# 7

# Further Consideration of Clearance, and Physiological Modelling

## 7.1 Introduction

Previous chapters have introduced the concept of clearance and its importance, along with apparent volume of distribution, as a determinant of the elimination half-life of a drug. The term 'clearance' is sometimes used to describe the phenomenon of removal of a drug from the body as a whole, when the term 'elimination' would be better. It should be remembered that clearance always refers to a volume of fluid from which a substance is removed in unit time, and thus will always have units of flow, such as $\mathrm{mL\,min^{-1}}$ or $\mathrm{L\,h^{-1}}$. However, there are times when it is normalized, for example to body weight, concentration of microsomal protein, or hepatocyte concentration. Clearance can be used to describe the behaviour of a drug *in vitro*, as well as in *in vivo* systems. The term can be applied to individual organs, for example, renal clearance, hepatic clearance, etc., or to the whole body when it may be referred to as systemic (or plasma) clearance.

## 7.2 Clearance *in vitro* (metabolic stability)

The study of renal clearance dates from the 1930s, when pioneering renal physiologists discovered that kidney function could be assessed in terms of the removal of drugs from the blood in the renal artery (Section 3.3.1.4). Pharmacokineticists have extended this to embrace all processes of drug elimination, and, in the case of the liver, to model it experimentally *in vitro*, in an experiment sometimes called 'metabolic stability'.

### *7.2.1 Microsomes*

Microsomal intrinsic clearance, $CL_{\mathrm{mic}}$, provides an assessment of the ability of the microsomal fraction of the liver to remove the drug from the biophase surrounding the enzyme surface in the absence of any delivery (by blood) or availability (e.g. restrictions imposed by protein binding) influences. The experimental measurement of microsomal intrinsic clearance *in vitro* involves incubation of drug in a fixed volume of fluid in which is suspended a known quantity of liver microsomes. The decay of drug concentration is monitored using a suitable analytical method. First-order decay is ensured by using an appropriately low drug concentration and an appropriately high microsome concentration. The first-order rate constant, $k$, is obtained from the

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slope of a semilogarithmic concentration–time plot:

$$\begin{aligned} CL_{\text{mic}} &= -\text{slope} \times \text{volume of incubation} \\ &= V.k \end{aligned} \tag{7.1}$$

Microsomes are considered to be 100% viable, and so the activity can be expressed in terms of the microsomal protein concentration. Normalizing $CL_{\text{mic}}$ to 1 mg of protein, gives units of mL min$^{-1}$ mg protein$^{-1}$. The rate of metabolism is $CL_{\text{mic}} \times$ C (see Equation 4.3)

At relatively high drug concentrations, when non-linear kinetics are seen, Equation 7.1 can be written in terms of Michaelis–Menten enzyme kinetics, as rate of reaction = $V_{\max} \times C/(K\text{m} + C)$ (see Equation 4.42):

$$CL_{\text{mic}} = \frac{V_{\max}}{k\text{m} + C} \tag{7.2}$$

where $C$ is the concentration in the biophase at the enzyme surface (in this case of *in vitro* work, in the fluid). At very high concentrations when the enzymes are saturated with drug, the rate of reaction is $V_{\max}$, so:

$$V_{\max} = CL_{\text{mic}} \times C \tag{7.3}$$

and

$$CL_{\text{mic}} = V_{\max}/C \tag{7.4}$$

which is analogous to the zero-order case, Equation 4.43. Obviously, the microsomes contain liver enzyme systems in which only microsomally catalysed chemical change occurs. However, microsomal reactions include oxidations, reductions, hydrolyses and some phase 2 reactions, so multiple chemical changes can occur. Only by measuring the concentrations of the different products can pure, single reaction kinetics be studied. This is not commonly done, as pharmacokineticists have, historically, measured disappearance of substrate, rather than appearance of products because interest was primarily in the disappearance of pharmacologically active molecules. Also, until metabolites have been identified, it is not possible to develop assays for them.

### *7.2.2 Hepatocytes*

Analogous experiments can be performed using hepatocytes instead of microsomes. The clearance is expressed in terms of the numbers of cells: mL min$^{-1}$ million cells$^{-1}$. Because hepatocytes are not necessarily 100% viable, a viability correction determined in a separate experiment with a compound whose properties are known may be needed. Also, hepatocytes reproduce a somewhat larger collection of metabolic reactions, microsomal and otherwise, so that the result with hepatocytes is a kinetic constant assessing a somewhat larger collection of product-formation reactions. Again, separate assays of products are needed if the kinetics of single reactions are to be studied.

Human hepatocytes contain an average of 52.5 mg of microsomal protein per g of liver and there are $\sim 120 \times 10^6$ hepatocytes per g of liver, so there is 0.44 mg of microsomal protein per 1 million hepatocytes *in vitro* and *in vivo*. The corresponding figure for the rat is 0.34 mg of microsomal protein per million hepatocytes. With *in vitro* work, the hepatocyte concentration is limited by the physical properties of the suspension – it is inconvenient if the hepatocyte concentration is such that it is difficult to achieve adequate mixing of the suspension without damaging the cells. Consequently, the suspension of hepatocytes must be relatively dilute. In contrast, microsomal suspensions can contain higher protein concentrations than is the case with the hepatocyte suspension. So, experimentally, drug half-life values

are often shorter in the conditions of the microsomal suspensions than in those of the hepatocyte suspensions, in spite of the fact that more reactions take place in the hepatocyte incubations. Normalization to hepatocyte concentration and to the microsomal protein concentration overcomes this small experimental difficulty, and:

- The clearance normalized for protein calculated in the hepatocyte experiment will usually be higher than that in the microsomal experiment.
- The half-life in a rat hepatocyte experiment may be longer than that in a human experiment with the same hepatocyte concentration, but, because *in vivo* the rat has more liver mass than the human per kg of body weight, the clearance when scaled up to *in vivo* expectations will be higher in the rat.

## 7.3 Clearance *in vivo*

A non-eliminating organ can remove drug molecules from the blood passing through it until equilibrium between the tissue and plasma concentrations is reached, after which elimination in the liver and kidney reduces the concentrations in both blood and tissue. In this situation:

$$\begin{aligned}\text{Rate of removal from plasma} &= QC_a - QC_v \\ &= Q(C_a - C_v)\end{aligned} \tag{7.5}$$

where $Q$ is blood flow, $C_a$ is the afferent arterial concentration and $C_v$ is the efferent venous concentration (see Figure 4.4). In this situation, the extraction ratio, $E$, can be viewed as assessing organ uptake.

$$E = \frac{C_a - C_v}{C_a} \tag{7.6}$$

This approach has been used in the search for an understanding of brain uptake in particular, where, in an appropriately designed experiment, carotid artery and jugular vein concentrations can be measured. For an eliminating organ, the organ clearance is the elimination rate divided by $C_a$, and this provides the basis for assessment of renal clearance in particular (see Section 3.3.1.4). The extraction ratio concept is of major importance in relation to the liver.

The concept of systemic clearance was introduced in earlier chapters, particularly Chapters 4 and 5, because of the importance of clearance as a determinant of the elimination half-life. However, it is worth emphasizing some key points. The relationship between elimination rate constant, apparent volume of distribution and clearance in a single-compartment model was demonstrated in Section 4.2.1, resulting in Equation 4.5:

$$CL = \lambda V \tag{4.5}$$

but it is rare that single-compartment models are applicable and the more useful equation is Equation 4.14:

$$CL = \frac{D}{AUC} \tag{4.14}$$

which has the advantage that it is generally applicable. It can be applied to multiple-compartment models (Section 5.1.1.4) or when a model has not been defined (Section 5.3.1). *AUC* is obtained using the trapezoidal method (see Appendix). It is, of course, necessary to define the terminal decay constant, $\lambda_z$, in order to extrapolate the area from the last time point to infinity.

### *7.3.1 Apparent oral clearance*

Following oral dosing the equation equivalent to Equation 4.14 is Equation 4.26:

$$CL = F\frac{D}{AUC} \tag{4.26}$$

Sometimes the value given by Equation 4.26 is referred to as 'oral clearance' or '*apparent* oral clearance' or sometimes even just 'clearance', a potential cause of confusion. Obviously, a value for $CL$ cannot be derived without knowing the proportion of the dose which reaches the systemic circulation, $F$. Because an accurate value of $F$ cannot be obtained without the use of i.v. doses, it would seem to be better to use the data from i.v. studies to obtain systemic clearance. To avoid ambiguity, any value obtained for $D/AUC$ from extravascular doses should be referred to as $CL/F$ or $CL_{\text{oral}}$. Apparent oral clearance is commonly used in studies of special populations, using literature values of $F$.

### *7.3.2 Two-compartment models*

Any of the appropriate equations in Chapter 5 may be used to calculate systemic clearance, including Equations 5.16 and 5.17 as they are mathematically related. However, Equation 4.14 is equally applicable, where $AUC$ is obtained from the trapezoidal method plus extrapolation to $t = \infty$. This obviates the need to (i) define the model and (ii) calculate the values of the microconstants. It is important that sufficient data are collected so that the extrapolation is not more that 5–10% of the total area.

### *7.3.3 Systemic clearance at steady-state*

For a drug infused at a constant rate, $R_0$, into a single compartment model until steady-state conditions apply (approximately $5 \times t_{1/2}$), $CL$ can be substituted for $\lambda V$ in Equation 4.34:

$$R_0 = C^{\text{ss}}\,CL \tag{7.7}$$

Rearrangement gives:

$$CL = \frac{R_0}{C^{\text{ss}}} \tag{7.8}$$

Similarly, for repeated i.v. doses, substitution into and rearrangement of Equation 4.38 results in:

$$CL = \frac{D}{C_{\text{av}}^{\text{ss}}\tau} = \frac{\text{Dosing rate}}{C_{\text{av}}^{\text{ss}}} \tag{7.9}$$

where dosing rate = dose/dosage interval ($D/\tau$). The usual problem arises with oral doses, if systemic availability is unknown then the clearance will be the apparent oral clearance, $CL/F$.

It might appear from Equation 7.7 that systemic clearance can be calculated from a single blood sample taken to measure the average steady-state concentration. While this is laudable from the point of view of generation of the maximum amount of information from minimal data, it should be remembered that an accurate value of $C_{\text{av}}^{\text{ss}}$ requires determination of the $AUC$ following a single dose (Section 4.2.6). Further, practical issues to be considered when calculating $CL$ after multiple doses, include:

- The assumption that $F$ does not change from single to multiple doses.
- That no enzyme induction or inhibition occurs.

