Journal of Pharmacokinetics and Biopharmaceutics, Vol. 7, No. 3, 1979

Sequential First-Pass Elimination of a Metabolite Derived from a Precursor

K. Sandy Pang[1,2] and James R. Gillette[1,3]

Received December 1, 1978—Final January 22, 1979

We examined data from our previous studies in which we not only delivered perfusate containing tracer concentrations of [^{14}C]phenacetin and its metabolite [^{3}H]acetaminophen under constant perfusate flow (10 ml/min/liver) into the rat liver preparation just once, but also recirculated fresh reservoir perfusate containing a tracer dose of [^{14}C]phenacetin through the same rat liver preparation. From the single-pass studies, estimates of f_m, the fractional rate of conversion for [^{14}C]phenacetin to form [^{14}C]acetaminophen, and $F_{(M,P)}$, the apparent availability of [^{14}C]acetaminophen, were obtained by determining the concentrations of [^{14}C]acetaminophen in the perfusate before and after incubation with Glusulase. These estimates were $f_m = 0.871 \pm 0.16$ and $F_{(M,P)} = 0.43 \pm 0.10$. These and the steady-state clearance values of phenacetin (9.1 ± 0.8 ml/min) and acetaminophen (6.7 ± 0.7 ml/min) from the single-pass studies were used to predict the concentrations of [^{14}C]acetaminophen in the reservoir perfusate on recirculation of [^{14}C]phenacetin. We found that the sequential first-pass elimination of the metabolite must be considered when the metabolite is highly extracted by the liver. If we had neglected to take this into account, the fractional rate of conversion of a precursor to form a metabolite and the rate of formation of the metabolite would have been underestimated by the factor $F_{(M,P)}$.

KEY WORDS: sequential first-pass elimination; metabolite; precursor; fractional rate of conversion; apparent availability.

INTRODUCTION

Although the concept of metabolite modeling is not new and there is growing concern about and recognition of metabolites as active and toxic substances, metabolite kinetics are seldom described in the literature (1–4). A predictive model for a metabolite becomes a necessity, particularly when

[1]Laboratory of Chemical Pharmacology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205.
[2]Present address: Department of Pharmaceutics, School of Pharmacy, University of Houston, Houston, Texas 77004.
[3]Address correspondence to J. R. G.

0090-466X/79/0600-0275$03.00/0

Glossary

Subscripts M, P, and (M,P) denote, respectively, the preformed metabolite, the precursor, and the metabolite derived from the precursor. Subscript ss denotes steady-state conditions. Subscripts i.v., i.p., and p.o. denote, respectively, the intravenous, the intraperitoneal, and the oral routes of administration.

$AUC_{(P)}$, $AUC_{(M)}$, and $AUC_{(M,P)}$ denote the area under the curve or the integral of the equation describing the concentration of a drug between the limits of $t = 0$ and $t = \infty$ for the precursor, for the preformed metabolite, and for the metabolite formed from a precursor, respectively.

C denotes concentration.

$C_{(P)}$, $C_{(P)L}$, $C_{In(P)}$, and $C_{Out(P)}$ denote, respectively, the concentrations of the precursor in central compartment, in the liver compartment, in blood entering the liver, and in blood leaving the liver.

$C_{(M,P)}$, $C_{(M,P)L}$, $C_{In(M,P)}$, and $C_{Out(M,P)}$ denote, respectively, the concentrations of the metabolite in the central compartment, in the liver compartment, in blood entering the liver, and in blood leaving the liver, after the administration of a precursor.

CL_P and CL_M denote, respectively, the intravenous or systemic clearance for the precursor and for the metabolite.

$C_{(M,P)formed}$ denotes the concentration of the metabolite formed from the precursor; i.e., $f_m E_P C_{In(P)}$.

$Dose_{(P)}$ and $Dose_{(M)}$ denote, respectively, the doses of the precursor and of the preformed metabolite.

E_P and E_M denote, respectively, the hepatic extraction ratios for the precursor and for the metabolite; i.e., $(1-F_P)$ and $(1-F_M)$, respectively.

f_m denotes the fractional rate of elimination of the precursor to form metabolite M; f_m denotes the rate of formation of metabolite/total rate of elimination of precursor.

$F_{(M)}$ denotes hepatic availability of preformed metabolite; i.e., $C_{Out(M)}/C_{In(M)}$.

$F_{(M,P)}$ denotes the apparent availability of a metabolite formed immediately from its precursor; i.e., $C_{Out(M,P),ss}/C_{(M,P)formed,ss}$; $C_{Out(M,P),ss}$ is the concentration of metabolite in blood leaving the liver; $C_{(M,P)formed,ss}$ is the concentration of the metabolite the concentration of the metabolite actually formed from the precursor in the liver under steady-state conditions.

k' denotes the rate constant for the formation of the metabolite.

k'' denotes the sum of the rate constants for the other elimination processes of the precursor in the liver.

k_P and k_M denote, respectively, the overall elimination rate constant for the precursor and for the metabolite.

