4

Absorption Kinetics and Bioavailability

Many pharmacokinetic studies are concerned principally with the *bioavailability* of the drug. Bioavailability, in simple terms, refers to the rate and extent of drug absorption. The rate at which a drug reaches the systemic circulation is an important consideration for drugs used to treat acute conditions, such as pain or insomnia, which can be ameliorated by a single dose. A drug that is absorbed slowly may not achieve sufficiently high concentrations at the site of action to elicit a desired effect or intensity of effect, even if the entire dose is absorbed. On the other hand, the extent of absorption is usually the more important factor for drugs that are administered repetitively for the treatment of subchronic or chronic conditions, such as infection, asthma, or epilepsy. The average drug concentration in plasma at steady state during repetitive administration is directly proportional to the amount absorbed from each dose but is independent of the rate of absorption. The rate of absorption does, however, influence the time course of drug concentration in plasma during a dosing interval at steady state. In some cases, very rapid absorption could produce transiently high drug concentrations in plasma that may be associated with adverse effects.

Comparative bioavailability refers to the relative bioavailability of a drug from two or more formulations. Comparative bioavailability studies are often carried out in place of clinical effect studies to determine whether two or more formulations containing the same active ingredients in the same amounts are therapeutically equivalent. It is assumed that two formulations that do not differ very much in the rate at which and extent to which they make the active ingredient available to the systemic circulation will not differ much in their therapeutic efficacy.

Pharmacokinetic theory is well developed and generally accepted for the determination of the extent or relative extent of absorption of a drug from a dosage form. Similar agreement does not exist with respect to characterizing the absorption rate of a drug. The results

of such analyses are usually dependent on the pharmacokinetic model that is assumed and are usually descriptive rather than rigorous. Characterization of absorption kinetics may be useful for determining relative differences in absorption rates between formulations in comparative bioavailability studies.

ABSORPTION RATE

Curve-Fitting

The most common method of evaluating absorption kinetics is to assume that the drug concentration-time data can be described by one of several pharmacokinetic compartment models and to fit the data to an equation consistent with the assumed model by means of the method of residuals (see Appendix C) or a nonlinear least-squares regression program and a digital computer (see Appendix H). The most common equations for a one-compartment model are

$$C = \frac{k_a F X_0}{V(k_a - K)} (e^{-Kt} - e^{-k_a t}) \tag{4.1}$$

which assumes first-order absorption and elimination,

$$C = \frac{k_a F X_0}{V(k_a - K)} [e^{-K(t-t_0)} - e^{-k_a(t-t_0)}] \tag{4.2}$$

which assumes a lag time t_0 before the onset of absorption,

$$C = \frac{k_0(e^{KT} - 1)e^{-Kt}}{VK} \tag{4.3}$$

which assumes zero-order absorption, where T = t during the absorption period and T = absorption time (a constant) during the post-absorption period, and

$$\frac{dX_u}{dt} = \frac{k_e k_a F X_0}{k_a - K} (e^{-Kt} - e^{-k_a t}) \tag{4.4}$$

which uses urinary excretion data. The output of the computer program contains estimates of the pharmacokinetic constants, including the absorption rate constant.

Ideally, one should have an independent estimate of K to differentiate the estimated rate constants and to avoid ambiguity in interpreting the results of such curve-fitting procedures. Serious problems are encountered if the absorption is complex rather than a simple first- or zero-order process. Sometimes most of the dose of a drug may be

relatively rapidly absorbed, but a small fraction of the dose is absorbed very slowly and absorption persists long after the time at which drug concentration in plasma reaches a maximum. In such cases the concentration-time curve may be apparently biexponential but the rate constant determined from the apparent postabsorption phase will be smaller than K. In this situation an independent estimate of K is needed. An example is shown in Fig. 4.1. Accurate estimates of k_a from urinary excretion data [see Eq. (4.4)] are possible only for drugs absorbed relatively slowly because urine collections cannot be made at very short intervals.

The absorption rate constants obtained by curve-fitting Eqs. (4.1) to (4.4) are at best estimates of the first-order loss of drug from the gastrointestinal tract, not of the first-order appearance of drug in the systemic circulation. If a drug undergoes simultaneous first-order absorption (rate constant k_{abs}) and first-order chemical or enzymatic degradation, k_d, in the gut, the apparent absorption

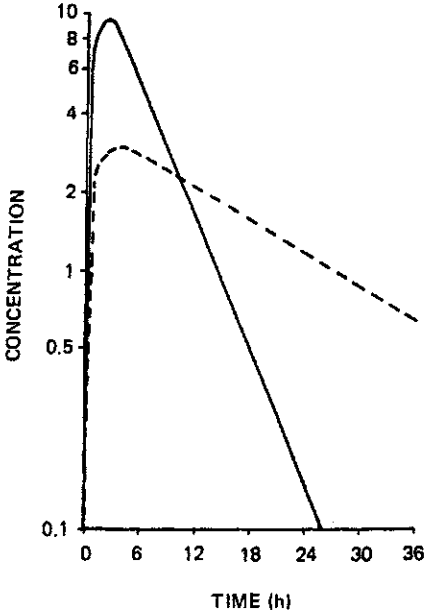

Fig. 4.1 Drug concentrations in plasma after oral administration of the same dose of drug as a conventional tablet (——) from which absorption is rapid and as a slowly dissolving tablet (---) from which absorption is slow. The half-life of the drug is 3.5 h, which is consistent with the value determined after giving the conventional tablet. The slow absorption found with the specialized dosage form results in an *apparent* half-life of 14 h.

rate constant, k_a, obtained on curve-fitting is actually the sum of k_{abs} and k_d [1]. Other factors that affect absorption, such as gastric emptying or gastrointestinal motility, can also distort the meaning of k_a [2,3]. In general, for any drug that is less than completely absorbed, it is unlikely that $k_a = k_{abs}$ [3].

Other problems in the estimation of k_a are encountered when curve-fitting concentration-time data to equations appropriate to a two-compartment model such as

$$C = Le^{-\lambda_1 t} + Me^{-\lambda_2 t} + Ne^{-k_a t} \tag{4.5}$$

[see Eq. (2.93)]. By definition $\lambda_1 > \lambda_2$ and it is likely for drugs that are rapidly absorbed that $k_a > \lambda_2$, but in all cases k_a may be smaller or larger than λ_1. There is no basis for assuming one or the other. Therefore, it is not possible to determine unambiguously k_a from drug concentration-time data obtained after oral administration. The dilemma may be resolved by independently estimating λ_1 and λ_2 after intravenous administration of the drug to the same subject. Some resolution may also be obtained by characterizing the pharmacokinetics of the drug after administration of a dosage form such as an oral solution, from which the drug is more rapidly absorbed. Most drug concentration in plasma-time data sets obtained after oral administration can be fitted with two exponential terms (i.e., a one-compartment model) rather than three exponential terms (i.e., a two-compartment model). However, intravenous administration of the same drug often suggests that the two-compartment model is more appropriate. Some reasons for this have been discussed in Chap. 2. Under these conditions, attempts to estimate the absorption rate constant from data obtained after oral administration can result in substantial error. It has been shown that if such data are fitted to Eq. (4.1), the larger of the two rate constants would not be equal to the absorption rate constant but, under certain conditions, may be equal to λ_1 [4]. Since for virtually all drugs the time course of concentration in plasma after intravenous administration shows multicompartment characteristics, and for most drugs a two- or three-compartment model is most appropriate, it follows that the estimate of an absorption rate constant from data obtained after oral administration of any drug, by assuming a one-compartment model, will be incorrect even if the drug were truly absorbed by apparent first-order kinetics.

Wagner [5] has proposed that although the absorption rate constant determined from a one-compartment fit of concentration-time data after oral administration of a drug that shows two-compartment characteristics after intravenous administration is incorrect, the ratio of the absorption rate constants calculated for two dosage forms using one-compartment analyses would be a good approximation of the actual ratio of the absorption rate constants. Ronfeld and Benet [4] ex-

amined the same question and concluded that the approximation error could be substantially larger than suggested by Wagner [5], but that a qualitative evaluation of the relative merits of different dosage forms could be accurately made with one-compartment fits.

Percent Absorbed-Time Plots

The problems associated with the characterization of absorption kinetics by curve-fitting have prompted many investigators to seek better methods of analysis. One of the most important of these alternative methods is based on the construction and evaluation of percent absorbed-time plots [6, 7], which do not require the assumption of zero- or first-order absorption.

One-Compartment Model (Wagner-Nelson Method). The amount of drug that has been absorbed into the systemic circulation, X_A, at any time after administration will equal the sum of the amount of drug in the body, X, and the cumulative amount of drug eliminated, X_E, by urinary excretion, by metabolism, and by all other routes at that time. Thus

$$X_A = X + X_E \tag{4.6}$$

which when differentiated with respect to time becomes

$$\frac{dX_A}{dt} = \frac{dX}{dt} + \frac{dX_E}{dt} \tag{4.7}$$

The term dX_E/dt (elimination rate of drug) is by definition equal to the product of the amount of drug in the body X and the apparent first-order elimination rate constant of drug from the body;

$$\frac{dX_E}{dt} = KX \tag{4.8}$$

Substitution of KX for dX_E/dt in Eq. (4.7) yields

$$\frac{dX_A}{dt} = \frac{dX}{dt} + KX \tag{4.9}$$

Since X equals VC, where V and C are the apparent volume of distribution and plasma concentration of drug, respectively, Eq. (4.9) may be written as

$$\frac{dX_A}{dt} = V\frac{dC}{dt} + KVC \tag{4.10}$$

Integration of Eq. (4.10) from time zero to T yields the following expression for the amount of drug absorbed to time T, $(X_A)_T$:

$$(X_A)_T = VC_T + KV\int_0^T C\,dt \tag{4.11}$$

where C_T is the plasma concentration of drug at time T and $\int_0^T C\,dt$ is the area under the plasma concentration versus time curve from time zero to T. An equation for the amount of drug ultimately absorbed, $(X_A)_\infty$, can be obtained by integrating (4.10) from time zero to infinity and recognizing that C equals zero at both times zero and infinity. Thus

$$(X_A)_\infty = KV\int_0^\infty C\,dt \tag{4.12}$$

where $\int_0^\infty C\,dt$ is the total area under the plasma concentration versus time curve. Dividing (4.11) by (4.12) and canceling common terms yields the expression for the fraction absorbed to time T:

$$\frac{(X_A)_T}{(X_A)_\infty} = \frac{C_T + K\int_0^T C\,dt}{K\int_0^\infty C\,dt} \tag{4.13}$$

Equation (4.13) relates the cumulative amount of drug absorbed after a certain time to the amount of drug ultimately absorbed, rather than to the dose administered. By collecting blood after a single oral dose and determining drug concentrations in plasma and the elimination rate constant, one can calculate the fraction absorbed for various times after administration. The calculations required to construct a percent absorbed-time plot are outlined in Table 4.1 and are based on the concentration-time data in columns 1 and 2. A plot of $C_T + K\int_0^T C\,dt$ versus time, as shown in Fig. 4.2, indicates that the curve is asymptotic and approaches the value of $K\int_0^\infty C\,dt$. After about 18 h $C_T + K\int_0^T C\,dt$ is independent of time and closely approximates $K\int_0^\infty C\,dt$, indicative of the fact that absorption is negligible and $(X_A)_T \simeq (X_A)_\infty$. The percent absorbed-time plot is shown in Fig. 4.3. The data suggest that absorption is relatively slow since at 2 h only about half of the absorption has taken place.

It is important to remember that percent absorbed-time plots tell us nothing about the extent of absorption. In principle one can obtain similar plots for two formulations of a drug that differ substantially in terms of how much of the drug is eventually absorbed. This difference will be reflected in the $C_T + K\int_0^T C\,dt$ versus time plots.

An important characteristic of the Wagner-Nelson method for evaluating absorption data is that no model is assumed for the absorption process. One often finds, however, that a plot of percent unabsorbed (i.e., $100\{1 - [(X_A)_T/(X_A)_\infty]\}$) versus time on semi-

Table 4.1 Calculation of Absorption Data Using the Wagner-Nelson Method

Time (h)	Drug Concentration (μg/ml)	$\int_0^T C\,dt$	$K\int_0^T C\,dt$	$C_T + K\int_0^T C\,dt$	Fraction Absorbed
0	0	0	0	0	0
1	1.88	0.94	0.08	1.96	0.29
2	3.05	3.41	0.29	3.34	0.49
3	3.74	6.80	0.59	4.33	0.64
5	4.21	14.75	1.27	5.48	0.81
7	4.08	23.04	1.98	6.06	0.90
9	3.70	30.82	2.65	6.35	
12	3.02	40.90	3.52	6.54	
18	1.86	55.54	4.78	6.64	
24	1.12	64.48	5.55	6.67	
36	0.40	73.60	6.33	6.73	
48	0.14	76.84	6.61	6.75	
60	0.05	77.98	6.71	6.76	
72	0.02	78.38	6.74	6.76	
∞	0	78.60	6.76	6.76	

Notes: The example concerns a drug absorbed and eliminated by first-order processes; a one-compartment model is assumed. The drug is eliminated with a half-life of 8 h ($K = 0.086\ h^{-1}$).

