymatic hydrolysis of esters than is the gut lumen. If pivampicillin, for example, is subject to hydrolysis in the fluids of the small intestine, this surely must represent only a small fraction of the dose because the blood levels of ampicillin are much higher after a dose of the prodrug than after an equivalent dose of ampicillin. This means that a significant fraction of the pivampicillin dose must be absorbed (penetrate the gut wall) as such and thereby evade preabsorptive metabolism.

Many different kinds of microorganisms are normal residents of the lower intestine. These bacteria, which constitute the intestinal microflora, can carry out a variety of metabolic processes, but they are particularly adept at reduction, including the reduction of double bonds, azo groups, aldehydes, ketones, and alcohols.[18]

Most drugs are absorbed before reaching the ileum and are not subject to metabolism by intestinal microorganisms. On the other hand, a substantial fraction of an oral dose of a slowly absorbed drug or a drug given in a prolonged-release dosage form may reach the lower intestines. When this occurs, preabsorptive metabolism by the intestinal microflora may affect the availability of the drug. This situation applies to digoxin.

In certain patients, about 10% of the population taking the drug, the availability of digoxin is unusually low. These patients also excrete large amounts of digoxin reduction products or DRPs in the urine. Moreover, there is a tendency in the general population to greater excretion of DRPs when poorly absorbed preparations are taken (Fig. 8–6). There is convincing evidence that digoxin is extensively inactivated by intestinal microorganisms in a minority of those receiving the drug and that this problem is more widespread with slowly absorbed preparations of the drug.[19,20]

The proposition that metabolism by intestinal microflora is more important for slowly-absorbed than for rapidly-absorbed drug products was tested by determining the effect of metoclopramide on digoxin absorption after a 0.5-mg dose of digoxin tablets or a 0.4-mg dose of a digoxin solution encapsulated in soft gelatin.[21] Digoxin is more rapidly and more completely absorbed from the soft gelatin capsules than from the tablets. Metoclopramide de-

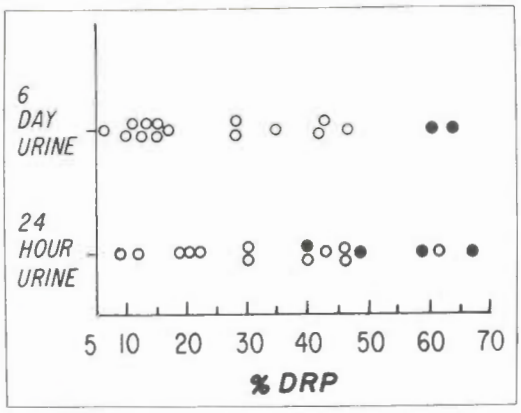

Fig. 8–6. Percent of drug-related material in the urine present as digoxin reduction products after a single oral dose of digoxin. ○: solutions or tablets with high dissolution rates. ●: tablets with low dissolution rates. (From Lindenbaum, J., et al.[19])

creased the bioavailability of digoxin tablets by about 25%, on the average, but had no effect on the bioavailability of digoxin following administration of soft gelatin capsules.

Another example is seen with acenocoumarol, an oral anticoagulant used outside the U.S. Acenocoumarol is converted by gut flora in vitro to amino and amido metabolites. Under typical clinical conditions, however, bacterial metabolism is of little importance because acenocoumarol is rapidly absorbed from its dosage form. Studies with commercial tablets indicate no measurable levels of reduced metabolites in plasma and less than 1% of the oral dose is excreted in urine as reduced metabolites. Administration of slowly-dissolving capsules containing relative coarse, crystalline acenocoumarol produced measurable plasma levels of both the amido and amino metabolites. Urinary recovery of reduced metabolites accounted for 6 to 12% of the dose.[22]

Certain oral antibiotics, including tetracycline and erythromycin alter the bacterial flora and decrease the inactivation of digoxin. Steady-state serum levels of digoxin in some patients have been found to increase 2-fold during oral antibiotic treatment, presenting the risk of toxicity.[24]

Other reports indicate that changes in gut bacteria as a result of treatment with antibiotics affect the disposition of sulfasalazine and oral contraceptives. Bacterial metabolism reduces the azo linkage in sulfasalazine to liberate sulfapyridine and 5-aminosalicylic acid (mesalamine) in the lower bowel. A 5-day course of oral ampicillin, 250 mg 4 times daily, significantly reduced gut bacteria-mediated conversion of sulfasalazine to sulfapyridine. AUC values for sulfapyridine after a single oral dose of sulfasalazine decreased from 370 μg-hr/ml under control conditions to 239 μg-hr/ml after ampicillin.[23]