Q denotes hepatic blood flow.

t denotes time.

V_P, $V_{R(P)}$, and $V_{L(P)}$ denote, respectively, the volume of distribution, the volume of the central compartment, and the volume of the liver compartment, for the precursor.

V_M, $V_{R(M)}$, and $V_{L(M)}$ denote, respectively, the volume of distribution, the volume of the central compartment, and the volume of the liver compartment for the metabolite.

drug effects are mediated solely through the metabolite as in prodrug administration.

The intent of the present article is to examine our previously published data (5) in which the metabolite of phenacetin, acetaminophen, was quantitated under both steady-state and quasi-steady-state as well as linear kinetic conditions after the administration of tracer amounts of [^{14}C]phenacetin in the perfused rat liver preparation. The existing

compartmental approach, which views the liver as a part of the central compartment, was used to predict, from the steady-state data, the concentrations of [^{14}C]acetaminophen in reservoir perfusate after the addition of a tracer amount of [^{14}C]phenacetin to the reservoir. We found that the predictions did not correlate with the observations; the concentrations of [^{14}C]acetaminophen were drastically overestimated. Therefore, we modeled the liver as a compartment separate from the central compartment and hence examined the effects of sequential first-pass elimination of the metabolite in prediction of metabolite concentrations.

THEORETICAL

It is assumed that the liver is the only eliminating organ for the precursor (P) and its metabolite (M) and that linear kinetic conditions occur whereby

1. The clearances of both the precursor and the metabolite, CL_P and CL_M, respectively, are concentration independent.
2. The unbound fractions of the precursor and the metabolite in blood are constant (6).
3. The ratio of the rate of conversion of a precursor to a metabolite to the total rate of precursor elimination, f_m, is a constant; f_m multiplied by the rate of elimination of P thus equals the rate of formation of M.

Only the simplest case is considered in the present analysis. It is assumed that the volume of liver is negligible compared to that of blood or plasma and that equilibration of the drug between the blood or plasma and the tissue occurs instantaneously.

The Liver as Part of the Central Compartment

In the simplest case, the drug in blood or plasma equilibrates so rapidly with the drug in organs and tissues that any changes in drug concentration would be reflected in the blood or plasma (central compartment) and the body behaves as a single compartment where elimination takes place. In such an instance, a one-compartment model describes the kinetics of a drug and a two-compartment model describes the kinetics of the metabolite (Fig. 1A).

Consider that a precursor, P, is converted to a metabolite, M, with a rate constant, k' (Fig. 1A). Biotransformation of the precursor to other metabolites and biliary excretion of the precursor proceed with rate constants whose sum is k''. The overall elimination rate constant for the precursor, k_p,

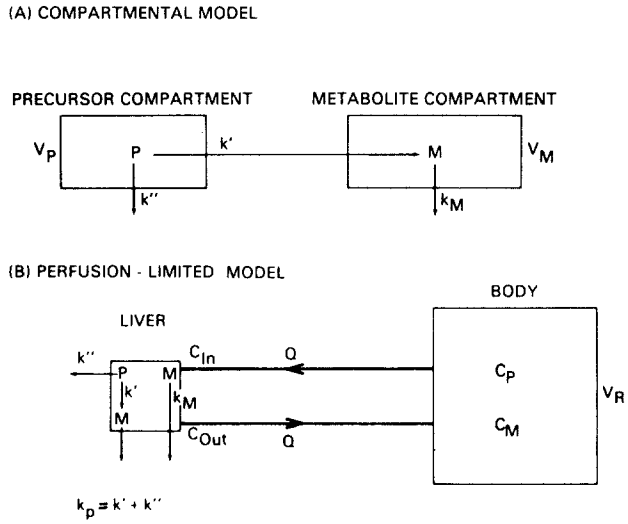

Fig. 1. A: One-compartment open model for the precursor and the metabolite. P and M denote the precursor and the metabolite, respectively. V_P and V_M denote the volumes of distribution of the precursor and the metabolite, respectively. k_M and k_P denote the overall elimination rate constants for the metabolite and the precursor, respectively; the latter is equal to the sum of the rate constant for the formation of the metabolite, k', and all other constants of elimination, k''. B: The liver as a discrete compartment. The liver, a drug-eliminating compartment, is connected to the reservoir, a noneliminating compartment, via hepatic blood flow (Q). C_{In} and C_{Out} denote the concentrations of the blood entering the liver and leaving the liver, respectively. C_P and C_M denote the concentrations of the precursor and the metabolite, respectively.

is the sum of k' and k'', and the overall elimination rate constant for the metabolite is denoted by k_M. By definition, the fraction of the dose of P which forms M, f_m, is given by

$$f_m = k'/k_P \tag{1}$$

or

$$k' = f_m k_P \tag{1A}$$

The rate equations which describe the change of material in each compartment after an intravenous (i.v.) dose of P [$\text{Dose}_{(P)\text{i.v.}}$] are

$$V_P \, dC_{(P)}/dt = -k_P V_P C_{(P)} \tag{2}$$

for precursor and

$$V_M\, dC_{(M,P)}/dt = k' V_P C_{(P)} - k_M V_M C_{(M,P)} \tag{3}$$

for metabolite, where V_P and V_M denote, respectively, the volumes of distribution of the precursor and the metabolite.