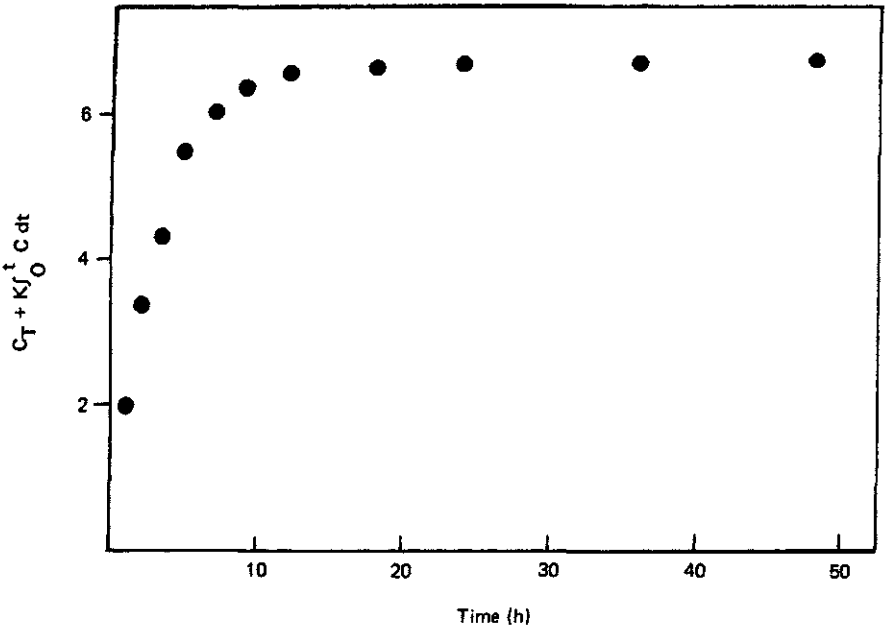

Fig. 4.2 Plot of the numerator of Eq. (4.13) (i.e., $C_T + K \int_0^t C\,dt$) versus time, based on the data in Table 4.1. Drug absorption is essentially complete after about 18 h. Thereafter, the value of $C_T + K \int_0^t C\,dt$ is a constant equal to $K \int_0^\infty C\,dt$ [i.e., the denominator of Eq. (4.13)].

logarithmic coordinates approximates a straight line. This suggests apparent first-order absorption and the apparent absorption rate constant may be estimated from the slope, which is equal to $-k_a/2.303$. A linear relationship between percent unabsorbed and time on rectilinear coordinates suggests apparent zero-order absorption. If sufficient data are available, one may be able to characterize more complex absorption kinetics (see Fig. 4.4).

Urinary excretion data can also be employed to construct percent absorbed-time plots. The excretion rate of intact drug in the urine, dX_u/dt, is given by

$$\frac{dX_u}{dt} = k_e X \tag{4.14}$$

where k_e is the apparent first-order excretion rate constant and X is the amount of drug in the body. Since X equals VC, it follows that

$$\frac{dX_u}{dt} = k_e VC \tag{4.15}$$

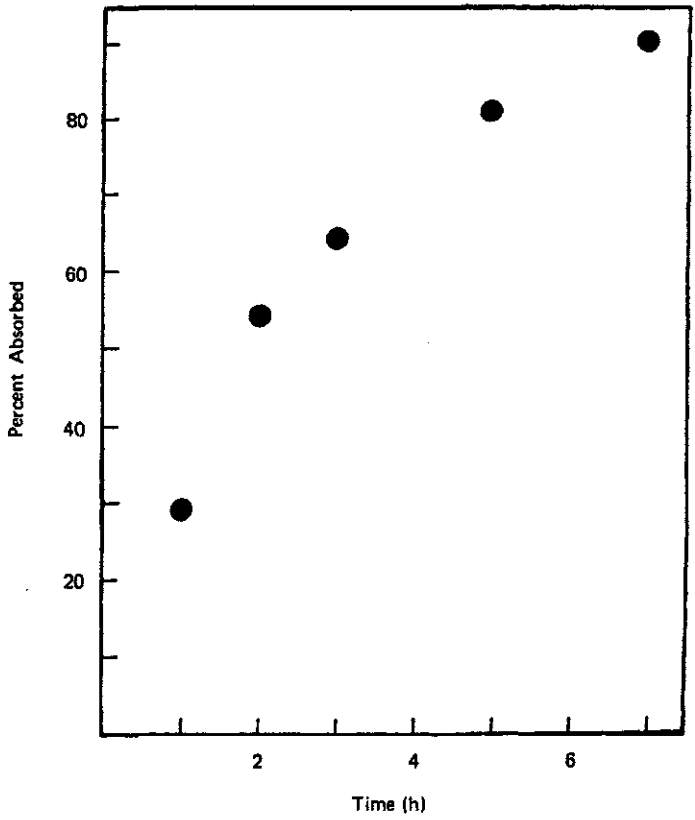

Fig. 4.3 Percent absorbed-time plot based on the data in Table 4.1. A plot of percent unabsorbed versus time on semilogarithmic coordinates would reveal apparent first-order absorption.

Rearranging terms yields

$$C = \frac{dX_u/dt}{k_e V} \tag{4.16}$$

Substituting this value of C in (4.10) and canceling common terms gives

$$\frac{dX_A}{dt} = \frac{1}{k_e}\frac{d(dX_u/dt)}{dt} + \frac{K}{k_e}\frac{dX_u}{dt} \tag{4.17}$$

which when integrated from time zero to T becomes

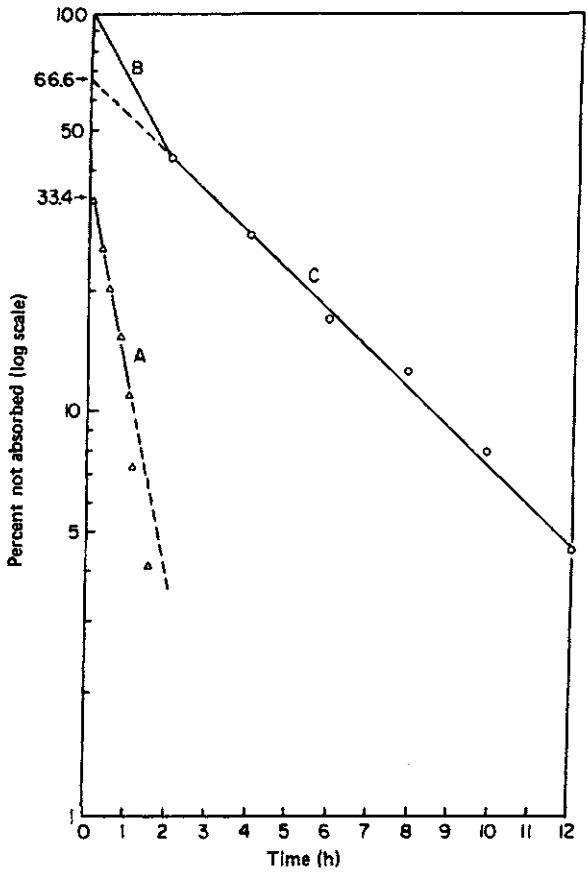

Fig. 4.4 Plot of percent sulfaethidole remaining to be absorbed (log scale) versus time after oral administration of a sustained-release suspension of the drug (see Ref. 6). The data show two components in the absorption phase and suggest that, under these conditions, drug absorption can be described by two parallel first-order processes.

$$(X_A)_T = \frac{1}{k_e}\left(\frac{dX_u}{dt}\right)_T + \frac{K}{k_e}(X_u)_T \qquad (4.18)$$

where $(dX_u/dt)_T$ is the excretion rate of intact drug in the urine at time T and $(X_u)_T$ is the cumulative amount of intact drug eliminated in the urine to time T. An equation for the total amount of drug ultimately absorbed, $(X_A)_\infty$, can be obtained by setting T equal to infinity in Eq. (4.18) and recognizing that dX_u/dt equals zero at time infinity. Thus,

$$(X_A)_\infty = \frac{K}{k_e} X_u^\infty \tag{4.19}$$

where X_u^∞ is the total amount of unchanged drug eliminated in the urine. The fraction absorbed at any time T, $(X_A)_T/(X_A)_\infty$, is determined by dividing (4.18) by (4.19) and canceling common terms:

$$\frac{(X_A)_T}{(X_A)_\infty} = \frac{(dX_u/dt)_T + K(X_u)_T}{KX_u^\infty} \tag{4.20}$$

Equation (4.20) indicates that, in principle, percent absorbed-time plots can be constructed based solely on urinary excretion data. Urine must be collected long enough to estimate K accurately but need not be collected to time infinity. A plot of $(dX_u/dt)_T + K(X_u)_T$ versus time is asymptotic, approximating KX_u^∞ when absorption is negligible.

In theory, percent absorbed-time plots may also be constructed from metabolite concentration in plasma versus time data or from urinary excretion rates of metabolite [8, 9], but the required assumptions make these methods of limited value.

The most serious limitation of the Wagner-Nelson method is that it applies rigorously only to drugs with one-compartment characteristics. In all other cases it is an approximation. It has been shown that the application of the Wagner-Nelson method to assess the absorption of drugs with multicompartment characteristics results in an underestimation of the time at which absorption ceases and an overestimation of the absorption rate [7]. The extent of error for a drug with two-compartment characteristics depends on the ratio of k_{10} or k_{el} to λ_2 [10]. If λ_2/k_{10} is ≥ 0.8, then in all likelihood the Wagner-Nelson method provides a reasonable approximation of the time course of absorption. Clearly, the Wagner-Nelson method should not be applied if drug concentration-time data after oral administration indicate multicompartment characteristics (see Fig. 2.15). A dilemma is encountered, however, when the concentration-time curve after oral administration of a drug that shows multicompartment characteristics on intravenous injection suggests a one-compartment model. Analysis of these data by the Wagner-Nelson method may produce incorrect results. One way of resolving this dilemma is to construct the percent absorbed-time plot using the Loo-Riegelman method, described in the next section. Unfortunately, this method requires concentration-time data obtained after both intravenous and oral administration and can be used in few instances. For this reason, the Wagner-Nelson method is likely to be applied in bioavailability studies for some time to come, despite the uncertainties.

Multicompartment Models (Loo-Riegelman Method). The Loo-Riegelman method requires drug concentration-time data after both oral and in-

travenous administration of the drug to the same subject. It can be applied generally to linear multicompartment pharmacokinetic models. The derivation that follows is based on a drug with two-compartment characteristics. The amount of drug absorbed into the systemic circulation at any time is given by

$$X_A = X_c + X_E + X_p \tag{4.21}$$

where X_E is the cumulative amount of drug eliminated by all pathways and X_c and X_p are the amounts of drug in the central and peripheral compartments, respectively. Differentiation of (4.21) with respect to time yields

$$\frac{dX_A}{dt} = \frac{dX_c}{dt} + \frac{dX_E}{dt} + \frac{dX_p}{dt} \tag{4.22}$$

The rate of elimination of drug, dX_E/dt, assuming first-order kinetics, is by definition

$$\frac{dX_E}{dt} = k_{10}X_c \tag{4.23}$$

where k_{10} is the apparent first-order elimination rate constant of drug from the central compartment. By substituting $k_{10}X_c$ for dX_E/dt in (4.22) and dividing both sides of the equation by the apparent volume of the central compartment, V_c, one obtains

$$\frac{1}{V_c}\frac{dX_A}{dt} = \frac{1}{V_c}\frac{dX_c}{dt} + \frac{1}{V_c}k_{10}X_c + \frac{1}{V_c}\frac{dX_p}{dt} \tag{4.24}$$

Since X_c/V_c equals the drug concentration in plasma, C, Eq. (4.24) can be written

$$\frac{1}{V_c}\frac{dX_A}{dt} = \frac{dC}{dt} + k_{10}C + \frac{1}{V_c}\frac{dX_p}{dt} \tag{4.25}$$

Integration of (4.25) from time zero to T yields the following expression for the amount of drug absorbed to time T:

$$\frac{(X_A)_T}{V_c} = C_T + k_{10}\int_0^T C\,dt + \frac{(X_p)_T}{V_c} \tag{4.26}$$

where $\int_0^T C\,dt$ is as defined previously in this chapter and C_T and $(X_p)_T$ are the plasma concentration and amount of drug in the peripheral compartment at time T, respectively. The expression for the amount of drug ultimately absorbed, $(X_A)_\infty$, is obtained by integrating (4.25) from time zero to infinity, which yields

$$\frac{(X_A)_\infty}{V_c} = k_{10}\int_0^\infty C\,dt \tag{4.27}$$

where $\int_0^\infty C\,dt$ is as defined previously. The fraction absorbed at any time T, $(X_A)_T/(X_A)_\infty$, is given by

$$\frac{(X_A)_T}{(X_A)_\infty} = \frac{C_T + k_{10}\int_0^T C\,dt + (X_p)_T/V_c}{k_{10}\int_0^\infty C\,dt} \tag{4.28}$$

Values for C_T, $\int_0^T C\,dt$, and $\int_0^\infty C\,dt$ are obtained from the oral absorption study. The rate constant k_{10} is estimated from a previous or subsequent intravenous study of the same subject. The amount of drug in the peripheral compartment as a function of time after oral administration divided by the volume of the central compartment can be estimated by a rather complicated approximation procedure requiring both oral and intravenous data.