- That linear kinetics apply after single and multiple dosing.
- That the subject is compliant in terms of dosage taking, including timing of doses.
- That enough multiples of the (unknown) half-life of the drug have elapsed to ensure that steady-state has been reached.

In reality, this approach is more likely to be successful with a drug for which the single dose clearance is known, and in testing to see if any of the changes bulleted above have indeed occurred.

### *7.3.4 Additivity of clearance*

One of the features of clearance is its 'additivity'. Thus, if a drug is eliminated only by the liver and kidney, systemic clearance must be the sum of the two:

$$CL = CL_R + CL_H \tag{4.2}$$

When this is the case, renal clearance can be obtained as described previously (Equation 3.6) and $CL_H$ obtained by difference. Note that Equation 3.6:

$$CL_R = \frac{U}{P} \times \text{urine flow rate} \tag{3.6}$$

is in keeping with the concepts of clearance discussed in this chapter because the urine concentration multiplied by the urine flow rate is the rate of elimination of the drug (e.g. mg min$^{-1}$) and $P$ is the plasma concentration at the midpoint of the period of collection.

If there are other mechanisms by which drug is being eliminated, pulmonary clearance, decomposition and metabolism by plasma esterases, for example, then the difference between $CL$ and $CL_R$ can only be described as *non-renal* clearance, $CL_{NR}$.

## 7.4 Hepatic intrinsic clearance

Hepatic clearance, $CL_H$ can be defined as:

$$CL_H = Q_H E \tag{7.10}$$

where $Q_H$ is blood flow through the liver and $E$ is the extraction ratio. The equations which follow arise from a concept of the liver behaving as a single homogeneous pool (the 'well-stirred' model), which is obviously an over-simplification of a complex body organ. There are several other models discussed in the literature, for example the 'parallel-tube model,' and the 'dispersion model,' and it is also possible to invoke multiple plate ideas as in chromatographic columns The homogeneous well-stirred pool concept has a simplicity that facilitates our understanding of a broad range of pharmacokinetic observations, and it is the one most generally used. It assumes that the drug metabolizing enzymes are distributed evenly throughout the liver, and that the hepatic portal vein and the hepatic artery are equivalent in providing blood flow and therefore drug delivery to the liver. There are also differences in blood pressure between the hepatic artery and the hepatic portal vein, and these physiological differences could affect the interaction between substrates and enzyme surfaces. Clearly, during drug absorption, the drug concentrations are much higher in the hepatic portal vein than they can ever be in the hepatic artery, and this would be expected to affect the drug concentrations at the enzyme surfaces, which could in turn reduce the likelihood of first-order metabolism occurring. Also, if there is any product inhibition in the mechanism, then there may be differences in the

extent to which this can occur in the two cases. Nevertheless, according to this model, the following holds true:

$$E = 1 - F \tag{7.11}$$

The applicability of this equation is dependent on there being no metabolism by the gastrointestinal mucosa. Equation 7.11 can be useful in scaling up from *in vitro* to *in vivo* (see later), and in understanding clearance calculations with data from oral doses, which are commonly exposed to the liver before they reach the remainder of the body.

Rane *et al.* (1971) predicted the hepatic extraction ratio from $V_{max}$ and $K$m estimates *in vitro* using rat liver homogenates [Figure 7.1(a)].

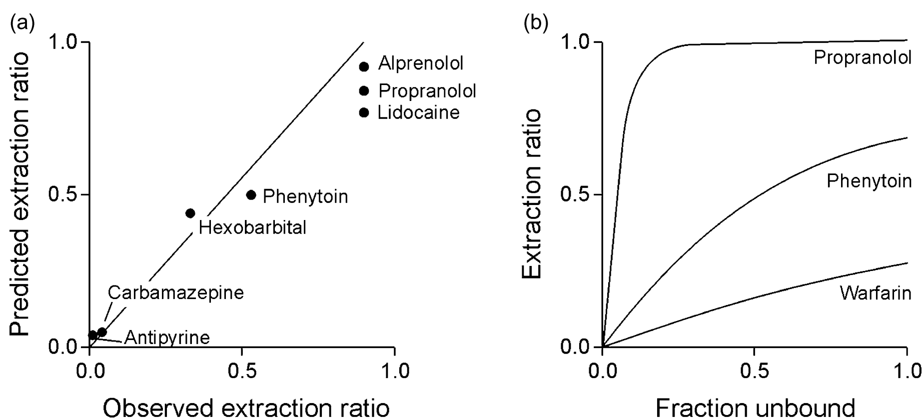



**Figure 7.1** (a) Relationship between observed extraction ratios in perfused rat liver and the values predicted using $V_{max}$ and $K$m values from metabolism in rat liver homogenates. The solid line is the line of idenity, slope = 1 (after Rane *et al.*, 1977). (b) Effect of plasma protein binding on the extraction of a highly extracted drug (propranolol), a poorly extracted drug (warfarin) and one with intermediate extraction (phenytoin) (from Shand *et al.*, 1976).

Hepatic intrinsic clearance, $CL_{int}$, is considered to be the maximal ability of the liver to remove drug irreversibly without any restrictions due to flow limitations or binding to proteins and so takes the form of Equation 7.2. However, when the substrate concentration is very low compared with $K$m, the equation can be written:

$$CL_{int} = \frac{V_{max}}{Km} \tag{7.12}$$

Note that the components of Equation 7.12 are measured with different units in different situations, most obviously in the case of $V_{max}$ which can have either mass/time or concentration/time units. Thus the expression $V_{max}/K$m is shown as identifying a first-order rate constant $k$ (when the units are reciprocal hours) in Chapter 3. Values will be 'real' or 'apparent' depending on whether purified enzymes are used. This becomes especially important when $V_{max}$ and $K$m concepts are applied to plasma concentrations of phenytoin (Chapters 5 and 19), and Equation 7.12 is the equation that is usually used for intrinsic clearance of a drug exhibiting first-order elimination kinetics. As hepatic blood flow increases, hepatic clearance increases to

a maximum, the value of which depends on $CL_{\text{int}}$:

$$CL_{\text{H}} = Q_{\text{H}} \frac{CL_{\text{int}}}{(Q_{\text{H}} + CL_{\text{int}})} \tag{7.13}$$

Comparing Equations 7.10 and 7.12, it follows that:

$$E = \frac{CL_{\text{int}}}{Q_{\text{H}} + CL_{\text{int}}} \tag{7.14}$$

Thus, the extraction ratio is a function of flow rate; the larger the blood flow, the smaller the extraction ratio. This relationship has been validated using compartmental and perfusion models (Perrier and Gibaldi, 1974; Rowland *et al.*, 1973). However, if the intrinsic clearance is small relative to the flow rate, then the denominator in Equation 7.13 approximates $Q_{\text{H}}$, so:

$$CL_{\text{H}} \approx CL_{\text{int}} \tag{7.15}$$

Such drugs are referred to as 'capacity-limited' or 'restricted'. For a drug with $CL_{\text{int}} > Q_{\text{H}}$, Equation 7.13 reduces to:

$$CL_{\text{H}} \approx Q_{\text{H}} \tag{7.16}$$

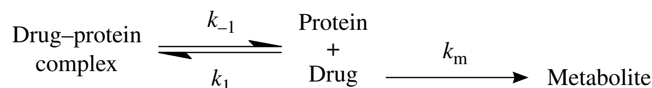
These drugs are referred to as 'flow rate-limited' drugs. Note that if a drug is entirely removed from the body by hepatic clearance, then $CL_{\text{H}} = CL$.

### *7.4.1 Effect of plasma protein binding on elimination kinetics*

There is little doubt that binding to plasma proteins can affect the rate of elimination of a drug; but in what way, and to what extent, may be difficult to predict. The equations in Section 2.4.3 apply to equilibrium between bound and unbound drug, and because plasma protein binding influences the apparent volume of distribution, it would be expected to affect elimination as:

$$t_{1/2} = \frac{0.693V}{CL} \tag{4.7}$$

provided that protein binding does not influence $CL$, and that is where there have been misunderstandings as to the influence of protein binding. At one time, a widely held belief was that plasma protein binding inevitably delayed elimination because less drug was available to the drug metabolizing enzymes. This erroneous generalization was 'supported' by some studies that demonstrated a negative correlation between percent bound and degree of metabolism. However, it was pointed out that metabolism is dynamic and when unbound drug is metabolized, bound drug dissociates to maintain the equilibrium:




so for binding to delay metabolism, $k_{-1}$ would have to be smaller than $k_{\text{m}}$ (Curry, 1977, 1980). In a series of theoretical calculations, Gillette (1973) reasoned that 'it seems probable that the rate of dissociation of the drug-protein complex seldom becomes rate limiting in the metabolism of drugs'; indeed he demonstrated that it is possible for plasma protein binding to hasten metabolism by the efficient transport of drug to the liver.

#### *7.4.1.1 Influence of protein binding on hepatic clearance*

Wilkinson and Shand (1975) showed that there is a delay only if protein binding is high and intrinsic clearance is low. They did this by a modification of the original equations (7.13 and 7.14):

$$E = \frac{f_u CL'_{int}}{Q_H + f_u CL'_{int}} \tag{7.17}$$

where $f_u$ is the fraction unbound and

$$CL'_{int} = \frac{EQ_H}{f_u(1-E)} \tag{7.18}$$

$CL'_{int}$ is the intrinsic clearance of the unbound drug. (Note that in some older literature the symbol $f_B$ was sometimes used for fraction unbound.) Modifying Equation 7.13 to take account of protein binding gives:

$$CL_H = Q_H\left(\frac{f_u CL'_{int}}{Q_H + f_u CL'_{int}}\right) \tag{7.19}$$

If the intrinsic unbound clearance is very small compared to the flow, $Q_H$, then Equation 7.19 approximates to:

$$CL_H = f_u CL'_{int} \tag{7.20}$$

Drugs with a low intrinsic clearance (capacity-limited) include warfarin and diazepam and, as predicted by Equation 7.20, the elimination of these drugs is affected by the degree of plasma protein binding. If the liver is the major route of elimination for these drugs, changes in $CL'_{int}$, resulting from enzyme induction or inhibition may markedly affect their elimination half-lives.