The equations which describe the concentration of P and M in the systemic blood at any time t [$C_{(P)}$ and $C_{(M,P)}$, respectively] are obtained by solving simultaneous equations 2 and 3:

$$C_{(P)} = \frac{\text{Dose}_{(P)\text{i.v.}}\, e^{-k_P t}}{V_P} \tag{4}$$

$$C_{(M,P)} = \frac{k'\text{Dose}_{(P)\text{i.v.}}[e^{-k_P t} - e^{-k_M t}]}{V_M(k_M - k_P)} \tag{5}$$

On substitution of equation 1A into equation 5, we obtain

$$C_{(M,P)} = \frac{f_m k_P \text{Dose}_{(P)\text{i.v.}}[e^{-k_P t} - e^{-k_M t}]}{V_M(k_M - k_P)} \tag{5A}$$

On integration of equations 4 and 5A between the limits $t = 0$ and $t = \infty$, the area under the curve for the precursor [$\text{AUC}_{(P)\text{i.v.}}$] and the area under the curve for the metabolite as formed from its precursor [$\text{AUC}_{(M,P)}$] are

$$\text{AUC}_{(P)\text{i.v.}} = \text{Dose}_{(P)\text{i.v.}}/k_P V_P = \text{Dose}_{(P)\text{i.v.}}/\text{CL}_P \tag{6}$$

$$\text{AUC}_{(M,P)} = f_m \text{Dose}_{(P)\text{i.v.}}/k_M V_M = f_m \text{Dose}_{(P)\text{i.v.}}/\text{CL}_M \tag{7}$$

where the intravenous or systemic clearances of the precursor and the metabolite, CL_P and CL_M, respectively, are given by the products of their rate constants and the volumes of distribution. The area under the curve of the metabolite [$\text{AUC}_{(M,P)}$] is the same for all routes of administration of P when P is absorbed completely and unchanged. Furthermore, the relationships among the area under the curve, clearance, and the dose hold regardless of the number of nonelimination compartments into which both the precursor and the metabolite become distributed; a nonelimination compartment is defined as a compartment that does not contain any system that eliminates the drug or the metabolite from the body.

The intravenous clearance of the metabolite can be obtained from an intravenous dose of the metabolite [$\text{Dose}_{(M)\text{i.v.}}$], and the area under the curve of the preformed metabolite [$\text{AUC}_{(M)\text{i.v.}}$] is

$$\text{CL}_M = \text{Dose}_{(M)\text{i.v.}}/\text{AUC}_{(M)\text{i.v.}} \tag{8}$$

After substituting equation 8 into equation 7 and rearranging the resulting

equation, we obtain the fraction of the dose of P which forms M:

$$f_m = \frac{\text{AUC}_{(M,P)}\text{Dose}_{(M)\text{i.v.}}}{\text{AUC}_{(M)\text{i.v.}}\text{Dose}_{(P)}} \tag{9}$$

Note that, according to the one-compartment model, f_m may be estimated after intravenous administration of M and of P by any route of administration, including intravenously.

The Liver as a Compartment Separate from the Central Compartment

The liver, an eliminating organ, is connected to the central compartment via blood flow, Q (Fig. 1B). When the liver is the only eliminating organ, and all other organs are rapidly equilibrated with the central compartment, a situation analogous to the liver perfusion system is established where the central compartment is equivalent to the reservoir. Elimination of a precursor and its metabolite occurs in the liver either by metabolism or by biliary excretion. After the introduction of a bolus of drug into the reservoir (equivalent to intravenous administration), the concentration of the drug in the reservoir is the same as that entering the liver (C_{In}), and the concentration of the drug entering the reservoir is the same as that leaving the liver (C_{Out}).