PRESYSTEMIC METABOLISM

After oral administration, a drug must pass sequentially from the gastrointestinal lumen, through the gut wall, then through the liver before reaching the systemic circulation (Fig. 8–7). This sequence is an anatomic requirement because blood perfusing the entire length of the gastrointestinal tract, with the exception of the buccal cavity and lower rectum, drains into the liver by way of the hepatic portal vein. Since the gut wall and liver are sites of drug metabolism, a fraction of the amount absorbed may be eliminated (metabolized) before reaching the bloodstream. Therefore, an oral dose of a drug may be completely absorbed but incompletely available to the systemic circulation because of presystemic or *first-pass* metabolism in the gut wall or liver.

Criteria have been developed to identify and quantify the extent of presystemic metabolism and to indicate where it is occurring. Its detection requires only that systemic availability is less than the fraction of the dose absorbed. The fraction absorbed may be determined from the urinary excretion of drug and metabolites, usually as total radioactivity, after oral administration of the drug (in a radiolabeled form), relative to that after intravenous administration. Many drugs undergoing presystemic metabolism in man have been identified on the basis of this type of information. Differentiation of the gut wall and liver as the site of presystemic metabolism is relatively simple in animals, but more difficult in man.

The theory and our understanding of hepatic presystemic metabolism is relatively advanced; our knowledge of gut wall metabolism is less well developed. Because an understanding of the hepatic first-pass effect is often useful in differentiating the sites of presystemic elimination, we will first consider the liver as the site of presystemic metabolism.

Hepatic Presystemic Metabolism

The liver is the most important site of presystemic elimination because of its high level of drug

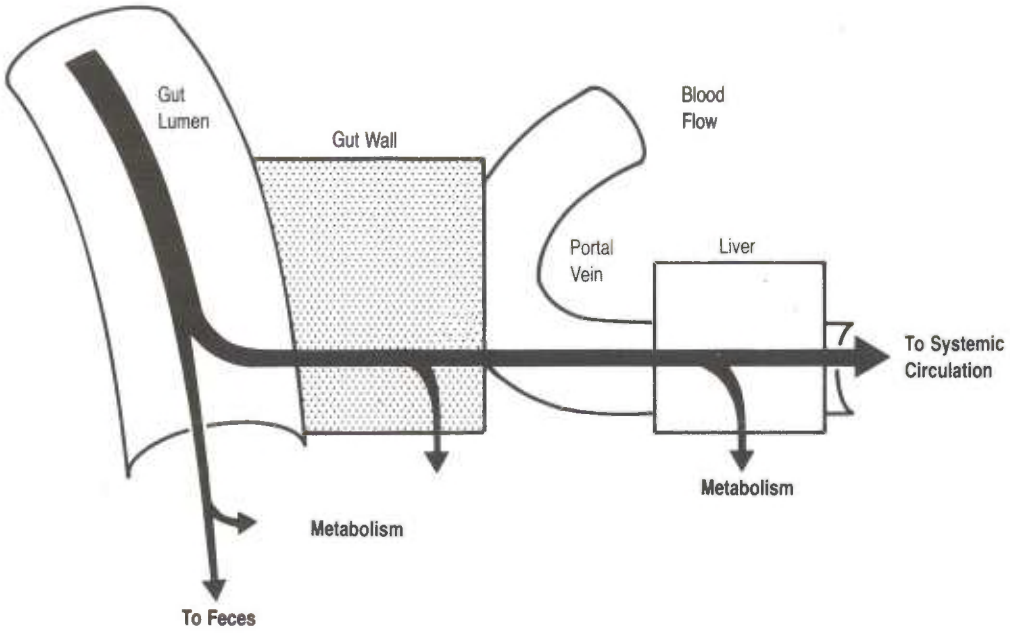

Fig. 8–7. After oral administration, a drug must pass sequentially from the gut lumen through the gut wall, then through the liver, before reaching the systemic circulation. Metabolism may occur in the lumen before absorption, in the gut wall during absorption, and/or in the liver after absorption but before reaching the systemic circulation. (From Rowland, M., and Tozer, T.N.[24])

metabolizing enzymes, its ability to rapidly metabolize many different kinds of drug molecules, and its unique anatomic location. A large number of drugs are subject to considerable hepatic first-pass metabolism; examples include β-blockers (propranolol and metoprolol), analgesics (propoxyphene, meperidine, and pentazocine), antidepressants (imipramine and nortriptyline), and antiarrhythmics (lidocaine and verapamil).