The differential equation for the rate of change in the amount of drug in the peripheral compartment with time is given by

$$\frac{dX_p}{dt} = k_{12}X_c - k_{21}X_p \tag{4.29}$$

where k_{12} and k_{21} are apparent first-order intercompartmental transfer rate constants. If one assumes that the amount of drug in the central compartment between two consecutive sampling periods can be approximated by a straight line, then

$$X_c = (X_c)_0 + \frac{\Delta X_c}{\Delta t}t \tag{4.30}$$

where $(X_c)_0$ and X_c are the amounts of drug in the central compartment at the time of the first of any two consecutive sampling periods (i.e., time t_0) and at time t, respectively; $(\Delta X_c/\Delta t)$ is the slope of this line; and t is any time within the sampling period and varies from 0 to Δt. Substitution for X_c in Eq. (4.29) yields

$$\frac{dX_p}{dt} = k_{12}(X_c)_0 + k_{12}\frac{\Delta X_c}{\Delta t}t - k_{21}X_p \tag{4.31}$$

the Laplace transform of which is

$$s\bar{X}_p - (X_p)_0 = \frac{k_{12}(X_c)_0}{s} + \frac{k_{12}(\Delta X_c/\Delta t)}{s^2} - k_{21}\bar{X}_p \tag{4.32}$$

where $(X_p)_0$ is the amount of drug in the peripheral compartment at time t_0 and s is the Laplace operator. Solving (4.32) for $\bar{X}_p$ yields

$$\bar{X}_p = \frac{(X_p)_0}{s + k_{21}} + \frac{k_{12}(X_c)_0}{s(s + k_{21})} + \frac{k_{12}(\Delta X_c/\Delta t)}{s^2(s + k_{21})} \tag{4.33}$$

By taking the anti-Laplace of this equation (see Appendix A), an expression for the amount of drug in the peripheral compartment as a function of time can be obtained. That is,

$$X_p = (X_p)_0 e^{-k_{21}t} + \frac{k_{12}(X_c)_0}{k_{21}}(1 - e^{-k_{21}t}) + \frac{k_{12}(\Delta X_c/\Delta t)}{k_{21}}t - \frac{k_{12}(\Delta X_c/\Delta t)}{k_{21}^2}(1 - e^{-k_{21}t}) \tag{4.34}$$

which may be simplified to

$$X_p = (X_p)_0 e^{-k_{21}t} + \frac{k_{12}(X_c)_0}{k_{21}}(1 - e^{-k_{21}t}) + \frac{k_{12}(\Delta X_c/\Delta t)}{k_{21}^2}(e^{-k_{21}t} + k_{21}t - 1) \tag{4.35}$$

Dividing Eq. (4.35) by V_c and setting time equal to the time between any two consecutive sampling periods, Δt, yields

$$\frac{(X_p)_T}{V_c} = \frac{(X_p)_0}{V_c} e^{-k_{21}\Delta t} + \frac{k_{12}C_0}{k_{21}}(1 - e^{-k_{21}\Delta t}) + \frac{k_{12}(\Delta C/\Delta t)}{k_{21}^2}(e^{-k_{21}\Delta t} + k_{21}\Delta t - 1) \tag{4.36}$$

If the sampling period is relatively short so that $k_{21}t \leq 0.5$ [11], the third term of Eq. (4.36) may be reduced by expressing the exponential term $e^{-k_{21}\Delta t}$ as a two-term Taylor expansion (i.e., $e^{-x} = 1 - x + x^2/2$). Equation (4.36) then simplifies to

$$\frac{(X_p)_T}{V_c} = \frac{(X_p)_0}{V_c} e^{-k_{21}\Delta t} + \frac{k_{12}C_0}{k_{21}}(1 - e^{-k_{21}\Delta t}) + \frac{k_{12}(\Delta C/\Delta t)(\Delta t)^2}{2} \tag{4.37}$$

The calculations involved in estimating values of $(X_p)_T/V_c$ as a function of time based on concentration-time data obtained after oral

administration and estimates of k_{12} and k_{21} obtained after an intravenous study are shown in Table 4.2. The values can then be used in Eq. (4.28) to generate percent absorbed-time data as shown in Table 4.3.

The Loo-Riegelman method can also be applied to urinary excretion data. In this case the equation analogous to Eq. (4.28) is

$$\frac{(X_A)_T}{(X_A)_\infty} = \frac{(dX_u/dt)_T + k_{10}(X_u)_T + k_e'(X_p)_T}{k_{10}X_u^\infty} \tag{4.38}$$

where

$$k_e'(X_p)_T = k_e'(X_p)_0 e^{-k_{21}\Delta t} + \frac{k_{12}(dX_u/dt)_0}{k_{21}}(1 - e^{-k_{21}\Delta t}) + \frac{k_{12}[\Delta(dX_u/dt)/\Delta t]}{k_{21}^2}(e^{-k_{21}\Delta t} + k_{21}\Delta t - 1) \tag{4.39}$$

Equation (4.39) is analogous to Eq. (4.36) and may be simplified by applying the two-term Taylor expansion if appropriate.

Although the application of the Loo-Riegelman method is limited because of the requirement for concentration-time data obtained after both oral and intravenous administration, it is a very useful and rigorous approach for the evaluation of absorption kinetics. The method can be used for drugs that distribute in any number of pharmacokinetic compartments. For example, the fraction absorbed equation for a drug that can be described after intravenous injection by a three-compartment model with linear elimination from the central compartment (see Fig. 2.17) is

$$\frac{(X_A)_T}{(X_A)_\infty} = \frac{C_T + k_{10}\int_0^T C\,dt + (X_2)_T/V_c + (X_3)_T/V_c}{k_{10}\int_0^\infty C\,dt} \tag{4.40}$$

where X_2 and X_3 are the amounts of drug in each peripheral compartment. Individual equations analogous to Eq. (4.36) must be written for the amount of drug in each peripheral compartment. For example,

$$\frac{(X_3)_T}{V_c} = \frac{(X_3)_0}{V_c} e^{-k_{21}\Delta t} + \frac{k_{13}C_0}{k_{31}}(1 - e^{-k_{31}\Delta t}) + \frac{k_{13}(\Delta C/\Delta t)}{k_{31}^2}(e^{-k_{31}\Delta t} + k_{31}\Delta t - 1) \tag{4.41}$$

Table 4.2 Calculation of Absorption Data Using the Loo-Riegelman Method

Time T	Drug Concentration in Plasma, C_T	ΔC	Δt	C_0	$(X_p)_0/V_c$
0	0.00	—	—	—	—
0.5	3.00	3.0	0.5	0.00	0.000
1.0	5.20	2.2	0.5	3.00	0.218
1.5	6.50	1.3	0.5	5.20	0.749
2.0	7.30	0.8	0.5	6.50	1.433
2.5	7.60	0.3	0.5	7.30	2.157
3.0	7.75	0.15	0.5	7.60	2.849
3.5	7.70	−0.05	0.5	7.75	3.471
4.0	7.60	−0.1	0.5	7.70	4.019
5.0	7.10	−0.5	1.0	7.60	4.469
6.0	6.60	−0.5	1.0	7.10	5.103
7.0	6.00	−0.6	1.0	6.60	5.442
9.0	5.10	−0.9	2.0	6.00	5.552
11.0	4.40	−0.7	2.0	5.10	5.318
15.0	3.30	−1.1	4.0	4.40	4.861

Notes: The estimation of $(X_p)_T/V_c$ following oral administration is based on Eq. (4.37). A two-compartment model and first-order disposition are assumed: $k_{12} = 0.29$, $k_{21} = 0.31$, and $k_{10} = 0.16$.

Although the Loo-Riegelman method was developed based on multicompartment models in which elimination takes place only from the central compartment, Wagner [12] has shown that the method is equally valid whether elimination occurs from the central compartment alone, from the peripheral compartment(s) alone, or from both (all) compartments.

An inherent limitation of the Loo-Riegelman method is the intrasubject variability in pharmacokinetic parameters such as k_{10}, k_{12}, and k_{21} between the intravenous and oral studies. The assumption must be made that the kinetics of drug distribution and elimination remain unchanged in the interval between doses. A method that eliminates intrasubject variability is the simultaneous administration

$\frac{(X_p)_0}{V_c} e^{-k_{21}\Delta t}$	$\frac{k_{12}(C)_0}{k_{21}}(1 - e^{-k_{21}\Delta t})$	$\left(\frac{k_{12}(\Delta t)^2}{2}\right)\frac{\Delta C}{\Delta t}$	$(X_p)_T/V_c$
—	—	—	0.000
0.000	0.000	0.218	0.218
0.187	0.402	0.160	0.749
0.642	0.697	0.094	1.433
1.228	0.871	0.058	2.157
1.849	0.978	0.022	2.849
2.442	1.018	0.011	3.471
2.976	1.039	−0.004	4.019
3.444	1.032	−0.007	4.469
3.276	1.900	−0.073	5.103
3.740	1.775	−0.073	5.442
3.989	1.650	−0.087	5.552
2.987	2.592	−0.261	5.318
2.861	2.203	−0.203	4.861
1.361	3.168	−0.638	3.891

of the oral and intravenous doses. The oral dose would consist of drug in the formulation to be evaluated and the intravenous dose would be a solution containing labeled drug (i.e., either a radioactive or a stable isotope) [13, 14]. The concentration of labeled drug in plasma must be determined by methods specific for unchanged drug.

Deconvolution Method

Deconvolution is a model-independent method for determining absorption rates. Our discussion will be limited to the application of, rather than the mathematical basis for, the method. It was introduced by Rescigno and Segre [15] in 1966, but its use has been limited. The

Table 4.3 Calculation of Absorption Data Using the Loo-Riegelman Method [see Eq. (4.28)]

T	C_T	$k_{10}\int_0^T C\,dt$	$(X_p)_T/V_c$	$(X_A)_T/(X_A)_\infty$	Percent Unabsorbed
0.5	3.00	0.12	0.22	0.165	83.5
1.0	5.20	0.45	0.75	0.316	68.4
1.5	6.50	0.92	1.43	0.437	56.3
2.0	7.30	1.47	2.16	0.540	46.0
2.5	7.60	2.06	2.85	0.618	38.2
3.0	7.75	2.68	3.47	0.687	31.3
3.5	7.70	3.30	4.02	0.742	25.8
4.0	7.60	3.91	4.47	0.790	21.0
5.0	7.10	5.08	5.10	0.854	14.6
6.0	6.60	6.18	5.44	0.901	9.9
7.0	6.00	7.19	5.55	0.926	7.4
9.0	5.10	8.96	5.32	0.958	4.2
11.0	4.40	10.48	4.86	0.976	2.4
15.0	3.30	12.95	3.89	0.996	0.4

Notes: A two-compartment open model and first-order disposition are assumed: $k_{10} = 0.16$. Values for $(X_p)_T/V_c$ are taken from Table 4.2, $\int_0^\infty C\,dt = 126.44$.

deconvolution method requires no assumptions regarding the number of compartments in the model or the kinetics of absorption. Linear distribution and elimination are assumed. Like the Loo-Riegelman method, deconvolution requires data obtained after both oral and intravenous administration in the same subject and assumes no differences in the pharmacokinetics of drug distribution and elimination from one study to the other. Drug concentrations must be measured at the same times following both oral and intravenous administration during the time that drug is absorbed after oral administration [16]. However, the deconvolution method does not require the determination of drug concentrations in plasma at equally spaced intervals during or after the absorption phase [17]. The accuracy of the method depends on the size of the sampling interval. The same applies to the Loo-Riegelman method [12].

Under these conditions the fraction unabsorbed or the fraction remaining FR in the gastrointestinal tract after a certain time, expressed in terms of the sampling interval, is given by [16]

$$(FR)_{n\Delta t} = \frac{H_{(n+1)\Delta t}}{H_{\Delta t}} - \sum_{\substack{i=2 \\ j=n}}^{\substack{j=1 \\ i=n+1}} \frac{F_i \Delta t}{F_{\Delta t}} [FR]_{(j-1)\Delta t} \tag{4.42}$$

where

$$(FR)_{n\Delta t} = 1 - \frac{(X_A)_T}{(X_A)_\infty} = 1 - \frac{(X_A)_{n\Delta t}}{(X_A)_\infty} \tag{4.43}$$

and $n\ \Delta t$ is the time after n sampling intervals equal to Δt. H is a function describing the drug concentration-time curve following oral administration and F is a function describing the drug concentration-time curve following intravenous bolus administration. $F_{n\Delta t}$ may be given by the drug concentration in plasma at $n\ \Delta t$ or the area under the drug concentration-time curve between $n\ \Delta t$ and $(n - 1)\ \Delta t$. $H_{n\Delta t}$ can only be expressed in terms of concentration. When both H and F are expressed in terms of drug concentrations in plasma, the method is termed point-point.

