ABSTRACT:

The theoretical basis of the use of the multiple indicator dilution technique to account for the heterogeneous distribution (or zonation) of enzymes in the liver was explored. The microcirculation was assumed to consist of identical capillaries perfused in parallel, with enzymatic activities for drug metabolism being distributed uniformly over the upstream half (periportal or pp) or the downstream half (perivenous or pv) of the flow path, whereas all other transport/removal processes were assumed to be homogeneously distributed. Outflow dilution profiles for parent drug and metabolite were estimated by inversion of Laplace transforms or by a finite difference method. The areas under the curves for parent and metabolite, the mean transit times of parent (MTT) and metabolite (MTTM, mean time from injection of parent to exit of metabolite from organ), and their relative dispersions (CV² or CVM²) were estimated from analytical expressions. When the influx-efflux ratio (or cellular-sinusoidal distribution ratio) for metabolite was equal to or smaller than that of the parent, the MTTM ranking was: pp < homogeneous < pv. The ranking was reversed when the influx-efflux ratio for metabolite greatly exceeded that for the parent. The presence of elimination pathways for the metabolite reduced its MTTM and CVM², more for pp than for homogeneous and pv cases.

The theory can be applied to determine enzyme zonation in multiple indicator dilution studies with use of the area under the curve for the metabolite and MTTM during prograde (from portal vein to hepatic vein) and retrograde (from hepatic vein to portal vein) perfusion.

The multiple indicator dilution (MID) technique has been used to assess the kinetics of transport and metabolism of substrates in intact organs such as the liver (Goresky, 1963; Goresky and Groom, 1984). It is based on bolus injection of labeled parent drug/metabolite and noneliminated reference indicators (labeled red blood cells, labeled albumin and sucrose, and labeled water to trace the sinusoidal blood volume, interstitial or Disse space, and cellular water space, respectively) at the inflow of the liver and has been used frequently to examine transport and metabolic processing in the dog liver (Goresky et al., 1973) or in the perfused rat liver preparation (Schwab et al., 1985; Pang et al., 1995). The theoretical basis of MID developed until now generally assumes constant enzyme activity along the sinusoidal flow path. In contrast to this, there is considerable experimental evidence that drug-metabolizing activities are heterogeneously distributed among the zonal regions of the acinus, the functional microcirculatory unit (Rappaport, 1958). The enzymes and their associated metabolic activities may be predominantly localized in the upstream [periportal (pp) or zone 1] or the downstream [perivenous (pv) or zone 3] region of the acinus (Pang and Terrell, 1981; Pang et al., 1983; Anundi et al., 1986; El Mouelhi and Kauffman, 1986; Morris et al., 1988; Xu and Pang, 1989; Pang and Chiba, 1994) or evenly distributed (Chiba et al., 1998).

A useful experimental protocol for probing enzyme zonation within the intact liver is perfusion of the liver in the prograde mode, with perfusate entering at the portal vein and leaving at the hepatic vein, versus the retrograde mode, with perfusate flowing in the opposite direction, from hepatic vein to portal vein (Pang and Terrell, 1981; Pang et al., 1983; St-Pierre et al., 1989). Retrograde perfusion effectively reverses the enzymic distributions. If outflow profiles or their moments are dependent on the localization of enzymes along the acinus, the observation of changes with reversal of the perfusion mode may be used to assess enzyme zonation.

The kinetics of drug elimination in the presence of enzyme zonation has been extensively studied in the steady state. Enzyme zonation will influence net rates of metabolism among sequential (Pang and Terrell, 1981; Pang and Stillwell, 1983; Xu and Pang, 1989) and parallel (Pang et al., 1983; Morris et al., 1988; Xu and Pang, 1989) pathways. However, the impact of enzyme zonation on the outflow profiles of formed metabolite after pulse injection of the drug has not been addressed previously. Although there is one such account on the use of metabolite data after pulse dosing to the liver preparation to ascribe enzyme zonation (Ballinger et al., 1995), the basis has been explained...
Theory

For simplicity, the microcirculation of an organ is represented by a single sinusoid, or alternately, by an array of identical sinusoids perfused in parallel. A two-zone model was developed with stepwise (incremental or decremental) change in enzymatic activity half way along the acinar flow path. Influx and efflux of parent drug and metabolite across cellular membranes of parenchymal cells, including the canalicular membrane for excretion, are assumed to occur uniformly over the whole sinusoidal flow path.