Hepatic presystemic metabolism is most easily understood when the liver is the sole organ of drug elimination. Under these conditions, the clearance of a drug, as determined after intravenous administration from the ratio of dose to area (AUC), is equal to hepatic clearance (Cl_H), which is given by:

$$Cl_H = Q_H ER_H \qquad (8–14)$$

where Q_H is hepatic blood flow and ER_H is the hepatic extraction ratio (see Chap. 2). Hepatic blood flow in man ranges from about 1.1 to 1.8 L/min, with an average of about 1.5 L/min. Hepatic extraction ratio may range from 0 to 1, depending on the liver's ability to metabolize the drug. The maximum clearance of a drug eliminated exclusively by hepatic metabolism is equal to hepatic blood flow; this occurs when $ER_H = 1.0$.

The fraction of drug eliminated from portal blood during absorption is given by the hepatic extraction ratio, ER_H; the remainder $(1 - ER_H)$ escapes into the systemic circulation, and is then cleared from the circulation by the liver, according to Equation 8–14. If a fraction (f) of the oral dose (D_o) is absorbed and then subjected to hepatic presystemic metabolism, the AUC after oral administration (AUC_o) is given by:

$$AUC_o = fD_o(1 - ER_H)/Q_H ER_H \qquad (8–15)$$

Since $Q_H ER_H$ is equal to hepatic clearance, which, under these conditions, is given by the ratio of intravenous dose (D_{iv}) to area (AUC_{iv}), we may rewrite Equation 8–15 as follows:

$$AUC_o/AUC_{iv} = fD_o(1 - ER_H)/D_{iv} \qquad (8–16)$$

The ratio of areas after oral and intravenous administration of equal doses of a drug is equal to its systemic availability (F). If we also assume that absorption is complete (f = 1), then:

$$F = (1 - ER_H) \qquad (8–17)$$

Equation 8–17 shows that systemic availability

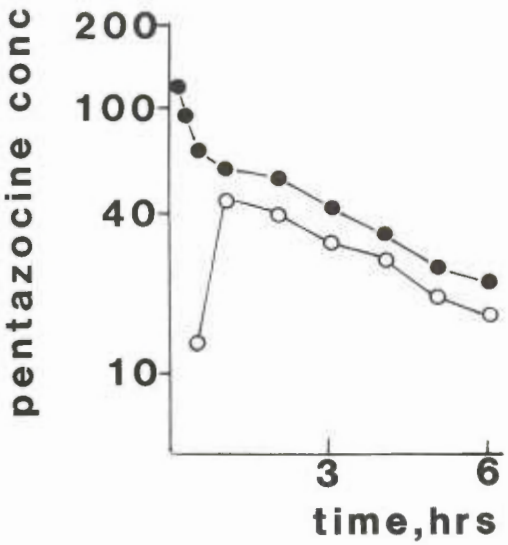

Fig. 8–8. Pentazocine concentrations in plasma (ng/ml) after administration of 100 mg orally (○) or 30 mg intravenously (●). (Data from Ehrnebo, M., Boréus, L.O., and Lönroth, U.[29])

depends on the hepatic extraction ratio. Drugs with low extraction ratios, such as antipyrine, warfarin, and tolbutamide, undergo little presystemic metabolism.

An estimate of the hepatic extraction ratio may be made by determining the clearance of the drug after intravenous administration and comparing this value to a mean value for liver blood flow, according to a rearrangement of Equation 8–14:

$$ER_H = Cl_H/Q_H \qquad (8–18)$$

The intravenous clearance of propranolol is about 1.05 L/min in man. Assuming an average liver blood flow of 1.5 L/min, we can calculate that $ER_H = 0.7$ and $F = 0.3$. Although propranolol is well absorbed, only 30% of an oral dose is available to the systemic circulation. This kind of information, in conjunction with experimental estimates of F, has been used to substantiate the predominantly hepatic presystemic elimination of several drugs, including propranolol,[25] lidocaine,[26] imipramine,[27] papaverine,[28] and pentazocine.[29]