Consider a situation where drug is administered intravenously and orally on two occasions and blood samples are obtained every 15 min (i.e., $\Delta t = 15$). Using the point-point method, the fraction remaining unabsorbed 15 min after oral administration is given by Eq. (4.42) as follows:

$$(FR)_{\Delta t} = \frac{C^{oral}_{2\Delta t}}{C^{oral}_{\Delta t}} - \frac{C^{i.v.}_{2\Delta t}}{C^{i.v.}_{\Delta t}} (FR)_0 \tag{4.44}$$

where $(FR)_{\Delta t}$ is the fraction unabsorbed 15 min after oral administration; $(FR)_0$ is the fraction unabsorbed at t = 0 and is equal to 1.0; $C^{oral}_{2\Delta t}$ and $C^{oral}_{\Delta t}$ are the drug concentrations in plasma 30 and 15 min, respectively, after oral administration; and $C^{i.v.}_{2\Delta t}$ and $C^{i.v.}_{\Delta t}$ are the drug concentrations in plasma 30 and 15 min, respectively, after intravenous bolus administration. The fraction remaining unabsorbed 30 min after oral administration is given by

$$(FR)_{2\Delta t} = \frac{C^{oral}_{3\Delta t}}{C^{oral}_{\Delta t}} - \frac{C^{i.v.}_{2\Delta t}}{C^{i.v.}_{\Delta t}}(FR)_{\Delta t} - \frac{C^{i.v.}_{3\Delta t}}{C^{i.v.}_{\Delta t}}(FR)_0 \qquad (4.45)$$

where $(FR)_{\Delta t}$ is obtained by first solving Eq. (4.44). Table 4.4 provides a numerical illustration of how the fraction remaining unabsorbed can be calculated by deconvolution using the point-point method.

For a one-compartment model with first-order absorption and first-order elimination, $(FR)_{n\Delta t}$ should be equal to $e^{-nk_a\Delta t}$. This is readily demonstrated by substituting the appropriate equations in Eq. (4.42). Under these conditions $(FR)_{\Delta t}$ is given by

Table 4.4 Calculation of Absorption Data Using Deconvolution (Point-Point Method) (see Ref. 16)

Time	$C^{i.v.}$	C^{oral}	FR[a]
0	100.0	0.0	1.00
1	84.0	58.6	0.35
2	70.6	69.9	0.12
3	59.9	65.9	0.05
4	49.4	57.9	
5	41.5	49.6	

[a]FR denotes the fraction remaining unabsorbed:

$$FR_1 = \frac{69.9}{58.6} - \frac{70.6}{84.0}(1.00) \qquad \text{(Eq. 4.44)}$$

$$FR_2 = \frac{65.9}{58.6} - \frac{70.6}{84.0}(0.35) - \frac{59.9}{84.0}(1.00) \qquad \text{(Eq. 4.45)}$$

$$FR_3 = \frac{57.9}{58.6} - \frac{70.6}{84.0}(0.12) - \frac{59.9}{84.0}(0.35) - \frac{49.4}{84.0}(1.00)$$

$$(FR)_{\Delta t} = \frac{A(e^{-2K\Delta t} - e^{-2k_a\Delta t})}{A(e^{-K\Delta t} - e^{-k_a\Delta t})} - \frac{Be^{-2K\Delta t}}{Be^{-K\Delta t}} \tag{4.46}$$

where $A = k_a FX_0/V(k_a - K)$ and $B = X_0/V$. Canceling common terms and rearranging terms yields

$$(FR)_{\Delta t} = \frac{e^{-2K\Delta t} - e^{-2k_a\Delta t} - e^{-K\Delta t}(e^{-K\Delta t} - e^{-k_a\Delta t})}{e^{-K\Delta t} - e^{-k_a\Delta t}} \tag{4.47}$$

which may be simplified to yield

$$(FR)_{\Delta t} = \frac{e^{-k_a\Delta t}(e^{-K\Delta t} - e^{-k_a\Delta t})}{e^{-K\Delta t} - e^{-k_a\Delta t}} = e^{-k_a\Delta t} \tag{4.48}$$

Benet and Chiang [18] recommend the use of the point-area method rather than the point-point method. In the point-area method, H is

Table 4.5 Calculation of Absorption Data Using Deconvolution (Point-Area Method) (see Ref. 16)

Time	$\int_0^t C^{i.v.}\,dt$	$\int_{t_1}^{t_2} C^{i.v.}\,dt$	C^{oral}	FR[a]
0	0.0	91.8	0.0	1.00
1	91.8	77.1	58.6	0.35
2	169.9	64.8	69.9	0.125
3	233.7	54.4	65.9	0.04
4	288.1	45.7	57.9	
5	333.8		49.6	

[a]FR denotes fraction remaining unabsorbed:

$$FR_1 = \frac{69.9}{58.6} - \frac{77.1}{91.8}(1.00)$$

$$FR_2 = \frac{65.9}{58.6} - \frac{77.1}{91.8}(0.35) - \frac{64.8}{91.8}(1.00)$$

$$FR_3 = \frac{57.9}{58.6} - \frac{77.1}{91.8}(0.125) - \frac{64.8}{91.8}(0.35) - \frac{54.4}{91.8}(1.00)$$

given by the drug concentration in plasma at time n Δt after oral administration and F is given by the area under the drug concentration versus time curve over the sampling interval after intravenous administration. The use of the point-area method to evaluate the time course of absorption is illustrated in Table 4.5.

Intercept Method

Vaughan [19] has proposed a method for evaluating the in vivo release rate constant of a drug from its oral formulations. The method is model independent but requires data after oral administration of both the formulation and a solution of the drug and assumes that absorption as well as distribution and elimination are first-order processes.

The drug concentration in plasma after a single oral dose D_s in solution can usually be described by a summation of exponential terms:

$$C_s = D_s \sum_{i=1}^{N} A_i e^{-\alpha_i t} \tag{4.49}$$

where A_i and α_i are constants and $\alpha_i > \alpha_{i+1}$. If after oral administration of the formulation containing a dose D_f, the drug is released from the formulation in a first-order fashion prior to absorption, drug concentrations in plasma are given by [19]

$$C_f = fD_f k_r \left(\sum_{i=1}^{N} \frac{A_i}{\alpha_i - k_r} \right) e^{-k_r t} + \sum_{i=1}^{N} \frac{fD_f k_r A_i e^{-\alpha_i t}}{k_r - \alpha_i} \tag{4.50}$$

where f is the fraction of the dose D_f that is absorbed relative to the amount absorbed after the solution and k_r is the first-order release rate constant from the formulation. Provided that $k_r > \alpha_N$ both C_s and C_f will, at some time after administration, be described by single exponential functions:

$$C_s = D_s A_N e^{-\alpha_N t} \tag{4.51}$$

and

$$C_f = \frac{fD_f k_r A_N e^{-\alpha_N t}}{k_r - \alpha_N} \tag{4.52}$$

where α_N is equal to λ_n for a multicompartment system or to K for a one-compartment system. The intercepts I of the extrapolations of the final exponential regressions of log C_s versus time and log C_f

versus time, with the concentration axis, are given by the coefficients of the terms on the right-hand side of Eqs. (4.51) and (4.52):

$$I_s = D_s A_N \tag{4.53}$$

and

$$I_f = \frac{fD_f k_r A_N}{k_r - \alpha_N} \tag{4.54}$$

Dividing Eq. (4.53) by (4.54), canceling common terms, and rearranging the resulting equation yields an expression for k_r:

$$k_r = \frac{\alpha_N}{1 - fD_f I_s / D_s I_f} \tag{4.55}$$

Hence k_r may be calculated from drug concentration-time data obtained after oral administration of a solution and a formulation. In principle, Eq. (4.55) may also be used with urinary excretion rate data. Vaughan [19] has provided an example based on urinary excretion rate data to illustrate the use of Eq. (4.55). A 15 mg dose of methylamphetamine was given as an aqueous solution and as a tablet formulation. The cumulative urinary excretion of unchanged drug was 50.4% of the dose after the solution and 50.9% of the dose after the tablet. Hence $f \simeq 1$. The final linear regressions of the log of urinary excretion rates against time had a half-life of 5 h corresponding to an α_N value of 0.1386 h^{-1}. The ratio of the intercepts was 0.7. Substitution of these values into Eq. (4.55) gives k_r as 0.462 h^{-1}. When release (dissolution) from the dosage is the rate-limiting step in drug absorption, this method gives an estimate of the absorption rate constant since under these conditions $k_r \simeq k_a$.

The usefulness of the intercept method is greatest when intravenous data are not available (so that the Loo-Riegelman or deconvolution method cannot be applied) and when the oral data clearly indicate that the drug distributes in a multicompartment manner (so that the Wagner-Nelson method may not be applied). The weaknesses of the method include the assumption of first-order absorption and the need for α_N to be essentially the same for both studies. Also, this method may yield unusual and misleading results if the drug precipitates in the gut after administration of the solution dosage form.

EXTENT OF ABSORPTION

Although the standard definition of bioavailability includes both rate and extent of drug absorption, bioavailability and the alternative terms, *availability* and *systemic availability*, are often used to signify solely the extent of absorption or the amount of drug reaching the

systemic circulation because this is often the principal concern of comparative bioavailability studies. Since the average steady-state concentration of drug in plasma on repetitive dosing is directly proportional to the amount absorbed, administering a drug in a formulation from which the extent of absorption is lower than from another formulation is the same as administering a lower dose.

The amount of drug reaching the systemic circulation after oral administration is often less than the administered dose. There are many reasons for this. Poor formulations may release only a part of the dose before reaching the colon. This is found most often with formulations of poorly water soluble drugs or with special formulations that are designed deliberately to delay release of the drug. However, oral administration of even the best formulation of a drug may result in less than completely availability. Some drugs are so polar that permeation of the gastrointestinal epithelium is limited. Other drugs are subject to chemical or enzymatic degradation before reaching the systemic circulation; this may occur in the gut lumen, in the gut wall, or in the liver during the first pass.

The systemic availability of a drug after oral administration of a formulation rarely exceeds that found with a solution. In almost all cases the performance of a dosage form or formulation can be evaluated by comparison with that of a solution. However, equivalent availability does not imply complete availability. For example, Wagner et al. [20] have shown that the availability of propoxyphene is the same after oral administration of a commercially available capsule and an aqueous solution, but the systemic availability of propoxyphene is less than 25% of the administered dose largely because of first-pass metabolism [21]. Although relative availability studies are useful for characterizing the formulation, one must determine absolute availability to characterize the drug.

Estimation of absolute availability after oral administration almost always requires comparison with data obtained after intravenous administration. In the case of water-soluble drugs, data after intramuscular administration may be acceptable as an absolute standard. Various oral standards have been used to determine relative availability. These include aqueous and nonaqueous solutions, carefully formulated suspensions and certain commercial formulations that are generally accepted as standards.

Almost all bioavailability studies are concerned with the systemic availability or relative availability of a drug after oral administration. However, the extent of absorption may also be of concern when administering a drug by any extravascular route, for example when giving a drug suspension intramuscularly or when giving a solution of drug that is likely to precipitate in the muscle depot on injection. Although it is reasonable to assume that the entire dose of an intramuscularly administered drug will be absorbed eventually, absorption may be so slow that, effectively, availability may be considered incomplete.

This may occur if the release of a fraction of the dose in the muscle depot is so slow as to give drug concentrations in plasma below that which one can measure. Availability is also a consideration after intravenous administration of a chemical derivative of a drug (a prodrug) that is intended to produce the drug itself in the body. If the prodrug is both converted to the drug and eliminated by other routes, the availability of the drug is less than complete. This is the case for chlorampehnical after intravenous administration of chloramphenical succinate [22].

Systemic or relative availability of a drug may be determined based on drug concentrations in plasma, urinary excretion of unmetabolized drug, or pharmacologic effects. The last mentioned is considered briefly in Chap. 6. In some instances availability estimates may be based on metabolite or total radioactivity in plasma or urine.

Drug Concentrations in Plasma

The most commonly used method for estimating availability is the comparison of the total area under the drug concentration in plasma versus time curve, AUC, after oral administration of the test formulation and after administration of the standard.

In referring to the availability of a drug after oral administration we will use the term *systemic availability* F when the standard is an intravenous solution and the term *relative availability* F_r when the standard is an oral formulation. An example of the results of a relative availability study is shown in Fig. 4.5. Formulation (a) is considered to be the reference standard.

By definition,

$$F = \frac{(\int_0^\infty C\,dt)_{oral}}{(\int_0^\infty C\,dt)_{i.v.}} = \frac{AUC_{oral}}{AUC_{i.v.}} \tag{4.56}$$

when equal doses are given intravenously and orally;

$$F = \frac{D_{i.v.} \cdot AUC_{oral}}{D_{oral} \cdot AUC_{i.v.}} \tag{4.57}$$

when different doses D are given intravenously and orally; and

$$F_r = \frac{AUC_{test}}{AUC_{standard}} \tag{4.58}$$

assuming that equal doses are given in the test formulation and the standard.

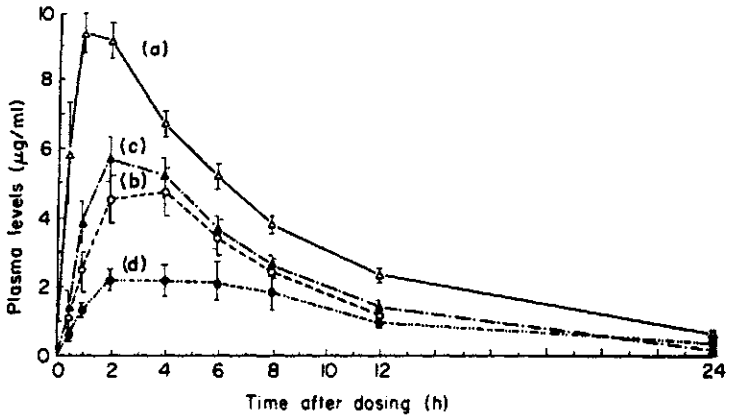

Fig. 4.5 Average chloramphenicol concentrations in plasma for groups of 10 healthy volunteers who received single 0.5 g oral doses of the drug in various commercial preparations (a, b, c, or d). Product (a) is considered the standard. (From Ref. 23.)