The specific model for the description of the hepatocellular entry of drug and formation of a metabolite product behind a membrane barrier is depicted in Fig. 1. The differential equations describing the transport and metabolism in a single hepatic sinusoidal flow path after pulse input have been presented previously (Goresky et al., 1993b). Due to the presence of ample fenestration of hepatic endothelial cells, the sinusoidal and the interstitial (Disse) spaces in the liver may be viewed as kinetically identical for solutes such that these are combined to form the expanded plasma space (Goresky and Groom, 1984). For a non-recirculating system (as in single pass liver perfusion), the differential equations are given as follows:

\[
\frac{\partial C_1}{\partial t} + \frac{\partial C_1}{\partial \tau} = k_{31}\theta C_3 - k_{13}C_1 + \frac{1}{Q} \delta(t) \delta(\tau)
\]

(1)

\[
\frac{\partial C_2}{\partial t} + \frac{\partial C_2}{\partial \tau} = k_{42}\theta C_4 - k_{24}C_2
\]

(2)

\[
\frac{\partial C_3}{\partial t} = \frac{k_{13}C_1}{\theta} - (k_{30} + k_{31} + k_{34})C_3
\]

(3)

\[
\frac{\partial C_4}{\partial t} = \frac{k_{24}C_2}{\theta} + k_{43}C_3 - (k_{42} + k_{40})C_4
\]

(4)

where \(C_1\) and \(C_2\) are dose-normalized tracer concentrations (fraction of dose per milliliter) of parent drug and metabolite, respectively, in the expanded (sinusoidal + interstitial) plasma space, \(C_3\), and \(C_4\) are the corresponding dose-normalized concentrations of drug and metabolite in hepatocytes, \(t\) is time, \(\tau\) is a space variable representing the cumulative transit time of a reference indicator from the entrance point of the sinusoid, or the ratio of the cumulative expanded plasma volume to sinusoidal flow, \(Q\) is the plasma flow through the liver, \(\theta\) is the ratio of the accessible cellular water space to the sinusoidal plasma volume, and \(\theta' = \theta(1 + \gamma_{ref})\), where \(\gamma_{ref}\) is the volume ratio of extracellular space of the reference indicator to the sinusoidal plasma volume. It must be noted that the reference indicator is one that occupies the same proportion of the combined sinusoidal and interstitial spaces as the tracer substance to be studied, but does not penetrate parenchymal cells (Goresky et al., 1992; Chiba et al., 1998). The value of \(\gamma_{ref}\) may differ for the parent drug and the metabolite due to differences in vascular binding. However, \(\gamma_{ref}\) is taken to be the same for drug and metabolite for this consideration, for the sake of simplification.

The transfer of the tracer label between the compartmental pools is described by a set of transfer coefficients \(k_{ij}\) each of which represents the fractional amount of tracer transferred from pool \(i\) to pool \(j\) per unit time in the source pool \(i\). Thus \(k_{11}\) and \(k_{12}\) are coefficients for influx of tracer precursor into, and efflux from, parenchymal cells; \(k_{23}\) and \(k_{43}\) are the corresponding coefficients for the metabolite; \(k_{34}\) is the coefficient for enzymic conversion of parent to metabolite; \(k_{30}\) is that for sequestration (removal) of the parent drug (other than formation of the metabolite), and \(k_{40}\) is that for sequestration of the metabolite (Fig. 1). The influx and efflux rate constants, \(k_1\) and \(k_{-1}\), have been defined previously by Goresky et al. (1973) as ratios of permeability-surface area products to the accessible cellular water space. They are related to the transfer coefficients defined in Fig. 1 according to the relations \(k_{11} = k_1\theta\) and \(k_{31} = \{k_{-1}, \) where \(k_2\) and \(k_4\) are the unbound fractions of drug in plasma and tissue, respectively. Similar relations apply to the transport coefficients for the metabolite, \(k_{34}\) and \(k_{42}\). The term \(1/Q(\theta(t)\theta(\tau))\) (where \(\theta\) is the unit impulse function) represents rapid bolus injection of the tracer dose. The solutions of eqs. 1 to 4 for the extracellular concentrations thus represent the impulse response of the system. Normalization of these solutions by multiplying by \(Q\) yields the unit impulse response per unit dose, which is equivalent to the frequency or permeability density function of transit times (Lassen and Perl, 1979; Bronikowski et al., 1987).

