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DRUG METABOLITE KINETICS

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1. INTRODUCTION

For many years the process of drug metabolism was considered synonymous with the inactivation of pharmacologically active and toxic compounds. However during the last decade it has become increasingly apparent that this traditional view of drug metabolism must be re-assessed. In 1976, a compilation of 58 drugs possessing metabolites with therapeutic and/or toxic activities was published (Drayer, 1976). Thus it may be essential to characterize quantitatively as well as qualitatively the different metabolic pathways available to a particular drug. A specific pathway may account for the main pharmacological action of a drug or other chemical, for its side effects and, in particular, for serious toxicities such as drug-induced tissue necrosis or carcinogenicity (Jollow *et al.*, 1977).

Full characterization of a metabolic route cannot be achieved from analysis of excreted products alone. Appropriate pharmacokinetic parameters, based on circulating blood or plasma concentration of the metabolite(s) in question, are necessary. Pharmacokinetic information together with activity data (albeit from animal models) will allow some assessment of the contribution of a particular metabolite to the overall therapeutic or adverse effect observed. Detailed pharmacokinetic information on a particular metabolic pathway of a drug is a rarity. The reasons for this paucity are not difficult to understand. Availability of pure standards and chemical stability of metabolites are obvious problems. There are also analytical problems, although in certain cases these have been partially overcome by recent developments in high performance liquid chromatography.

Metabolic pathways of drugs are frequently complex. It is the rare exception when one metabolite accounts for the metabolic fate of a drug. Parallel pathways are a common feature of metabolic profiles and often each pathway consists of a catenary sequence of metabolites. In addition, crossover between parallel pathways may occur, resulting in more than one source of formation for a particular metabolite. However complex the pathway, the time course for any metabolite can be described by the general relationship—

Rate of change of metabolite in body = Rate of formation − Rate of elimination.

This relationship provides the basis of a general model for drug metabolite kinetics which is the starting point for this review. The model serves to introduce certain principles relevant to metabolite kinetics and is then expanded to include a number of situations. These include plasma metabolite concentration–time profiles after single and chronic drug administration, the influence of route of administration and first pass metabolite production. A summary of metabolite kinetic parameters and the methods available for their calculation is also presented. Finally, some applications of metabolite kinetics which have not specifically been covered in the preceding sections are discussed.

2. A GENERAL MODEL FOR METABOLITE KINETICS

To demonstrate certain principles pertinent to metabolite kinetics, the simplest metabolite model will be considered. In this model an intravenously administered drug

is converted entirely to a single metabolite which is eliminated in urine without further metabolism. Although this model is not realistic, it may be expanded easily to encompass the more complex situations encountered in modelling metabolite kinetic data. It will be seen that the expanded versions of this model differ quantitatively but not qualitatively from the simple model.

$$A \xrightarrow{k} A(m) \xrightarrow{k(m)} Ae(m)$$

Scheme 1. A general model for metabolite kinetics.

A is the amount of drug in the body; $A(m)$ and $Ae(m)$ are the amounts of metabolite in the body and excreted in the urine, respectively; k and $k(m)$ are the first-order rate constants for elimination of drug and metabolite, respectively.

The assumptions which apply to this and the expanded models, introduced later in this review, are as follows:

(a) Linear kinetics prevail, that is, rate constants and volume terms are independent of the amounts of drug and metabolite in the body.
(b) Distribution is much more rapid than elimination for both drug and metabolite. Hence, mono-exponential equations adequately describe the disposition of both species.
(c) All doses, amounts and concentrations are molar and no corrections for differences in molecular weight between drug and metabolite are necessary.
(d) Metabolite formation occurs only in the liver.
(e) All metabolite reactions are unidirectional. No metabolite can undergo a back conversion to the parent drug.

For the model shown in Scheme 1

$$\text{Rate of metabolite formation} = k \cdot A \tag{1}$$

$$\text{Rate of metabolite elimination} = k(m) \cdot A(m). \tag{2}$$

Therefore, according to mass balance considerations, the rate of change of metabolite in the body may be described by

$$\frac{dA(m)}{dt} = k \cdot A - k(m) \cdot A(m). \tag{3}$$

Substituting for the amount of drug in the body at any time

$$A = D \cdot e^{-k \cdot t} \tag{4}$$

where D is the dose of drug administered, Eqn (3) becomes

$$\frac{dA(m)}{dt} = k \cdot D \cdot e^{-k \cdot t} - k(m) \cdot A(m). \tag{5}$$

This may be integrated with respect to time (Gibaldi and Perrier, 1975):

$$A(m) = \frac{k \cdot D}{k(m) - k} [e^{-k \cdot t} - e^{-k(m) \cdot t}]. \tag{6}$$

The above relationships provide the following information on the metabolite time course in the body (Cummings *et al.*, 1967). At zero time there is no metabolite and during the accrual phase the amount of metabolite in the body will increase as the formation rate exceeds the elimination rate. At the time when the amount of the metabolite in the body is maximal, the formation rate equals the elimination rate. Once the elimination rate exceeds the formation rate, the amount of metabolite in the body will decline.

Figure 1 illustrates a number of metabolite-time profiles which have been simulated using the simple model and a range of k and $k(m)$ values. In each panel, Curve 1 is the disposition–time curve for parent drug. Curves 2–6 are metabolite time courses.

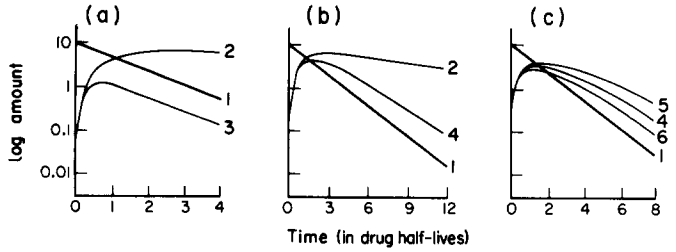


FIG. 1. Drug and metabolite–time profiles simulated according to the general model in Scheme 1. Curve 1 refers to parent drug [Eqn (4)] and Curves 2–6 to metabolite [Eqn (6)]. $D = 10$, $k = 1$, $k(m) = 0.2$ (Curve 2), 5 (Curve 3), 1 (Curve 4), 0.7 (Curve 5) and 1.3 (Curve 6).

Curve 2 represents the situation where $k(m)$ is 5 times slower than k. The amount of metabolite in the body rapidly exceeds the amount of parent drug and for a considerable period of time the metabolite is the only species measurable. In Panel a, no elimination phase is apparent in Curve 2 within the time span of the simulation. The amount of metabolite rises to an asymptotic value approximately equal (on a molar basis) to the amount of drug administered. In Panel b, using an extended time scale, the decline of metabolite in the body is now observable. However, it is only after a time period equivalent to 5 half-lives of the parent drug that the terminal elimination phase is reached. The slope of this decline is considerably different from the slope of the drug decline.

In Curve 3, $k(m)$ exceeds k by 5 fold. The accrual is much reduced and the maximum is attained earlier than in the previous case. The amount of metabolite is always lower than the amount of drug, and the declining slope for the metabolite is the same as that for the drug, after a time period equivalent to 2 half-lives of the drug.

The mathematical basis for these effects can be ascertained from Eqn (6). When $k > k(m)$, then at some time after drug administration $e^{-k \cdot t}$ will approach zero while $e^{-k(m) \cdot t}$ still has a finite value. Consequently

$$A(m) \rightarrow \frac{k \cdot D}{k(m) - k} e^{-k(m) \cdot t} \tag{7}$$

and in the logarithmic form

$$\log A(m) \rightarrow \log\left[\frac{k \cdot D}{k(m) - k}\right] - \frac{k(m) \cdot t}{2.3}. \tag{8}$$

Therefore, a plot of $\log A(m)$ versus time will eventually become linear with a slope of $k(m)/2.3$ (Curve 2).

Similarly when $k(m) > k$, Eqn (6) reduces to

$$A(m) \rightarrow \frac{k \cdot D}{k(m) - k} e^{-k \cdot t} \tag{9}$$

and

$$\log A(m) \rightarrow \log\left[\frac{k \cdot D}{k(m) - k}\right] - \frac{k \cdot t}{2.3}. \tag{10}$$

Under these circumstances (Curve 3) the terminal slope of a plot of $\log A(m)$ versus time is $k/2.3$.

Curve 3 may be described as showing a *Formation Rate Limitation* (FRL). It should be noted that the fastest terminal half-life attainable for a metabolite is that of the parent drug. In the FRL case the metabolite is eliminated almost as soon as it is formed. Hence the elimination rate is approximately equal to the formation rate:

$$k(m) \cdot A(m) \simeq k \cdot A. \tag{11}$$

Thus the ratio of metabolite to drug in the terminal phase will depend upon the ratio of the rate constants,

$$A(m) \simeq \frac{k}{k(m)} \cdot A. \tag{12}$$

Curve 2 is an example of an *Elimination Rate Limitation* (ERL). There is no upper limit for the terminal half-life of a metabolite.

It is evident from Fig. 1 that the maximum amount of metabolite in the body $[A_{max}(m)]$ and the time taken to attain this value $[t_{max}(m)]$ is a function of the two rate constants (Gibaldi and Perrier, 1975):

$$t_{max}(m) = \frac{2.3}{k(m) - k} \log\left[\frac{k(m)}{k}\right] \tag{13}$$

$$A_{max}(m) = \frac{k \cdot D}{k(m) - k} (e^{-k \cdot t_{max}(m)} - e^{-k(m) \cdot t_{max}(m)}) \tag{14}$$

$$= D \cdot e^{-k \cdot t_{max}}. \tag{15}$$

Therefore, as the ratio of $k(m)$ to k increases, $t_{max}(m)$ increases and $A_{max}(m)$ decreases.

Frequently $k(m)$ and k may be of the same order of magnitude. The closer these rate constants become the more difficult it is to delineate a terminal linear slope from the logarithmic plot. When the two rate constants are equal, Eqn (6) does not apply. The appropriate equation describing the time course for the metabolite in this case is

$$A(m) = k \cdot D \cdot t \cdot e^{-\lambda \cdot t} \tag{16}$$

where $\lambda = k = k(m)$. In the logarithmic form Eqn (16) becomes

$$\log A(m) = \log(k \cdot D) + \log t - \frac{k \cdot t}{2.3}. \tag{17}$$

Curve 4 in Fig. 1b illustrates an example of a metabolite–time profile which conforms to the above situation. It can be seen that no linear terminal phase is attained despite the long time span of the simulation (12 half-lives). The curvilinear nature of this relationship is due to the $\log t$ term. The rate constant λ may only be obtained graphically from parent drug data.

When $k(m)$ equals k the equations for $t_{max}(m)$ and $A_{max}(m)$ become (Gibaldi and Perrier, 1975):

$$t_{max}(m) = 1/\lambda \tag{18}$$

$$A_{max}(m) = D \cdot e^{-1}. \tag{19}$$

The non-linear nature of a metabolite–time profile may not be readily apparent. In Panel c of Fig. 1, Curves 5 and 6 have ratios for $k(m)/k$ of 0.7 and 1.3, respectively. In the experimental situation the scatter in the metabolite data may delude the investigator

TABLE 1. *Comparison of Actual Metabolite Elimination Rate Constants and Apparent Rate Constants Obtained from the Terminal Phase of the Simulated Metabolite–Time Plots shown in Fig. 1*

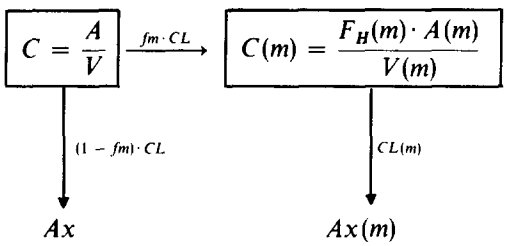
Curve	Actual $k(m)$	Apparent $k(m)$[a]	Percentage underestimation of actual $k(m)$
2	0.2	0.19	5
5	0.7	0.60	14
4	1.0	0.79	21
6	1.3	0.91	30

[a]Obtained by a least squares fit of metabolite–time data determined over the time period between 5–8 drug half-lives.

into fitting a linear terminal phase to these curves. Table 1 lists the rate constants obtained by a least-squares fit of the metabolite data determined over the time period between 5–8 drug half-lives. For comparison, the same exercise was carried out on the other metabolite curves. It can be seen that the least-squares fit underestimates $k(m)$ significantly when the $k(m)/k$ is in the unity region.