When elimination is perfusion rate limited (that is, there is no diffusional barrier for a drug or a metabolite to reach its site of elimination), the drug in the blood emerging from the organ is in equilibrium with that in the organ (7). The rate equations which describe the rates of change of material in the reservoir (of volume V_R) and the liver (of volume V_L) are

$$V_{R_{(P)}}\, dC_{(P)}/dt = QC_{\text{Out}_{(P)}} - QC_{\text{In}_{(P)}} \tag{10}$$

$$V_{L_{(P)}}\, dC_{(P)_L}/dt = QC_{\text{In}_{(P)}} - QC_{\text{Out}_{(P)}} - k_P V_{L_{(P)}} C_{(P)_L} \tag{11}$$

for precursor and

$$V_{R_{(M)}}\, dC_{(M,P)}/dt = QC_{\text{Out}_{(M,P)}} = QC_{\text{In}_{(M,P)}} \tag{12}$$

$$V_{L_{(M)}}\, dC_{(M,P)_L}/dt = f_m F_{(M,P)} QE_P C_{\text{In}_{(P)}} + QC_{\text{In}_{(M,P)}} - QC_{\text{Out}_{(M,P)}} - QE_M C_{\text{In(M,P)}} \tag{13}$$

rate of change of drug liver	net contribution from precursor (after sequential first-pass elimination of metabolite)	rate in	rate out	elimination on recirculation of preformed metabolite

for metabolite, where $F_{(M,P)}$ is the apparent availability of the metabolite derived immediately from the precursor and is the ratio of the steady-state concentration of the metabolite in the hepatic venous blood to the steady-

state concentration of the metabolite which is actually formed in the liver (5). Hence the total concentration of the metabolite formed immediately from its precursor is higher than the concentration of the metabolite which is detected in the hepatic venous blood when there is sequential first-pass elimination of the metabolite.

When the volume of the liver is negligible compared to the volume of the reservoir, the terms $V_{L_{(P)}}\, dC_{(P)_L}/dt$ and $V_{L_{(M)}}\, dC_{(M,P)_L}/dt$ may be set to zero. By solving equations 10–13 simultaneously, we obtain the following equations which describe the concentrations of the precursor and the metabolite in the central compartment (reservoir) at any time:

$$C_{(P)} = \frac{\text{Dose}_{(P)\text{i.v.}}\, e^{-QE_P t/V_{R_{(P)}}}}{V_{R_{(P)}}} \tag{14}$$

$$C_{M,P} = \frac{f_m F_{(M,P)} \text{Dose}_{(P)\text{i.v.}}}{V_{R_{(M)}}} \left[\frac{QE_P/V_{R_{(P)}}}{QE_M/V_{R_{(M)}} - QE_P/V_{R_{(P)}}}\right] \times \left[e^{-QE_P t/V_{R_{(P)}}} - e^{-QE_M t/V_{R_{(M)}}}\right] \tag{15}$$

Since the volume of the liver (V_L) is small compared to the volume of the central compartment (V_R), the volume of the central compartment becomes the volume of distribution of the drug and the metabolite. Also, as the product QE is clearance, QE/V becomes the overall elimination rate constant. Therefore, equations 14 and 15 can be expressed as

$$C_{(P)} = \frac{\text{Dose}_{(P)\text{i.v.}}\, e^{-k_P t}}{V_P} \tag{14A}$$

$$C_{(M,P)} = \frac{f_m k_P F_{(M,P)} \text{Dose}_{(P)\text{i.v.}} [e^{-k_P t} - e^{-k_M t}]}{V_M (k_M - k_P)} \tag{15A}$$

Note that equation 14A is the same as equation 4. However, the equation for the concentration of the metabolite (equation 15A) according to this approach (the liver as a separate compartment), in contrast to that for the former approach (the liver as part of the central compartment, equation 5A), reflects the sequential first-pass elimination of the metabolite.

On integration of equations 14A and 15A between the limits of $t = 0$ and $t = \infty$, we obtain the area under the curve for the precursor [$\text{AUC}_{(P)\text{i.v.}}$] and for the metabolite [$\text{AUC}_{(M,P)}$] (5):

$$\text{AUC}_{(P)\text{i.v.}} = \text{Dose}_{(P)\text{i.v.}}/k_P V_P = \text{Dose}_{(P)\text{i.v.}}/\text{CL}_P \tag{16}$$

$$\text{AUC}_{(M,P)} = \frac{f_m F_{(M,P)} \text{Dose}_{(P)\text{i.v.}}}{k_M V_M} = \frac{f_m F_{(M,P)} \text{Dose}_{(P)\text{i.v.}}}{\text{CL}_M} \tag{17}$$

The area under the curve of the metabolite is the same for all routes of administration of P when all of P is completely absorbed unchanged. Furthermore, the relationships among the area under the curve, clearance, and the dose hold regardless of the number of nonelimination compartments and the volume of the liver considered for both the precursor and the metabolite.