Some drugs such as propranolol and lidocaine have intrinsic clearances greater than liver blood flow and when the intrinsic unbound clearance is very large compared with the hepatic flow,

$$CL_H = Q_H \tag{7.21}$$

According to Equation 7.21 the clearance of these drugs will be unaffected by changes in plasma protein binding, but will be affected by changes in hepatic blood flow, as might occur with heart or liver disease or drugs that affect cardiac output. Enzyme induction or inhibition should have less impact on the kinetics of these drugs. However, for a constant rate infusion the steady-state concentrations will be:

$$C^{ss} = \frac{R_0}{Q_H} \tag{7.22}$$

by rearrangement of Equation 7.6 and substitution of Equation 7.21. A clinically important point is that Equation 7.22 predicts that the steady-state total concentrations will be unaffected by alterations in protein binding. There may be some change due to redistribution between plasma and tissue concentrations, but it is possible that total concentrations may remain reasonably constant when the unbound concentration increases. Thus dosing should be based on the unbound concentrations. Most drugs fall between the extremes of capacity-limited and flow-limited [Figure 7.1(b)].

Capacity-limited and flow rate-limited drugs may be referred to as lowly and highly extracted drugs, respectively, indicating the relationship with $E$. This is the case for the equations presented so far which consider the liver to be a homogeneous solution of the drug. An alternative model is the parallel-tube model

of the liver which assumes an exponential gradient exists between arterial and venous blood such that:

$$CL_{\mathrm{H}} = Q_{\mathrm{H}}[1 - \exp(-f_{\mathrm{u}} CL'_{\mathrm{int}}/Q_{\mathrm{H}})] \tag{7.23}$$

Now, rather than $E$, the critical term is $f_{\mathrm{u}} CL'_{\mathrm{int}}$. For example when this is larger than $Q_{\mathrm{H}}$, Equation 7.23 approximates to Equation 7.21.

#### *7.4.1.2 Influence of protein binding and volume of distribution on half-life*

Wilkinson and Shand also examined the significance of tissue distribution and protein binding on half-life by using the following definition of volume of distribution:

$$V = V_{\mathrm{b}} + V_{\mathrm{t}} \frac{f_{\mathrm{u}}}{f_t} \tag{7.24}$$

where $V_{\mathrm{b}}$ is the blood volume, $V_{\mathrm{t}}$ is the apparent volume of distribution made up of other tissues of the body, and $f_{\mathrm{u}}$ and $f_{\mathrm{t}}$ are the fractions of unbound drug in the blood and tissues, respectively. It was shown that the half-life is a function of volume of distribution, hepatic blood flow, fraction unbound, and unbound intrinsic clearance:

$$t_{1/2} = 0.693\left(\frac{V}{Q_{\mathrm{H}}} + \frac{V}{f_{\mathrm{u}} CL'_{\mathrm{int}}}\right) \tag{7.25}$$

Increases in the left-hand term within the brackets (increased volume of distribution or reduced liver blood flow) will tend to increase $t_{1/2}$, as might be expected. An increase in intrinsic clearance will decrease $t_{1/2}$. The effect of binding is more complex, depending on whether the drug has a high or low intrinsic clearance. Basically however, with increased binding (i.e. decreased $f_{\mathrm{u}}$) the right hand term of the part of Equation 7.25 in brackets will tend to increase $t_{1/2}$. For a drug with low intrinsic clearance, as $f_{\mathrm{u}}$ decreases from unity, half-life increases to become very long as $f_{\mathrm{u}}$ approaches zero whereas for a drug with high intrinsic clearance, the increase in $t_{1/2}$ is very much less marked (Figure 7.2). Taking the effect of tissue

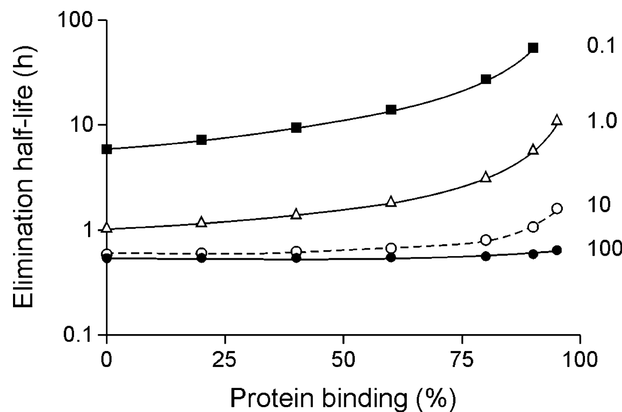



**Figure 7.2** Effect of plasma protein binding and elimination half-life for four values of intrinsic clearance. Calculated from Equation 7.25, using $Q_{\mathrm{H}} = 1.5\,\mathrm{L\,min^{-1}}$, $V = 70\,\mathrm{L}$ and intrinsic clearance = 100, 10, 1 and 0.1 times $Q_{\mathrm{H}}$.

binding into account gives:

$$t_{1/2} = 0.693\left(\frac{V_{\mathrm{b}}}{f_{\mathrm{u}}CL'_{\mathrm{int}}} + \frac{V\mathrm{t}}{f_{\mathrm{t}}CL'_{\mathrm{int}}}\right) \tag{7.26}$$

The precise effect on half-life will be determined in each case by the interplay of binding, intrinsic clearance, hepatic blood flow, and tissue binding. At one extreme, the half-life of propranolol was shown to be relatively short in the presence of high protein binding. In contrast, for drugs at the other extreme (e.g. warfarin and tolbutamide), the consequence of high plasma protein binding will be a long half-life. It should be noted that the combination of a low value for $CL'_{\mathrm{int}}$ and $V$, and a high value for percent plasma protein binding, is likely to be rare, because of the significance of the physical properties of drugs leading to the expectation that high $V$, low $f_{\mathrm{u}}$, and high $CL'_{\mathrm{int}}$ will occur in parallel. The combination of low $V$, low $f_{\mathrm{u}}$, and low $CL'_{\mathrm{int}}$ occurs with tolbutamide, warfarin, and non-steroidal anti-inflammatory drugs, and it is only with such drugs that protein binding effects on elimination are recorded.

### 7.4.2 *First-pass metabolism*

For a drug taken orally that is *completely absorbed* from the gastrointestinal tract and *only metabolized by the liver*, the fraction of the oral dose that reaches the systemic circulation, $F$, is given by rearranging Equation 7.11:

$$F = 1 - E \tag{7.27}$$

In this case $F$ can be considered to be the fraction of the dose that escapes first-pass metabolism. It is possible to show that:

$$CL_{\mathrm{oral}} = CL_{\mathrm{int}} \tag{7.28}$$

It must be noted that this only applies when the strict caveats stated above apply. The ratio of $AUC$ values is commonly used to evaluate $F$ and Equation 7.27 used to calculate $E$.

However, oral and i.v. doses reach the liver by different routes. Oral doses more or less completely pass through the hepatic portal vein (which thus behaves like an artery), while i.v. doses pass through the hepatic artery (Figure 7.3). Once both doses are fully equilibrated within the body the hepatic artery becomes the

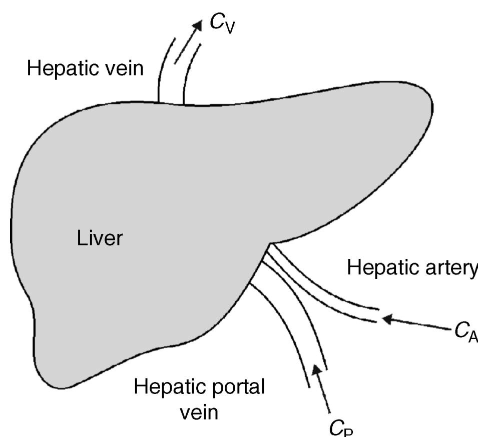



**Figure 7.3** Approximately 75–80% of blood supplying the liver enters via the hepatic portal vein which carries deoxygenated blood containing substances that have been absorbed from the GI tract. Oxygenated blood from the heart enters via the hepatic artery. Blood leaves via the hepatic vein which drains into the vena cava.

major route for both. One can envisage two values of $E$, one for the hepatic artery/hepatic vein transfer, and another for the hepatic portal vein/hepatic vein transfer. Because the products of these two transfers intermingle in the hepatic vein, these two $E$s are not readily accessible (they can be assessed in heroic experiments in which radioactive doses are used, and different isotopes are incorporated into the i.v. and oral doses which are given together – and these experiments are most successful if the various blood vessels are separately sampled).

Failure to recognize that Equation 7.27 only applies when the liver is the only site of metabolism will result in erroneous conclusions as illustrated in Figure 7.4 which shows data relating $CL_{mic}$ and $F$ for a series of compounds. There is no useful correlation. In spite of this, $1 - F$ has been used for estimating $E$, and hence $CL_H$, and then back calculating from $CL$ to intrinsic microsomal clearance.

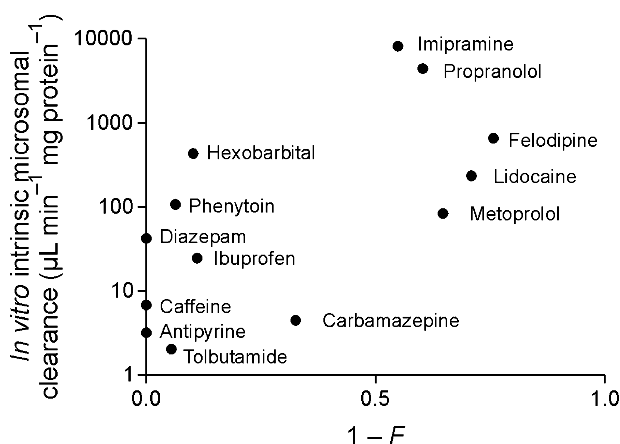



**Figure 7.4** Comparison of *in vitro* microsomal intrinsic clearance from a variety of literature sources with assessment of bioavailability ($F$) for 13 representative drugs, showing only the most slender of relationships. Bioavailability data are from Goodman and Gilman (1996, 2001 and 2005), and *in vitro* data from 13 individual papers located by means of a literature search.

The literature concerned with intrinsic clearance creates potential for confusion concerning what is what. This occurs because the concepts were created on the basis of protein-free incubations *in vitro*, and assays of plasma concentrations (including protein-bound material) *in vivo*. There can be only one *in vivo* or hepatic 'intrinsic clearance' (the ability of the liver to metabolize the drug in the absence of delivery or availability restrictions). This should be given the symbol $CL'_{int}$. There is however also microsomal intrinsic clearance, which may or may not (usually not) reproduce hepatic intrinsic clearance – the symbol $CL_{mic}$ has been used for this. This is measured experimentally *in vitro* using microsomes.

The calculation of clearance in hepatocytes can be of $CL'_{int}$, from the experimentally measured clearance in the incubation making use of the data on the hepatocyte concentration. This experiment may or may not reproduce the intrinsic clearance that occurs *in vivo*, as it involves hepatocyte reactions in the absence of blood flow or availability restrictions.