It is easily shown for any multicompartment model with linear processes that the ratio of areas after intravenous and oral administration is equal to F. Since

$$AUC_{oral} = \frac{FD_{oral}}{(V_\beta \lambda_n)_{oral}} \tag{4.59}$$

and

$$AUC_{i.v.} = \frac{D_{i.v.}}{(V_\beta \lambda_n)_{i.v.}} \tag{4.60}$$

it follows that

$$\frac{AUC_{oral}}{AUC_{i.v.}} = \frac{FD_{oral}(V_\beta \lambda_n)_{i.v.}}{D_{i.v.}(V_\beta \lambda_n)_{oral}} \tag{4.61}$$

Assuming that the same dose was given intravenous and orally, and the clearance of the drug, $V_\beta \lambda_n$, was the same in each study, Eq. (4.61) can be reduced to Eq. (4.56).

The proximity of the estimated average value of F or F_r as derived from either Eq. (4.56) or (4.58) to the true value of F or F_r depends on the assumption that average drug clearance is the same in each of the comparative studies. This is unlikely to be the case if different panels of subjects are used for each trial since intersubject variability in drug clearance can often be pronounced. This

variability can be reduced (but not eliminated) by carefully matching the subjects with respect to sex, body weight, age, health status, and other factors. A still better solution is to use the same subjects in both trials. Furthermore, by using the same subjects and by alternating the order of drug administration (i.e., a crossover study), we can avoid subject effects and period effects.

Today, almost all bioavailability studies are carried out in a crossover fashion with a single panel of subjects. Hence the average values of F or F_r determined from these studies are usually good estimates of the true value. However, these studies are still sometimes plagued by intrasubject variability; that is, an individual's ability to clear a drug may differ demonstrably from one administration of drug to the next. It is likely that the larger the intrasubject variability in drug elimination, the larger the standard deviation associated with the estimated value of F or F_r. Large standard deviations make it difficult to differentiate products, an important purpose of bioavailability studies. Differentiation at an appropriate level of confidence under conditions where there is considerable intrasubject variability may require a very large panel of subjects.

There is considerable interest in reducing the effect of intrasubject variability in bioavailability studies. This would be accomplished if we could somehow account for differences in clearance in the same individual from one treatment to another [see Eq. (4.61)]. Unfortunately, this is not possible because one cannot determine clearance without making some assumption concerning bioavailability. Alternatively, one can assume that the apparent volume of distribution in a given individual is invariant from trial to trial and then correct for differences in half-life [24].

Rearranging Eq. (4.61), assuming that $(V_\beta)_{i.v.} = (V_\beta)_{oral}$, and recognizing that $t_{1/2} = 0.693/\lambda_n$ yields

$$F = \frac{D_{i.v.}(t_{1/2})_{i.v.} \cdot AUC_{oral}}{D_{oral}(t_{1/2})_{oral} \cdot AUC_{i.v.}} \tag{4.62}$$

This so-called half-life correction method assumes that a change in $t_{1/2}$ from one study to the next in the same subject reflects solely a change in clearance and is not mediated by a change in apparent volume of distribution. It is probably reasonable to attempt the half-life correction in most bioavailability studies but to accept it only when it results in a substantial decrease in the standard deviation of the mean value of F or F_R. The half-life correction method must never be used when a change in $t_{1/2}$ reflects more persistent or prolonged absorption of drug from one dosage form than another [25].

An alternative correction for intrasubject variability called the Kwan-Till method is based on variability in renal clearance and requires both plasma concentration and urinary excretion data [26]. This method assumes that changes in total clearance are solely the result

of changes in renal clearance and that nonrenal clearance remains constant from study to study. It appears to be most useful for but not limited to drugs that are substantially excreted unchanged in the urine. The total plasma or systemic clearance is given by

$$Cl_s = \frac{D_{i.v.}}{AUC_{i.v.}} \tag{4.63}$$

but may also be expressed as

$$Cl_s = Cl_r + Cl_{nr} \tag{4.64}$$

that is, as the sum of renal clearance Cl_r and nonrenal clearance Cl_{nr}. The renal clearance of a drug is given by

$$Cl_r = \frac{f_u D}{AUC} \tag{4.65}$$

where f_u is the fraction of the administered dose that is ultimately excreted unchanged in the urine;

$$f_u = \frac{X_u^\infty}{D} \tag{4.66}$$

Equation (4.61) may be rearranged and expressed as

$$F = \frac{(Cl_s)_{oral} D_{i.v.} \cdot AUC_{oral}}{(Cl_s)_{i.v.} D_{oral} \cdot AUC_{i.v.}} = \frac{(Cl_{nr} + Cl_r)_{oral} D_{i.v.} \cdot AUC_{oral}}{(Cl_{nr} + Cl_r)_{i.v.} D_{oral} \cdot AUC_{i.v.}} \tag{4.67}$$

Assuming that nonrenal clearance is the same for both the oral and intravenous study, and recognizing that Cl_{nr} is equal to the difference between Eqs. (4.63) and (4.65), we can state that

$$F = \frac{(D_{i.v.}/AUC_{i.v.} - f_{u,i.v.} D_{i.v.}/AUC_{i.v.} + f_{u,oral} D_{oral}/AUC_{oral}) AUC_{oral} D_{i.v.}}{(D_{i.v.}/AUC_{i.v.} - f_{u,i.v.} D_{i.v.}/AUC_{i.v.} + f_{u,i.v.} D_{i.v.}/AUC_{i.v.}) AUC_{i.v.} D_{oral}} \tag{4.68}$$

which can be simplified to

$$F = \left(\frac{D_{i.v.}}{AUC_{i.v.}} - \frac{f_{u,i.v.} D_{i.v.}}{AUC_{i.v.}} + \frac{f_{u,oral} D_{oral}}{AUC_{oral}} \right) \frac{AUC_{oral}}{D_{oral}}$$

$$= \frac{AUC_{oral} D_{i.v.} (1 - f_{u,i.v.})}{AUC_{i.v.} D_{oral}} + f_{u,oral} \tag{4.69}$$

Even if the assumption regarding the constancy of nonrenal clearance from one study to the next were incorrect, it would be of little consequence if the drug were substantially excreted unchanged, since Cl_{nr} would represent a small fraction of Cl_s. This method has recently been used for estimating the availability of fluoride from tablets [27]. Calculating F by means of Eq. (4.57) and (4.69) yielded values of 107.8 ± 27.2 and 100.8 ± 9.2 %, respectively. Applying the correction factor reduced the apparent variability of the estimate. In this case, nonrenal clearance was about 60% of total clearance. The Kwan-Till method outlined above applies exactly only when an intravenous reference is available. In the absence of such data, an approximation has been proposed and evaluated [26, 28].

The correction method described above assigns the variability in total plasma clearance to renal clearance and assumes no variability in nonrenal clearance. Since there is no way to measure nonrenal clearance independently, one may alternatively assume that the nonrenal clearance varies in direct proportion to changes in renal clearance, so that

$$F = \frac{(Cl_r)_{oral} D_{i.v.} \cdot AUC_{oral}}{(Cl_r)_{i.v.} D_{oral} \cdot AUC_{i.v.}} \tag{4.70}$$

Calculating the availability of fluoride for the example cited above [27] using Eq. (4.70) yields F = 101.5 ± 24.0%. The correction reduces the average bioavailability to a more realistic absolute estimate, but is has no effect on the standard deviation. Although this method is not useful for fluoride, it may apply to other drugs and may be used in the absence of an intravenous reference.

A systemic (integrated) approach to the estimation of bioavailability using both model-independent (Kwan-Till) and pharmacokinetic (half-life correction) techniques has been presented [29]. The methods of Kwan-Till [26] and Wagner-Nelson [6] or Loo-Riegelman [7] are integrated such that one is able to check many of the assumptions inherent in these techniques and make adjustments for apparent deviations. This integrated approach as well as the Kwan-Till method requires that plasma and urine be obtained during the bioavailability study; the half-life correction method requires one or the other, not both.

In the typical single-dose bioavailability study, blood sampling is usually terminated before the entire drug concentration in plasma versus time curve is characterized (see Fig. 4.6). In such cases the estimation of $\int_0^\infty C\,dt$ or AUC requires an extrapolation. The available data are first used to calculate $\int_0^t C\,dt$, where t is the time the last sample was obtained, using the trapezoidal rule (see Appendix D) or some other method [30]. The data are then plotted on semilogarithmic coordinates to estimate K or λ_n (see Fig. 4.6). It is assumed that the drug concentration-time curve after time t is described by

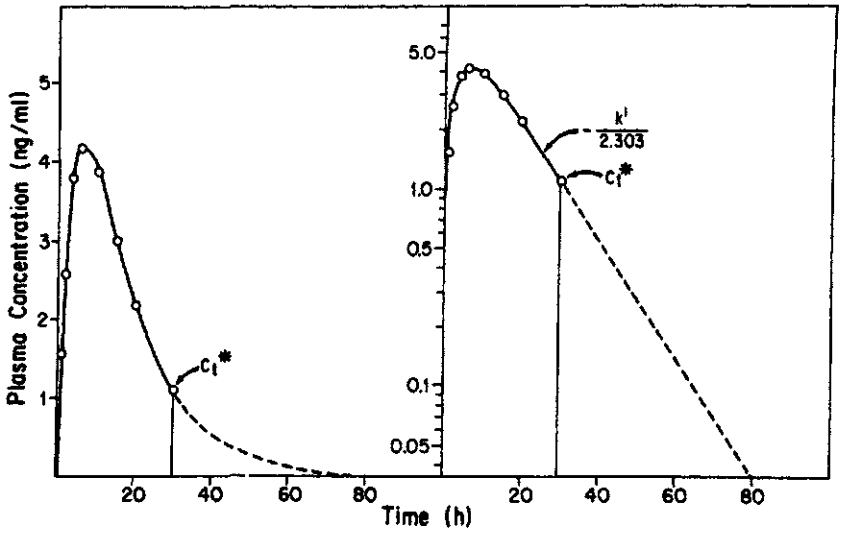

Fig. 4.6 Rectilinear and semilogarithmic plots of drug concentration in plasma versus time after a single oral dose. The last blood sample was taken before drug concentration had declined to a negligible level, requiring that part of the total area under the curve be estimated [see Eq. (4.75).]

$$C = C_0 e^{-Kt} \tag{4.71}$$

or

$$C = C_0 e^{-\lambda_n t} \tag{4.72}$$

Integrating these expressions from t to infinity yields

$$\int_t^\infty C\,dt = \frac{C_t}{K} \tag{4.73}$$

or

$$\int_0^t C\,dt = \frac{C_t}{\lambda_n} \tag{4.74}$$

where C_t is the concentration at the last sampling time. The total area under the drug concentration in plasma versus time curve for a multicompartment model is given by

$$AUC = \int_0^{\infty} C\,dt = \int_0^{t} C\,dt + \frac{C_t}{\lambda_n} \tag{4.75}$$

This technique is useful but does not reduce the need for obtaining blood samples for as long as possible after dosing. The smaller the contribution of the extrapolation area term (C_t/K or C_t/λ_n) to the total area, the more accurate the estimation of total area.

The treatment described above suggests that the minimum time required for sampling in a bioavailability study is that which assures a reliable estimate of the elimination rate constant (i.e., three to four elimination half-lives after dosing). In some instances, however, considerably shorter sampling periods appear adequate. Lovering et al. [31] determined for different formulations of many drugs that the ratio of areas under the drug concentration-time curve changed little between the apparent end of the absorption period and the time when blood sampling was terminated. They concluded that for a wide range of conditions the area ratios for any two formulations at a time equal to about twice that required for the apparent termination of absorption are within a few percentage points of the area ratios at infinite time. The theoretical basis for these observations is complex, but the work of Kwan and colleagues [32–34] provides some insight. In general, we can state for all cases that sampling should not be terminated until some time after absorption is complete. For a one-compartment model with first-order absorption and elimination, the closer the values of the absorption rate constants for two formulations, the shorter is the sampling time required for the ratio of areas to approximate the ratio at infinite time. For the same model the greater the difference between k_a and K, the shorter is the time required to determine an area ratio that approximates the ratio at infinite time. For example, for two formulations each with $k_a/K \geq 5$, the area ratio after a time equal to one elimination half-life is usually within 80% of the ratio at infinite time. When the values of the absorption rate constants are closer, the approximation is better. When the sampling interval is equal to two elimination half-lives, the area ratio is within 90% of the ratio at infinite time if $k_a/K \geq 5$ for both formulations. It appears that the use of partial areas in comparative bioavailability studies will be most successful for drugs with long half-lives and for formulations from which these drugs are relatively rapidly absorbed. Although we can rationalize results such as those found with digoxin [35], where for certain formulations the area measured over the interval 0 to 5 h correlates extremely well with the area measured over the interval 0 to 96 h, the prospective use of partial areas cannot be encouraged. One is always faced with the uncertainty of deciding when absorption has effectively stopped. If sampling is terminated before absorption is complete, comparison of partial areas will be misleading. For this reason we favor sampling as long as possible after administration and the application of Eq. (4.75) where appropriate.