When the apparent availability of the metabolite $F_{(M,P)}$ is identical to the availability of the preformed metabolite, $F_{(M)}$, the area under the curve of the metabolite becomes (4)

$$\mathrm{AUC}_{(M,P)} = \frac{f_m F_{(M)} \mathrm{Dose}_P}{\mathrm{CL}_M} \tag{17A}$$

The area under the curve following either an intraperitoneal (i.p.) or oral (p.o.) dose of the metabolite [$\mathrm{Dose}_{(M)}$] when the metabolite is totally absorbed unchanged via the portal venous blood is

$$\mathrm{AUC}_{(M)\text{i.p. or p.o.}} = \frac{F_{(M)} \mathrm{Dose}_{(M)\text{i.p. or p.o.}}}{\mathrm{CL}_M} \tag{18}$$

After substitution of equation 17A into equation 18 and on rearrangement of the terms, the fraction of the dose of P which is converted to M, f_m, is given by (8)

$$f_m = \frac{\mathrm{AUC}_{(M,P)} \mathrm{Dose}_{(M)\text{i.p. or p.o.}}}{\mathrm{AUC}_{(M)\text{i.p. or p.o.}} \mathrm{Dose}_{(P)}} \tag{19}$$

Note that P may be administered by any route but that M should be administered either orally or intraperitoneally but not intravenously when $F_{(M,P)}$ is significantly less than 1.0. When $F_{(M,P)}$ nearly equals 1.0, M may be administered by any route.

DATA ANALYSIS

Data analysis was performed on our published results (5) in which we passed perfusate containing tracer concentrations of both [^{14}C]phenacetin (specific activity 55.7 mCi/mmole) and its metabolite [^{3}H]acetaminophen (specific activity 937.5 mCi/mmole) for 40 min only once through the rat liver preparations (single pass) under constant perfusate flow (10 ml/min/liver). Additionally, we recycled fresh perfusate containing a tracer dose of [^{14}C]phenacetin in the reservoir (equivalent to intravenous administration) for the subsequent 120 min under constant perfusate flow (10 ml/min/liver) through the same rat liver preparation (Fig. 2). The validity of all the assumptions stated previously was verified in the system.

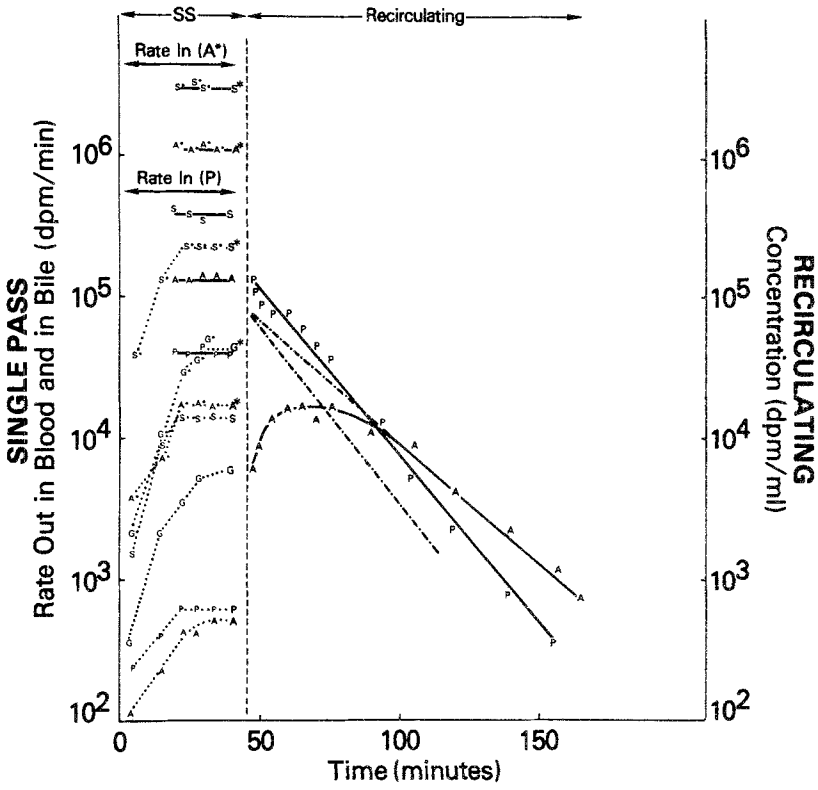

Fig. 2. Design of the experiment—a single pass (40 min) followed by recirculation of perfusate (120 min). Perfusate containing a constant input concentration of [^{3}H]acetaminophen and [^{14}C]phenacetin was delivered under constant perfusate flow (10 ml/min) to the rat liver preparation. The rates of input of [^{3}H]acetaminophen [Rate In (A*)] and of [^{14}C]phenacetin [Rate In (P)] represent the product of flow and their respective input concentrations. The rate out (flow × output concentration) for [^{3}H]acetaminophen (A*), [^{3}H]acetaminophen sulfate conjugate (S*), [^{14}C]phenacetin (P), and [^{14}C]acetaminophen sulfate conjugate (S) in the effluent perfusate are represented by solid lines (——). The rates of appearance of [^{3}H]acetaminophen and its metabolites and of [^{14}C]phenacetin and its metabolites in bile are shown by dotted lines (· · · ·). G and G* represent [^{14}C]acetaminophen glucuronide conjugate and [^{3}H]acetaminophen glucuronide conjugate, respectively. The recirculating design consisted of recirculation of perfusate containing a dose of [^{14}C]phenacetin in the reservoir. The dashed lines (– · – ·) represent the extrapolated line of the logarithmic terminal linear portion of the [^{14}C]acetaminophen (metabolite) curve and the residual line obtained by taking the difference between the extrapolated line and the metabolite curve.