Plasma concentrations of pentazocine after administration of 100 mg orally and 30 mg intravenously are shown in Figure 8–8. Although the intravenous dose is smaller, it results in higher plasma levels. The systemic availability of pentazocine after oral administration, calculated after taking into account the difference between intravenous and oral doses in 5 subjects, varied from 11 to 32%, with a mean value of 18%. This low systemic availability of pentazocine is consistent with its high hepatic clearance, in the order of 1.2 L/min.[29]

Table 8–1. Relationship Between Steady-State Concentration of Alprenolol on 200 mg Twice a Day and Single Dose Data After Oral or Intravenous Administration*

Rank No.	Steady-state concn. (ng/ml)	Bioavailability (oral)	Clearance (iv)
1	37.0	0.15	0.71
2	32.1	0.13	0.52
3	14.1	–	1.37
4	13.2	0.07	0.94
5	12.0	0.05	0.78
6	3.9	0.03	0.41
7	2.7	0.01	2.03

*Data from Alván, G., et al.[30]

With many drugs, presystemic metabolism and systemic availability vary markedly from one person to another. The variability contributes to the interindividual differences in steady-state concentrations of the drug. Studies with the β-blocker alprenolol show a 14-fold range in steady-state concentrations in healthy subjects taking oral doses of 200 mg twice a day. Intravenous studies in the same subjects indicate only a 4-fold range of clearance values.

Additional studies reveal that the rank order for individual steady-state plasma concentrations of alprenolol is the same as that for the relative bioavailability of the 200-mg oral dose; no correlation is found between steady-state levels and individual clearance values (Table 8–1). These results demonstrate that differences in first-pass metabolism contribute substantially to interindividual variability in steady-state plasma concentrations of a drug with a high hepatic extraction ratio.[30]

Presystemic metabolism after oral administration of a drug results in the formation of a bolus of metabolites during the drug's first pass through the liver. Accordingly, we would expect to see higher peak levels of metabolites after oral administration of a drug with a high hepatic extraction ratio than after parenteral administration. Figure 8–9 shows mean plasma concentrations of nortriptyline (NT) and its 10-hydroxy metabolite after oral and intramuscular administration of the same dose of NT. Lower concentrations of NT occur after oral than after intramuscular administration. In contrast, initial plasma concentrations of the metabolite (up to 10 hr) are much higher after oral than after intramuscular doses.[31]

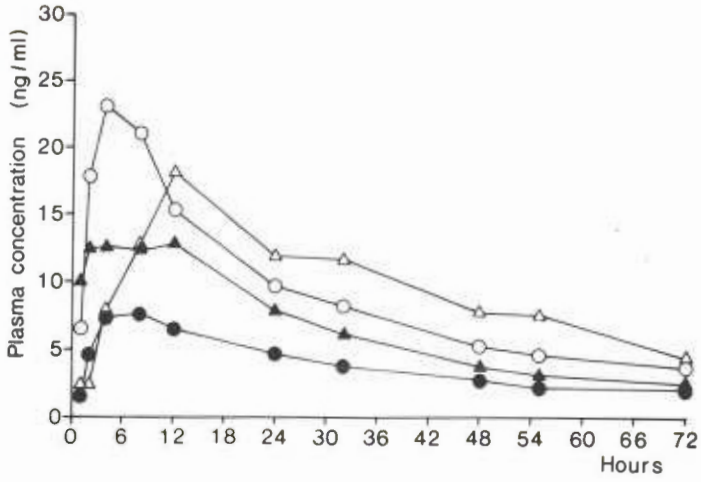

Fig. 8–9. Nortriptyline (▲,●) and 10-hydroxynortriptyline (△,○) concentrations in plasma after oral (●,○) and intramuscular injection (▲,△) of a 40-mg dose of nortriptyline. (From Alván, G., et al.[31])

Gut Wall Presystemic Metabolism

Presystemic metabolism in the gut wall and liver can be differentiated in animals by comparing drug concentration after oral and intraportal administrations to assess the contribution of the gut wall, and after intraportal and intravenous administrations to assess the contribution of the liver.