*In vivo* data starting with the experimental observation of systemic clearance, $CL$ (using plasma assays that include protein-bound drug) is first used to calculate hepatic clearance (probably less than $CL$ because of the renal, and other, contributions). Hepatic clearance, $CL_H$, can then be used to 'back-calculate' an equivalent of microsomal intrinsic clearance (using data for the number of hepatocytes per gram of liver, and again using the data for microsomal protein concentration per gram of liver) but it should be recognized that this does not

calculate $CL_{mic}$. Rather, it calculates $CL_{int}$, which is equal to $f_u CL'_{int}$. Note that, because $f_u$ is a fraction, $CL'_{int} > CL_{int}$, in keeping with the definition that $CL'_{int}$ is the maximum hepatic activity in the absence of availability, that is protein binding and delivery, restrictions.

## 7.5 *In vitro* to *in vivo* extrapolation

It is desirable to use intrinsic clearance to help determine the expected systemic clearance, and hence the half-life, in human investigations. The strategy for this *in vitro/in vivo* scaling is relatively straightforward:

- Measure the half-life of metabolism of the drug *in vitro*.
- Calculate microsomal *in vitro* intrinsic clearance.
- Calculate microsomal *in vivo* intrinsic clearance from *in vivo* $CL_{mic}$ = *in vitro* $CL_{mic}$ × microsomal protein (mg per g of liver) × g liver per kg of body weight.
- Calculate hepatic clearance using hepatic blood flow (use the Wilkinson–Shand equation, Equation 7.13, and literature values for hepatic blood flow; note that this 'labels' microsomal intrinsic clearance as the only contributor to hepatic clearance).
- If the volume of distribution is known calculate the hepatic contribution to $CL$ and hence the contribution to the half-life.
- If the percent of the dose that is excreted unmetabolized is known use the additivity of clearance to calculate the anticipated $CL$.
- Correct the calculation for protein binding using $CL_{int} = f_u CL'_{int}$.

In regard to the fifth point above, if the apparent volume of distribution is known then an *in vivo* experiment and assessment of half-life assessment has already been done. In fact, it is likely that the *in vivo* kinetics in a suitable animal species, including renal clearance, and the approach described above to determine the microsomal intrinsic clearance contribution to total clearance, will have been carried out. This collection of data can be used to make predictions for human beings, in combination with allometric scaling approaches (Chapter 15). A selection of scaling factors for the rat is given in Table 7.1.

**Table 7.1** Scaling factors for the rat

| Property | Value | Scaling values for standard weight rat (250 mg) |
|---|---|---|
| Liver weight | 45 g kg body weight$^{-1}$ | 11 g |
| Liver blood flow | 1.8 mL min$^{-1}$ g liver$^{-1}$ | 20 mL min$^{-1}$ |
| Hepatocyte number[a] | $1.35 \times 10^{8}$ cells g liver$^{-1}$ | $1.5 \times 10^{9}$ cells |
| Microsomal protein yield[a] | 45 mg protein g liver$^{-1}$ | 500 mg protein |

[a] Literature averages.

Ideally, the intrinsic clearance obtained *in vitro* would equal that observed *in vivo*. Various investigators have studied correlations between *in vitro* and *in vivo* values, for both rats and humans. Typically, in quite complex studies, *in vitro* microsomal intrinsic clearance accounted for, on average, only about one-fifth of *in vivo* intrinsic clearance in humans (Naritomi *et al.*, 2001). Similarly, *in vitro* hepatocyte intrinsic clearance accounted for, on average, one fifth to one quarter of *in vivo* intrinsic clearance in rats (Lavé *et al.*, 1997). Further, *in vitro* hepatocyte intrinsic clearance accounted for, on average, approximately one fifth of *in vivo* intrinsic clearance in humans (Lavé *et al.*, 1997). Among the possible

reasons for these results were:

- Intrinsic clearance *in vivo* includes all processes of elimination, including renal excretion, and non-hepatocyte, non-microsomal metabolism.
- While the calculations can allow for blood flow and protein binding effects, they do not allow for variations in fine detail of liver perfusion local to enzyme surfaces.
- Non-specific binding effects could reduce the actual drug concentrations at enzyme surfaces.
- There could be lack of homogeneity of distribution of the enzymes through the liver.

As the result, *in vivo* intrinsic clearance is found in fact to correlate quite well with extraction ratio. The best *in vitro* predictor of human *in vivo* data appears to be human hepatocytes.

## 7.6 Limiting values of clearance

Conceptually, it seems to be obvious that clearance numbers will relate to blood flow properties of organs, and will have upper limits, such as

- $CL$ cannot exceed cardiac output: 5.3 L min$^{-1}$ (or 75 mL min$^{-1}$ kg$^{-1}$).
- $CL_H$ cannot exceed hepatic blood flow: 1.5 L min$^{-1}$.
- $CL_R$ cannot exceed renal blood flow: 1.5 L min$^{-1}$.
- $CL_R$ for drugs for which there is no renal tubular membrane transfer cannot exceed glomerular filtration rate (125 mL min$^{-1}$ plasma $\cong$ 230 mL min$^{-1}$ blood).

Many measurements of clearance conform to these concepts and indeed the clearance of some compounds may be used to estimate plasma/blood flows, for example *p*-aminohippuric acid to measure renal plasma flow (Section 3.3.1.4). However, Table 7.2 shows a selection of clearance values, together with data for apparent volume of distribution and half-life, which show that in certain cases systemic clearance can exceed cardiac output. Although it might be expected, there is no obvious correlation between the values of clearance, volume of distribution, and half-life among the compounds in Table 7.2.

**Table 7.2** Values of systemic clearance, apparent volume of distribution and elimination half-life for selected drugs

| Drug | Systemic clearance, *CL* | | *V* | Half-life |
|---|---|---|---|---|
| | (mL min$^{-1}$ kg$^{-1}$) | (L min$^{-1}$) | (L kg$^{-1}$) | (h) |
| Glyceryl trinitrate | 230 | 16.1 | 3.3 | 2.3 (min) |
| Prazepam | 140 | 9.8 | 14.4 | 1.3 |
| Triametrine | 63 | 4.4 | 13.4 | 4.2 |
| Azathioprine | 57 | 4.0 | 0.81 | 0.16 |
| Hydralazine | 56 | 3.9 | 1.5 | 0.96 |
| Isosorbide | 45 | 3.2 | 1.5 | 0.8 |
| Cocaine | 35 | 2.5 | 2.1 | 0.71 |
| Desipramine | 30 | 2.1 | 34 | 18.0 |
| Nicotine | 18.5 | 1.3 | 2.6 | 2.0 |
| Propranolol | 12 | 0.84 | 3.9 | 3.9 |
| Diltiazem | 11.5 | 0.81 | 5.3 | 3.2 |
| Chlorpromazine | 8.6 | 0.60 | 21.0 | 30.7 |

Examples of very high clearance drugs also include physostigmine, esmolol, loratidine, misoprostol, spironolactone, and, according to some reports, selegiline. Some of the explanations as to why *CL* can appear to, or actually, exceed cardiac output could include:

- Experimental errors in measurement of *CL* or calculation errors resulting from use of inappropriate models.
- Widespread non-enzymatic chemical degradation of the drugs throughout the body.
- A major contribution from non-hepatic and non-renal elimination. This seems to occur with glyceryl trinitrate, which is extensively metabolized in blood vessel walls and something similar could occur with drugs metabolized by plasma esterases, e.g. physostigmine. In these cases metabolism or chemical degradation occurs continuously, independent of blood flow to any particular organ of the body.
- Also, we should not lose sight of the fact that, when clearance is calculated from *D*/*AUC* for a drug with a very high apparent volume of distribution and therefore a very low *AUC*, the errors in the result of the calculation will be relatively high.

A major consequence of the risk that exists of an erroneously calculated value for systemic clearance is that use of Equation 4.2 could lead to seriously incorrect estimates of hepatic clearance as it is based on the difference between systemic clearance and renal clearance.

## 7.7 Safe and effective use of clearance

- *In vitro* work can and should involve the measurement of microsomal intrinsic clearance for all drugs of interest. This is the core process of drug metabolism reduced to fundamentals free of blood flow and protein-binding influences.
- *In vitro* work can and should involve the measurement of hepatocyte clearance, and calculation of intrinsic clearance from the data involved, so that the contribution to clearance from the presence of enzymes in the intact liver cells can be evaluated.
- Use of perfused liver (*in vitro* or *in vivo*) is a valuable research technique, which can provide data on hepatic activity towards the drug with physiological and biochemical processes intact but with delivery controlled; the same could be said of perfused kidney, or renal artery/renal vein sampling, but this is not commonly attempted.
- Systemic clearance from *D*/*AUC* should be calculated in all *in vivo* work, but care must be taken in its interpretation, in particular it is important not to view it as an assessment of hepatic activity.
- Comparison of members of a series of compounds in drug development or clinical pharmacology (including Phase I) can use clearance values as operational numbers (for comparative purposes) provided due allowance is made for the potential ‘noise’ in such work.
- Dosing adjustments with digoxin and other drugs can be based on clearance rather than half-life.
- Clinical dosing must respect the phase of the kinetics that is in effect when dosing regimens or adjustments are made. For example, gentamicin dosing regimens are based on the distribution phase of a biexponential decay, while most dosing regimens are based on the terminal phase of a bi- or multi-exponential decay – clearances measured appropriate to the clinical objective must be used. This is considered in more detail in Chapter 19.

## 7.8 Physiological modelling

The concepts of organ clearances can be applied to what are known as ‘physiological models’. In the previous chapters most of the pharmacokinetic models have been based on the concept of the body as one or more

'compartments' or 'pools' which are treated as if they contain homogeneous solutions of the drug. These models are useful for such things as deriving dosing schedules; however these compartments may have little or no relationship to anatomical spaces or organs. Furthermore, tissues in the same compartment can have markedly different concentrations, for example, the plasma and liver concentrations depicted in Figure 2.14(a). In physiologically based pharmacokinetic modelling (PBPK), the tissues and organs that play a role in the disposition of the drug being investigated are included in the model. Thus, there is no general physiological model; individual models will be dictated by the nature of the drug and to some extent by the route of administration. The various organs are connected by arterial and venous blood flows. Because of their importance in drug disposition the liver and kidney are usually included. If the lungs are included in the model then they are placed in series with the right and left heart (Figure 7.5).