Also, biliary excretion of [^{14}C]phenacetin and of [^{3}H]acetaminophen was negligible (<1%) compared to the amount of drug and preformed metabolite infused (5).

Single Pass of Perfusate

The concentrations of [^{14}C]phenacetin and [^{3}H]acetaminophen in the input perfusate and the concentrations of [^{14}C]phenacetin, [^{14}C]acetaminophen, and [^{3}H]acetaminophen in the output perfusate were assayed before and after incubation with Glusulase. Assays after treatment with Glusulase, an enzyme preparation which contains β-glucuronidase and arylsulfatase and thus catalyzes the hydrolysis of both [^{14}C]acetaminophen sulfate conjugate and [^{14}C]acetaminophen glucuronide conjugate, furnished the steady-state concentrations of the metabolite actually formed [$C_{(M,P)\text{formed}}$].

The fractional conversion of phenacetin to form acetaminophen, f_m, is given by (5)

$$f_m = C_{(M,P)\text{formed,ss}}/[C_{\text{In}_{(P),\text{ss}}} - C_{\text{Out}_{(P),\text{ss}}}] \tag{20}$$

The apparent availability of the metabolite, $F_{(M,P)}$, is given by the ratio of the steady-state concentration of acetaminophen in perfusate leaving the liver to the steady-state concentration of acetaminophen actually formed (5):

$$F_{(M,P)} = \frac{C_{\text{Out}_{(M,P)\text{ss}}}}{C_{(M,P)\text{formed,ss}}} \tag{21}$$

The rate constants for the elimination of phenacetin and acetaminophen, k_P and k_M, respectively, were calculated by the relationship $k = QE/V$.

These rate constants, together with f_m and $F_{(M,P)}$ for the individual experiments, are shown in Table I.

Table I. Summarized Data from the Single-Pass Design Which Were Utilized for Prediction of the Recirculating Design

Study No.	f_m	$F_{(M,P)}$	k_P[a] (min^{-1})	k_M[b] (min^{-1})
A	0.599	0.535	0.0364	0.0248
B	0.814	0.359	0.0495	0.0405
C	1.05	0.280	0.0582	0.0473
D	1.03	0.447	0.0514	0.0461

[a] k_P is calculated by $QE_P/V_{R(P)}$.
[b] k_M is calculated by $QE_M/V_{R(M)}$.

Recirculation of Perfusate

The volume of distribution was virtually identical to the volume of the reservoir perfusate for both phenacetin and acetaminophen in the system (5). Data from the recirculating experiments for both phenacetin and acetaminophen were fitted simultaneously (equations 4 and 5A or 15A) by the nonlinear curve-fitting computer program MLAB (9). Weights (1/concentration) were incorporated into the curve-fitting procedure. Four parameters were estimated: the concentration of the precursor at zero time, k_P, k_M, and either f_m (equation 5A) or the product $f_m F_{(M,P)}$ (equation 15A). The values for f_m in the latter product were calculated by dividing the product $[f_m F_{(M,P)}]$ by the experimentally derived values of $F_{(M,P)}$ which were obtained in the single-pass experiment.

The measured area under the curve of the metabolite $[AUC_{(M,P)}]$ was also used to estimate f_m according to both equations 7 and 17. The CL_M and $F_{(M,P)}$ values used in the calculations were obtained from the single-pass experiment in the same rat preparation.

RESULTS

Predictions from the Data of the Single-Pass Experiment

On modeling the liver as a part of a single compartment (Fig. 1A), good predictions were obtained for the concentrations of the precursor [^{14}C]phenacetin in the reservoir perfusate. But the predicted concentrations of the metabolite [^{14}C]acetaminophen were drastically overestimated (Fig. 3A–D). By contrast, on modeling the liver as a discrete compartment separate from the central compartment (Fig. 1B), good predictions were obtained for the concentrations of both the precursor and the metabolite (Fig. 3A–D).

Estimation of f_m from the Recirculating Experiment

The results of the curve-fitting procedure according to either equations 4 and 5A or equations 4 and 15A were generally satisfactory. The f_m values obtained by the curve-fitting procedure and those calculated from the area under the curve of the metabolite $[AUC_{(M,P)}]$ (equation 7 or equation 17) were compared to the experimentally derived values obtained during the single-pass experiments (Table II).