Glucuronidation of morphine, naloxone, and buprenorphine by the liver and intestine has been compared in rats.[32] The drugs were given by peripheral intravenous (iv) and hepatic portal vein (hpv) injection, and instilled into the duodenum (id). AUC decreased in the following order: iv > hpv > id. The results suggest that these related compounds are subject to presystemic metabolism, in both the gastrointestinal wall and the liver. For each drug, hepatic extraction was more efficient than intestinal extraction.

Another experimental model was developed to determine the site of first-pass metabolism of midazolam, a benzodiazepine with high presystemic extraction after oral administration.[33] Domestic pigs received single intravenous and oral doses of the drug. Multiple blood samples were simultaneously drawn from the portal vein and from a systemic vein during the first 8 hr after the dose. Differences in AUC at the two sampling sites after oral administration indicate hepatic extraction; differences after iv administration indicate gut wall extraction.

After iv administration, midazolam had a high systemic clearance value, suggesting the likelihood of first-pass metabolism. AUC values for systemic vs portal sites were nearly identical, suggesting little, if any, metabolism in the gut wall. After oral administration the systemic/portal AUC ratio averaged only 0.15, suggesting a high degree of hepatic extraction. The portal AUC after oral administration was similar to the systemic AUC after iv administration, again suggesting little gut wall metabolism. The investigators concluded that the extensive presystemic extraction of oral midazolam is largely the result of hepatic biotransformation rather than metabolism either within the gastrointestinal tract or during absorption into the portal circulation.

Despite the importance of understanding the site of presystemic extraction of drugs, human studies are limited by the necessarily invasive experimental techniques. Sampling of portal blood is generally possible only in patients in whom portal catheterization is otherwise clinically indicated.

An example is found in a report on the concentrations of phenacetin and its metabolite, acetaminophen, in portal and hepatic venous blood after intragastric or intraduodenal administration of phenacetin to patients with portal hypertension.[34] The concentration ratio of metabolite to drug in portal blood soon after drug administration was low, ranging from 0.01 to 0.11. Furthermore, at each sampling time, the concentration ratio in the portal vein was much lower than in the hepatic vein or in peripheral blood. The hepatic extraction ratio of phenacetin was estimated to be about 0.6 to 0.8, consistent with the low bioavailability of the drug.[35]

These results indicate that O-dealkylation of phenacetin occurs mainly in the liver and only to a limited extent in the gut wall.

A similar study in patients with portal hypertension was carried out with flurazepam.[36] High concentrations of the mono- and didesethyl metabolites of flurazepam were found in portal vein blood soon after intraduodenal administration of the drug, consistent with intestinal wall metabolism. Efficient hepatic extraction of both flurazepam and its metabolites, however, was also observed. The results suggest that presystemic metabolism of flurazepam in man occurs in the gut wall as well as in the liver.

More direct evidence of gut wall metabolism in man is found in a report on the concentrations of ethinyl estradiol and its conjugated metabolite in portal and peripheral vein blood following oral administration to postsurgical patients.[37] In each patient, for about 40 to 50 min after administration, the concentration of conjugated ethinyl estradiol in the portal vein was considerably higher than in the peripheral vein. Back and co-workers calculated that about 44% of the absorbed dose undergoes presystemic metabolism in the gut wall;[37] an additional 25% of the dose is subjected to hepatic first-pass metabolism.

In vitro studies show that ethinyl estradiol is extensively metabolized by human jejunal mucosa, obtained by biopsy from healthy subjects, to form the sulfate conjugate.[38] The degree of conjugation of mestranol and levonorgestrel, two other contraceptive steroids, was much lower than for ethinyl estradiol. The results with levonorgestrol are consistent with the high systemic availability of the steroid.[39]

Changes in metabolite excretion patterns may provide indirect evidence for gut wall metabolism. Intravenous isoproterenol is excreted largely unchanged in man. On the other hand, the sulfate conjugate accounts for 80% of the drug in the urine after oral administration. No sulfate conjugate is found after intravenous administration. The results suggest that the presystemic metabolism of isoproterenol in man is confined to the mucosal surface of the gut wall.[40]

Albuterol (salbutamol), a potent beta-adrenergic agonist used widely in the treatment of bronchial asthma, is subject to substantial presystemic metabolism after oral administration. Morgan et al.[41] studied the kinetics of albuterol and its sulfate conjugate metabolite, in plasma and urine, after intravenous and oral administration.