3. METABOLITE PLASMA CONCENTRATION–TIME PROFILES FOLLOWING SINGLE DOSE OF DRUG

Before the general metabolite kinetic model may be applied to metabolite plasma concentration–time data, a number of additional factors must be considered. These factors are shown in Scheme 2 for the disposition of a primary metabolite.



Scheme 2. Primary metabolite model.

Previously undefined terms included in Scheme 2 are: C, concentration of drug in plasma at any time, which is equal to amount of drug in the body divided by its volume of distribution (V); CL, the total body clearance of the drug; fm, the fraction of drug converted to $A(m)$; $(1 - fm)$, the remaining fraction of drug eliminated via an unspecified species (Ax); $C(m)$, the concentration of metabolite in plasma at any time, which is dependent on both the amount of metabolite in the body and the volume of distribution for the metabolite [$V(m)$], as well as the systemic availability of the metabolite [$F_H(m)$]; $Ax(m)$, the unspecified species via which $A(m)$ is eliminated; $CL(m)$, the clearance of the metabolite.

Two of the most important amendments to the general model which are incorporated into Scheme 2 are fm and $F_H(m)$. First, since only a prescribed fraction of dose is converted to the metabolite, fm, metabolite formation no longer equals drug elimination. Second, this scheme deals with apparent metabolite formation (i.e. metabolite appearance as viewed from the plasma). A certain fraction of the primary metabolite formed may undergo sequential metabolism to a secondary metabolite without leaving the liver. Hence, there may be a deficiency between the amount of metabolite formed and the amount of metabolite detected based on plasma concentrations. The systemic availability of the metabolite, $F_H(m)$, may be conceived as the ratio of the amount of metabolite leaving the liver to the amount of metabolite formed.

For Scheme 2, the rate equations for metabolite appearance and elimination become

$$\text{Rate of metabolite appearance} = fm \cdot F_H(m) \cdot CL \cdot C \tag{20}$$

$$\text{Rate of metabolite elimination} = CL(m) \cdot C(m). \tag{21}$$

Thus at any time,

$$\text{Rate of change of metabolite in body} = V(m) \cdot \frac{dC(m)}{dt}$$

$$= fm \cdot F_H(m) \cdot CL \cdot C - CL(m) \cdot C(m). \tag{22}$$

Equations (20) and (22) may be expanded to different forms depending on what function describes the time course for C. In the following subsections the cases of

intravenous and extravascular administration of a single dose of parent drug will be examined.

3.1. INTRAVENOUS DRUG ADMINISTRATION

When a drug is administered by a rapid intravenous bolus its elimination from the plasma may be described by

$$C = \frac{D}{V} \cdot e^{-k \cdot t}. \tag{23}$$

Substitution into Eqns (20) and (22) yields the appropriate rate equations for a primary metabolite formed from an intravenously administered drug:

$$\text{Rate of metabolite appearance} = fm \cdot F_H(m) \cdot k \cdot D \cdot e^{-k \cdot t} \tag{24}$$

$$V(m) \cdot \frac{dC(m)}{dt} = fm \cdot F_H(m) \cdot k \cdot D \cdot e^{-k \cdot t} - CL(m) \cdot C(m). \tag{25}$$

Equation (25), when integrated with respect to time, may be rearranged to give

$$C(m) = \frac{fm \cdot F_H(m) \cdot k \cdot D}{V(m) \cdot [k(m) - k]} [e^{-k \cdot t} - e^{-k(m) \cdot t}]. \tag{26}$$

Comparison of Eqns (26) and (6) will show that the exponents are the same and only the coefficient includes the new terms of Scheme 2. Thus the same rate limiting cases exist with $C(m)$ data as with $A(m)$ data. ERL will occur when $k(m) < k$. FRL will occur when $k < k(m)$. In the latter situation the terminal slope is still controlled by the elimination rate constant for the parent drug. The presence of parallel pathways does not alter the upper limit for the half-life of declining $C(m)$. As will be discussed later, the actual metabolite formation rate constant cannot be obtained from the slope of metabolite plasma concentration–time profiles.

In the primary metabolite model, the maximum $C(m)$ attained is now defined as

$$C_{max}(m) = \frac{fm \cdot F_H(m) \cdot D}{V(m)} e^{-k \cdot t_{max}(m)}. \tag{27}$$

Therefore, whether $C(m)$ will exceed C is no longer as clear cut as with the simple model. The terms fm and $F_H(m)$ are invariably less than one and will tend to lower $C(m)$ relative to C. Thus in the FRL $C(m)$ may be very low and not detectable by many assay techniques.

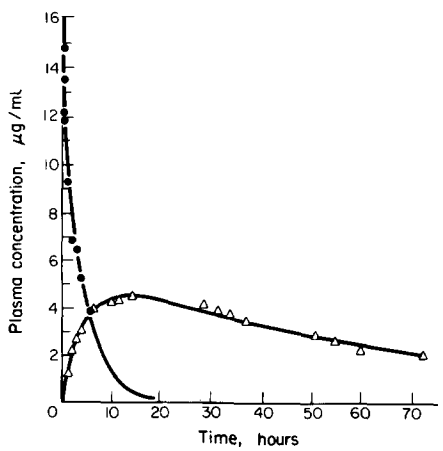


FIG. 2. Plasma concentration–time profiles for primidone (●) and its metabolite phenobarbitone (△) following intravenous administration of primidone to a rabbit. From Hunt and Miller (1978).

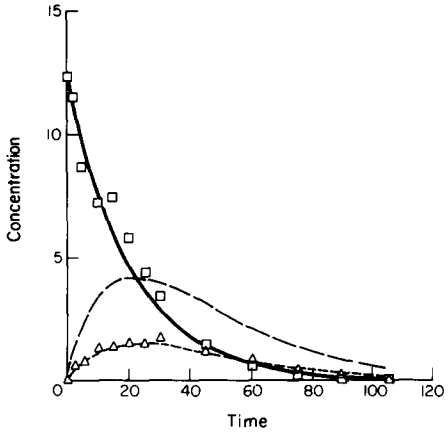


FIG. 3. Blood concentration–time profiles for phenacetin (□) and its metabolite paracetamol (△) in an isolated perfused rat liver preparation following administration of phenacetin. The metabolite concentration–time curves shown were simulated using Eqn (26) where $F_H(m) = 0.36$ (·····) and 1 (– –). From Pang and Gillette (1979).

For ERL, the combination of a slow terminal half-life and a low *fm* may result in $C(m)$ only exceeding C some considerable time after drug administration. An example of this situation can be seen with primidone and its metabolite phenobarbitone (see Fig. 2). In rabbit, primidone has a half-life of approximately 2 hr, therefore more than 90 per cent of the drug has been eliminated in the 8 hr period that it takes for metabolite concentrations to exceed drug concentrations in the plasma. This particular example of metabolite kinetics may be explained by the *fm* (0.28) and the long half-life (30 hr) for phenobarbitone elimination.

The importance of metabolite availability as an additional factor which lowers plasma metabolite concentrations can be seen in Fig. 3. The data presented refers to phenacetin and its metabolite paracetamol. The *fm* for this pathway in rat is 0.81. The simulated metabolite concentration–time curves were produced using Eqn (26) with the appropriate parameters (Pang and Gillette, 1979). The dotted line represents the case where $F_H(m) = 0.36$ and the dashed line where $F_H(m) = 1$. Clearly the dotted line is the more appropriate for the metabolite data indicating significant sequential metabolism of paracetamol prior to leaving the liver.

On the other hand, metabolite plasma concentrations may approach or even exceed drug concentrations due to differences in volume of distribution. The volume of distribution for a metabolite may be considerably smaller than that of their parent drug as a consequence of its polar nature. Consequently high plasma concentrations may be achieved rapidly for metabolites showing both FRL and ERL. Figure 4 illustrates the

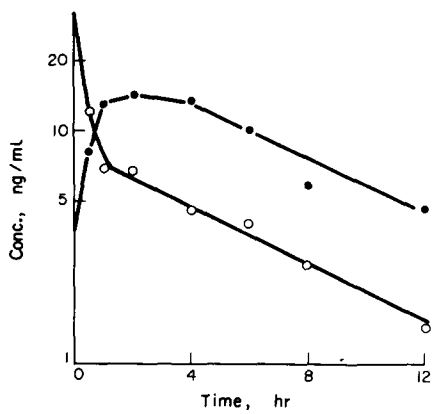


FIG. 4. Plasma concentration–time profiles for propranolol (○) and its metabolite naphthoxylactic acid (●) following intravenous administration of propranolol in man. From Walle *et al.* (1979a).

situation for propranolol and one of its metabolites, napthoxylactic acid. Metabolite and drug show terminal half-lives of approximately 4 hr and metabolite concentrations exceed drug concentrations by 2–3 fold.

The area under the metabolite concentration–time curve may be calculated by integrating Eqn (22) between time zero and infinity. Since the integral of $dC(m)/dt$ is zero, the resulting equation may be written as

$$fm \cdot F_H(m) \cdot CL \cdot \mathrm{AUC} = CL(m) \cdot \mathrm{AUC}(m), \tag{28}$$

where AUC and AUC(m) are the areas under the plasma concentration–time curve between zero and infinity for drug and metabolite respectively.

When the drug is administered by intravenous bolus,

$$CL \cdot \mathrm{AUC} = D. \tag{29}$$

Therefore, substituting into Eqn (28) and rearranging gives an expression for AUC(m),

$$\mathrm{AUC}(m) = \frac{fm \cdot F_H(m) \cdot D}{CL(m)}. \tag{30}$$

Equation (28) may also be rearranged to give a ratio of areas,

$$\frac{\mathrm{AUC}(m)}{\mathrm{AUC}} = \frac{fm \cdot F_H(m) \cdot CL}{CL(m)}. \tag{31}$$

This equation may be useful to provide relative information on $CL(m)$ when fm and $F_H(m)$ are not known. If the left hand side of Eqn (31) is greater than 1 then $CL(m)$ must be slower than CL as neither of the fractions can exceed 1 (Rowland and Tozer, 1980).

3.2. Extravascular Drug Administration

Drug administration by an extravascular route may produce quite a different primary metabolite concentration–time profile to that observed following intravenous administration of drug. The rate and extent of absorption of parent drug, together with its disposition characteristics, determine the drug concentration–time course, which in turn influence the metabolite concentration–time course. This catenary sequence of events is illustrated in Scheme 3.

$$Aa \xrightarrow{ka} C \xrightarrow{CL} C(m) \xrightarrow{CL(m)} Ax(m)$$

Scheme 3. Catenary metabolite model for extravascular drug administration.

Aa is the amount of drug at the absorption site and ka is the rate constant for absorption. In Scheme 3 no allowance is made for first pass metabolite formation. This complication will be dealt with later.

The plasma concentration–time profile for a drug administered by an extravascular route which involves no first pass metabolism may be described as

$$C = \frac{ka \cdot fa \cdot D}{V \cdot (ka - k)} (e^{-k \cdot t} - e^{-ka \cdot t}) \tag{32}$$

where fa is the fraction of dose which is absorbed.