There are times when the estimation of the availability of a drug after a single dose is difficult. For example, single-dose bioavailability studies in patients who require the drug necessitate stopping drug therapy. Also, the usual dose of some drugs produce such low drug concentrations in plasma after a single dose that it may be impossible to determine concentrations for more than a few hours after administration. In such cases it may be desirable to estimate bioavailability after repetitive dosing. Drug concentrations in plasma at steady state are often considerably higher than those found after a single dose. We have shown in Chap. 3 that the area under the drug concentration in plasma versus time curve over a dosing interval at steady state after repetitive dosing of a fixed dose at a fixed interval is equal to the total area resulting from that dose in a single-dose study. Therefore,

$$F_r = \frac{(\int_0^\tau C_{ss}\, dt)_{test}}{(\int_0^\tau C_{ss}\, dt)_{standard}} \qquad (4.76)$$

where C_{ss} denotes drug concentrations at steady state and τ is the dosing interval. Equation (4.76) assumes that the dosage regimen was the same for both studies. One advantage of this method is that fewer data points are required to characterize the area because the time course of change in drug concentrations in plasma at steady state is less precipitous than after a single dose and sampling times are bounded by the dosing interval. A second advantage is that patients or normal subjects may be crossed over from one formulation to another without a drug washout period. It is necessary, however, upon a change of formulation that the drug be given for four to seven elimination half-lives before estimating $\int_0^\tau C_{ss}\, dt$ to assure attainment of the new steady state. Table 4.6 summarizes the results of a steady-state bioavailability study with digoxin [36]. The study consisted of a randomized crossover design, in six healthy volunteers, with three 2-week treatment periods. Digoxin was given once daily at 8:00 a.m. Drug concentrations in plasma were determined during the final dosing interval (day 14). Figure 4.7 shows average drug concentration-time curves during a dosing interval at steady state for three quindine formulations [37].

Attempts to estimate availability by comparing single steady-state drug concentrations in plasma after different formulations rather than areas over the dosing interval may lead to incorrect results. For example, consider a one-compartment model with first-order absorption and elimination. The drug concentration at the end of any dosing interval at steady state is given by

Table 4.6 Estimation of Digoxin Bioavailability Using Steady-State Plasma or Urine Data (see Ref. 36)

Dosage Form	$\int_0^\tau C_{ss}\,dt$ (ng-h/ml)	F	X_u^{ss} (mg)	F
Intravenous solution	37.6	1.00	105.0	1.00
Oral solution	25.6	0.68	94.7	0.90
Oral tablet	21.8	0.58	89.7	0.85

Notes: In each case, 0.25 mg of digoxin was given every 24 h for 2 weeks. Each value represents the mean of six subjects.

$$C_{min}^{ss} = \frac{k_a F X_0}{V(k_a - K)} \frac{1}{1 - e^{-K\tau}} e^{-K\tau} \tag{4.77}$$

assuming that each dose is given postabsorption. The ratio of trough or minimum concentrations at steady state for two formulations in the same individual is given by

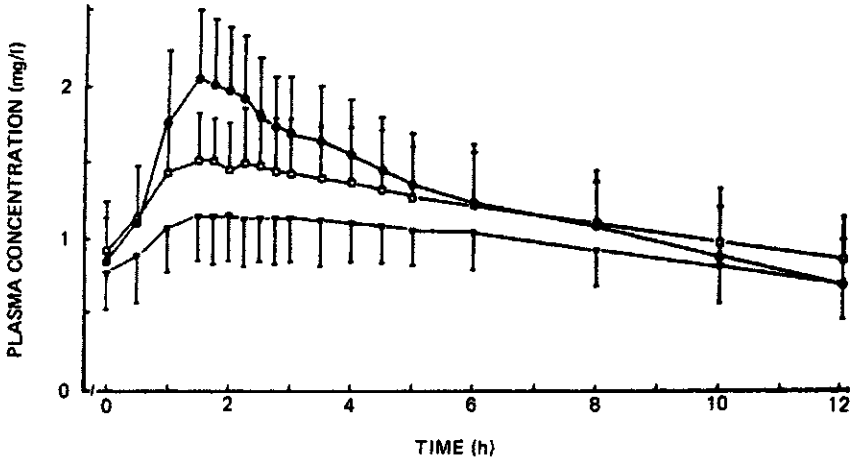

Fig. 4.7 Mean quinidine concentrations in plasma for three different products during a dosing interval at steady state. The drug was given every 12 h for 6 days before sampling. Comparison of the areas under the curves during the dosing interval, adjusted for administered dose, permits an assessment of relative bioavailability. (From Ref. 37, reprinted with permission.)

$$\frac{(C_{min}^{ss})_{test}}{(C_{min}^{ss})_{standard}} \approx \frac{[k_a F/(k_a - K)]_{test}}{[k_a F/(k_a - K)]_{standard}} \tag{4.78}$$

assuming that K, V, τ, and X_0 are the same in both cases. Clearly, the ratio of trough concentrations is equal to F_r (i.e., $F_{test}/F_{standard}$) only if the absorption rate constants for each formulation are the same or if absorption from both formulations is such that $k_a >> K$ in each case. Further examination of Eq. (4.78) reveals that if drug is absorbed from two formulations to the same extent but at different rates, the ratio of trough levels cannot be unity. For example, if the standard were absorbed faster than the test formulation but to the same extent, the ratio will exceed unity and one could incorrectly conclude that the test formulation is better absorbed.

The principal disadvantage of the steady-state method for estimating availability is that the clinical aspects are much more difficult to control and execute. It may take many days to achieve steady state. In any prolonged study, the potential lapses in subject compliance increase with time. As an alternative, Kwan and colleagues [32–34] have proposed a comprehensive method to permit bioavailability estimates under quasi- or non-steady state conditions. The basic strategy is to effect sufficient drug accumulation to facilitate assessment of bioavailability without unduly prolonging the clinical phase of the study. Only one aspect of this method will be considered here. The reader is directed to the original publications for mathematical derivations.

Consider that two treatments of the same drug are to be compared by administering sequentially ℓ doses of a standard formulation followed immediately by m doses of a test formulation according to the same dosage regimen. Under certain conditions it can be shown that [32]

$$F_r = \frac{F_{test}}{F_{standard}} = \left[\frac{(\int_0^\tau C\,dt)_{m+\ell}}{(\int_0^\tau C\,dt)_{\ell}} - e^{-mK\tau}\right] \frac{1 - e^{-\ell K\tau}}{1 - e^{-mK\tau}} \tag{4.79}$$

where the integral term in the numerator represents the area under the drug concentration in plasma versus time curve over the dosing interval after the last dose of the test formulation and the one in the denominator represents the area over the dosing interval after the last dose of the standard. If $m = \ell$, Eq. (4.79) reduces to

$$F_r = \frac{(\int_0^\tau C\,dt)_{m+\ell}}{(\int_0^\tau C\,dt)_{\ell}} - e^{-mK\tau} \tag{4.80}$$

For a multicompartment system K is replaced by λ_n. The derivation of Eq. (4.79) is based on a linear model with first-order absorption,

and requires that $k_a >> K$ or λ_n for both formulations, where K or λ_n represents the slope of the terminal linear phase of a semilogarithmic plot of plasma concentration versus time. Alternatively, it requires that k_a is the same for both formulations. If neither of these conditions is satisfied, Eqs. (4.79) and (4.80) are approximations. The validity of the approximation depends on (1) the difference between the two absorption rate constants (the smaller the difference, the better the approximation); (2) the difference between the absorption rate constant for each formulation and the elimination rate constant of the drug (the larger these differences, the better the approximation); and (3) the proximity of $\ell\tau$ and $(m + \ell)\tau$ to the time required to achieve steady state (the closer one is to steady state, the better the approximation). Kwan presents several strategies to improve the approximations as well as alternative strategies to compare different formulations under a variety of quasi- and non-steady state conditions [32–34]. Based on his experience with this method, Kwan [33] concludes: "In general, the relative bioavailability between two formulations in a crossover study is a function of the ratio of respective mean plasma concentration at quasi- and nonsteady-state. Appropriate correction factors may be introduced to compensate for the effects of dose, dosing sequence, half-life, sampling interval, and residuals. Each of these elements can be readily identified in the equations developed for each design variation."

Although it is widely accepted that the absolute availability F of a drug after oral administration can be determined only by reference to results obtained after intravenous administration, there is an interesting exception. A method has been proposed for estimating the absolute availability of drugs with renal clearances that are reproducibly perturable, without reference to an intravenous dose [38].

Consider the oral administration of a drug under two conditions, X and Y, which results in different renal clearances. These conditions may be the coadministration of agents that acidify or alkalinize the urine or that inhibit tubular secretion. Total clearance is the sum of renal and nonrenal clearances. We shall assume that nonrenal clearance and the fraction of dose absorbed are the same under both conditions. Therefore,

$$(Cl_s)_X = (Cl_r)_X + (Cl_{nr})_X \tag{4.81}$$

and

$$(Cl_s)_Y = (Cl_r)_Y + (Cl_{nr})_Y \tag{4.82}$$

where $(Cl_{nr})_X = (Cl_{nr})_Y$. Subtracting Eq. (4.82) from (4.81) yields

$$\Delta Cl_s = \Delta Cl_r \tag{4.83}$$

where $\Delta Cl_s = (Cl_s)_X - (Cl_s)_Y$ and $\Delta Cl_r = (Cl_r)_X - (Cl_r)_Y$. For each condition, total clearance is given by

$$(Cl_s)_X = \frac{FD}{AUC_X} \tag{4.84}$$

and

$$(Cl_s)_Y = \frac{FD}{AUC_Y} \tag{4.85}$$

where $F = F_X = F_Y$ and the dose $D = D_X = D_Y$. It follows that

$$\Delta Cl_r = \frac{FD}{AUC_X} - \frac{FD}{AUC_Y} \tag{4.86}$$

and

$$F = \frac{\Delta Cl_r}{D} \frac{AUC_X \cdot AUC_Y}{AUC_Y - AUC_X} \tag{4.87}$$

Since all the terms on the right-hand side of Eq. (4.87) can be determined from the two experiments, it is evident that under certain conditions F can be determined without resorting to an intravenous study.

This method was tested using intravenous furosemide data from a furosemide-probenecid interaction study [39]. If the method were valid, an F value of unity should be obtained. A mean value of $F = 1.05 \pm 0.11$ was determined. The method has also been used to estimate the availability of tocainide [40] and lithium [41].

Urinary Excretion Data

It is sometimes advantageous or necessary to determine systemic or relative availability from urinary excretion data. The basis for this determination is that the ratio of the total amount of unchanged drug excreted in the urine after oral administration to that following intravenous administration of the same dose is a measure of the absorption (systemic availability) of the drug. This relationship is valid for all linear models. Since

$$X_u^\infty = \frac{FDCl_r}{Cl_s} \tag{4.88}$$

it follows that

$$F = \frac{(X_u^\infty)_{oral}(Cl_s)_{oral}(Cl_r)_{i.v.}D_{i.v.}}{(X_u^\infty)_{i.v.}(Cl_s)_{i.v.}(Cl_r)_{oral}D_{oral}} \tag{4.89}$$

If we assume that there is a crossover design with a single panel of subjects and that there is no intrasubject variability in Cl_r and Cl_s from one study to the next, Eq. (4.89) reduces to

$$F = \frac{(X_u^\infty)_{oral} D_{i.v.}}{(X_u^\infty)_{i.v.} D_{oral}} \tag{4.90}$$

or

$$F = \frac{(X_u^\infty)_{oral}}{(X_u^\infty)_{i.v.}} \tag{4.91}$$

when equal doses are administered intravenously and orally. In a similar manner we can show under similar conditions that

$$F_r = \frac{(X_u^\infty)_{test}}{(X_u^\infty)_{standard}} \tag{4.92}$$

An example of the data required to estimate relative bioavailability from urinary excretion studies is shown in Fig. 4.8.