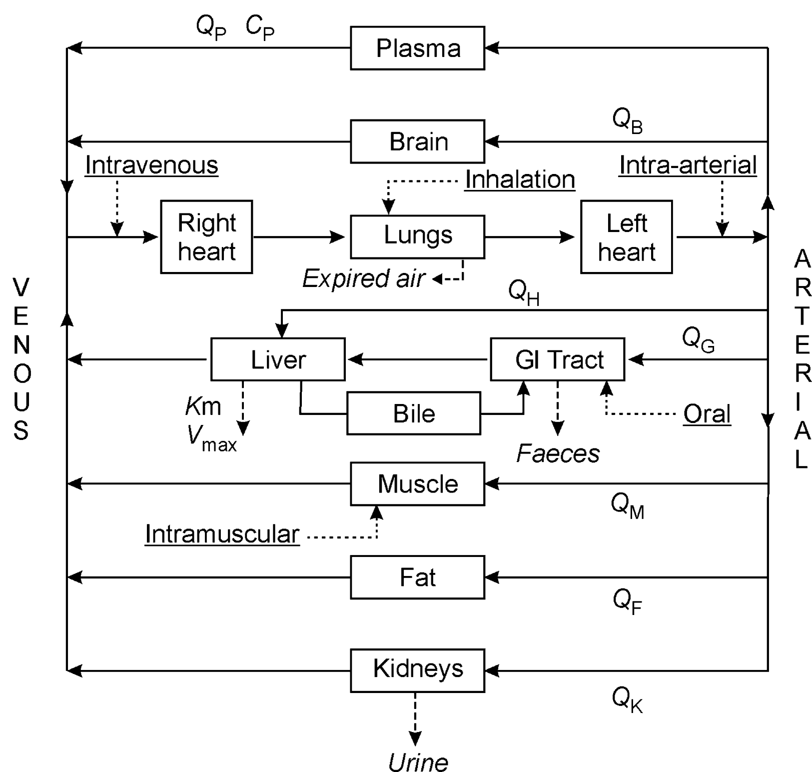



**Figure 7.5** Hypothetical physiological model demonstrating how relevant organs are connected by arterial and venous blood flows and how various routes of administration can be depicted as required by the model.

Each organ or tissue type has an associated blood or plasma flow, $Q_t$ and volume $V_t$. A further complexity is that each organ is modelled as consisting of plasma, interstitial and intracellular components. Thus many physiological factors can be incorporated in the model including the effects of plasma and tissue binding and the effects of the drug on blood flows to the organs, should that be appropriate; for example the effect of propranolol on cardiac output. *In vitro* data such as *K*m and $V_{max}$ values from metabolism studies and partitioning between the components of a tissue can be incorporated.

In many situations the distribution of drug between tissue and blood is flow-limited. As blood flows through a tissue, drug is extracted so that at equilibrium the tissue to blood concentration is given by

a partition coefficient, $R_t$:

$$R_t = C_t/C_B \tag{7.29}$$

The differential equation describing the rate of change in the tissue is:

$$\begin{aligned}\frac{dC_t}{dt} &= \frac{Q_t C_B - Q_t C_t/R_t}{V_t} \\ &= \frac{Q_t(C_B - C_t/R_t)}{V_t}\end{aligned} \tag{7.30}$$

where $V_t$ is the apparent volume of distribution of the tissue. However, if the tissue is an eliminating organ, such as the liver, then drug is also removed by elimination (rate = concentration × clearance) and Equation 7.30 becomes:

$$\frac{dC_t}{dt} = \frac{Q_t(C_B - C_t/R_t)}{V_t} - C_t.CL_t \tag{7.31}$$

If the kinetics of elimination are non-linear then $CL_t$ can be related to the apparent $K$m and $V_{max}$ values using an equation analogous to Equation 4.42.

Having decided which tissues and organs should be included in the model, the rate of change of drug concentration in the blood (or plasma) can be modelled by summing all the component terms. If the modelling is done in terms of plasma concentrations, then the overall apparent volume of distribution of the drug at steady-steady is given by all the apparent volumes of distribution of the tissues plus the volume of plasma, $V_P$:

$$V_{SS} = V_P + \sum V_t R_t \tag{7.32}$$

The situation is more complex when the tissue distribution is membrane-limited, rather than flow-limited. Under these circumstances, equations describing the net flux of drug through the membrane have to be derived. The movement may be by simple diffusion or saturable carrier-mediated transport.

### *7.8.1 Practical considerations*

To utilize PBPK models is necessary to know the volumes of the appropriate tissues, the partition coefficients of the drug between blood and those tissues, as well as the blood flows though them, for the species under investigation. It may be possible to use published data for these values (Table 7.3) or it may be necessary to measure them. Sometimes allometric scaling is used (Chapter 15). Blood flows may be determined using such techniques as microsphere, laser Doppler velocimetry or tracer dilution techniques. It should be remembered that the total blood flow through the tissues cannot exceed cardiac output. Values of $R_t$ can be obtained by infusing drug to steady-state conditions, after which the animals are killed for analysis of tissue concentrations so that Equation 7.29 can be used. Non-linearity of $R_t$ values with increasing drug doses indicates binding or complex diffusion in the tissue being studied.

Once all the parameters have been obtained these can be used in the model. The plasma concentration data are not fitted statistically as in other models but the physiological parameters are adjusted to obtain the most appropriate model. Tissues with large blood flows and volumes will have the greatest influence on the model while smaller tissues may have little influence on the overall quality of 'fit'. Thus models, unsurprisingly, are likely to be heavily dependant on the liver and kidney.

**Table 7.3** Physiological parameters for several species[a]

| Parameter | Mouse | Rat | Monkey | Dog | Man |
|---|---|---|---|---|---|
| Body weight (g) | 22 | 200 | 5000 | 17 000 | 70 000 |
| ***Volume (mL)*** | | | | | |
| Plasma | 1.0 | 9.0 | 220 | 650 | 3000 |
| Muscle | 10 | 100 | 2500 | 7500 | 35 000 |
| Kidney | 0.34 | 1.9 | 30 | 76 | 280 |
| Liver | 1.3 | 8.3 | 135 | 360 | 1350 |
| Gut | 1.5 | 11 | 230 | 640 | 2100 |
| ***Plasma flow rate (mL min$^{-1}$)*** | | | | | |
| Muscle | 0.5 | 3.0 | 50 | 140 | 420 |
| Kidney | 0.8 | 5.0 | 74 | 190 | 700 |
| Liver | 1.1 | 6.5 | 92 | 220 | 800 |
| Gut | 0.9 | 5.3 | 75 | 190 | 700 |

[a]Bischoff *et al*., 1971.

## 7.9 Inhomogeneity of plasma

Plasma is not necessary homogeneous with regard to drug concentration while absorption of oral or intramuscular doses is continuing. There will be a concentration gradient through the blood stream with the highest concentration just beyond the absorption site and the lowest concentration in blood arriving at the absorption site. This has been demonstrated for ethanol (Table 7.4). Blood ethanol concentrations were measured at five locations after oral administration. During absorption highest concentrations occurred in arteries and lowest in veins but when absorption was more or less complete (90–150 min) blood concentrations were almost homogeneous. It should be recognized, therefore, that pharmacokinetic analysis and concentration–effect studies may be markedly affected by time and site of sampling.

**Table 7.4** Ethanol content of blood drawn simultaneously from various parts of the body: dog (15 kg) given 3 g kg$^{-1}$ into stomach

| Time (min) | Concentrations in blood (g L$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | Artery Left heart | Jugular vein | Femoral vein | Right heart | Skin capillary |
| 30 | 2.31 | 2.00 | 1.09 | | 2.13 |
| 60 | 2.86 | 2.65 | 2.10 | 2.85 | |
| 90 | 2.91 | 2.80 | 2.60 | | 2.74 |
| 150 | 2.58 | 2.50 | 2.46 | | |
| 210 | 2.22 | 2.17 | 2.09 | 2.21 | 2.14 |
| 270 | 1.9 | 1.88 | 1.82 | | |
| 330 | 1.65 | 1.63 | 1.6 | 1.65 | 1.62 |

Haggard and Greenberg, 1934.

The issues associated with arteriovenous differences in drug concentrations have been discussed in a two-part review by Chiou (1989a,b), which he described as ‘critical or even provocative’. Generally, when a drug is administered, whether it be injected or taken orally, it is transported to the heart and enters the arteriolar circulation (Figure 7.5). In the early phases uptake by tissues reduces the venous concentrations relative to the

afferent arterial ones [Figure 7.6(a)]. There comes a time when the arteriolar and venous concentrations become equal. At later times, when the plasma concentrations have been reduced by elimination, the tissues release drug into the blood flowing through them so that the venous concentrations are now higher than the arterial ones [Figure 7.6(b)].

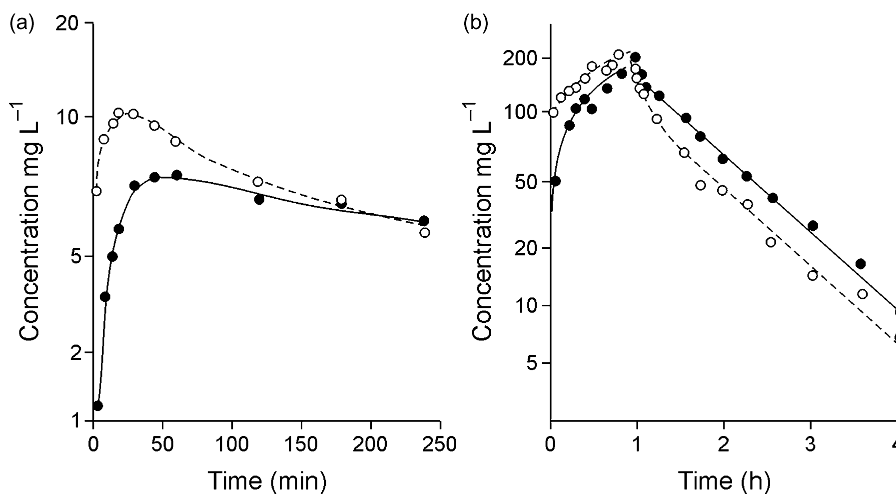



**Figure 7.6** (a) Mean arterial (○) and peripheral venous (•) plasma concentrations of lidocaine in 10 patients after extradural injection of 400 mg. (b) Femoral arterial (○) and venous (•) plasma concentrations of propranolol in a 18.3 kg dog given a constant intravenous infusion (15.3 mg h$^{-1}$) for 60 minutes. (Redrawn from Chiou, 1989a.)