Substitution of Eqn (32) into Eqns (20) and (22) provides the appropriate rate equations of metabolite appearance, and metabolite body concentration, respectively. For example—

$$V(m) \cdot \frac{dC(m)}{dt} = \frac{fm \cdot F_H(m) \cdot k \cdot ka \cdot fa \cdot D}{(ka - k)} (e^{-k \cdot t} - e^{-ka \cdot t}) - CL(m) \cdot C(m). \tag{33}$$

The integrated equation which describes the metabolite plasma concentration–time

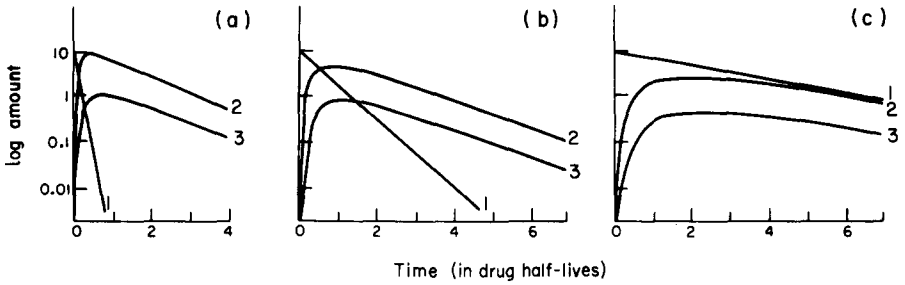


FIG. 5. Drug and metabolite–time profiles simulated according to the catenary metabolite model for extravascular drug administration in Scheme 3. The amount of drug at the absorption site (Curve 1), the amount of drug in the body (Curve 2) and amount of metabolite in the body (Curve 3) is shown. $D = 10$, $k = 0.1$, $k(m) = 0.5$, $ka = 2$ (Panel a), 0.25 (Panel b) and 0.05 (Panel c).

course is a complex function. However, from above considerations it is apparent that it must be tri-exponential to incorporate the exponents which describe drug absorption, drug disposition and metabolite disposition. It has the following general form:

$$C(m) = A \cdot e^{-k \cdot t} + B \cdot e^{-k(m) \cdot t} - C \cdot e^{-ka \cdot t} \tag{34}$$

where the coefficients A, B and C will be functions of the various rate constants and fractions. In practice all three exponents may not be resolved from the plasma concentration–time data.

Figure 5 shows a series of computer simulations for amount of drug at the absorption site (Curve 1), amount of drug in the body (Curve 2) and amount of metabolite in the body (Curve 3). In each case $k(m)$ exceeds k by a factor of 5 while ka is varied.

In Panel a, ka is the fastest of the three rate constants. The amount of drug at the absorption site decreases rapidly producing an early peak in the amount of drug in the body. The metabolite kinetics display FRL. In fact Curve 3 in Fig. 5a is almost identical to Curve 3 in Fig. 1a, which was simulated using the same k and $k(m)$ parameters with intravenous administration of drug.

In Panel b of Fig. 5, ka is decreased to a value midway between k and $k(m)$. Curve 3 still shows FRL, but a broad peak is apparent and the terminal slopes of metabolite and drug only become parallel after a time period equivalent to three drug half-lives.

In Panel c, ka has been decreased to the slowest rate constant. The kinetics of both drug and metabolite now show an absorption rate limitation. Curves 1, 2 and 3 have the same terminal slope. An example of this type of metabolite kinetics is shown in Fig. 6 for the acetyl conjugate of 5-aminosalicylic acid. After intravenous administration

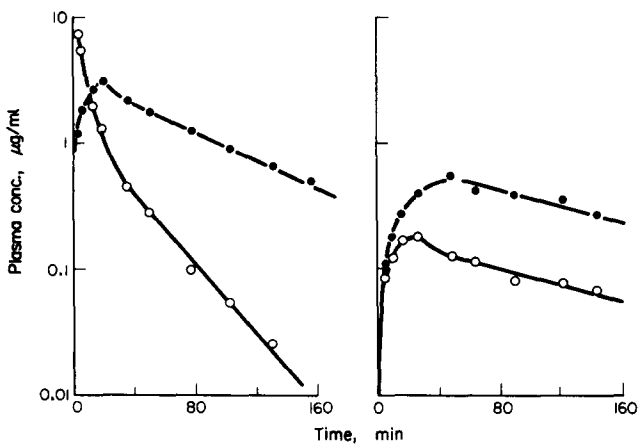
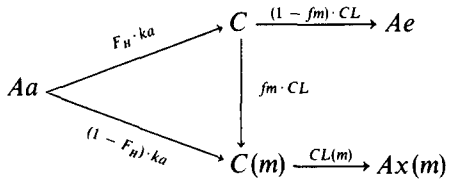


FIG. 6. Plasma concentration–time profiles for 5-aminosalicylic acid (○) and its metabolite acetylaminosalicylic acid (●) following intravenous (Panel a) and intraduodenal (Panel b) administration of 5-aminosalicylic acid in rat. Cassidy, M. K. and Houston, J. B. (unpublished data).

the metabolite kinetics show ERL. Terminal half-lives for metabolite and parent drug are 55 and 26 min, respectively. When administered intraduodenally, aminosalicylic acid terminal half-life increases to 95 min and its acetyl conjugate kinetics become absorption rate limited.

After oral administration, drugs which possess high hepatic extraction ratios will undergo a certain degree of metabolism prior to reaching the systemic circulation. Therefore, when viewed from the plasma it appears as if a mixture of metabolite and drug is absorbed into the body. Scheme 4 illustrates this situation.



Scheme 4. Model for first pass metabolite kinetics following oral administration of drug.

In the above Scheme, F_H and $(1 - F_H)$ are the fractions of absorbed drug dose which escape or are removed by the liver during the absorption phase, respectively.

When first pass metabolism occurs, it is necessary to modify the equations presented earlier in this sub-section. The rate of first pass metabolism may be described by

$$\text{Rate of first pass metabolite appearance} = fm \cdot F_H(m) \cdot CL \cdot \Delta C \tag{35}$$

where ΔC is the difference between the drug concentration in the hepatic portal vein and in the arterial blood supply which occurs during the absorption process. This concentration difference may be conceived as being equal to the absorption rate divided by the hepatic portal venous flow, Q, where

$$\text{Absorption rate} = ka \cdot Aa \tag{36}$$

$$= ka \cdot fa \cdot D \cdot e^{-ka \cdot t}. \tag{37}$$

Therefore,

$$\Delta C = \frac{ka \cdot fa \cdot D \cdot e^{-ka \cdot t}}{Q}. \tag{38}$$

Substitution of Eqn (38) for ΔC and $Q \cdot (1 - F_H)$ for CL, enables Eqn (35) to be written as

$$\text{Rate of first pass metabolite appearance} = fm \cdot F_H(m) \cdot (1 - F_H) \cdot ka \cdot fa \cdot D \cdot e^{-ka \cdot t}. \tag{39}$$

For comparison, the rate of systemic metabolite appearance following oral drug administration is

$$\text{Rate of systemic metabolite appearance} = \frac{fm \cdot F_H(m) \cdot k \cdot ka \cdot fa \cdot F_H \cdot D}{(ka - k)} (e^{-k \cdot t} - e^{-ka \cdot t}). \tag{40}$$

Equations (39) and (40) may be used to obtain an expression which describes the time course of metabolite plasma concentration when first pass metabolism of drug occurs. As with the previously discussed extravascular situation, the metabolite concentration–time equation will be tri-exponential of the general form of Eqn (34). However, the contribution of first pass metabolism will alter the coefficients A, B and C and therefore change the shape of the curve considerably, as shown in Fig. 7, by means of computer simulation. In each simulation the three rate constants concerned with drug absorption, drug disposition and metabolite disposition are held constant. The extent of absorption from the gastrointestinal tract is complete, fm is set to unity, while F_H is varied.

In Panel a, F_H is 0.99 and as discussed previously only two exponential processes may be discerned. When F_H is 0.75 (Panel b) the metabolite (Curve 2) peaks at the same time

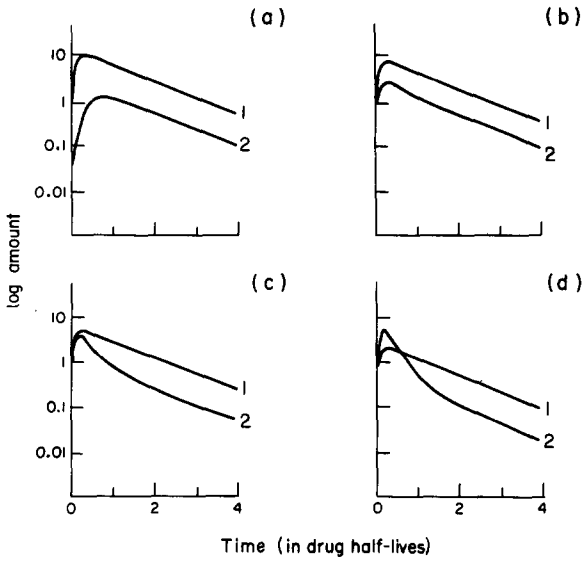


FIG. 7. Drug and metabolite–time profiles simulated according to the model for first pass metabolite kinetics following oral administration of drug in Scheme 4. Curves 1 refer to parent drug and Curves 2 to metabolite. $D = 10$, $ka = 2$, $k = 0.1$, $k(m) = 0.5$, $F_H(m) = 0.99$ (Panel a), 0.75 (Panel b), 0.5 (Panel c) and 0.25 (Panel d).

as the drug (Curve 1) and after a time period equivalent to one drug half-life the curves decline in parallel. A further decrease in F_H to 0.50 as shown in Panel c. In this case the tri-exponential nature is quite evident. When F_H is 0.25 (Panel d) the tri-exponential relationship becomes more pronounced, the metabolite peak is greater than the drug peak and it occurs earlier.

An example of a metabolite concentration–time profile where first pass metabolism contributes significantly to metabolite production is the 4-hydroxylation of propranolol (Fig. 8). The tri-exponential nature of the metabolite data is evident and the concentration of metabolite peaks before the drug (Walle *et al.*, 1980). Earlier reports on the kinetics of this propranolol metabolite stated erroneously that metabolite half-life was shorter than the drug half-life (Paterson *et al.*, 1970). It would appear that the assay sensitivity in the early study was inadequate to characterize fully the time course for 4-hydroxypropranolol.

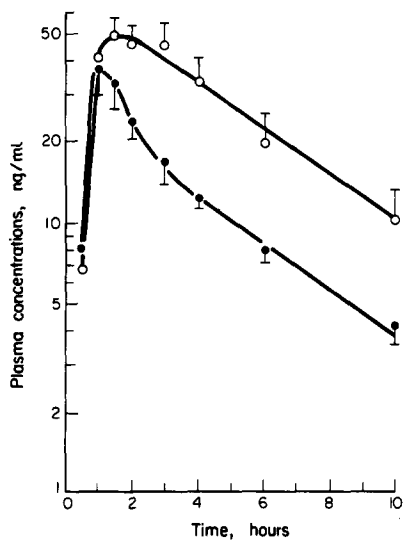


FIG. 8. Plasma concentration–time profiles for propranolol (○) and its metabolite 4-hydroxypropranolol (●) following oral administration of propranolol in man. From Walle *et al.* (1980).

It should be noted that the identity of any rate constant obtained from concentration–time data following oral administration of drug can only be made by comparison. It is necessary to have not only parent drug data for the oral route but also drug and metabolite data from drug intravenous administration.

Although route of administration of drug may alter the shape of the metabolite plasma concentration–time profile it will not alter the total area under the curve. The AUC(m) is independent of first pass metabolism. The rate of change of metabolite in body when formation may occur both systemically and via a first pass effect may be written by incorporating Eqn (39) with Eqn (22), that is

$$V(m)\cdot\frac{dC(m)}{dt} = fm\cdot F_H(m)[(1 - F_H)\cdot ka\cdot fa\cdot D\cdot e^{-ka\cdot t} + CL\cdot C] - CL(m)\cdot C(m). \quad (41)$$

Integrating between time zero and infinity and rearranging gives

$$fm\cdot F_H(m)\cdot[(1 - F_H)\cdot fa\cdot D + CL\cdot \mathrm{AUC}] = CL(m)\cdot \mathrm{AUC}(m). \quad (42)$$

Substituting

$$CL\cdot \mathrm{AUC} = fa\cdot F_H\cdot D \quad (43)$$

and rearranging, results in an expression for AUC(m) when the drug is given orally:

$$\mathrm{AUC}(m) = \frac{fm\cdot F_H(m)\cdot fa\cdot D}{CL(m)}. \quad (44)$$

Equation (44) will be equivalent to Eqn (30) providing the extent of absorption is complete. Combining Eqns (30) and (44) gives an expression for fraction of drug absorbed:

$$fa = \frac{\mathrm{AUC}_{po}(m)}{\mathrm{AUC}_{iv}(m)}. \quad (45)$$

Thus, by using the above area under the curve relationships it is possible to resolve a low bioavailability figure (F) into a first pass metabolism component and an incomplete absorption component, since

$$F = fa\cdot F_H. \quad (46)$$

Figure 9 presents data on lignocaine and its de-ethylated metabolite which illustrates this principle. Panel a shows that the bioavailability of orally administered lignocaine, based on AUC considerations is approximately 0.2 over the dose range studied. Panel b provides evidence that first pass metabolism can explain this low bioavailability, since the AUC(m)–dose relationship is independent of route of administration of parent drug.