Algebraic Expressions for Laplace Transforms and Moments. Analytical solutions of eqs. 1 to 4 in the Laplace domain for the homogeneous case were the same as found previously (Goresky et al., 1993b; Mellick et al., 1997). The formulations of parent and metabolite in the extracellular space are:

\[
\tilde{C}_1(s) = \frac{1}{Q} e^{-k_1\theta s} \tag{5}
\]

\[
\tilde{C}_2(s) = \frac{k_1k_2k_3l_1(s+k_{40})e^{-k_{42}l_1s} - e^{-k_{31}l_1s}}{Q(\tilde{k}_1s + \tilde{k}_{42})}\tag{6}
\]

where \(\tilde{C}_1(s)\) and \(\tilde{C}_2(s)\) are the Laplace transforms of \(C_1\) and \(C_2\), respectively, \(s\) is the Laplace variable, and the exponential coefficients, \(\lambda_1\) and \(\lambda_2\), are:

\[
\lambda_1(s) = s + k_{30} - \frac{k_{31}k_{41}}{s + k_{30} + k_{31} + k_{34}}\tag{7}
\]

\[
\lambda_2(s) = s + k_{42} - \frac{k_{24}k_{42}}{s + k_{40} + k_{42}}\tag{8}
\]

The area under the curve (AUC) or zeroth moment is obtained from the following equation:

\[
\text{AUC} = \int_0^\infty \tilde{C}_1(t) dt = \lim_{s \to 0} \tilde{C}_1(s) = \frac{\lambda_1}{\lambda_2}\tag{9}
\]

The recovery (survival fraction, or availability, \(F\)) is obtained as:

\[
F = \text{AUC} \times Q\tag{10}
\]
The mean transit time (MTT) or first moment is obtained from the following equation:

\[ MTT = \frac{1}{AUC} \int_0^{\infty} tC_i(t)dt \]

\[ = -\frac{1}{AUC} \lim_{\tau \to 0} \frac{\partial \tilde{C}_i(s)}{\partial s} \quad \text{(11)} \]

Finally, the variance of the transit time (VTT) or the second moment is obtained from the following equation:

\[ VTT = \frac{1}{AUC} \int_0^{\infty} t^2 C_i(t)dt - MTT^2 \]

\[ = \frac{1}{AUC} \lim_{\tau \to 0} \frac{\partial^2 \tilde{C}_i(s)}{\partial s^2} - MTT^2 \quad \text{(12)} \]

The relative dispersion (the square of the coefficient of variation) defined as:

\[ CV^2 = \frac{VTT}{MTT^2} \]

(13)

Similar relations are obtained for the corresponding moments for the metabolite, \( AUC_{M,MTT} \), \( VTT \), and \( CV^2 \) from the metabolite curve, \( C_f(t) \).

Substitution of eqs. 5 and 6 into eqs. 9, 11, and 12 yields the following analytical expressions for the zeroth, first, and second moments of the parent drug:

\[ AUC = \frac{1}{Q} e^{\frac{k_1(k_3 + k_4)}{(k_3 + k_4)}} \]

(14)

\[ MTT = \left[ 1 + \frac{k_1k_{34}}{(k_30 + k_31 + k_34)} \right] \tau \]

(15)

\[ VTT = \frac{2k_1k_{34} \tau}{(k_30 + k_31 + k_34)} \]

(16)

Equations 14 and 15 are equivalent to those presented previously (Goresky et al., 1993b). Corresponding expressions for a preformed metabolite (administered to the liver through the portal vein) are equivalent to those for the parent drug, and are obtained by replacing \( k_{34} \) by \( k_{24}, k_{31} \) by \( k_{43}, k_{30} \) by \( k_{40} \), and setting \( k_{34} \) to zero.