After iv administration, total plasma clearance was 480 ml/min and the elimination half-life was about 4 hr. Urinary excretion of unchanged albuterol accounted for 64% of the dose and the sulfate metabolite accounted for 12%. After oral administration, systemic availability was only 50%, and urinary excretion of unchanged drug and metabolite accounted for 32% and 48% of the dose, respectively.

Total urinary recovery of drug-related material was similar after each route of administration, indicating that although oral albuterol has a low bioavailability, it is well absorbed from the gastrointestinal tract. The data also indicate that the fraction of the dose of albuterol eliminated on the first pass could be accounted for entirely as sulfate conjugate formed, presumably, in the gut wall.

Commonly, the existence of gut wall metabolism is inferred when the degree of presystemic metabolism of drug exceeds the hepatic extraction ratio. For example, the hepatic extraction ratio of terbutaline, determined after intravenous administration, is only about 0.08. This means that if the entire oral dose were absorbed, a systemic availability of 92% should result. In fact, the availability of terbutaline is only 10%. Determination of free terbutaline in the feces suggests that only 55% of the drug is absorbed. Under these conditions, we expect a systemic availability of 0.55×0.92 or 51%. Clearly, incomplete absorption and hepatic presystemic metabolism cannot account for the low systemic availability of terbutaline. We must conclude that a large fraction of the dose of terbutaline is metabolized by another presystemic route, most likely the gut wall.[42]

REGULATORY AND CLINICAL CONSIDERATIONS

Both biopharmaceutic and metabolic factors influence the bioavailability of drugs. Although there is usually little we can do to alter unfavorable metabolic characteristics, this is not true for biopharmaceutic factors that limit the availability of a drug. During the last decade there has been a heightened awareness of the role of the dosage form on the bioavailability and clinical efficacy of drugs; the general result has been better dosage forms.

For some time now, the U.S. Food and Drug Administration has required some degree of characterization of bioavailability for all new drugs intended for oral use. Some attention has also been given to dosage forms intended for other routes of

administration. These requirements have established a standard of performance.

More recently, the FDA has required secondary (or generic) manufacturers who are interested in marketing a drug after a patent or period of exclusive-use has lapsed to demonstrate bioequivalence (comparable bioavailability) with the innovator's dosage form before approval to market is granted. The Congress directed the FDA to apply these criteria to generic products through the passage of the Drug Price Competition and Patent Restoration Act in 1984. Before this landmark legislation, the only way a secondary manufacturer could market a drug was to carry out clinical trials demonstrating comparable efficacy to the innovator's product.

A bioequivalence trial generally consists of a comparison of the area under the drug concentration-time curve, peak concentration, and time to peak concentration after a single dose of the generic and "standard" product using a randomized, two-way crossover design. Urinary excretion data may also be useful, particularly for drugs that are substantially excreted unchanged. The FDA bioequivalence guidance for hydrochlorothiazide recommends a urinary excretion study.

Panels of healthy human subjects are almost always used in bioequivalence studies. The FDA recognizes the possibility that some conditions found only in special populations (patients, elderly, etc.) could affect bioavailability and is prepared to modify its guideline calling for the use of normal subjects if the need is adequately documented for a given drug.

The Agency also requires the determination of metabolite kinetics if the drug is metabolized to a clinically important biotransformation product. This requirement is controversial. Some scientists believe that a metabolite should be followed only as an alternative when it is difficult to measure unchanged drug in the plasma.

Can dissolution testing assure bioequivalence? This question has been widely debated. The FDA and most pharmaceutical scientists believe that there is not yet evidence to show that a dissolution test will assure bioequivalence. Dissolution testing is important in assuring lot-to-lot uniformity of a drug product and supporting minor changes (e.g., a change in color) in the product. Also, it is FDA policy that if a product meets in vivo bioequivalence requirements at one dosage strength and the formulations of other strengths are proportional to the strength tested and meet dissolution requirements, then no further in vivo studies are needed for approval.

The usual criteria for bioequivalence calls for the mean AUC and C_{max} values for the two products to be within 20%, but the FDA also applies a 90% confidence interval test based on the two one-sided t-test approach,[43] one test to verify that the bioavailability of the test product is not too low, and the other to show that it is not too high. The entire 90% confidence interval must also lie within the limits of plus-or-minus 20%.