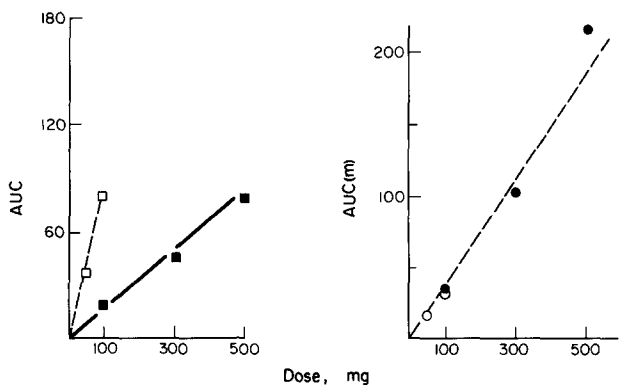
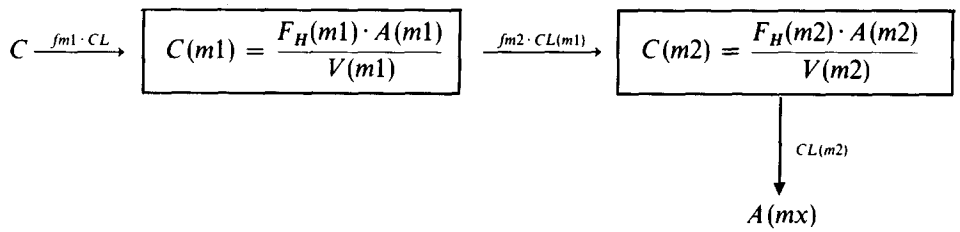


FIG. 9. Area under the curve–dose relationships for lignocaine and its de-ethylated metabolite after intravenous (□, ○) and oral (■, ●) administration of lignocaine to a human subject. Bennett, P. N., Aarons, L. J., Bending, M. R., Steiner, J. A., Rowland, M. R. (unpublished data).

3.3. Secondary and Other Intermediate Metabolites

In the previous sub-sections only primary metabolite kinetic models have been considered. However, these models and the relationships derived provide a general form on which secondary and other intermediate metabolite models may be based.

A catenary chain of metabolites is a commonly encountered situation. For example, Scheme 5:



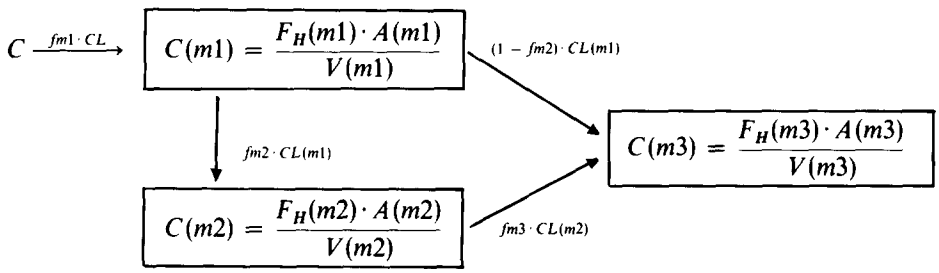
Scheme 5. Catenary metabolite kinetic model.

In this scheme $m1$ refers to the primary metabolite and $m2$ to the secondary metabolite in the chain.

By analogy with the catenary extravascular model (Scheme 3), the following expression for rate of appearance of $m2$ can be written

$$\text{Rate of } m2 \text{ appearance} = fm2 \cdot F_H(m2) \cdot CL(m1) \cdot C(m1).$$

Parallel sources of formation for a particular intermediate metabolite is another situation which may occur. For example, Scheme 6:



Scheme 6. Parallel metabolite formation model.

In the above Scheme $m1$ and $m2$ are precursors for $m3$.

By analogy with the first pass metabolite formation model (Scheme 4) the following rate equations for the appearance of $m3$ may be written:

$$\text{Rate of } m3 \text{ appearance from } m1 = (1 - fm2) \cdot F_H(m3) \cdot CL(m1) \cdot C(m1) \quad (48)$$

$$\text{Rate of } m3 \text{ appearance from } m2 = fm3 \cdot F_H(m3) \cdot CL(m2) \cdot C(m2). \quad (49)$$

Clearly the development of both models will yield complex polyexponential functions to describe the time course of these intermediate metabolites. However, the terminal phase of any log metabolite concentration–time profile will reflect the slowest step in the sequence. All metabolites which are formed beyond the rate limiting step will decline proportionally with the half-life of this slowest step (Rowland and Tozer, 1980).

3.4. Estimation of Metabolite Disposition Parameters

Many of the parameters important in metabolite disposition cannot be readily estimated from metabolite data obtained following drug administration. It is necessary also to have metabolite data obtained following administration of the metabolite *per se*.

TABLE 2. *Metabolite Kinetic Parameters which may be Obtained After Administration of Different Compounds*

Compound administered to obtain metabolite data	Metabolite kinetic parameters obtained
Parent drug	$k(m)$, AUC(m)
Parent drug, preformed metabolite	fm, km, $CL(m)$
Preformed metabolite (intravenously)	$CL(m)$, $V(m)$
Preformed metabolite (intravenously and orally)	$F_H(m)$

In the following section parameters calculated from data on the preformed metabolite will be identified by a prime notation, for example AUC$'(m)$.

Table 2 lists the sources of data necessary to calculate certain metabolite parameters. Only two parameters may be calculated directly from metabolite data obtained after parent drug administration. The remaining parameters require metabolite data from administration of the performed metabolite.

The following discussion is limited to primary metabolite parameters. Similar approaches may be applied for intermediate metabolite parameter calculation.

3.4.1. *Metabolite Elimination Rate Constant* [k(m)]

Pang and Gillette (1980) have recently reviewed a number of procedures which may be used to calculate metabolite elimination rate constants. The slope of a metabolite concentration–time curve will be dependent upon whether the metabolite kinetics show FRL or ERL. In the former case the metabolite terminal phase parallels that of the parent drug. In the latter case the metabolite terminal slope may be used to calculate $k(m)$. Similarly the choice of graphical procedures suggested by Pang and Gillette (1980) will depend upon whether FRL or ERL applies.

An expression for the ratio of metabolite to drug plasma concentrations can be written by dividing Eqn (26) by Eqn (23)—

$$\frac{C(m)}{C} = \frac{fm \cdot F_H(m) \cdot CL}{V(m) \cdot [k(m) - k]} \{1 - \exp[k - k(m)] \cdot t\}. \tag{50}$$

When $k(m) > k$(FRL), the exponent is negative and the curve of $C(m)/C$ versus time is convex (see Fig. 10c). When $k > k(m)$ (ERL), the exponent is positive and the curve concave (Fig. 10b). In the special case where $k = k(m) = \lambda$ the metabolite plasma concentration–time course is described by

$$C(m) = fm \cdot F_H(m) \cdot \lambda \cdot D \cdot t \cdot e^{-\lambda \cdot t}. \tag{51}$$

The ratio $C(m)/C$ obtained by dividing Eqn (51) by Eqn (23) is

$$\frac{C(m)}{C} = \frac{fm \cdot F_H(m) \cdot \lambda \cdot V \cdot t}{V(m)} \tag{52}$$

and the ratio–time plot is linear (see Fig. 10a).

The time period over which plasma samples are required will depend on how dissimilar k and $k(m)$ are. As stressed earlier, when metabolite kinetics show ERL it may be necessary to sample for a time period in excess of five drug half-lives. For FRL the plateau in $C(m)/C$ will be approached within approximately two drug half-lives. Panels b and c illustrate the effect of varying fm and $F_H(m)$. In all cases the curves are not altered in any qualitative fashion.

Equation (50) may be transformed to a linear function by taking the logarithm of the derivative, an approximation of which is

$$\log\left[\frac{\Delta C(m)/C}{\Delta t}\right] = \log\left[\frac{fm \cdot F_H(m) \cdot CL}{V(m)}\right] + \frac{[k - k(m)] \cdot t_{\text{mid}}}{2.303} \tag{53}$$

where t_{mid} is the mid-point of the time interval Δt and $\Delta[C(m)/C]$ is the difference in the

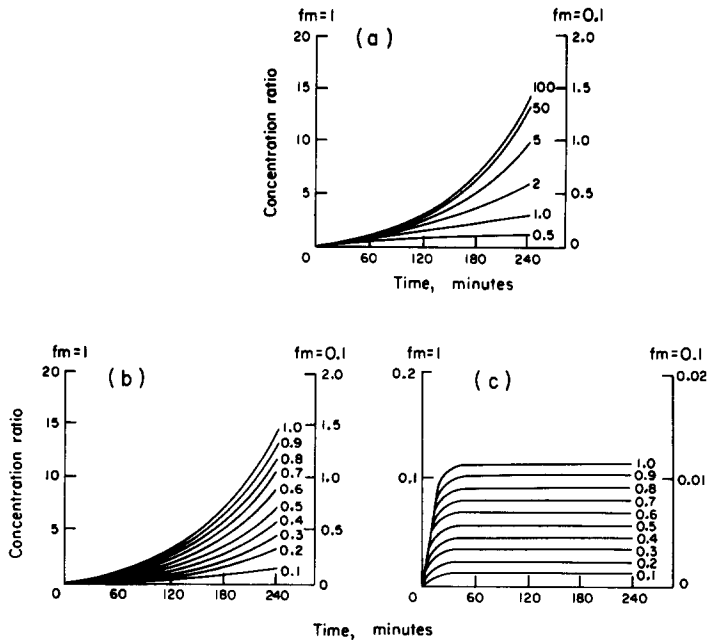


FIG. 10. Simulated metabolite: drug plasma concentration ratio–time profiles. In all cases $k = 0.01155\ \text{min}^{-1}$ and $V = V(m)$. In Panel a, $C(m)/C$ vs time was simulated with Eqns (50) and (52) for $F_H(m) = 1$ when $fm = 1$ (left ordinate), and when $fm = 0.1$ (right ordinate) for different values of $k(m)$, such as $k/k(m) = 0.5$, 1, 2, 5, 50 and 100. In Panels b and c Eqn (50) was used for an ERL case $[k/k(m) = 100]$ and a FRL case $[k/k(m) = 0.1)$. Other conditions as Panel a except that $F_H(m)$ is varied between 0.1 and 1 as shown. From Pang and Gillette (1980).

ratio over the time interval. Thus the slope will be dependent on the two rate constants—negative for FRL and positive for ERL. When k is known $k(m)$ is easily calculated. As reviewed by Pang and Gillette (1980), alternative procedures for calculating $k(m)$ are applicable for either FRL or ERL.

3.4.1.1. *Formation rate limitation*

When metabolite kinetics are FRL $k(m)$ may be obtained by curve stripping the metabolite concentration–time profile (Gibaldi and Perrier, 1975). Extrapolation of the linear terminal slope $(k/2.3)$ allows the difference between observed and extrapolated metabolite concentrations to be calculated for each time point. Plotting these differences on semilogarithmic paper allows $k(m)$ to be obtained from the slope—$k(m)/2.3$ (see Fig. 11a).

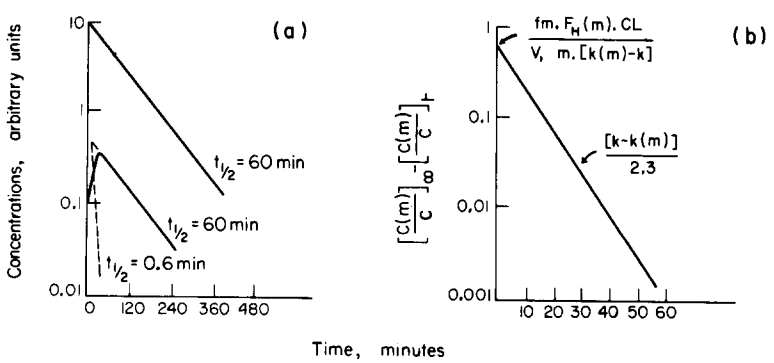


FIG. 11. Estimation of $k(m)$ in the formation rate limitation case. Panel a, curve stripping a metabolite concentration–time curve. Panel b the same data as Panel a plotted in the form of Eqn (55). From Pang and Gillette (1980).