Using a physiology-based principle, the ratio of arterial to venous concentrations can be calculated:

$$\text{ratio} = \frac{1-\lambda R}{\dot{Q}} \tag{7.33}$$

where $\lambda$ is the first-order elimination rate constant, $R$ is the apparent partition coefficient and $\dot{Q}$ is the blood flow per unit weight of tissue. Therefore, short half-lives (larger $\lambda$) and extensive tissue binding will result in a large arteriovenous difference, whereas an increase in blood flow will tend to reduce the difference. Not only will there be differences in the arterial and venous concentrations but the concentrations in blood from different sampling sites may be different. Capillary blood concentrations should lie between those of arterial and venous blood, but may be affected by blood flow to the sampling site, for example finger-tip samples had greater ethanol concentrations than those collected from the big toe.

The consequences of inhomogeneity and sampling site on derived pharmacokinetic parameters have been largely ignored. The terminal half-lives may be the same [Figure 7.6(b)] but volume of distribution data based on intercepts on a concentration axis and even *AUC* estimations are likely to be in error. (Chiou, 1989a,b), throwing into doubt some of the fundamental concepts of the subject.

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# 8

# Drug Formulation: Bioavailability, Bioequivalence and Controlled-Release Preparations

## 8.1 Introduction

There was a time when it was believed that if a tablet contained its labelled quantity of active drug, then it was as clinically effective as a pure solution of that drug. Also, chemical equivalence was historically equated with clinical equivalence. However, it is now well established that differences in product formulation can lead to large differences in speed of onset, intensity and duration of drug response.

The study of formulation factors in pharmacological response is described as the science of 'biopharmaceutics'. The word 'bioavailability' may be used to describe the extent to which a drug is released from its pharmaceutical dosage form to be available to exert an effect. Regulatory authorities have defined bioavailability as:

> The rate and extent to which the therapeutic moiety is absorbed and becomes available to the site of drug action (Chen *et al*., 2001).

Clearly, no single pharmacokinetic assessment measures both rate and extent, and some authorities would prefer the definition to refer to only extent. Of course, what is normally studied is *systemic availability*, that is, the appearance of the drug in the *general* circulation, which has the potential to vary in both rate and extent (Section 8.3). This is a composite result of pharmaceutical and biological factors. Note the potential for conflict between 'absorption' and 'bioavailability'. Also, the term 'bioequivalence' is commonly used, implying that two or more products are comparable, to some standard, in their release of active medication *into the blood* (see later).

Variations in bioavailability were first observed in the 1960s and 1970s as the result of:

- Therapeutic failures on changing to new suppliers of certain drugs (presumably as the result of lesser availability of the drugs in the second preparations).
- Increased incidence of unwanted effects and toxicity on changing to new suppliers of certain drugs (presumably as the result of greater availability of the drug in the second preparation).
- Observation of other differences, such as in clinical response.
- Observation of differences in drug concentrations in blood and blood fractions.

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Scientific investigation is not straightforward. There is a hierarchy of testing methods for bioavailability. In descending order of accuracy, sensitivity and reproducibility, the methods are:

- *In vivo* tests in humans with plasma concentration measurements.
- *In vitro* tests (e.g. dissolution) that have been correlated with human *in vivo* data from the first bullet point.
- *In vivo* tests in animals that have been correlated with human *in vivo* data from bullet point 1.
- An *in vivo* test in humans based on urinary excretion of the active drug substance (not metabolites).
- An *in vivo* test in humans using measurement of pharmacological effec.
- A well-controlled clinical trial in humans testing therapeutic outcome, specifically conducted to test bioavailability, involving comparison of two products.
- A validated *in vitro* test, without the support of *in vitro*/*in vivo* correlation studies.

By far the most satisfactory bioavailability investigations are conducted *in vivo* in humans by the study of drug concentrations in blood and blood fractions. In practice, studies of the first bullet point are required for new products and for new formulations of existing drugs, and they should be supported and extended by batch testing with dissolution tests (second bullet point). Only when such evaluations are not possible will other methods be acceptable.

## 8.2 Dissolution

Strictly speaking dissolution is the process of active medicament dissolving in the fluid around it. However, it has long been recognized that the release of a drug from a tablet involves at least five steps in sequence. These are wetting of the dosage form, penetration of the dissolution medium into the dosage form, disintegration of the tablet, deaggregation of the dosage form and dislodgement of the drug-containing granules (see later) almost universally necessary in tablet production, and, finally, dissolution of the active medicament (as defined above). Pharmaceutical dissolution tests, such as those using Wood's Apparatus, which is a rotating disc system, attempt to reproduce this sequence of events. They do not reproduce the biological factors that play a part in transfer of drug to the systemic circulation. In bioavailability testing a correlation will be sought between *in vivo* (plasma concentration) data and dissolution data. This correlation may be seen as the same rank order of a number of different formulations in the *in vivo* and *in vitro* test results, and/or reproducibility of *in vivo* and *in vitro* data, such that the dissolution test can be applied to future batches in the quality control process, in the expectation that reproducible dissolution test data can be taken as assurance that the *in vivo* data will be reproducible. It is not practicable to test every batch *in vivo*.

## 8.3 Systemic availability

Bioavailability assessments using plasma concentrations rely on three fundamental descriptive pharmacokinetic observations: the maximum concentration, $C_{max}$, the time of the maximum concentration, $t_{max}$, and the area under the plasma concentration–time curve, $AUC$. $C_{max}$ and $AUC$ evaluate the extent to which drug becomes bioavailable, whilst $t_{max}$ evaluates the rate at which a drug becomes available.

Measuring the proportion or fraction of a dose of drug which appears in the general circulation is relatively simple to assess by comparing $AUC$ following the test dose with the $AUC$ following an intravenous dose ($AUC_{i.v.}$). For a drug that is eliminated according to first-order kinetics, the area under the blood concentration curve–time curve from $t = 0$ to $t = \infty$, is directly proportional to the amount of drug that enters the systemic circulation. However, plasma concentrations are usually measured on the assumption that there is also a direct relationship between blood and plasma concentrations of the drug. When a drug is injected intravenously, the entire dose enters the circulation and so the $AUC_{i.v.}$ can be used

to estimate what fraction, *F*, of a dose given by an alternative route, reaches the systemic circulation. For an oral dose:

$$F = \frac{AUC_{\text{p.o.}}}{AUC_{\text{i.v.}}} \times \frac{Dose_{\text{i.v.}}}{Dose_{\text{p.o.}}} \tag{8.1}$$

where $AUC_{\text{p.o.}}$ is the area under the curve flowing an oral dose. If equal sized doses are given then Equation 8.1 simplifies to:

$$F = \frac{AUC_{\text{p.o.}}}{AUC_{\text{i.v.}}} \tag{8.2}$$

In sequential experimental designs, the doses of drugs to be compared are given to the same subjects with a suitable time interval between the doses to ensure that all of the first dose has been removed before the second is given. The *AUC* values are usually calculated using the trapezoidal method (see Appendix).

### *8.3.1 Effect of bioavailability on plasma concentration–time curves*

It should be obvious that a reduction in bioavailability, say for an orally administered dose, will reduce the plasma concentrations relative to those of an equal size intravenous dose. However it is worth considering how the concentration–time curves are affected. Figure 8.1(a) shows typical curves for an intravenous injection and an oral administration for which the systemic availability, $F = 1$. Note that the concentration

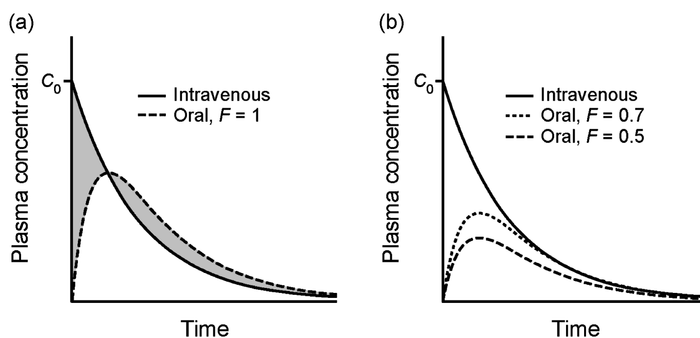


**Figure 8.1** Comparison of plasma concentration–time curves for various values of *F*. (a) $F = 1$. At later times the concentrations after the oral dose must be higher than the i.v. and the areas of shaded areas must be equal. (b) $F = 0.7$ concentrations at later times are superimposed, whereas $F = 0.5$ all the oral concentrations are less that the i.v. ones. $C_0$ is the initial plasma concentration after the i.v. injection.

following the i.v. injection declines very rapidly, as would be expected for a drug eliminated according to first-order kinetics. The concentrations after the oral dose increase from zero to the maximum concentration ($t_{\max}$) – the point at which the rate of absorption equals the rate of elimination – and then decline because from this point the rate of elimination is greater than the rate of absorption. The $AUC_{(0-\infty)}$ is the same for each route of administration (although of course infinite time cannot be shown on the figure). Part of the area under the curves is common to both routes but the shaded areas represent areas that are only under the i.v. or the oral curve. These areas must be equal. It is, at least in theory, possible for the concentrations to be superimposable at later times. This occurs when $F \sim 0.7$ [Figure 8.1(b)] whereas for lower values *all* the oral concentrations are less than those after the i.v. injection.

Although the curves in Figure 8.1 are labelled 'oral' the principles apply equally to other extravascular routes of administration such as intramuscular, subcutaneous and inhalation.

## 8.4 Formulation factors affecting bioavailability

### *8.4.1 Origins of variation*

As implied earlier, the simplest oral preparation would be a pure solution of a drug in water, and oral solutions are sometimes used in comparisons with tablets in studies of 'relative bioavailability'. Solution preparations are used in therapeutics, but the bitter taste, instability and insolubility of many compounds necessitate complex formulations with solubilizing agents, flavouring agents and antioxidants. These difficulties could be overcome by packing the dry powdered drugs in gelatin capsules. However, machine filling of gelatin capsules without additives is difficult because of the small size of many drug doses. Hence the use of tablets, but as tablets have no outer gelatin shell, they must be made durable by compression. Various other inactive constituents (excipients) are needed for a variety of purposes, and they can lead to a considerable range of bioavailability problems. Apart from the active constituent it is generally necessary to include the following:

#### *8.4.1.1 Diluents*

As already mentioned, the weight of medicament is often too small for easy handling. This problem is commonly overcome by dilution of the active material with an inert material, such as lactose, starch, calcium phosphate or calcium sulfate, to increase the bulk. These substances can form complexes with the active ingredient, and affect solution of the latter in biological fluids once the preparation has disintegrated. They can also directly affect the drug absorptive process. The use of lactose in this context is waning because of the risk of lactose intolerance in some patients.