This confidence interval requirement ensures that the difference in mean values for AUC and C_{max} will be much less than 20%. The experience to date in reviewing bioequivalence studies with generic products indicates that 80% of the approvals had AUC values within 5% of the reference product. In view of this experience, some scientists believe that the FDA should be more stringent, requiring the mean values for AUC to be within 10% rather than 20%. On the other hand, some believe that the current requirements for C_{max} values are too stringent, considering the difficulty in accurately estimating this value, and the typical finding for most products (generic or brand name) that C_{max} values are more variable than AUC values.

The approval process for generic products has worked remarkably well for conventional oral dosage forms. Almost no documented examples of clinically important differences between generic and original products have been reported. The one class of drugs that continues to be put forward (often with scant evidence) as a challenge to the sufficiency of bioequivalence studies to assure the performance of a generic product is the anticonvulsants.

A case for bioinequivalence of a generic drug product has been made in a report concerning a 16-year-old girl with severe cerebral palsy and seizures since birth.[44] During treatment with primidone and other medication, her usual seizure frequency was one to two seizures per week. Serum levels of both primidone and phenobarbital, its metabolite, are frequently monitored in patients receiving primidone.

The patient had been taking the same antiepileptic medication for 9 years. Within 3 weeks of switching her to a generic primidone, there was a rise in seizure frequency and she was switched back to the original dosage form. With this change, the seizure frequency decreased to baseline. Serum

drug concentrations were not measured during this period.

The patient's condition remained stable until 3 months later, when she was admitted to hospital for feeding problems. Before admission, she was taking her usual medication and serum trough levels were 10.8 mg/L for primidone and 19.1 mg/L for phenobarbital. During hospitalization, she was again switched to the primidone product that caused a problem 3 months earlier. After 6 days of receiving this product, morning trough levels were 5.1 mg/L for primidone and 15.9 mg/L for phenobarbital.

On day 6, the daily dose of primidone was increased from 500 to 625 mg, but despite this change, serum levels continued to fall and the patient had more frequent seizures. On day 10, serum primidone was less than 2.0 mg/L and serum phenobarbital was 10.4 mg/L. At this time, the patient was returned once again to the original primidone product. After 6 days of receiving this product at a dose of 500 mg/day, primidone levels were 9.0 mg/L and phenobarbital levels were 12 mg/L, and the patient's seizure frequency returned to baseline.

The evidence is clear that the two primidone products used in this patient were not bioequivalent. This observation raises concern that an initial determination of bioequivalence may change with time because of subtle changes in manufacturing or lot-to-lot variability. This problem seems to call for some stringent dissolution criteria. In any event, the investigators urged that product substitution be cautiously considered in patients who have already been titrated and maintained on an antiepileptic preparation.

Controlled-Release Medication

A basic question in developing a controlled-release product of a drug that has been used in a conventional dosage form is whether a formal clinical evaluation of the new dosage form's safety and efficacy is needed, or whether a pharmacokinetic evaluation will suffice. The FDA's position is that if there is a well-defined relationship between plasma concentration of drug and/or active metabolite and clinical response, it may be possible to rely on plasma concentration data alone as a basis for the approval of a product.

On the other hand, "where the therapeutic effect is indirect, where irreversible toxicity can occur, where there is evidence of functional (pharmacodynamic) tolerance, where peak to trough differences of the immediate release form are very large, or where there is any other reasonable uncertainty concerning the relationship between plasma concentration and therapeutic and adverse effects, it will probably be necessary to carry out clinical studies."[45]

For the development of a controlled-release oral dosage form of a drug marketed in an immediate-release form for which an extensive base of pharmacodynamic-pharmacokinetic data exists, the following pharmacokinetic studies are usually required. A single dose, three-way crossover study where the immediate-release and the controlled-release products are given to fasted subjects, and the controlled-release form is also given after a high fat meal.

The fasting comparison permits an estimation of the extent of absorption from the controlled-release form relative to the immediate-release form. The food study is essentially a drug interaction assessment. If there are no differences in AUC and peak concentration following administration of the controlled-release form to fed and fasted subjects, then no further food studies are needed. If a decrease or an increase in the extent of absorption is found after a meal, it may be necessary to determine the cause of the food effect as well as the effect of time on the food-drug effect (i.e., would absorption be affected if the dosage form were given 1 or 2 hr after a meal rather than with a meal).