An alternative procedure is based on Eqn (50). When FRL occurs the ratio $C(m)/C$ approaches a plateau as time approaches infinity,

$$\frac{C(m)}{C} \rightarrow \frac{fm \cdot F_H(m) \cdot CL}{V(m) \cdot [k(m) - k]}. \tag{54}$$

Thus Eqn (50) may be transformed to

$$\log\left\{\left[\frac{C(m)}{C}\right]_\infty - \left[\frac{C(m)}{C}\right]_t\right\} = \log\left\{\frac{fm \cdot F_H(m) \cdot CL}{V(m) \cdot [k(m) - k]}\right\} + \frac{[k - k(m)] \cdot t}{2.3} \tag{55}$$

where $[C(m)/C]_\infty$ and $[C(m)/C]_t$ are the plasma concentration ratios at the plateau and at any time, t, respectively. Thus $k(m)$ may be obtained after determining the slope of this linear plot (see Fig. 11b).

3.4.1.2. *Elimination rate limitation*

When metabolite kinetics display FRL, $k(m)$ may be obtained from the terminal slope of the plot of log $C(m)$ against time (see Fig. 12a).

Also $k(m)$ may be calculated from Eqn (50) expressed in its logarithmic form, that is

$$\log\left[\frac{C(m)}{C}\right] = \log\left[\left[\frac{fm \cdot F_H(m) \cdot CL}{V(m) \cdot [k(m) - k]} \times \{1 - \exp[k - k(m)] \cdot t\}\right]\right] \tag{56}$$

or

$$\log\left[\frac{C(m)}{C}\right] = \log\left\{\frac{fm \cdot F_H(m) \cdot CL}{V(m) \cdot [k - k(m)]}\right\} + \log\{\exp[k - k(m)] \cdot t - 1\}. \tag{57}$$

The slope of the semilogarithmic plot of $[C(m)/C]$ against time will increase to a constant value, $\{\exp[k - k(m)] \cdot t - 1\}$ will approach $\exp[k - k(m)] \cdot t$ with time, and therefore the terminal slope of the plot will approach $[k(m) - k]/2.3$. Figure 12b is a plot of the data presented in Fig. 12a in the form of Eqn (57).

3.4.2. *Area under the Metabolite Plasma Concentration–Time Curve following Drug Administration* [AUC(m)]

The area under the metabolite concentration–time curve between time zero and the last sampling point (T) may be calculated by the trapezoidal rule (Gibaldi and Perrier,

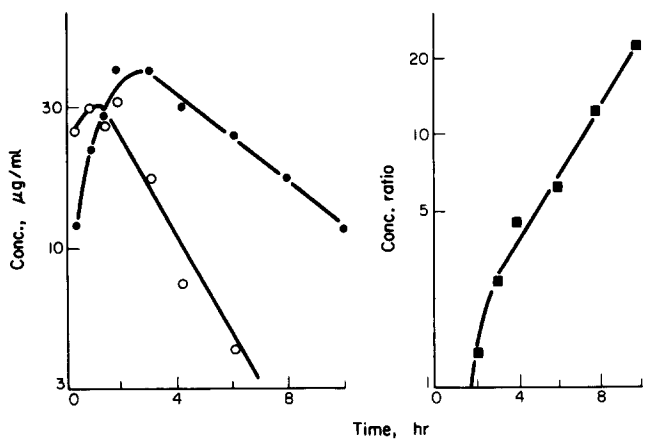


FIG. 12. Estimation of $k(m)$ in the elimination rate limitation case. Panel a, plasma concentration–time profiles for acetohexamide (○) and its metabolite hydroxyhexamide (●) in a human subject. k and $k(m)$ calculated from linear regression of the terminal phases of the drug and metabolite concentration–time curves are equal to 0.496 and 0.187 hr^{-1}, respectively. Data from Galloway *et al.* (1967). Panel b, the same data as Panel a, plotted in the form of Eqn (57). Slope of terminal phase equals 0.127 and therefore $k - k(m)$ equals 0.292 and $k(m)$ equals 0.204 hr^{-1}.

1975). Calculation of the area under the curve from the last sampling point to infinity may be more difficult. When a good estimate of the terminal slope is available then the decline in metabolite plasma concentration post T may be predicted from

$$C(m) = C_T(m) \cdot e^{-\lambda_z \cdot t} \tag{58}$$

where $C_T(m)$ is the last plasma metabolite concentration assayed and λ_z is the terminal rate constant.

Integration of Eqn (58) from T to infinity provides the extrapolated portion of the area $[AUC_{T\to\infty}(m)]$:

$$AUC_{T\to\infty}(m) = \frac{C_T(m)}{\lambda_z}. \tag{59}$$

In clear cases of FRL and ERL, Eqn (59) may be used with confidence. However, when k and $k(m)$ are of a similar order of magnitude the terminal linear slope of a logarithmic plot may not be readily apparent. In such a situation there is a strong possibility that λ_z will be underestimated and hence $AUC(m)$ overestimated.

When it is apparent that $k(m) \simeq k$ then the extrapolated area is best estimated by integrating Eqn (51) from T to infinity:

$$AUC_{T\to\infty}(m) = \frac{D(T+1)e^{-\lambda T}}{CL(m)}. \tag{60}$$

If the metabolite data are such that no information is available on the rate-limiting step then an alternative approach is available. Equation (22) may be integrated between T and infinity to give

$$V(m) \cdot C_T(m) = k \cdot V \cdot AUC_{T\to\infty} - k(m) \cdot V(m) \cdot AUC_{T\to\infty}(m). \tag{61}$$

This may be arranged to give

$$AUC_{T\to\infty}(m) = \frac{CL \cdot AUC_{T\to\infty}}{CL(m)} - \frac{C_T(m)}{k(m)} \tag{62}$$

which, by substituting $AUC_{T\to\infty} = C_T/k$, becomes

$$AUC(m)_{T\to\infty} = \frac{V \cdot C_T}{CL(m)} - \frac{C_T(m)}{k(m)}. \tag{63}$$

3.4.3. *Availability of Metabolite* $[F_H(m)]$

The systemic availability of a metabolite can only be determined by administration of the preformed metabolite. It is necessary to know the $AUC'(m)$ for both intravenous $AUC'_{iv}(m)$ and oral $AUC'_{po}(m)$ administration:

$$AUC'_{iv}(m) = \frac{M}{CL'(m)} \tag{64}$$

$$AUC'_{po}(m) = \frac{F_H(m) \cdot M}{CL'(m)} \tag{65}$$

where M is the dose of metabolite administered by both routes.

Dividing Eqn (65) by Eqn (64) produces

$$F_H(m) = \frac{AUC'_{po}(m)}{AUC'_{iv}(m)} \tag{66}$$

providing that the absorption of metabolite by the oral route is complete.

The availability of metabolite is dependent on its hepatic extraction ratio $E_H(m)$, which in turn is dependent on the metabolite hepatic clearance $CL_H(m)$ and hepatic

blood flow (Q_H). Hence

$$F_H(m) = 1 - E_H(m) \tag{67}$$

$$= 1 - \frac{CL_H(m)}{Q_H}. \tag{68}$$

Therefore, lowly cleared metabolites [low $E_H(m)$] will tend to exhibit good systemic availability and highly cleared metabolites [high $E_H(m)$] will be poorly available.

3.4.4. *Fraction of Drug Converted to Metabolite* (fm)

The fraction of drug converted to a particular metabolite may also be calculated from area under the curve considerations. It is necessary to know the metabolite area following both drug and preformed metabolite administration. The ratio of the two metabolite areas provide an estimate of *fm* (Kaplan *et al.*, 1973a). The metabolite area following drug administration is independent of route of administration (see Section 3.2) providing absorption is complete. However the route of administration of preformed metabolite used to obtain AUC′(*m*) is critical in determining *fm*.

When the intravenous route is used and Eqn (30) is divided by Eqn (64),

$$\frac{\text{AUC}(m)}{\text{AUC}'_{\text{iv}}(m)} = \frac{fm \cdot F_H(m) \cdot D \cdot CL'(m)}{M \cdot CL(m)}. \tag{69}$$

Therefore, if D and M are equivalent in molar terms and $CL(m)$ equals $CL'(m)$

$$\frac{\text{AUC}(m)}{\text{AUC}'_{\text{iv}}(m)} = fm \cdot F_H(m). \tag{70}$$

Oral administration of preformed metabolite requires Eqn (30) to be divided by Eqn (65):

$$\frac{\text{AUC}(m)}{\text{AUC}'_{\text{po}}(m)} = \frac{fm \cdot F_H(m) \cdot D \cdot CL'(m)}{F_H(m) \cdot M \cdot CL(m)} \tag{71}$$

$$= fm. \tag{72}$$

Clearly only Eqn (72), where the oral route is used to administer the preformed metabolite, provides the true estimate of *fm*. Equation (70) will underestimate *fm* by a factor equal to $F_H(m)$. However, for certain needs it may not be necessary to obtain estimates of *fm* and $F_H(m)$ *per se*. When only the product of the two fractions is required, Eqn (70) will suffice (Pang *et al.*, 1979).

Under certain conditions *fm* may be calculated from urinary recovery of metabolite. However, this method will only be useful if the complete metabolic profile has been characterized and the contribution of all the sequentially formed metabolites can be assessed. An accurate estimate of *fm* via urinary data necessitates the summing of all the metabolites produced via the primary metabolite of interest.

3.4.5. *Clearance of Metabolite* [CL(m)]

Metabolite clearance can be calculated from plasma data from intravenous preformed metabolite administration by rearranging Eqn (64), that is

$$CL'(m) = \frac{M}{\text{AUC}'_{\text{iv}}(m)}. \tag{73}$$

Metabolite data obtained from parent drug administration may be used by rearranging Eqn (30)—

$$CL(m) = \frac{fm \cdot F_H(m) \cdot D}{\text{AUC}(m)}. \tag{74}$$

Although this latter equation requires a knowledge of *fm* and $F_H(m)$ it is valid for any route of administration for parent drug. It is also independent of whether metabolite formation or metabolite elimination is rate limiting or if both processes occur at the same rate.

3.4.6. *Volume of Distribution of Metabolite* [V(m)]

The volume of distribution for a metabolite may only be obtained directly from intravenous administration of preformed metabolite,

$$V(m) = \frac{M}{C'_0(m)} \tag{75}$$

where $C'_0(m)$ is the concentration of metabolite at zero time.

Alternatively $V(m)$ may be calculated from the metabolite clearance and the metabolite elimination rate constant:

$$V(m) = \frac{CL(m)}{k(m)}. \tag{76}$$

3.4.7. *Metabolite Formation Clearance* (CL_m)

Metabolite formation clearance is a derived parameter which may be calculated if a knowledge of total body clearance for parent drug and the fractional conversion to the metabolite is available:

$$CL_m = fm \cdot CL. \tag{77}$$

The calculation of this parameter is based on the principle that clearance terms are additive. In view of method of calculation some investigators use the term partial clearance for this parameter.

3.4.8. *Metabolite Formation Rate Constant* (*km*)

Only when one metabolic pathway is operative and rate of drug elimination equals rate of metabolite formation can the formation rate constant for the metabolite be obtained directly from plasma data.

In the vast majority of cases *km* may only be obtained using an analogous equation to Eqn (77), that is

$$km = fm \cdot k. \tag{78}$$

4. METABOLITE PLASMA CONCENTRATION–TIME PROFILES DURING CHRONIC DRUG ADMINISTRATION

The implications of chronic drug administration on the kinetics of its metabolites will be considered in this section. Drug administration via an intravenous infusion will be examined initially. Then the added complications involved with multiple oral dosing will be considered. These complications include incomplete absorption, first pass metabolism and fluctuations in metabolite plasma concentrations during the dosing interval.

4.1. Steady-state Metabolite Plasma Concentrations

At steady-state, by definition, the metabolite formation rate equals the metabolite elimination rate. Therefore Eqn (22) may be written as

$$fm \cdot F_H(m) \cdot CL \cdot C_{ss} = CL(m) \cdot C_{ss}(m) \tag{79}$$

where C_{ss} and $C_{ss}(m)$ are the steady-state plasma concentrations for drug and metabolite respectively.