#### *8.4.1.2 Granulating and binding agents*

The drug, or drug–diluent mixture, cannot usually be pressed into a tablet of sufficient strength to survive buffeting in bottles. It is more satisfactory to first prepare granules by mixing the dry powder with a natural gum or mucilage, such as solution of acacia or tragacanth, or with syrup (sucrose), gelatin, povidone, various cellulose products or partially hydrolysed starch. The particles of powder are moistened with the granulating agent until they aggregate in relatively large angular granules, which have a very large surface area. The next stage is sieving, to control the granule size, and thorough drying of the now uniform granules.

The dry granules are commonly at this stage given a further external coating of the granulating solution, this time as a binding agent. The combined effect of the large surface area, the angular properties of the granules, and the adhesive properties of the binding agent then leads to a cohesive tablet when the mixture is divided into quantities of the required size and compressed into tablets in the tablet-making machine. These materials are present to preserve the structural integrity of the tablet, and inevitably retard drug release.

#### *8.4.1.3 Lubricants and surfactants*

The use of machinery in tablet making necessitates the use of lubricants. It is desirable that the granules should adhere to themselves but not to the tablet punches, and this ideal is achieved by the incorporation of a small amount of talc or other dry consumable lubricant in the powder. Materials used include stearic acid and various stearates, hydrogenated vegetable oils, polyethylene glycol and sodium lauryl sulfate. The term 'glidant' is sometimes used in this context. Some of the materials may be water repellent and so affect the 'wettability' of the disintegrating tablet. Equally, surfactants may be included to increase dissolution.

#### *8.4.1.4 Disintegrating agents*

The manufacturer may be content with a robust tablet, but the patient wants all of the manufacturers' work undone in order to effect rapid disintegration. Three types of agent can be incorporated into the tablet for this purpose.

- Substances (e.g. starch) that swell up on contact with moisture.
- Substances (e.g. cocoa butter) that melt at body temperature.
- Substances (e.g. a dry mixture of sodium bicarbonate and tartaric acid) that effervesce on contact with water.

Of these, the last is the most popular. Also used to aid disintegration are alginic acid, microcrystalline cellulose and colloidal silicates.

#### *8.4.1.5 Miscellaneous*

Apart from the above, it is sometimes necessary to include antioxidants to prevent decomposition, or substances (such as potassium carbonate) to control the pH of the tablet in the event of attack by moisture. Dyes will be included if coloured tablets are required, and dyes may be adsorbed on to aluminium hydroxide, providing another adsorbing surface for the drug and, in this case, another opportunity for a pH influence. If appropriate, flavouring may be included.

#### *8.4.1.6 Coated tablets*

Coating of tablets is carried out for both cosmetic and practical purposes. Sugar-coated tablets can be produced in bright colours and polished to a high degree for the purpose of making them attractive to the eye (a dubious virtue). Coating involves solutions of sucrose, as well as various coloured materials. The sugar and colours are dried on to the compressed tablets and the dry residue is mechanically polished. A practical purpose of coating is to ensure that the tablet reaches the intestine before disintegrating. This is achieved by including materials in the coating which are not attacked by the acid in the stomach, but which are attacked in the less acidic intestine. Control of tablet disintegration in this particular way *can* involve further additives. Finally, coated tablets are often marked with an identification symbol. This involves a small amount of an edible ink. Coating can involve the use of sugars, starch, calcium carbonate, talc, titanium dioxide, acacia, gelatin, wax, shellac, cellulose acetate, other cellulose materials and polyethylene glycol.

#### *8.4.1.7 Capsules*

Some of the above additives are required in capsules, although obviously not granulating agents. Particularly with capsules, colours, opaquants (e.g. titanium dioxide), dispersants, hardening agents (e.g. sucrose), fillers, lubricants and glidants are needed. There is of course, also the gelatin, which is available in a variety of types based on bone and skin waste from other industries. In particular, successful products using soft gelatin capsules are now common. There is some concern that gelatin will have to be phased out because its use is not acceptable to all patients.

### ***8.4.2 Examples of drugs showing bioavailability variations***

A number of important bioavailability examples have been studied. Some were potentially life-threatening. Some of the drugs that were reported as having problems of bioavailability are no longer available, or rarely

prescribed, but they serve to illustrate the issues that needed to be considered. The features of a compound, or those materials mixed with it, which will lead to a bioavailability influence include:

- Particle size, in that smaller particles of drugs when released from tablets dissolve more quickly (phenacetin – now obsolete, nitrofurantoin, griseofulvin, sulfadiazine, spironolactone and aspirin).
- Crystalline form, salt form and complexing with tablet constituents, affecting the rate of solution in a similar way (chloramphenicol existed in various polymorphic forms).
- Low aqueous solubility leading to slow solution at the best of times.
- Wetting agents and other materials in the tablet affecting the interaction of constituent drug and aqueous media.
- Variations in tablet making consequent on granule compression, humidity, etc.

It is obvious that solubility in aqueous media is the key factor and it has been suggested that this is especially so when one of the following apply:

- A sparingly soluble drug is used in relatively high doses.
- Absorption only occurs in the upper part of the gastrointestinal tract.
- A steady and prolonged release of the drug is required.
- High early plasma levels are required for the desired action.
- The gastrointestinal contents or gut flora exert a destructive effect on the drug.
- The drug is absorbed by carrier-mediated mechanism.

Solubility, and particularly the rate at which solids dissolve, will be influenced by the particle size of the active ingredient (Figure 8.2).

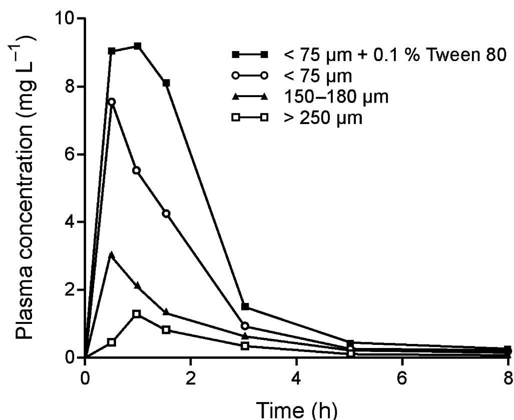


**Figure 8.2** Mean plasma concentrations of phenacetin (acetophenetidin) in six human subjects after 1.5 g doses in suspensions of different particle sizes and with and without Tween 80 (after Prescott *et al.*, 1970).

Apparently simple differences such as using capsules rather than tablets, or vice versa, may result in marked changes in bioavailability and consequently clinical effects. For example, the urinary excretion rate of triamterene was greater following administration of tablets rather than capsules and this was accompanied by corresponding increases in sodium excretion (Figure 8.3).

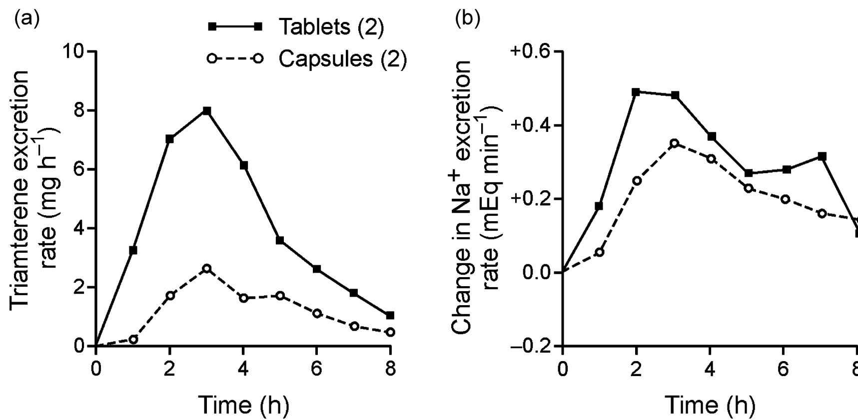


**Figure 8.3** (a) Mean rate of excretion of triamterene in eight subjects following oral doses as capsules and tablets. (b) Mean rate of sodium excretion. (From the data of Tannanbaum *et al.*, 1968).

Considering bioavailability from the clinical point of view, Turner (1974) emphasized the facts that the possibility of a bioavailability problem achieving clinical significance would be enhanced with:

- Sparingly soluble drugs for which there is a close relationship between dissolution rate and plasma levels, and where different formulations with similar disintegration times show marked differences in dissolution times.
- In replacement therapy, such as for thyroid and adrenal cortical deficiency, and in diabetes mellitus. The clinical effects of small changes in the bioavailability of replacement drugs in conditions such as hypothyroidism and Addison's disease may develop only slowly and insidiously, and may not, therefore, be easily recognized until a serious condition has developed.
- In the control of serious clinical conditions in which there is a narrow range of optimum plasma concentrations of drugs for correct therapy.
- Therapies requiring the use of drugs with a very small therapeutic ratio, so that relatively small changes in plasma concentrations may lead to the development of signs of toxicity.

Unsurprisingly, the most dramatic bioavailability demonstrations have been with drugs with small therapeutic windows and marked toxicity. Problems with digoxin toxicity were explained by differences in tablet dissolution (Figure 8.4).

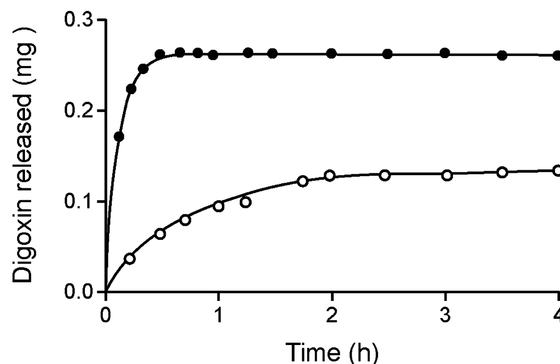


**Figure 8.4** Dissolution *in vitro* of two formulations of digoxin (redrawn from Fraser *et al.*, 1972).

Also, in Australia in particular, a number of patients showed phenytoin toxicity in 1968 when the manufacturer concerned changed one of the excipients from calcium sulfate to lactose. The lactose was more easily wetted, allowing faster dissolution and higher concentrations in plasma (Figure 8.5).

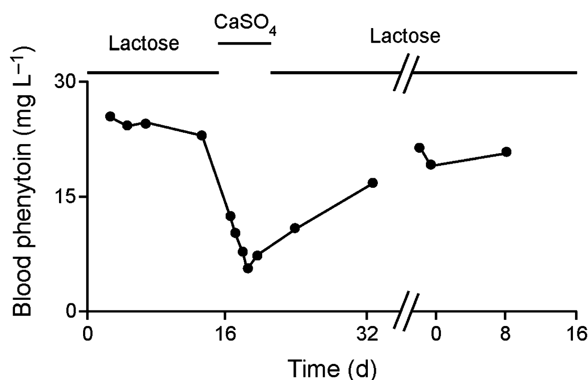


**Figure 8.5** Influence of lactose and calcium sulfate as excepients on the concentrations of phenytoin in blood in a patient taking 400 mg per day. (Redrawn after Tyrer *et al.*, 1970.)