The f_m values obtained on modeling the liver as a part of the central compartment were underestimated in both instances; those obtained on modeling the liver as a discrete compartment separate from the central compartment were in close agreement with the observations (Table II). The

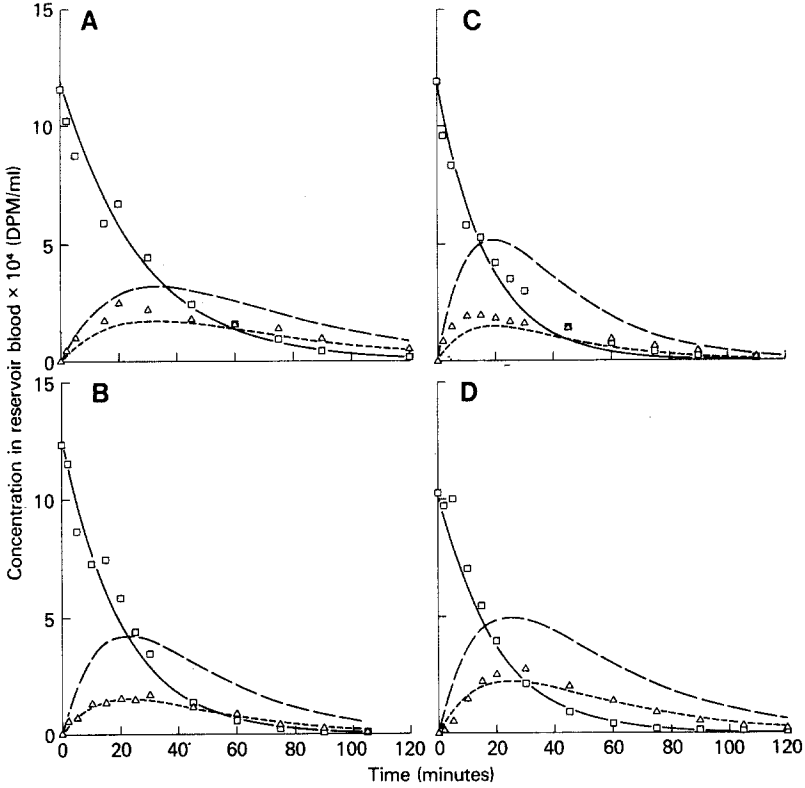

Fig. 3. Discrimination between the models. The decline of [^{14}C]phenacetin according to both models (——) and the appearance of [^{14}C]acetaminophen according to the model with the liver as a separate compartment (- - - -) and according to the model with the liver as a part of the central compartment (– – –) were simulated for studies A and B based on the data obtained from the single-pass design (Table II) and equations 4, 5A, and 15A; □ and △ denote the observed concentrations of [^{14}C]phenacetin and [^{14}C]acetaminophen, respectively.

difference between the estimated f_m values according to the two models is the factor $F_{(M,P)}$.

DISCUSSION

The examination of the simplest approach, a one-compartment model for the precursor, and a two-compartment model for the metabolite, as exemplified by phenacetin and acetaminophen in the perfused rat liver preparation, revealed that while the time course of the precursor is well described, the time course for the metabolite is poorly indicated (Fig.

Table II. Fraction of the Dose of the Precursor, f_m, as Estimated by the Compartment Model and the Perfusion Model

Study No.	Observed f_m	f_m estimated from the curve-fitting procedure		f_m estimated from the area under curve	
		Compartment model[a]	Perfusion model[b]	Compartment model[c]	Perfusion model[d]
A	0.599	0.362	0.677	0.380	0.710
B	0.814	0.339	0.944	0.304	0.847
C	1.05	0.333	1.189	0.319	1.139
D	1.03	0.381	0.852	0.448	1.003

[a]Equation 5A.
[b]Equation 15A.
[c]Equation 7.
[d]Equation 19.

3A–D). On adopting the alternate approach in which the liver is viewed as a compartment separate from the central compartment and considering sequential first-pass elimination of the metabolite good predictions were obtained for the time course of both the precursor and the metabolite (Fig. 3A–D). Deviations from perfect correlation in the data may be due to a decreased viability of the liver preparation; the recirculation part of each experiment always followed the single-pass part of the experiment.

The estimated f_m obtained from the curve-fitting procedure and with the area under the curve of the metabolite reveals that when the liver is incorporated into the central compartment, this fraction (f_m) may be drastically underestimated if there is a significant sequential first-pass elimination of the metabolite (Table II). The alternate approach with the liver as a separate compartment accounts for this effect and hence furnishes a better estimate of f_m. Again, deviations from perfect correlations suggest a decreased viability of the liver preparation.

All our findings support the view that sequential first-pass elimination of the metabolite acetaminophen occurs. Indeed, sequential first-pass elimination of the metabolite occurred, as evidenced by the presence of the secondary metabolite [^{14}C]acetaminophen sulfate conjugate in the outflow perfusate on passage of [^{14}C]phenacetin only once through the liver preparation (Fig. 2). Moreover, there is a significant sequential first-pass effect of the metabolite [$F_{(M,P)} = 0.42$] (5). When this effect is not considered in the metabolite data, the fractional rate of conversion of the precursor to form the metabolite, f_m, and the rate constant of formation of the metabolite, k', will be underestimated by the factor $F_{(M,P)}$.