For the formed metabolite, the following analytical expressions are obtained:

\[ AUC_{M,x} = \frac{k_1k_{34}k_{12}e^{\frac{k_1k_{34}+k_{34}}{k_30+k_31+k_34}} - e^{\frac{k_1k_{34}}{k_30+k_31+k_34}}}{k_2k_{42}(k_30+k_31+k_34) - k_1(k_30+k_31+k_34)(k_40+k_42)}Q \]

(17)

\[ MTT_{M,x} = \frac{k_1(k_30 + k_{34} + k_{40} + k_{42}) - k_2(k_30 + k_{34} + k_{40})}{k_1(k_30 + k_{34})} \]

\[ + \left[ 1 + \frac{k_3k_{34}}{(1-\alpha)(k_30 + k_31 + k_34)} + \frac{k_3k_{42}}{(1-\alpha)(k_40 + k_42)} \right] \tau \]

(18)

\[ VTT_{M,x} = \frac{2(k_{34} - k_{31})}{k_1(k_30 + k_{34})(k_40 + k_{42}) - k_2k_{42}(k_30 + k_{34} + k_{40})}
\]

\[ + \left[ k_3(k_30 + k_{34} + k_{40} + k_{42}) - k_2(k_30 + k_{34} + k_{40}) \right] \]

\[ + \left[ k_3k_{34} \tau + 2k_1k_{34}(k_30 + k_{34} + k_{31}) \right]
\]

\[ + \frac{1}{(1-\alpha)(k_30 + k_{34} + k_{31})} \]

\[ \tau \]

(19)

where

\[ \alpha = e^{\frac{k_1(k_{34}+k_{42})}{(k_30+k_{34})}} \]

(20)

The above algebraic expressions were obtained using MathView software (Waterloo Maple Inc., Waterloo, Ontario, Canada) on a Power Macintosh computer. Outflow profiles and their moments for a single sinusoid with uniform enzyme distribution are obtained by setting \( \tau \) equal to the total transit time for the reference indicator, \( MTT_{ref} \). The latter is determined as the ratio of volume to flow, \( MTT_{ref} = V_{ref}/Q \), where \( V_{ref} \) is the distribution volume of the reference indicator, and reflects the distribution of the tracer in the extracelullar space when entry into the hepatocellular space is negligible.

**Enzyme Zonation.** The transfer coefficient for metabolic transformation is treated as a function of \( \tau \) to represent enzyme zonation, whereas rate constants for transmembrane transport are treated as constants. For the formulation of Laplace transforms and moments of outflow profiles, the sinusoid was considered as two half sinusoids arranged in series, each with \( \tau = 0.5 \) MTT_{ref}. A stepwise change at \( \tau = 0.5 \) was assumed such that

\[ k_{34}(\tau) = (1 + \tau) \tilde{k}_{34}, \quad 0 < \tau < 0.5 \text{ MTT}_{ref} \]

(21)

\[ k_{34}(\tau) = (1 - \tau) \tilde{k}_{34}, \quad 0.5 \text{ MTT}_{ref} < \tau < \text{MTT}_{ref} \]

(22)

where \( \tilde{k}_{34} \) is the length-averaged value of \( k_{34}(\tau) \) and \( \tau \) is a heterogeneity parameter with values between \(-1\) and \(1\). In our designation, positive values of \( \tau \) denote predominantly pp enzyme distribution and negative values predominantly pv enzyme distribution. The special cases considered included the even or uniform enzyme distribution with \( \tau = 0 \), the exclusively pp enzyme distribution with \( \tau = 1 \), and the exclusively pv enzyme distribution with \( \tau = -1 \). Various values of \( \tau \), ranging from \(-1\) to \(1\), were used in the above expressions (eqs. 21 and 22) to explore the impact of intermediate enzyme zonation on the moments of drugs and metabolites.