Similarly major differences in serum glucose concentrations after tolbutamide were shown to be due to formulation differences (Figure 8.6)

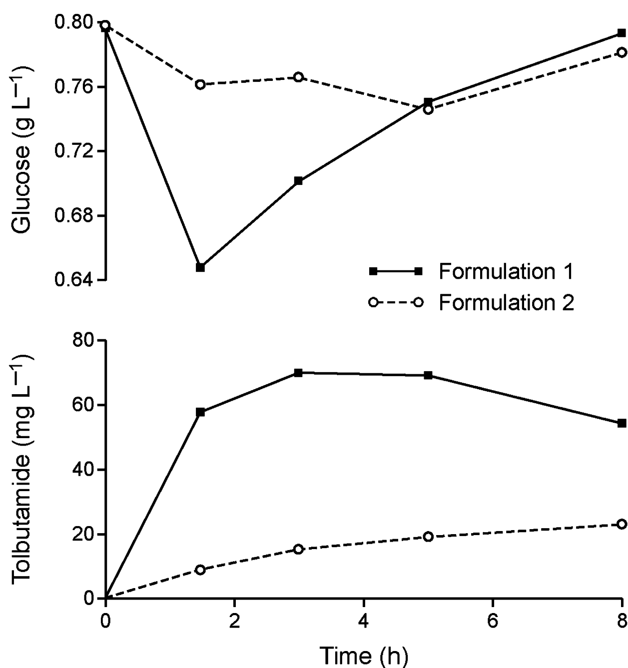


**Figure 8.6** Mean serum tolbutamide and glucose concentrations in 10 subjects given identical dose in two different formulations (after Varley, 1968).

## 8.5 Bioequivalence

Bioequivalence studies are initiated to investigate differences between products; usually so called 'generics' (an unfortunate term) are compared with established preparations. For example, a generic diazepam might be compared with a proprietary brand such as Valium. The aim is not to show that the test compound is better than the established one but to show equivalence to it. Thus, if the innovator product (the proprietary brand) has low bioavailability then the new generic product must also have low bioavailability. In the United States the Food and Drug Administration (FDA) has defined bioequivalence as, 'the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.'

Typically a bioequivalence study will involve *in vivo* testing of the generic drug against the standard drug in a cross-over design using 24 to 36 healthy, normal volunteers. Sometimes the study will call for the experiments to be conducted after meals but usually the subjects are fasted before they are given the drugs. Sufficient blood samples must be collected so that the $C_{max}$, $t_{max}$ and $AUC$ can be measured. The FDA usually considers two products bioequivalent if the 90% confidence intervals (CI) of the relative mean $C_{max}$, $AUC_{(0\text{--}t_z)}$ and $AUC_{(0-\infty)}$ of the generic formulation to reference is within 80% to 125% in the fasting state. Similar requirements were designed to apply in Australia, but the 90% CI values are based on log-transformed data. This is because $\ln(AUC)$ data are usually normally distributed. Protocols for bioequivalence evaluation will be designed with strict statistical control so that they adequately test for, say, $\pm 20\%$ differences between pairs of products. Much of the modern bioavailability literature is devoted to the design of such protocols, including detailed statistical control and analysis, as well as to debate on such issues as crossover and sequential study designs. The exact standard in any particular case will depend on what is practicable and desirable, as well as such considerations as the likely clinical result of, say, a 10% difference between products, and whether therapeutic objectives would be best met by emphasis on one, two or all three of the pharmacokinetic assessments commonly made, or on other criteria. Thus, there are commonly tighter requirements for drugs with a narrow therapeutic window (e.g. thyroxine and digoxin) and/or those with saturable metabolism (phenytoin, for instance).

In addition to requirements for new products, bioequivalence studies are required when a manufacturer changes the formulation of an existing product. Bioequivalence concepts are rarely applied to competing controlled-release preparations, or to immediate-release and controlled-release products with the same active constituent. Controlled-release products face very little generic competition.

## 8.6 Controlled-release preparations

Controlled-release preparations are either those that provide a sustained-release of drug or a delayed-release. The latter are usually enteric-coated oral preparations, not *designed* for delayed-release, but to avoid breakdown in the acid environment of the stomach, which of course will delay release to some extent depending on gastric emptying.

The principle of sustained-release is to ensure the rate constant of release and, hence, absorption ($k_a$) is less than the elimination rate constant ($k$) (Figure 8.7). As with any sequential reaction, the rate constant of the slowest step is rate limiting (Section 1.5.1.1). Therefore the half-life of elimination of the drug from plasma is determined by $k_a$ not $k$, i.e. $t_{1/2} = 0.693/k_a$ (i.e. flip-flop, Section 4.2.4.2). This results in a longer duration of action which in turn means less frequent dosing, more convenient dosing and better patient compliance.

Sustained-release preparations are available for most routes of administration, including sublingual, oral, subcutaneous, intramuscular, transdermal and rectal. Oral preparations make use of wax matrices or tablets with different layers disintegrating at different rates, and capsules containing hundreds of pellets of different types (and often of different colours), each type disintegrating at a different rate. Transdermal preparations

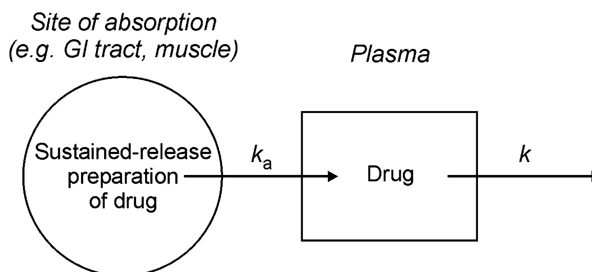


**Figure 8.7** Principle of sustained-release: $k_a << k$ so the overall rate is determined by $k_a$.

include glyceryl trinitrate, hyoscine and nicotine patches where the patch includes a rate-limiting membrane to ensure a steady release of drug. Several preparations of insulin are available for subcutaneous injection, each giving different rates of release. Sustained-release preparations for intramuscular injection include microcrystalline salts of penicillin G and esters of the antischizophrenic drug, fluphenazine. In these preparations, the drug is slowly released from the injection site and so these injections are sometimes referred to as depot injections. The way in which the rate of release controls the time course of the drug in plasma is illustrated in Figure 8.8. Fluphenazine was quickly absorbed after intramuscular injection and the plasma half-life was approximately 12 h. However, when fluphenazine enanthate, dissolved in sesame oil, was injected fluphenazine was only slowly released, resulting in low but sustained plasma concentrations with a half-life of ~3.5 days. Note that although the *rate constant* of elimination is some seven times greater than the *rate constant* of absorption, initially all the dose is in the muscle and none in the plasma, so the *rate* of absorption is greater than the rate of elimination. It is only later, when the plasma concentration is greater, that the rate of elimination becomes greater than the rate of absorption.

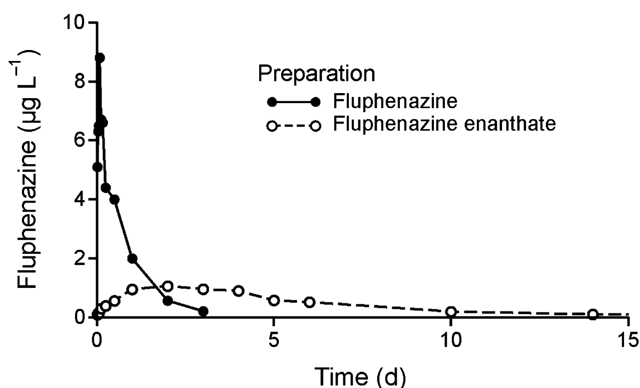


**Figure 8.8** Fluphenazine concentrations in plasma after i.m. injections of fluphenazine, as the hydrochloride salt, and as the enanthate ester (after Curry *et al.*, 1979).

Fluphenazine enanthate and fluphenazine decanoate, which is even longer acting ($t_{1/2}$ of release ~12 days), are prodrugs, as the esters are hydrolysed to release the active drug. Long-acting i.m. preparations of penicillin G are microcrystalline salts; procaine penicillin G acts for approximately 3 days, whereas benzathine penicillin G acts for up to 7 days.

Enteric-coated preparations are used to reduce gastric disturbances, such as bleeding after oral administration of non-steroidal anti-inflammatory dugs such as aspirin and diclofenac, or ferrous sulfate. The delay in onset is not a problem when drugs are being used chronically. Coated tablets are also useful for drugs that are unstable in gastric acid, for example pancreatic enzymes given to sufferers of cystic fibrosis.

## 8.7 Conclusions

There is no doubt that bioavailability is a real and important factor in drug response, but it can now be considered to be under control. Thanks to legislation that has required higher standards of quality control, improved analytical methods, easier availability of human volunteer research facilities, a greater sense of responsibility within the industry, better scientific data on excipient factors in product performance, and more diligent use of dissolution testing, there should never again be therapeutic failures, product variations or toxicity induction on the scale seen in the 1960s and 1970s. It should be noted that bioavailability problems were most obvious with phenytoin, digoxin, thyroxine and tolbutamide, drugs with low therapeutic indices, and for which analytical methods were available at the time for the purpose of therapeutic monitoring. Modern research is designed to bring to the market drugs with better margins of safety.

There have, however, been two long-term social consequences of the enlightenment initiated by the bioavailability scares of the 1960s and 1970s, and of the improvements in the relevant science that ensued:

- A long-term distrust of generic drugs.
- A long-term practice of physicians, patients and pharmacists on insisting that thyroid hormone products, anticonvulsants (especially phenytoin), and, to some extent, digoxin, continue to be dispensed as the brand-named drug.

As noted earlier, both phenacetin and tolbutamide, used as examples here, and also chloramphenicol (which was the subject of extensive bioavailability research at one time) are now virtually obsolete, although not, primarily for bioavailability reasons. The other key examples, thyroxine, phenytoin and digoxin remain just too important in medicine for bioavailability considerations to adversely affect their positions in the pharmaceutical armamentarium.

The consequences continue to fuel a vigorous debate concerning the effectiveness and safety of *all* generic prescribing with different interest groups promoting their particular philosophies to the confusion of a distrustful consumer population. It can now be presumed that, unless proved otherwise for a specific case, that products that have been adequately tested for their bioequivalence, and are thus certified to be bioequivalent, are clinically equivalent.

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