This equation may be rearranged to give

$$C_{ss}(m) = \left[\frac{fm \cdot F_H(m) \cdot CL}{CL(m)}\right] C_{ss}. \tag{80}$$

When the drug is given by intravenous infusion, steady-state plasma drug concentrations are governed by infusion rate and clearance, that is

$$C_{ss} = \frac{R_0}{CL} \tag{81}$$

where R_0 is the rate of infusion.

Substitution into Eqn (80) gives

$$C_{ss}(m) = \frac{fm \cdot F_H(m) \cdot R_0}{CL(m)}. \tag{82}$$

Drug administration via an extravascular route does not usually result in a constant rate of input of drug into the body. Within the dosing interval, the concentration of drug in the body will fluctuate according to rates of absorption and disposition of the drug. The average concentration during interval once steady-state has been achieved will depend upon the systemic availability in addition to clearance and dosing interval:

$$C_{ss,av} = \frac{F \cdot D}{CL \cdot \tau} \tag{83}$$

where $C_{ss,av}$ is the average plasma concentration at steady-state during the dosing interval (τ). F is the fraction of drug which is systemically available, that is, the product of fa and F_H [Eqn (46)].

The average plasma concentration of metabolite during the dosing interval at steady-state will represent the sum of systemically formed metabolite and first pass formed metabolite:

$$\text{Average concentration of systemically formed metabolite} = \frac{fm \cdot F_H(m) \cdot fa \cdot F_H \cdot D}{CL(m) \cdot \tau} \tag{84}$$

$$\text{Average concentration of first pass formed metabolite} = \frac{fm \cdot F_H(m) \cdot fa \cdot (1 - F_H) \cdot D}{CL(m) \cdot \tau}. \tag{85}$$

Summing Eqns (84) and (85) produces

$$C_{ss,av}(m) = \frac{fm \cdot F_H(m) \cdot fa \cdot D}{CL(m) \cdot \tau}. \tag{86}$$

Equations (82) and (86) are equivalent, differing only in mode of drug administration. The intravenous infusion rate (R_0) is merely replaced by an oral dosing rate ($fa \cdot D/\tau$). This rule may be applied to all subsequent equations to convert from infusion to multiple oral administration.

The fluctuations observed in metabolite plasma concentrations at steady-state when the drug is administered extravascularly will depend upon whether the kinetics of the metabolite show FRL or ERL. In the case of FRL the difference between maximum and minimum steady-state concentrations of the metabolite will be similar to that of the parent drug. The elimination rate constant for the drug controls the decline in both plasma concentrations within the dosing interval. Such a situation occurs with at least two propranolol metabolites, namely naphthoxylactic acid (Walle *et al.*, 1979a) and propranolol glucuronide (Walle *et al.*, 1979b).

In the case of ERL, the difference between maximum and minimum concentrations at steady-state will be less for the metabolite than for the parent drug, since by definition

$k(m) < k$. For example, the fluctuations within a dosing interval are greater for diazepam than for its desmethyl metabolite (Kaplan *et al.*, 1973b).

4.2. Predictions of Steady-state Metabolite Concentrations from Single Dose Data

A useful expression relating steady-state metabolite concentrations to AUC(m) from a single dose may be obtained substituting Eqn (30) into Eqn (82):

$$C_{ss}(m) = \frac{R_0 \cdot \mathrm{AUC}(m)}{D}. \tag{87}$$

A similar expression may be obtained for the average steady-state metabolite plasma concentration when the drug is given via the oral route:

$$C_{ss,\,av,\,po}(m) = \frac{\mathrm{AUC}(m)}{\tau}. \tag{88}$$

This single dose metabolite data may be used to predict metabolite plasma concentrations at steady-state. This approach may be extended to predict steady-state concentrations for one particular route of drug administration from single dose data obtained using a different route of drug administration (Lane and Levy, 1980). When the same dose of drug is administered by both intravenous and oral route, the areas under the plasma metabolite concentration–time curve are related in the following manner:

$$\mathrm{AUC}_{iv}(m) = \frac{\mathrm{AUC}_{po}(m)}{fa}. \tag{89}$$

Therefore the average steady-state metabolite plasma concentration after oral drug administration may be predicted from the AUC(m) obtained after an intravenous single drug dose:

$$C_{ss,\,av,\,po}(m) = \frac{\mathrm{AUC}_{iv}(m) \cdot fa}{\tau}. \tag{90}$$

In an analogous fashion, the ratio of steady-state concentrations of metabolite and drug may also be predicted from single dose data (Lane and Levy, 1980). Rearrangement of Eqn (79) provides an expression for the plasma concentration ratio for metabolite and drug at steady-state:

$$\frac{C_{ss}(m)}{C_{ss}} = \frac{fm \cdot F_H(m) \cdot CL}{CL(m)}. \tag{91}$$

The right hand side of this equation is identical to Eqn (31) from Section 3.1. Thus we can write

$$\frac{C_{ss}(m)}{C_{ss}} = \frac{\mathrm{AUC}(m)}{\mathrm{AUC}}. \tag{92}$$

Once again the prediction of steady-state behaviour from single dose considerations is not restricted to a common route of administration. The areas under the plasma concentration–time curve for intravenous and oral administration of the same dose of drug are related thus:

$$\mathrm{AUC}_{iv} = \frac{\mathrm{AUC}_{po}}{fa \cdot F_H}. \tag{93}$$

Therefore the average steady-state concentration ratio for oral drug administration may be predicted from areas obtained following an intravenous single dose of drug:

$$\frac{C_{ss,\,av,\,po}(m)}{C_{ss,\,av,\,po}} = \frac{\mathrm{AUC}_{iv}(m)}{\mathrm{AUC}_{iv} \cdot F_H}. \tag{94}$$

4.3. METABOLITE PLASMA CONCENTRATIONS DURING AND AFTER DRUG INFUSION

The plasma concentration–time profile for drug during an infusion may be characterized by

$$C = \frac{R_0}{CL}(1 - e^{-k \cdot t}). \tag{95}$$

Thus the time taken to reach steady-state is independent of infusion rate and clearance and is governed solely by the drug half-life.

During a drug infusion the rate of appearance of metabolite will be

$$\text{Rate of metabolite appearance} = \frac{fm \cdot F_H(m) \cdot R_0}{V(m)}(1 - e^{-k \cdot t}). \tag{96}$$

Therefore, the time course for metabolite concentration at any time during drug infusion may be described by

$$C(m) = \frac{fm \cdot F_H(m) \cdot R_0}{CL(m)}\left\{1 - \frac{k(m) \cdot e^{-k \cdot t}}{[k(m) - k]} - \frac{k \cdot e^{-k(m) \cdot t}}{[k - k(m)]}\right\}. \tag{97}$$

Equation (91) predicts that a time plot for metabolite concentrations during a drug infusion will be S-shaped. Figure 13 illustrates this point for both FRL (Panel a) and ERL (Panel b) by means of computer simulations. It is also apparent from Eqn (91) that the time taken for the metabolite to reach steady-state will depend on the smallest rate constant. Hence when FRL prevails the drug elimination half-life governs the time to steady-state. Consequently both metabolite and drug reach steady-state within the same time period (Fig. 13a). If the metabolite kinetics are ERL, the metabolite elimination half-life will control the time to steady-state. In this case the metabolite takes much longer to achieve steady-state than the drug (Fig. 13b).

When steady-state has been achieved and the infusion of drug is stopped, the wash out of drug from the body will be controlled by the drug elimination rate constant. Hence the decline in drug plasma concentrations post infusion can be described by

$$C = \frac{R_0}{CL} e^{-k \cdot t}. \tag{98}$$

During this wash out period the appearance rate of metabolite can be written as

$$\text{Rate of metabolite appearance} = fm \cdot F_H(m) \cdot R_0 \cdot e^{-k \cdot t}. \tag{99}$$

Therefore, using this equation, the time course for metabolite plasma concentrations

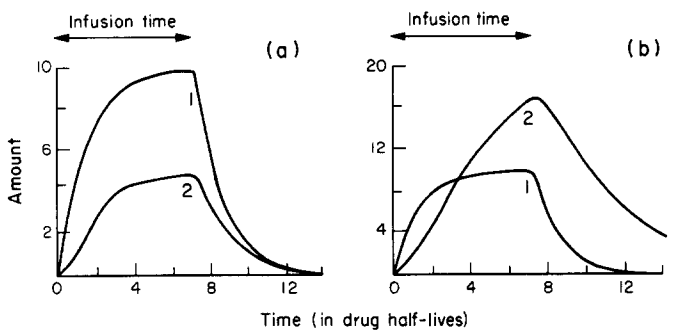


FIG. 13. Drug and metabolite–time profiles during and after drug infusion. Curve 1 refers to parent drug and Curve 2 to metabolite for the FRL case (Panel a) and the ERL case (Panel b). For this simulation the general model in Scheme 1 was used where drug is infused over a time period equivalent to 7 drug half-lives at a rate of 7 units per half-life, $k = 1$ and $k(m) = 2$ (Panel a) and 0.5 (Panel b).

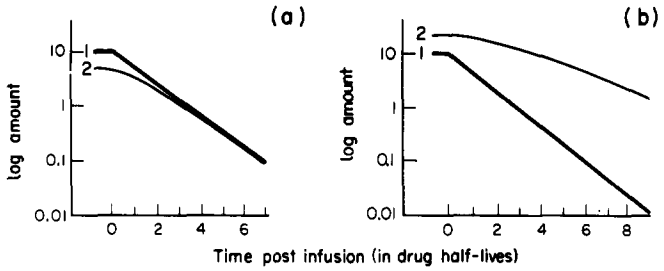


FIG. 14. Semi-logarithmic drug and metabolite–time profiles after stopping a drug infusion where steady-state drug and metabolite levels were achieved. Other conditions as Fig. 13.

at any time after ceasing drug infusion may be written in the following form

$$C(m) = \frac{fm \cdot F_H(m) \cdot R_0}{CL(m) \cdot [k(m) - k]} [k(m) \cdot e^{-k \cdot t} - k \cdot e^{-k(m) \cdot t}]. \quad (100)$$

Therefore, once the infusion has been stopped the decline in metabolite from steady-state will be biphasic. The convex nature of a semilogarithmic plot of this decline is shown in Fig. 14. For both FRL (Panel a) and ERL (Panel b) the terminal phase is reached within a time period equivalent to three drug half-lives. In the former case the terminal half-life is that of the parent drug and in the latter case it is the metabolite's own half-life.

If the drug infusion is stopped before the metabolite reaches steady-state then the subsequent time course of metabolite will differ from the above situation. As shown in Fig. 13b for the ERL case, the metabolite does not immediately decline at the end of the infusion. It continues to accrue until its formation route equals its elimination rate and then the decline is apparent.

4.4. Metabolite–Drug Interactions

Chronic regimens frequently lead to an accumulation of both drug and its metabolites in the body. High and relatively constant amounts of both species may result in an interaction between a metabolite and its parent drug which is not apparent at single small doses. Two types of interaction will be discussed briefly here—auto-induction and end product inhibition. Both of these interactions alter the conversion of drug to metabolite and may be envisaged as adaptive responses of the body to prevent further accumulation of either drug, metabolite or both species.

4.4.1. *Auto-induction*

Enzyme induction is a well documented phenomenon (Conney, 1967) by which certain compounds, which are substrates for the hepatic microsomal enzyme systems, can promote the drug metabolizing capacity of the liver. Synthesis of *de novo* protein is initiated by a high concentration of inducer at the enzyme site. A number of drugs are known to induce their own metabolism. An example is the anticonvulsant carbamazepine. Figure 15 shows the plasma concentrations of this drug and one of its metabolites, carbamazepine epoxide, in a monkey during a four day infusion of carbamazepine. The elimination half-life for both drug and metabolite is approximately one hour in this animal species. Hence steady-state is achieved within the first eight hours of the infusion. During the subsequent three days however, the carbamazepine plasma concentrations fall to a new steady-state which is only 60 per cent of the original. A similar decrease occurs in the carbamazepine epoxide steady-state concentrations which suggests that the elimination of this metabolite (to the diol) is enhanced to a similar degree as its formation.