For the protocols used in indicator dilution studies, the principles of linear systems analysis apply. In particular, the overall impulse response of subsystems connected in series is the convolution of the individual unit impulse responses (Lassen and Perl; 1979; Bronkowksi et al., 1987). Laplace transforms and AUCs are obtained as the products of those of the outflow profiles of the subsystems, whereas MTTs and VTTs are their sums. Because convolution is commutative, the overall impulse response is independent of the order in which the subsystems are connected.

In the pp case (\( \tau = 1 \)), the overall outflow profile of the metabolite is the convolution of the outflow profile of the metabolite formed from the parent drug from an upstream partial sinusoid (where \( k_{34} \) is twice the average value) and the outflow profile of an existing (preformed) metabolite for a downstream partial sinusoid. In the pv case (\( \tau = -1 \)), the overall outflow profile of the metabolite is the convolution of the outflow profile of the parent drug from an upstream partial sinusoid where no conversion takes place (\( k_{34} \) is set to zero), and the outflow profile of the metabolite formed from the parent drug from a downstream partial sinusoid (where \( k_{34} \) is set to twice the average value). With stepwise increasing or decreasing enzyme activity, the moments for the metabolite outflow curves are evaluated as follows:

\[ AUC_{M} = AUC_{M,pp} + AUC_{M,pv} + AUC_{pp} + AUC_{pv} \]

(23)

\[ MTT_{M} = \frac{(MTT_{M,pp} + MTT_{M,pv})AUC_{M,pp}AUC_{M,pv}}{AUC_{M,pp}AUC_{M,pv} + AUC_{M,pp}AUC_{M,lim}} \]

(24)
MULTIPLE INDICATOR-DILUTION AND ENZYME HETEROGENEITY

Fig. 2. Outflow profiles of parent drug from a single sinusoid with and without enzyme zonation.

Fractions recovered per unit transit time of the reference indicator, C(t) × Q × MTTref, were plotted versus normalized time, t/MTTref. Transfer coefficients for influx (k11), outflux (k13), and enzymic conversion (k14) were k11 = k13 = k14 = 4 × MTTref⁻¹ (Data Set A), k11 = 4 × MTTref⁻¹, k13 = k14 = 0.25 × MTTref⁻¹ (Set B), and k11 = k13 = k14 = 0.25 × MTTref⁻¹ (Set C). Only the returning components are shown. Each throughput component consists of an impulse function ("spike") at unit normalized time (t/MTTref = 1) with an integral of 0.018 (Sets A and B) or 0.78 (Set C).

\[ VTT_M = \frac{(VTT_{M,pp} + VTT_{pp,pp})\text{AUC}_{M,pp}\text{AUC}_{pp,pp} + \text{AUC}_{M,pp}\text{AUC}_{pp,pp}}{(VTT_{pp} + VTT_{M,pp})\text{AUC}_{pp,pp}\text{AUC}_{M,pp}} + \frac{(VTT_{M,pp} + VTT_{pp,pp})\text{AUC}_{pp,pp}\text{AUC}_{M,pp} + VTT_{M,pp}\text{AUC}_{pp,pp}}{(VTT_{pp} + VTT_{M,pp})\text{AUC}_{pp,pp}\text{AUC}_{M,pp}} + \frac{(\text{MTT}_{M,pp} + \text{MTT}_{pp,pp} - \text{MTT}_{pp} - \text{MTT}_{M,pp})^2\text{AUC}_{M,pp}\text{AUC}_{pp,pp}\text{AUC}_{pp,pp}}{(\text{AUC}_{M,pp}\text{AUC}_{pp,pp}\text{AUC}_{pp,pp} + \text{AUC}_{M,pp}\text{AUC}_{pp,pp})^2} \]  

where AUC_{pp,pp}, AUC_{M,pp}, and AUC_{pp,pp} are expressions for the AUC of the parent drug, the formed metabolite (subscript M), and the preformed metabolite (subscript PM), respectively, for the pp part of the acinus, and AUC_{M,pp}, AUC_{M,pp}, and AUC_{pp,pp} are the corresponding values for the pv part. The similar expressions for the mean transit times and variances were evaluated according to eqs. 15, 16, 18, and 19 with the appropriate values for k_{M,pp} and \tau = 0.5 MTTref. The moments of the formed metabolite (eqs. 23–25) are thus influenced by those of the parent and the preformed metabolite in the first half and second half of the liver acinus.