The Kwan-Till method may be used in conjunction with urinary excretion data to reduce the standard deviation of the mean value of F or F_r. One of two corrections based on experimental estimates of renal clearance may be applied. First, one may assume that although renal clearance is different from one study to the next, this change is compensated for by changes in nonrenal clearance so that total (systemic) clearance is the same. In this case Eq. (4.89) reduces to

$$F = \frac{(X_u^\infty)_{oral}}{(X_u^\infty)_{i.v.}} \frac{D_{i.v.}}{D_{oral}} \frac{(Cl_r)_{i.v.}}{(Cl_r)_{oral}} \tag{4.93}$$

Alternatively, one may assume that nonrenal clearance Cl_{nr} remains the same. In this case Eq. (4.89) may be written as

$$F = \frac{(X_u^\infty)_{oral}}{(X_u^\infty)_{i.v.}} \frac{D_{i.v.}}{D_{oral}} \frac{(Cl_{nr} + Cl_r)_{oral}(Cl_r)_{i.v.}}{(Cl_{nr} + Cl_r)_{i.v.}(Cl_r)_{oral}} \tag{4.94}$$

Recognizing that nonrenal clearance is the difference between Cl_s and Cl_r and that $(Cl_{nr})_{oral} = (Cl_{nr})_{i.v.}$, we obtain

$$F = \frac{(X_u^\infty)_{oral}}{(X_u^\infty)_{i.v.}} \frac{D_{i.v.}}{D_{oral}} \frac{(Cl_s)_{i.v.} - (Cl_r)_{i.v.} + (Cl_r)_{oral}}{(Cl_s)_{i.v.} - (Cl_r)_{i.v.} + (Cl_r)_{i.v.}} \frac{(Cl_r)_{i.v.}}{(Cl_r)_{oral}} \tag{4.95}$$

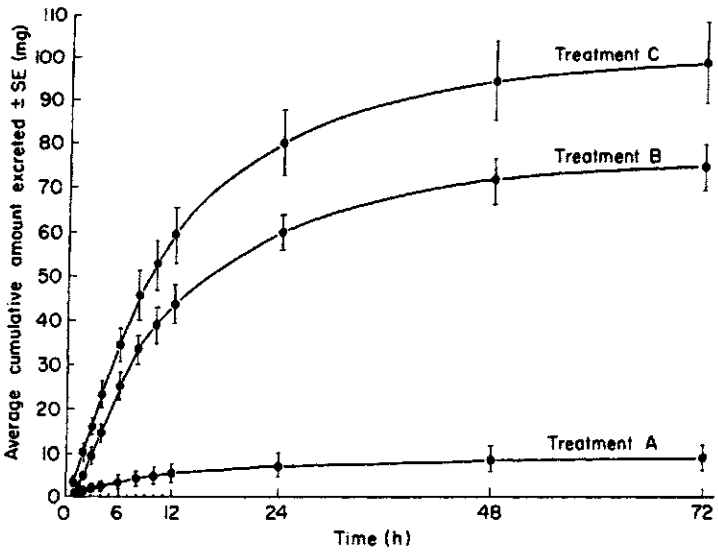

Fig. 4.8 Average cumulative amounts of tetracycline excreted in the urine of six subjects after a single 250 mg dose of the drug (see Ref. 42). The upper curve (C) was the result of administering an oral aqueous solution of the drug to fasting subjects. The middle curve (B) was observed after oral administration of the solution to the same subjects after breakfast. Curve A was obtained after rectal administration of the aqueous solution.

Rearrangement of Eq. (4.88) yields

$$(Cl_s)_{i.v.} = \frac{(Cl_r)_{i.v.}}{(f_u)_{i.v.}} \tag{4.96}$$

where $(f_u)_{i.v.} = (X_u^\infty)_{i.v.}/D_{i.v.}$. Substituting for Cl_s in Eq. (4.95) according to (4.96) and simplifying, we obtain

$$F = \frac{(X_u^\infty)_{oral}}{(X_u^\infty)_{i.v.}} \frac{D_{i.v.}}{D_{oral}} \frac{(Cl_r/f_u)_{i.v.} - (Cl_r)_{i.v.} + (Cl_r)_{oral}}{(Cl_r/f_u)_{i.v.}} \times \frac{(Cl_r)_{i.v.}}{(Cl_r)_{oral}} \tag{4.97}$$

which further simplifies to

$$F = \frac{(X_u^\infty)_{oral}}{(X_u^\infty)_{i.v.}} \frac{D_{i.v.}}{D_{oral}} \frac{(Cl_r/f_u)_{i.v.} - (Cl_r)_{i.v.} + (Cl_r)_{oral}}{(Cl_r)_{oral}/(f_u)_{i.v.}} \tag{4.98}$$

The principal drawback in using urinary excretion data for estimating availability is the need for collecting urine until virtually all of the drug has been excreted. With some drugs this may require several days of collection. Some investigators have observed with certain drugs that the ratio of amounts excreted over a relatively short period of time after administration of two formulations is similar to the ratio obtained on prolonged urine collection. For example, Greenblatt et al. [43] found that the 1-day and 6-day excretion of digoxin after intravenous and oral administration of many preparations were highly correlated ($r = 0.94$) and the overall variability in the two measures was nearly identical, despite the fact that less than half of the cumulative 6-day urinary digoxin excretion was recovered on the first day of collection. A similar observation has been made by Bates and Sequeira [44] with respect to the urinary excretion of total 6-desmethylgriseofulvin after administration of more than 20 formulations of griseofulvin which varied about fourfold in availability (see Fig. 4.9). Theory predicts that the ratio of amounts excreted in the urine in a comparative bioavailability study are asymptotic with time. For drugs with long half-lives and for formulations from which these drugs are relatively rapidly absorbed, the ratio will closely approximate the asymptotic value long before the drug is completely excreted. The use of partial urine collections for estimating comparative bioavailability may be appropriate if the pharmacokinetics of the drug are well characterized, but the prospective use of this method requires too many assumptions to be reasonable.

Systemic or relative availability of a drug may also be estimated from urinary excretion data at steady state. In theory the amount excreted over a dosing interval at steady state is equal to the total amount excreted to infinity after a single dose of the drug. Therefore,

$$F_r = \frac{(X_u^{ss})_{test}}{(X_u^{ss})_{standard}} \tag{4.99}$$

where X_u^{ss} denotes the amount of drug excreted in the urine from time zero to τ during any dosing interval at steady state. Equation (4.99) assumes that the dosage regimen was the same for both studies. A principal advantage of steady-state studies compared to single-dose studies is that the urine collection period is bounded by the dosing interval. Patients or normal subjects may be crossed over from one formulation to another without a drug washout period but, on a change of formulation, the drug must be given for four to seven elimination half-lives before determining X_u^{ss} to assure that the new steady state has been reached. Table 4.6 compares the bioavailability of digoxin from different formulations as estimated from the area under serum

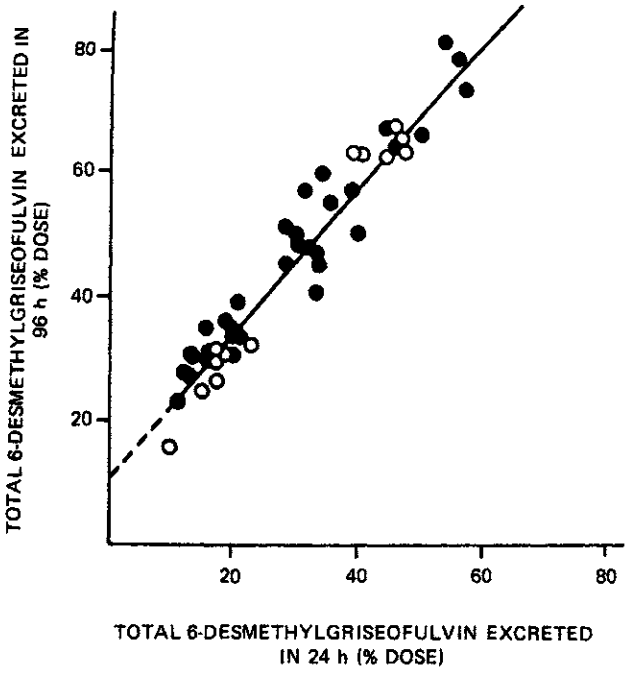

Fig. 4.9 Relationship between 24 h and 96 h cumulative urinary excretion of 6-desmethylgriseofulvin after a single 500 mg dose of griseofulvin in various products to healthy volunteers. In the case of griseofulvin it appears that bioavailability estimates based on a 24 h urine collection are equivalent to those based on a complete (96 h) collection of urine. $y = 1.20x + 11.2$, $n = 47$, $r = 0.965$, $P < 0.001$. (From Ref. 44, reprinted with permission.)

digoxin concentration-time curves over a dosing interval at steady state and from steady-state digoxin excretion in urine.

Bioavailability Estimates Based on Radioactivity, Nonspecific Assays, or Metabolite Levels

In the early studies of a new drug candidate, a specific assay may not be available at a time when one wishes to evaluate the absorption of the drug from test formulations. In this case it is not uncommon for investigators to use nonspecific assays which detect drug as well as one or more metabolites (i.e., "apparent" drug) or to administer radiolabeled drug and to determine total radioactivity in plasma or urine. Nonspecific assays have also been applied to drugs that are used in very small doses and have relatively large apparent volumes

of distribution, so that drug concentrations in plasma are unusually low and below the sensitivity of common assay methods. Some bioavailability studies have been based on the appearance of a major metabolite of the drug in plasma or urine. This is often the case when a drug is very rapidly metabolized and intact drug is difficult or impossible to detect.

For linear pharmacokinetic systems, estimates of relative availability based on the area under the concentration of total radioactivity, apparent drug, or metabolite in plasma versus time curve or based on cumulative urinary excretion of total radioactivity, apparent drug or metabolite may provide a useful measure of the relative performance of the test formulation. The use of nonspecific assays is not appropriate for nonlinear systems. In such cases the total area under the intact drug concentration in plasma-time curve is a function of the rate of absorption and the amount absorbed, and estimates of availability based on total radioactivity or other nonspecific methods may be misleading. Nonspecific assays should never be used for estimating systemic or absolute availability. The approach fails to detect presystemic metabolism in the gut or liver during absorption since drug and metabolites are not differentiated. Consequently, systemic availability will be overestimated.

Many other useful comments regarding the use of isotopes in bioabailability studies are found in a scientific commentary by Riegelman et al. [45].

STATISTICAL CONSIDERATIONS IN COMPARATIVE BIOAVAILABILITY STUDIES

An aspect of bioavailability testing that is of concern to the scientist and that has broad socioeconomic implications is the interpretation of the results. Metzler notes that very often bioavailability is a problem in equivalence [46]. Is the test formulation equivalent to the standard? What constitutes inequivalence? The answers to these questions must be based on a consideration of pharmacokinetics, clinical implications, and statistics. An extensive discussion of the subject is beyond the scope of this text, but a limited consideration is appropriate. The reader is referred to commentaries by Metzler [46] and Westlake [47] for a more detailed treatment.

The traditional statistical methodology which has been applied to scientific experiments is designed to show that a difference exists between two treatments. The null hypothesis of no difference is formulated in the expectation that the results of the experiment will be inconsistent with the null hypothesis and the alternative hypothesis of some difference could be accepted. If this is not the case, we *accept* the null hypothesis, which is quite different from proving it.

Bioavailability studies present some nontraditional problems. Sometimes we are interested in proving that the test formulation is

Table 4.7 Comparison of Confidence Interval and Hypothesis Testing (see Ref. 46)

Test Formulation	Results of Experiment: Comparative Bioavailability of Test Formulation to Standard Formulation with 95% Confidence Limits (Statistical)	Confidence Limit (95%) Criterion for Acceptance and Decision Reached by a Knowledgeable Pharmacologist, Physician, etc.	Decision Based on Hypothesis Testing, $\alpha = 0.05$
		Drug A: *Lower limit must be 80% or more*	*One-sided test*
A-1	92% (82% or more)	Acceptable	Acceptable
A-2	92% (85% or more)	Acceptable because lower limit exceeds 80%	Unacceptable
A-3	100% (55% or more)	100% looks good, but data are insufficient, unacceptable	Acceptable

A-4	95% (78% or more)	Unacceptable because the lower limit is less than 80%; may become acceptable with more data	Acceptable
A-5	120% (105% or more)	Acceptable, even though more available than standard, because lower limit is greater than 80%	Acceptable
		Drug B: *Lower limit must be greater than 85%, and upper limit must be less than 115%*	*Two-sided test*
B-6	105% (97% and 113%)	Acceptable	Acceptable
B-7	110% (95% and 125%)	Unacceptable because upper limit is greater than 115%	Acceptable
B-8	96% (93% and 99%)	Acceptable	Unacceptable

different from the standard, but at other times we are interested in "proving" that they are equivalent. Obviously, the most expedient approach to accepting the null hypothesis is poorly designed experiments with few subjects and large variability. Even in the more traditional situation where we are seeking differences between formulations we may find statistically significant differences that are in fact trivial from a clinical point of view. What we really want to learn from all bioavailability studies, irrespective of our expectation, is the difference between the test formulation and the standard and whether or not the difference is acceptable. The latter is largely a clinical question but also of concern to compendias and others who are interested in establishing standards. Thus it appears reasonable to conclude that the evaluation of bioavailability data should be based on a confidence interval method rather than hypothesis testing [46, 47]. The clinician or some other appropriate party can specify that the bioavailability of the new formulation relative to the standard must be within a certain range and that this must be known with a certain level of confidence. For example, it might be specified that, with 95% confidence, the new formulation should be between 80 and 120% as available as the standard. A comparison of decisions based on confidence intervals and hypothesis testing for several comparative bioavailability studies is presented in Table 4.7. If it is known that the standard formulation is completely available, it is only necessary to specify lower limits for the formulation (see drug A in Table 4.7). In most cases both lower and upper limits would be specified (see drug B in Table 4.7). The confidence interval method is gaining wide acceptance as the appropriate statistical approach for evaluating comparative bioavailability studies.