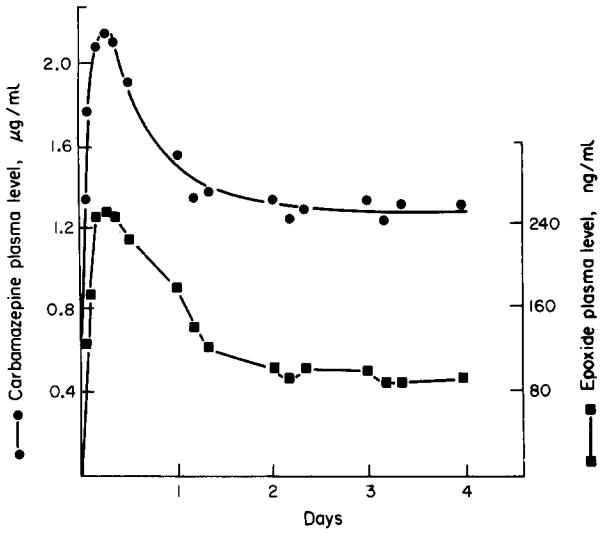


FIG. 15. Plasma concentration–time profiles for carbamazepine (●) and its metabolite carbamazepine-10,11-epoxide (■) during a 4 day infusion of carbamazepine to a monkey. From Patel *et al.* (1978).

4.4.2. *End Product Inhibition*

Chronic administration of diazepam results in a prolongation of the terminal half-life of this drug. As illustrated in Fig. 16 this inhibitory effect is associated with accumulation of the desmethyl metabolite. It has been postulated (Klotz *et al.*, 1976) that this phenomenon is an example of end product inhibition. As supporting evidence these investigators report that administration of desmethyldiazepam *per se* for a similar period of time produces an identical inhibitory effect.

Four years earlier, Ashley and Levy (1972) had proposed a similar hypothesis to explain the non-linearity of phenytoin disposition in the rat. In the case of phenytoin a prolongation of terminal half-life was achieved both by administering 4-hydroxyphenytoin exogenously and by producing a build up of the same metabolite endogenously. This latter effect was produced by administration of salicylamide as a selective inhibitor of glucuronidation, the pathway responsible for the clearance of the 4-hydroxy metabolite (Levy and Ashley, 1973).

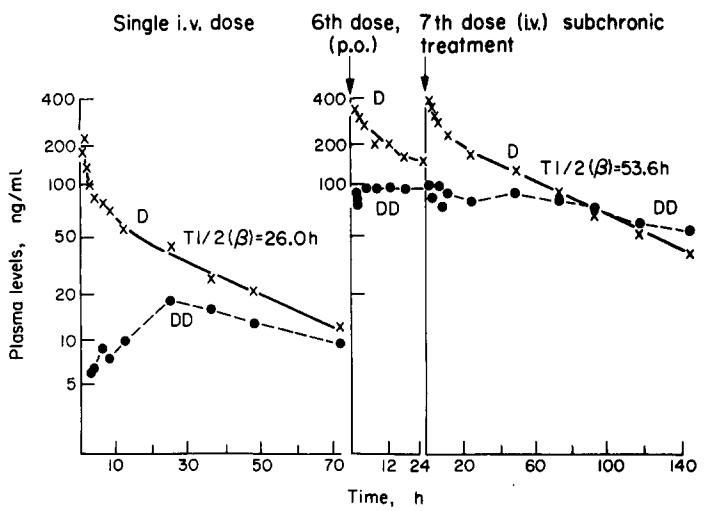


FIG. 16. Plasma concentration–time profiles for diazepam (×) and its desmethyl metabolite (●) during a 7 day treatment of diazepam to a human subject. The first and seventh doses were given intravenously and the other five doses orally. From Klotz *et al.* (1976).

5. SOME APPLICATIONS OF METABOLITE KINETICS

5.1. Characterisation of Drug Elimination

The most frequently used and, in the majority of cases, the most satisfactory method of characterising the overall elimination of a drug is by the analysis of drug plasma concentration–time data. Under certain circumstances this approach may not be practicable.

Drugs with large volumes of distribution display low plasma concentrations which may not be readily assayed. However, if one of its metabolites or a measure of "total" drug (for example, radioactivity following administration of a radio-labelled drug) can be determined then an estimate of the drug half-life can be obtained. When the metabolite(s) display FRL kinetics the estimate will be quite accurate. When it is not known whether FRL or ERL govern the kinetics, the half-life obtained must be regarded as an upper limit which may be a gross overestimation of the drug half-life.

The protocol of certain pharmacokinetic studies may not allow invasive samples, for example paediatric or geriatric studies. In such circumstances the analysis of excreta, usually urine, may offer a method for determining overall drug elimination. For a number of reasons it may be preferable to assay urine for metabolite(s) rather than for drug since the former is frequently excreted in larger amounts and less prone to changes in urine flow and pH.

The rate of urinary excretion of a metabolite may be described by

$$\frac{\mathrm{d}Ae(m)}{\mathrm{d}t} = fe(m) \cdot CL(m) \cdot C(m) \tag{101}$$

where $fe(m)$ is the fraction of the drug dose converted to metabolite and excreted without further metabolism.

As the metabolite excretion rate is directly proportional to the plasma metabolite concentration, an excretion rate–time profile will show a similar shape to a plasma metabolite concentration–time profile, several examples of which were discussed in Section 3. Therefore, when the metabolite kinetics show FRL, the terminal slope of a metabolite urinary excretion rate–time profile reflects the drug half-life. The urinary excretion rates determined experimentally are obviously not instantaneous rates $[\mathrm{d}Ae(m)/\mathrm{d}t]$ but average rates over a finite time period $[\Delta Ae(m)/\Delta t]$. However, the average rate closely approximates the instantaneous rate at the mid-point of the urine collection period as long as the latter does not exceed the half-life of the drug (Gibaldi and Perrier, 1975).

Figure 17 illustrates this relationship for amylobarbitone and two of its metabolites—amylobarbitone N-glucuronide and 3′-hydroxyamylobarbitone. The kinetics of both metabolites show FRL and hence the terminal phases of the logarithm of the urinary excretion rate–time plots parallel the terminal phase for the amylobarbitone plasma concentration–time profile.

Similarly, if the metabolite is volatile and excreted via the lungs then the exhalation rate–time profile may be of use to determine the parent drug kinetics. For example, [N-$^{14}CH_3$]-antipyrine is demethylated to yield the nor metabolite and ^{14}C-formaldehyde. The latter metabolite is rapidly converted to $^{14}CO_2$ which mixes with the endogenous carbon dioxide prior to exhalation. Time plots for $^{14}CO_2$ exhalation rate and antipyrine plasma concentrations following administration of [N-$^{14}CH_3$]-antipyrine are shown in Fig. 18. Again the metabolite kinetics are FRL, and the terminal half-life of the $^{14}CO_2$ exhalation rate–time plot reflects the half-life of antipyrine.

The classic example of salicylate metabolism (Levy *et al.*, 1972) illustrates how much information may be obtained from metabolite excretion rate–time profiles. This drug is eliminated completely in the urine as unchanged drug and four metabolites. Two of the metabolic pathways are readily saturable and the other elimination processes

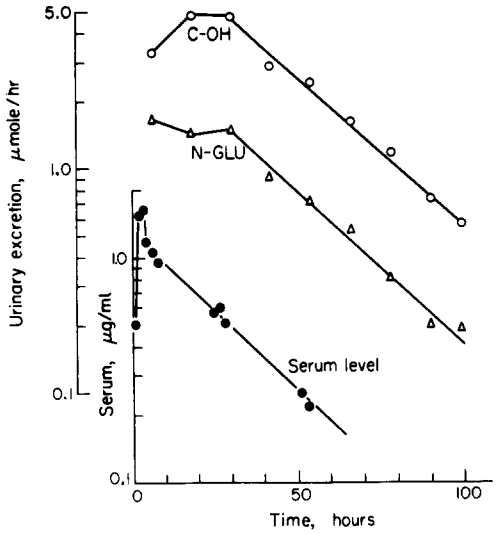
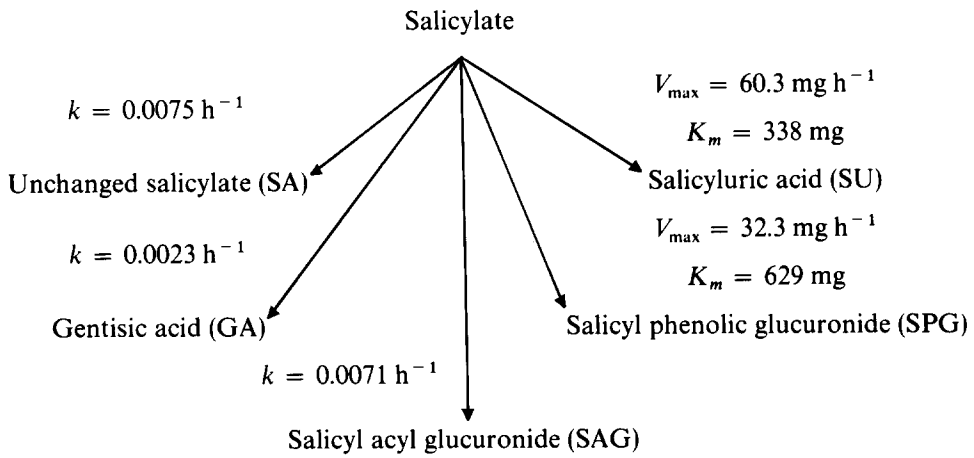


FIG. 17. Serum concentration–time profiles for amylobarbitone (●) and urinary excretion rate–time profiles for amylobarbitone N-glucuronide (△) and 3'-hydroxy-amylobarbitone (○) following oral administration of amylobarbitone to a human subject. From Kalow *et al.* (1978).

apparently first order. Scheme 7 summarizes the pharmacokinetic model which describes the fate of salicylate in man.



Scheme 7. Pharmacokinetic model for the fate of salicylate in man.

The above scheme was characterized from urinary excretion data in the following manner. Each of the metabolites of salicylate are excreted so rapidly that the amount of unmetabolized drug remaining in the body at any time after absorption can be determined accurately from the difference between the dose and the "total" salicylate excreted in the urine. Since the metabolites show FRL, the rate of excretion for a given metabolite reflects the rate of formation. Therefore by using the following linear transformation for the Michaelis–Menten equation the non-linear pathways were identified:

$$\frac{A}{v} = \frac{K_m}{V_{max}} + \frac{1}{v} \cdot A \tag{102}$$

where A is the amount of salicylate in the body, v is the formation rate (excretion rate), K_m is the Michaelis–Menten constant and V_{max} the maximum formation rate for the particular metabolite. Figure 19a shows the SPG data plotted according to Eqn (96), and demonstrates the limited capacity of this reaction. In contrast, the SAG data

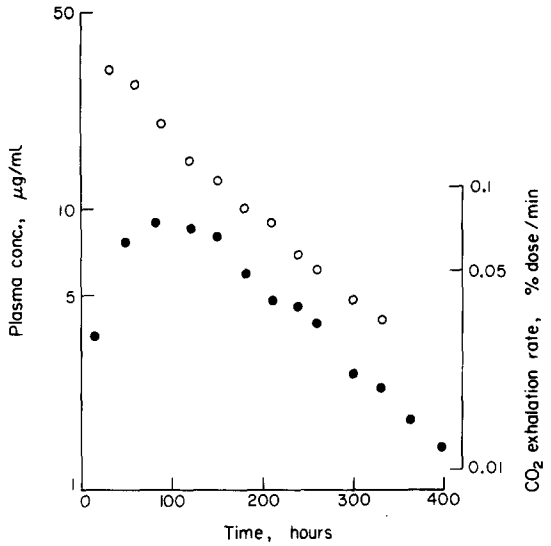


FIG. 18. Plasma concentration–time profile for antipyrine (○) and $^{14}CO_2$ exhalation rate–time profile (●) following administration of [N-$^{14}CH_3$]-antipyrine to a rat. Rhodes, J. C. and Houston, J. B. (unpublished data).

(Fig. 19b) shows a slope of zero indicating the first order nature of this metabolite production.

The time course for the elimination of salicylate from the body can be described by

$$\frac{dA}{dt} = -(ke_{SA} + km_{SAG} + km_{GA})A - \frac{V_{max,SPG} \cdot A}{K_{m,SPG} + A} - \frac{V_{max,SU} \cdot A}{K_{m,SU} + A}. \quad (103)$$

This equation, together with the parameter values listed in Scheme 7, were used to predict successfully the kinetics of salicylate elimination over a 15-fold dose range. The model was also consistent with the time course of the metabolites and the quantitative composition of the urinary metabolite over the same dose range.