Calculations. The outflow profiles (impulse responses) shown in Figs. 2 and 3 were calculated according to eqs. 1 to 4 using a finite difference method with analytical evaluation of discontinuities along the front as described previously (Schwab, 1984). Alternatively, eqs. 5 and 6 were used to calculate Laplace transforms, which were then used with numerical Laplace inversion using a Fortran subroutine Inlap from IMSL (Visual Numerics, Inc., Houston, TX). The results were found to be the same with reasonable accuracy (generally <1% error). Both procedures were further verified by comparing the results for uniform enzyme distribution with those obtained with published analytical solutions (Goresky et al., 1973; 1993b). These methods provide only the returning component of the parent drug. The throughput component is denoted as an impulse function of the form \(1/Q e^{-k_{M,pp}}delta(t - MTT_{ref})\), where \(delta(t - MTT_{ref})\) is the delta or unit impulse function at \(t = MTT_{ref}\).

According to eqs. 5 and 6, the calculated outflow profiles depend on the values of the transfer coefficients and on the transit time of the reference indicator MTTref. In reporting calculated outflow profiles, transfer coefficients were normalized by multiplying by MTTref time was normalized by dividing by MTTref, and concentrations were normalized as \(C(t)MTT_{ref} = C(t)/V_{ref}\). With the normalization, outflow profiles become independent of MTTref or Q. AUCs, MTTs, and VTTs were obtained by numerical integration of the calculated curves and monoeponential extrapolation between the last calculated point and infinity. In the case of the precursor, the calculated moments were adjusted to include the throughput component, as follows:

\[ AUC = \int_{MTT_{ref}}^{\infty} C(t)dt + \frac{1}{Q} e^{-k_{M,pp}}MTT_{ref} \]  
\[ MTT = \frac{1}{AUC} \int_{MTT_{ref}}^{\infty} tC(t)dt + \frac{1}{Q} e^{-k_{M,pp}}MTT_{ref} \]  
\[ VTT = \frac{1}{AUC} \int_{MTT_{ref}}^{\infty} t^2C(t)dt + \frac{1}{Q} e^{-k_{M,pp}}MTT_{ref} - MTT^2 \]

The values obtained in this way were compared with those obtained from the analytical expressions in eqs. 14 to 19. The two values generally agreed within <1% (<2% for VTT). Because they were obtained independently, the close agreement provided confidence in the accuracy of the numerical method.

Numerical calculations were performed on a Hewlett-Packard 9000 Model 712/80 work station (Hewlett-Packard, Palo Alto, CA) equipped with a 64-bit RISC processor.

Results

For the sake of simplification, emphasis was given to representative outflow profiles and values of moments (F, MTT, and CV²) for the cases on homogeneous (even), exclusively pp (with enzymes within the first half of the liver, \(r = 1\)), and exclusively pv (with enzymes within the second half of the liver, \(r = -1\)) enzymic distributions are reported in Fig. 2 and Table 1 for the parent drug, and in Fig. 3 and Table 2 for the formed metabolite; the effect of the elimination constant of the metabolite, \(k_{4,pp}\), on the MTTM² is summarized in Fig. 4. For other enzymic distributions that were intermediate between the exclusively pp and exclusively pv cases (defined within the limits, \(-1 < r < 1\)), values for AUC_{M,pp}, MTT_{M,pp}, and CV_{M,pp}² are further shown in Fig. 5.