The FDA also requires a multiple dose, steady-state, crossover comparison of the controlled-release and immediate-release products as part of the pharmacokinetic evaluation. Ordinarily, the same daily dose is used for each regimen but the immediate-release form is given more frequently than the controlled-release form (e.g., 3 times a day versus once a day). Concentrations over at least one dosing interval should be measured in each leg of the crossover. Some investigators favor measurements over 24 hr in each leg of the study, to account for diurnal variation.

The controlled-release product should produce an AUC equivalent to the immediate release product, and the degree of fluctuation at steady-state [i.e., $(C_{max} - C_{min})/C_{av}$] for the controlled-release product should be similar to, or less than, that for the immediate release form. If appropriate, levels of major active metabolites should also be measured. For racemic drugs, consideration should be given to measurement of individual enantiomers.

Since the passage of the Drug Price Competition and Patent Restoration Act in 1984, attention has

also been given to criteria needed to demonstrate the equivalence of a generic product to an approved controlled-release product. The current position of the FDA on this matter is as follows: "the new generic formulation must be comparable with respect to AUC, C_{max}, and C_{min} in a cross-over steady-state study *vs* the standard controlled-release product using the accepted Agency criteria for equivalence. In some cases, it may also be necessary to match the concentration-time profile of the approved controlled-release dosage form. The food studies described previously are also needed."[45]

SPECIFIC DRUGS

The following discussion is a summary of reports of poor bioavailability or "inequivalences" of marketed products, listed alphabetically by drug. That most of the material has been taken from previous editions of this text and that comparatively few examples of bioinequivalence have been reported in the past five years are encouraging signs, indicative of the attention given to the development of dosage forms today.

Acetazolamide

Most of the reports on differences in bioavailability of marketed products have concerned prolonged-release dosage forms. Clinical studies with acetazolamide, a carbonic anhydrase inhibitor used in treating glaucoma, provide an example.[46] Acetazolamide was only 60% available from a sustained-release capsule, Diamox Sequels, compared to that observed after an aqueous suspension. Consistent with these results, steady-state concentrations of acetazolamide for the prolonged-release capsules were about half the values observed for an immediate-release dosage form. Since Diamox Sequels is considered to be an effective product, the results suggest that lower doses of acetazolamide in rapid-release dosage forms may be useful for treating glaucoma.[47]

Aminosalicylate

Studies in Canada with various dosage forms of aminosalicylic acid (PAS), which is used, usually in combination, in the treatment of pulmonary and extrapulmonary tuberculosis, indicated large differences in drug absorption.[48] The availability of a prolonged-release product, estimated from cumulative urinary recovery of the drug in 8 subjects, was only 42% compared to that observed following administration of a standard capsule containing drug and lactose. The relative availability of PAS from two different lots of an enteric-coated tablet and from a powder containing a polyamine resin complex of the drug was 51%, 64%, and 66%, respectively. Another investigation found no absorption of PAS in 8 subjects after administration of an enteric-coated tablet.[49]

Ampicillin

Concern for differences in bioavailability of the widely used antibiotic ampicillin was stimulated by a report from Canada demonstrating that two brands of ampicillin capsules produced lower serum concentrations than did ampicillin capsules manufactured by a third company.[50] Products B and C were only 78% and 72% as available as product A, based on the area under the serum concentration versus time curves. A second bioavailability study comparing product A with a reformulated product C indicated bioequivalence.[51] The reformulation involved a minor change in the amount of a dispersing agent. The bioavailability monograph on ampicillin published by the American Pharmaceutical Association in 1975 concluded that it is unlikely that possible differences in bioavailability among the current major United States suppliers are of clinical importance.[52] The same holds true today.

Aspirin

Poor bioavailability of aspirin has been reported only with enteric-coated products. Less than 25% of the dose was absorbed in 3 of 4 subjects after administration of a certain brand of enteric-coated aspirin tablets.[53] A clinical study with this enteric-coated product in arthritic patients showed erratic and low concentrations of salicylate, compared to those observed after regular administration of conventional aspirin tablets.[54] This problem has all but disappeared with the materials in use today to provide enteric protection.

Ascorbic Acid

This vitamin has been widely used since the claim in 1970 that daily consumption of large quantities of ascorbic acid may be beneficial for reducing the frequency and duration of the common cold. Ascorbic acid absorption was investigated in 4 subjects who received different oral dosage forms containing 1 g of vitamin C.[55] About 85% of a 1-g intravenous dose was recovered in the urine as as-