The model in which the liver is part of the central compartment, however, was shown by other investigators to simulate very adequately

metabolite concentrations such as salicylic acid formed from acetylsalicylic acid in man (1), biotransformed products from chlordiazepoxide in the dog (2), and metabolites from N_4-ethoxyacetylsulfamethazole in the monkey (3), even when the effects of sequential first-pass elimination of the metabolite were ignored. But a close examination of the data revealed that the liver extraction ratios of the metabolites were low. The intravenous clearance of salicyclic acid in man (2) was calculated to be 0.024 liters/min, and thus the hepatic extraction ratio should be about 0.016 [liver blood flow in man was assumed to be 1.5 liters/min, and the contributions of renal clearance (10) and renal metabolism (11) had been ignored in the calculation]. The intravenous clearances of the metabolites of chlordiazepoxide (2), RO-550883/1 and RO-520921, were 0.64 and 0.79 ml/min/kg, respectively; hence their hepatic extraction ratios should be about 0.018 and 0.023, respectively (assuming the perfusion rate to the liver was 1 ml/min/g liver). The intravenous clearances of the three N_4-ethoxyacetylsulfamethoxazole metabolites (N_4-hydroxyacetylsulfamethoxazole, sulfamethoxazole, and N_4-acetylsulfamethoxazole) were 17.03, 2.23, and 11.11 ml/min, respectively; hence their maximal hepatic extraction ratios should be about 0.12, 0.016, and 0.14, respectively (assuming that liver weight was 3.5% body weight and hepatic perfusion rate was 1 ml/min/g liver). For metabolites such as salicylic acid, RO-50883/1, and RO-520921, whose hepatic availability approaches unity, the rate constants of formation (k') estimated according to equation 5 approximate the true values, and hence the f_m values (equation 5A) would correspondingly be in agreement with the true values. Because the $F_{(M,P)}$ values equal nearly 1.0 in these experiments, the simulated metabolite curves are in close agreement with the observed data and the estimation of the f_m values by a ratio of the areas of the metabolite after the intravenous administration of the metabolite and its precursor (equation 9) would be valid. The metabolites of N_4-ethoxyacetylsulfamethoxazole, however, have appreciable extraction ratios (0.12 and 0.14). After accommodating the disposition curve of the parent drug, the simulated metabolite curves are higher than that for the observed data (3). The error of underestimation of f_m for a terminal metabolite from a given drug will be augmented when the metabolites are formed in sequence, for example, the fractional conversion (f_m) of isoniazid to isonicotinuric acid via acetylisoniazid and isonicotinic acid (12); the factor by which the overall f_m is underestimated is the overall apparent availability of the terminal metabolite $F_{(M,P)\text{OVERALL}}$ or the product of the apparent availability of the terminal metabolite and all the apparent availabilities of the preceding metabolites.

For a metabolite which has a significant sequential first-pass effect, underestimation of the rate constant of the formation of the metabolite (k')

and the fractional rate of conversion (f_m) by a factor of $F_{(M,P)}$ is anticipated. The conventional method with a comparison of the areas under the metabolite curve after intravenous administration of the metabolite and of the precursor (equation 9) would also underestimate the parameters by the same factor. By contrast, incorporation of $F_{(M,P)}$ would overcome such an inadequacy. It is frequently assumed that the availability of the preformed metabolite and the availability of a metabolite immediately derived from a precursor are identical, that is, $F_{(M)} = F_{(M,P)}$. The assumption, however, is not always valid (5). The values of $F_{(M)}$ may be greater than $F_{(M,P)}$ because of the presence of diffusional barriers; a preformed metabolite has to gain access into hepatocytes while the metabolite derived immediately from a precursor need not cross the barrier before it is metabolized. The value of $F_{(M)}$ may also be less than $F_{(M,P)}$ because of incomplete mixing of the precursor and the metabolite in the liver (5). If indeed $F_{(M)} = F_{(M,P)}$, as it would when the liver behaves as a well-mixed compartment (7), the parameter f_m (hence k') can be estimated by the ratio of the areas of the metabolite following the intraportal administration of a metabolite and the administration of the precursor by any route (8).

This aspect of the sequential first-pass elimination of the metabolite in the modeling of metabolite kinetics occasionally may be important in therapy.

For a prodrug that is transformed into a drug that is pharmacologically active or toxic, a significant sequential first-pass elimination of the drug would lead to a lower estimate of f_m (or k') or a higher estimate for the concentrations of the metabolite. In these instances, the existing approach for the prediction of drug levels after the administration of a prodrug, based on the disposition of intravenous drug administration (13), would need to be modified.

ACKNOWLEDGMENTS

The authors would like to thank Dr. K. C. Kwan, Merck Sharp and Dohme Laboratories, West Point, Pennsylvania, for invaluable discussions.

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