SUSTAINED RELEASE

The therapeutic index TI of a drug has classically been defined as the ratio of the median toxic or lethal dose to the median effective dose. For clinical purposes, a better definition is the ratio of the maximum drug concentration in plasma at which the patient is free of adverse effects of the drug to the minimum drug concentration in plasma required to elicit a minimally adequate therapeutic response. In principle, a drug should be given with sufficient frequency so that the ratio of maximum to minimum drug concentrations in plasma at steady state is less than the therapeutic index and at a high enough dose to produce effective concentrations [48]. For a linear, one-compartment system with repetitive intravenous dosing (constant dose, constant dosing interval τ) the ratio of maximum to minimum drug concentrations in plasma at steady state is given by

$$\frac{C_{max}^{ss}}{C_{min}^{ss}} = e^{K\tau} \tag{4.100}$$

where K is the first-order elimination rate constant. It follows that

$$e^{K\tau} \leq TI \tag{4.101}$$

and

$$\tau \leq t_{1/2} \frac{\ln TI}{\ln 2} \tag{4.102}$$

where TI is the therapeutic index. When the therapeutic index of a drug is 2, the dosing interval should be equal to no more than one biologic half-life of the drug. For drugs with short half-lives ($t_{1/2} \leq 6$ h) and low therapeutic indices (TI ≤ 3), the proper dosing schedule requires the drug to be given unreasonably frequently. This situation prevails with theophylline and procainamide, among other drugs. Sustained-release dosage forms may alleviate this problem, since the slower the absorption of a drug, the smaller the ratio of C_{max} to C_{min} over a dosing interval at steady state. In theory a drug that must be given every 3 h at a dose of 100 mg can be given every 6 h (D = 200 mg), every 12 h (D = 400 mg), or every 24 h (D = 800 mg) simply by reducing the absorption rate constant of the drug to maintain the C_{max} to C_{min} ratio. This may be accomplished by modifying the formulation to reduce the release rate of drug relative to that of a conventional formulation. Many sustained-release products are commercially available from which drug is absorbed in an apparent first-order fashion but at a considerably lower rate than observed after conventional tablets or capsules (see Fig. 4.10).

Although mathematical theory sets no limit as to how infrequently we can give a drug in a sustained-release formulation, a very stringent limit is imposed on oral formulations by the finite time over which a drug may be absorbed in the gastrointestinal tract after administration. The literature on drug absorption, gastric emptying, and intestinal motility suggests to us that within 9 to 12 h after administration of most prolonged-release dosage forms, the drug will be at a site in the intestine from which absorption is poor and ineffective. With this effective absorption time range in mind, it follows that the maximum absorption half-life should be 3 to 4 h. Formulations that release drug more slowly are likely to result in unacceptably low availability in a significant number of patients. In principle, a formulation that releases a well-absorbed drug in a first-order fashion with a half-life of 4 h will result in bioavailabilities ranging from about 80 to 90% of the dose if absorption time is limited to 9 to 12 h. A formulation with a 3 h half-life for drug release yields availabilities of about 90 to 95% of the dose over these absorption times. Shorter effective absorption times require still more conservative estimates of maximum half-lives.

Assuming maximum absorption half-lives of 3 or 4 h, to ensure adequate availability, we have calculated C_{max} to C_{min} ratios at

Table 4.8 Calculated Steady-State Data for Drugs with Different Elimination Half-Lives Given in One of Two Sustained-Release Formulations (see Ref. 50)

Elimination Half-Life (h)	Dose	τ	t_{max}^{ss}	C_{max}^{ss}	C_{min}^{ss}	$C_{max}^{ss}/C_{min}^{ss}$
		Formulation A: release half-time = 3 h				
1.98	400	8	2.6	17.3	9.5	1.8
	600	12	3.1	20.7	6.0	3.4
	1200	24	3.5	35.6	0.9	41.0
3.15	400	8	2.9	25.8	17.5	1.5
	600	12	3.6	29.4	12.8	2.3
	1200	24	4.3	46.4	3.0	15.3
4.01	400	8	3.0	31.9	23.5	1.4
	600	12	3.8	35.6	18.4	1.9
	1200	24	4.8	53.2	5.8	9.2
4.95	400	8	3.0	38.7	30.2	1.3
	600	12	4.0	42.4	24.8	1.7
	1200	24	5.2	60.3	9.8	6.2
5.97	400	8	3.1	46.1	37.5	1.2
	600	12	4.1	49.8	31.9	1.6
	1200	24	5.5	67.1	15.0	4.5

		Formulation B: release half-time = 4 h				
1.98	400	8	2.7	16.5	10.6	1.6
	600	12	3.3	19.1	7.6	2.5
	1200	24	3.9	30.4	1.9	15.8
3.15	400	8	3.0	25.0	18.7	1.3
	600	12	3.8	27.7	14.8	1.9
	1200	24	4.9	40.4	4.9	8.2
4.01	400	8	3.1	31.2	24.8	1.3
	600	12	4.1	33.9	20.6	1.6
	1200	24	5.4	47.6	8.2	5.8
4.95	400	8	3.2	38.0	31.5	1.2
	600	12	4.2	40.7	27.1	1.5
	1200	24	5.9	54.7	12.8	4.3
5.97	400	8	3.2	45.4	38.9	1.2
	600	12	4.4	48.1	34.2	1.4
	1200	24	6.2	62.2	18.4	3.4

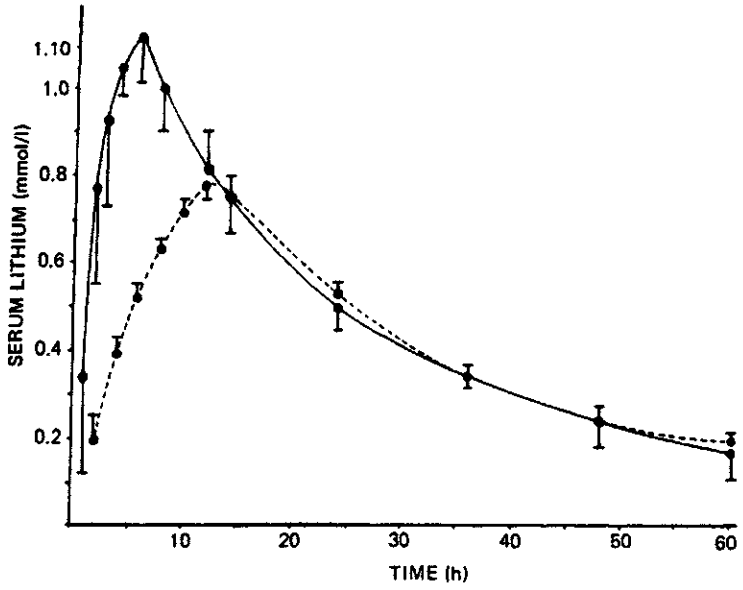

Fig. 4.10 Mean serum lithium concentrations after administration of a single 1.8 g dose to four manic patients (see Ref. 49). The drug was given either as a conventional preparation (——) or as a sustained-release preparation (---).

steady state for drugs with elimination half-lives ranging from 1 to 6 h given at a dosing rate of 50 mg/h at intervals of 8, 12, or 24 h. The maximum concentration in plasma at steady state was determined from

$$C_{max}^{ss} = \frac{D}{V} \frac{1}{1 - e^{-K\tau}} e^{-Kt_{max}^{ss}} \tag{4.103}$$

assuming complete absorption, where

$$t_{max}^{ss} = \frac{2.3 \log [k_a(1 - e^{-K\tau})/K(1 - e^{-k_a\tau})]}{k_a - K} \tag{4.104}$$

and the minimum concentration in plasma at steady state from

$$C_{min}^{ss} = \frac{k_a D}{V(k_a - K)} \left(\frac{1}{1 - e^{-K\tau}} e^{-K\tau} - \frac{1}{1 - e^{-k_a\tau}} e^{-k_a\tau} \right) \tag{4.105}$$

The results are summarized in Table 4.8. It is evident, in general, that drugs with short half-lives and low therapeutic indices must be given no less frequently than twice a day. Once-a-day dosing with sustained-release dosage forms is appropriate for drugs with higher therapeutic indices or with longer half-lives. However, the need for sustained release formulations of such drugs is not as great since adequate therapy can be achieved at reasonable dosing intervals.

Drugs with pronounced multicompartment characteristics after oral administration often show large C_{max} to C_{min} ratios. Some must be dosed at intervals considerably less than the biologic half-life to avoid adverse effects that are associated with high drug concentrations in plasma (central compartment). A relatively modest reduction in the absorption rate constant of such drugs by appropriate formulation may substantially reduce the maximum to minimum drug concentrations in plasma at steady state and may permit considerably less frequent administration of the drug. In essence, the reduced absorption rate may eliminate the "spike" of drug concentration in plasma associated with rapid absorption and slow distribution [50]. The principal advantage of less frequent drug administration is the potential improvement in patient compliance with the prescribed regimen.

Pharmacokinetic theory suggests that the ultimate method for reducing the C_{max} to C_{min} ratio is to have zero-order absorption. Once steady state is achieved under these conditions, drug concentration in plasma is constant as long as absorption persists. Several

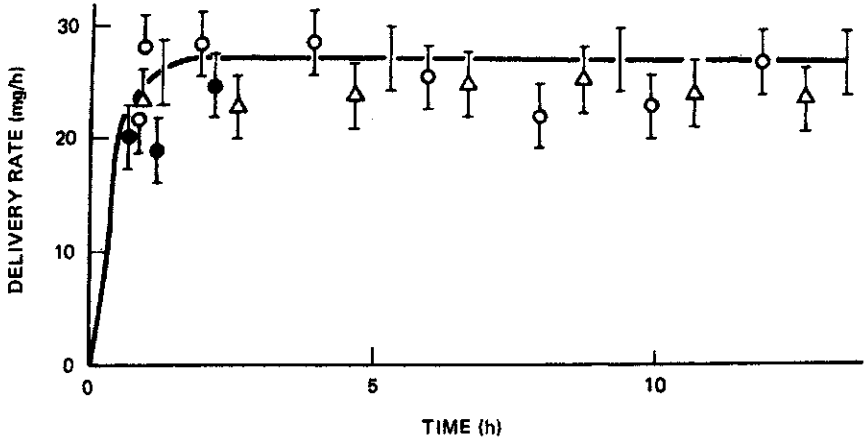

Fig. 4.11 In vitro (——) and in vivo (△, ○, ●) release rates of potassium chloride from a dosage form that utilizes the principle of the elementary osmotic pump. The in vivo data were obtained in three different dogs. Bars show experimental error. (From Ref. 54, reprinted with permission.)

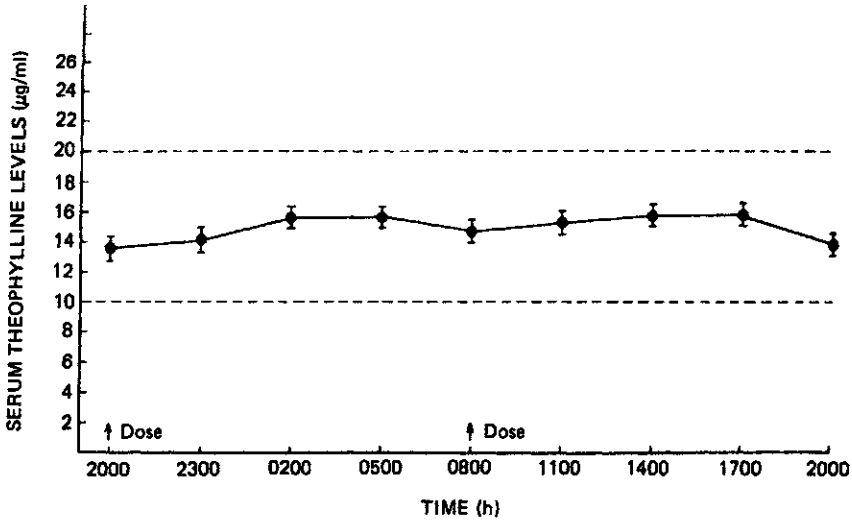

Fig. 4.12 Mean steady-state serum levels of theophylline in 20 asthmatic children who were receiving an oral sustained-release preparation of the drug every 12 h. The very small difference between peak and trough concentrations suggests that absorption of the drug from this dosage form can be described, at least on the average, by zero-order kinetics. (From Ref. 55. © 1980 American Academy of Pediatrics.)

investigators have discussed the application of pharmacokinetic principles to the design of sustained-release formulations that release drug in a zero-order fashion [51–53]. An example of such a system is the elementary osmotic pump [54]. The in vivo release rate of KCl from this dosage form in the gastrointestinal tract of dogs is shown in Fig. 4.11. Such dosage forms, however, are still limited by considerations of effective residence time of drug at absorption sites in the gastrointestinal tract. Accordingly, a drug with a short half-life must usually be given no less frequently than twice a day.

In our view the most important criteria for the evaluation of sustained-release products are bioavailability and C_{max} to C_{min} ratios at steady state. It is certainly desirable to have a bioavailability of at least 80% relative to the conventional dosage form. Where appropriate, the peak-to-trough ratio at steady state should be no greater than the therapeutic index of the drug. In all cases, this ratio should not exceed that observed after repetitive administration of the conventional dosage form at shorter intervals. The data in Fig. 4.12 indicate exemplary performance of a sustained-release product of theophylline. The C_{max} to C_{min} ratio at steady state resulting from

administration of this dosage form every 12 h is smaller than that found on administration of a conventional dosage form of theophylline every 6 h.

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