5.2. Rationalization of Inconsistent Drug Plasma Concentration—Response Correlations

The relationship between a drugs plasma concentration and its pharmacological action may become complicated when active metabolites are involved. The observed response, whether therapeutic or toxic, will represent a composite of activity of both drug and the

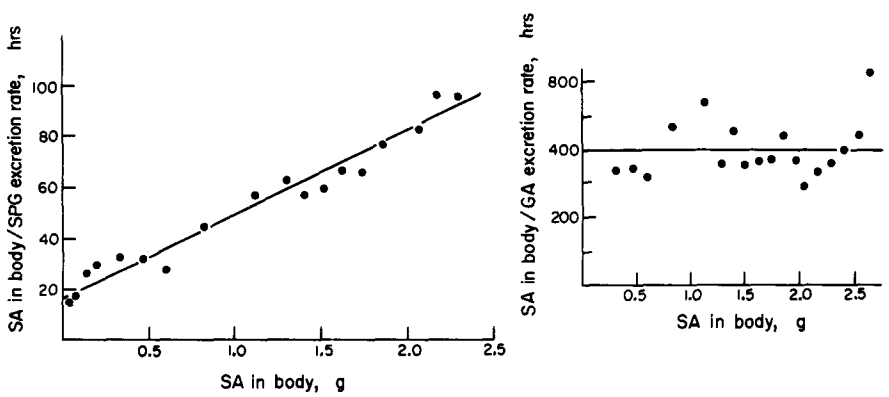


FIG. 19. Michaelis–Menton based plots [Eqn (96)] for salicyl phenolic glucuronide and salicyl acyl glucuronide using urinary excretion data from a human subject who ingested salicylate orally. From Levy *et al.* (1972).

metabolite(s) involved. A recent review (Drayer, 1976) has tabulated fifty-eight examples of drugs with active metabolites. Three well documented examples are discussed below.

5.2.1. *Alprenolol*

This beta-adrenergic receptor blocker is highly cleared and extensively metabolized. The 4-hydroxylated metabolite achieves higher concentrations in the plasma than its parent drug on oral but not intravenous administration (Ablad *et al.*, 1974). This phenomenon becomes less marked at higher doses of alprenolol, probably due to a saturation of first pass hydroxylation (Collste *et al.*, 1979). Figure 20 shows the correla-

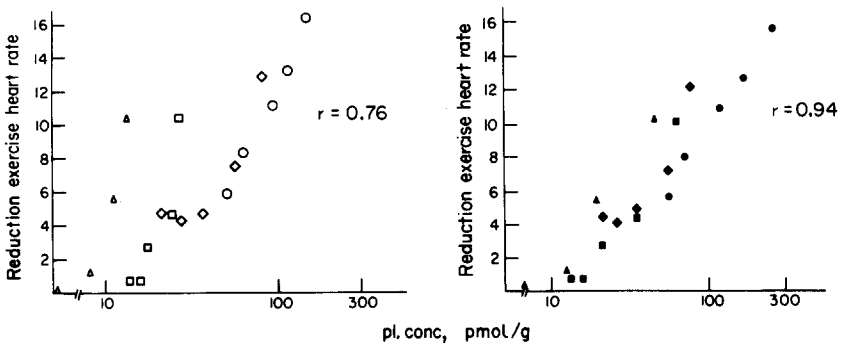


FIG. 20. Correlation between percentage reduction in exercise tachycardia and plasma concentrations of alprenolol (open symbols, Panel a) and alprenolol + 4-hydroxyalprenolol (closed symbols, Panel b). Doses of alprenolol administered were 50 mg (△), 100 mg (□) and 200 mg (○) orally, and 10 mg (◇) intravenously. From Collste *et al.* (1979).

tion between the reduction in exercise tachycardia, and plasma concentrations of drug and hydroxylated metabolite. The data shown was obtained following administration of three oral doses (50, 100 and 200 mg) and a 10 mg intravenous dose. Panel a in Fig. 20 shows close correspondence between the response–alprenolol concentration relationship for the 200 mg oral and the intravenous doses. However, the analogous relationship for the two lower oral doses appear displayed to the left, that is, they show a larger response for a given plasma concentration. Inclusion of 4-hydroxyalprenolol plasma concentrations to give a response–(alprenolol + 4-hydroxyalprenolol) concentration relationship effectively reduces the slope of the line and the four sets of data converge to produce a much better overall correlation (Panel b, Fig. 20). Further analysis of this data (Collste *et al.*, 1979) provides evidence that alprenolol and its 4-hydroxylated metabolite are equipotent as beta adrenergic receptor blocking agents.

5.2.2. *Glutethimide*

Glutethimide is also hydroxylated to an active metabolite which has been shown to account for the complex clinical course of coma induced by overdosing this drug. Figure 21 shows that the changes in clinical status of a patient who ingested 12 g of drug correlate more closely with the concentration–time profile for the metabolite than for the parent drug. The investigators were able, by using comparative pharmacological data from animal studies, to construct an activity index from the plasma concentrations of both species. The good agreement between both coma grade and respiratory status and this index is evident from Fig. 21.

5.2.3. *Procainamide*

Procainamide is extensively acetylated and this metabolite possesses similar anti-arrhythmic activity to the parent drug. Since the dosage regimen for procainamide was

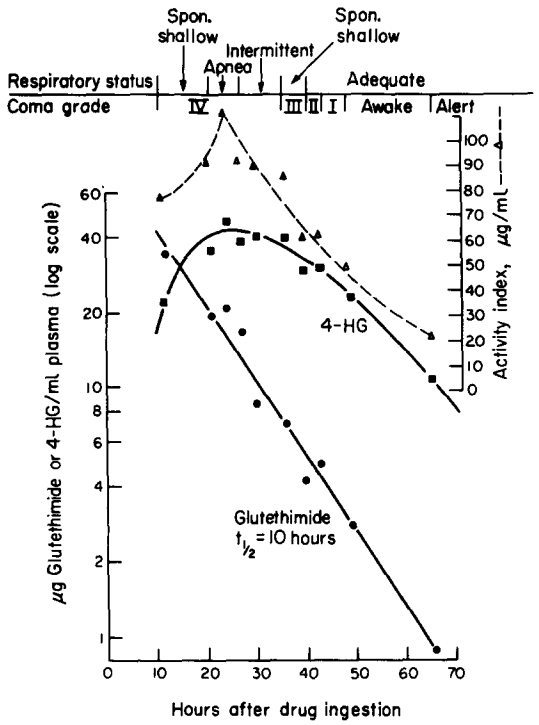


FIG. 21. Clinical course of a patient who ingested a 12 g dose of glutethimide. Respiratory status and grade of coma are correlated with an index of total pharmacological activity (△) calculated by doubling the 4-hydroxyglutethimide plasma concentrations (■) and adding them to the parent drug plasma concentrations (●). From Hansen *et al.* (1975).

derived empirically rather than on the basis of the pharmacokinetics of the parent drug, some allowance for the pharmacological activity and the long half-life of acetylprocainamide has been made. However, in fast acetylators and patients with renal insufficiency, the plasma concentrations of acetylated metabolite will be particularly high and individual dosage adjustment may prove difficult (Atkinson and Strong, 1977).

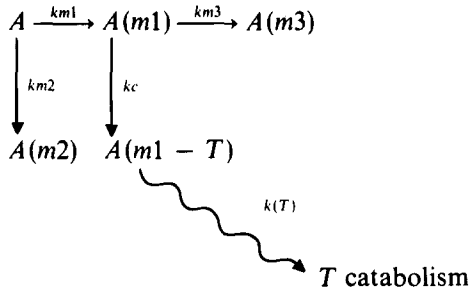
The acetyl conjugate is excreted essentially unchanged and hence extensive accumulation occurs in renal failure (Drayer *et al.*, 1977). The relationship between the accumulation of pharmacologically active drug metabolites in renal failure and the incidence of adverse drug effects have been discussed by Drayer (1976).

5.3. Kinetics of Covalent Binding of Reactive Metabolites

Many chemicals, including certain drugs, are converted in the body to chemically reactive metabolites that react with various cellular components. These reactive metabolites may cause various toxic effects including cancer, mutagenesis, cellular necrosis, blood dyscrasias and foetal damage (Jollow *et al.*, 1977).

The kinetics of reactive metabolites, or more precisely the kinetics of covalent binding, differ in a number of aspects from the kinetics of reversibly acting drugs. These differences have been discussed at length by Gillette (1974, 1977). The relationship between the extent of covalent binding of a reactive metabolite and toxicity will depend not solely on the rates of formation and inactivation of the reactive metabolite. The rate at which the target molecule (for example, DNA, protein or lipid) is repaired or replaced is also of importance.

Scheme 8 provides a model for the kinetics of covalent binding of a reactive metabolite which is an extension of the previously discussed metabolite models.



Scheme 8. Model for the kinetics of covalent binding of a reactive metabolite.

In this scheme, $A(m1)$ is the amount of reactive metabolite capable of covalently binding with the target molecule to give $(m1 - T)$ or undergoing inactivation to another metabolite, $m3$; $A(m2)$ represents an alternative route of elimination for the drug, A, which involves non-reactive metabolites; $km1$, $km2$ and $km3$ are the formation rate constants for the above pathways; kc and $k(T)$ are the rate constants for the formation and elimination of the covalently bound $m1$.

In Scheme 8 the overall elimination rate constant for drug, k, and $m1$, $k(m1)$, may be considered as

$$k = km1 + km2 \tag{104}$$

$$k(m1) = kc + km3. \tag{105}$$

The fraction of the dose of drug A converted to $m1$ will be given by the ratio

$$fm1 = \frac{km1}{k}. \tag{106}$$

Similarly the fraction of $m1$ covalently bound is the ratio

$$fm2 = \frac{kc}{k(m1)}. \tag{107}$$

Therefore, the fraction of the dose of drug A which is covalently bound, fc, is

$$fc = fm1 \cdot fm2 \tag{108}$$

$$= \frac{km1}{(km1 + km2)} \frac{kc}{(kc + km3)}. \tag{109}$$

In other words the proportion of the dose that becomes covalently bound may be regarded as a series of ratios (Gillette, 1974), the length of which will depend upon the number of sequential reactions concerned. In the above case the primary metabolite is reactive, therefore only two reactions are involved.

It should be noted that each fraction (ratio) depends not only on the formation rate of the reactions involved in the covalent binding pathway but also on the formation rate of the innocuous pathways. The former is the numerator in Eqns (100) and (101) and the sum of all the rate constants contributing to the overall elimination of drug or reactive metabolite is the denominator. These considerations explain a number of apparent inconsistencies in comparative studies on drug kinetics and extent of covalent binding. For example, pre-treatment with inducers or inhibitors of the enzyme responsible for the formation of the reactive metabolite may not greatly change the extent of covalent binding if they also cause parallel changes in the other pathways. A degree of specificity is necessary before either fraction, and therefore the magnitude of covalent binding, changes. Hence enzyme perturbations may have dramatic effects on drug clearance without altering the fractions and no change in drug kinetics may be accompanied by large changes in the extent of covalent binding (Gillette, 1977).

6. CONCLUSION

In this re-appraisal of the role of kinetics in drug metabolism it has become evident that many drug-related phenomena cannot be solely rationalized from parent drug data. The need to consider drug metabolite data in addition to parent drug data necessitates the use of metabolite kinetic models. The present review provides a commentary on the current status of drug metabolite kinetics. Certain of the principles of metabolite kinetics have obvious parallels in drug kinetics. The catenary nature of a precursor–successor relationship for a metabolite is analogous to the absorption–disposition relationship for a drug, for example. However, other phenomena kinetics are quite unique. Both aspects have been integrated and discussed in the present review.

In 1963 the first paper specifically concerned with metabolite kinetics was published (Cummings and Martin, 1963). However, in the following decade the development of this area of pharmacokinetics was slow and erratic. It is only since the mid-1970s that a regular flow of publications with experimental data and theoretical considerations on metabolite kinetics has been forthcoming. It is likely that investigations in this area will continue to increase in number and hence the present approaches to metabolite kinetics will be refined.

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