Outflow Profiles of Parent Drug. The outflow profiles after pulse injection for uniform enzyme distribution have been described previously for a sequestered tracer (Goresky et al., 1973; 1993b) and are now compared with those of pp and pv enzymic distributions. Normally, the outflow profiles for the parent drug consisted of two parts: the throughput component and the returning component. However, for pulse injection, the throughput component consisted of an impulse function (a “spike”) at unit normalized time (t/MTTref = 1), with an integral of 0.78 for Sets 6 and 6A, and of 0.018 for all other sets. Only the returning component was shown in Fig. 2. As expected for
irreversible conversion, the dilution outflow profiles for the parent
drug were independent of the transfer coefficients for the metabolite.
Values for AUC, MTT, and CV² were found to be always larger in the
exclusive pp or pv enzyme cases than in the homogeneous enzyme
case, but the outflow profiles for exclusively pp and pv enzyme
distributions were necessarily identical because for the cases exam-
inied, the exclusive pp and pv enzyme distributions were mirror
images of each other (Table 1). The MTTs of the parent drug in-
creased with increasing influx-efflux ratio (or cellular-sinusoidal dis-
tribution ratio, $k_{13}/k_{31}$). For example, increasing $k_{13}/k_{31}$ from 1 to 16
(compare Sets A or C to B, Fig. 2) resulted in concentrative uptake
of the drug and yielded protracted outflow profiles for the parent drug
and increased MTTs. Decreasing the influx and efflux coefficients
while maintaining their ratios constant (compare Sets A and C) led to
an increased throughput component and yielded a reduced and pro-
tracted returning component without changing the overall MTT of the
drug (Table 1).

**Metabolite Outflow Profiles for Even, pp, and pv Cases in the
Absence of Metabolite Elimination ($k_{40} = 0$).** For all cases, the
condition where formation of the metabolite from the parent drug is
the only elimination pathway of the parent drug was examined ($k_{30} =
0$, all data sets in Table 2, Fig. 3). With lack of elimination of
metabolite (Sets 1 to 6, $k_{40} = 0$), the sum of the venous recoveries
of parent drug and metabolite equals unity. AUC M was always smaller
when enzyme heterogeneity was present, and there was no difference
between the examples on the exclusive pp and pv enzyme distribu-
tions.

The MTT M s increased with increasing influx-efflux ratio ($k_{13} /
 k_{31}$ or cellular-sinusoidal distribution ratio) for the parent drug, a trend
also observed for MTT (compare Set 1 with Set 4 or Set 2 with Set 5,
Table 2). Similarly, the MTT M s increased with increasing influx-
efflux ratio for the metabolite, $k_{24}/k_{42}$ (compare Set 1 with Set 2, or
Set 4 with Set 5, Table 2). A special situation arises when the transport
parameters of the parent drug and the metabolite are equal ($k_{13} =
 k_{24}$ and $k_{31} = k_{42}$; Sets 1 and 5). In this case, the metabolite outflow
profile was necessarily identical for pp and pv enzyme distribu-
tions.

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 k_{31}$ or cellular-sinusoidal distribution ratio) for the parent drug, a trend
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 k_{24}$ and $k_{31} = k_{42}$; Sets 1 and 5). In this case, the metabolite outflow
profile was necessarily identical for pp and pv enzyme distribu-
tions.

The behavior of the parent drug in the upstream half of the sinusoid
with pv enzyme zonation was identical with that of the metabolite in

**TABLE 1**

<table>
<thead>
<tr>
<th>Zeroth, first, and second moments of parent drug that is metabolized by enzymes that are heterogeneously distributed in liver ($k_{30} = 0$)</th>
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<tbody>
<tr>
<td>Data Sets</td>
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<tr>
<td>Transfer coefficients$^a$</td>
</tr>
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<td>pv</td>
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$^a$ Transfer coefficients were normalized by multiplying by the transit time of the whole sinusoid.
$^b$ MTTs were normalized by dividing by the transit time of the whole sinusoid.
$^c$ Variances were normalized by dividing by the square of the transit time of the whole sinusoid.

Fractions recovered per unit transit time of the reference indicator, $C(t) \times Q \times MTT_{ref}$, were plotted versus normalized time, $t/MTT_{ref}$. Data Sets 1 to 6 correspond to different sets of transfer coefficients for membrane transport and enzymic conversion, as compiled in Table 2.

**Fig. 3.** Outflow profiles of metabolites from a single sinusoid, with and without enzyme zonation.
the downstream half of the sinusoid with pp enzyme zonation, because in both instances, transport coefficients were equal and removal did not occur. With linear kinetics, the overall outflow profile was independent of the order in which the two regions were connected, and the metabolite outflow profile was therefore identical in both cases.

A weaker correlation was found when membrane permeabilities differed between the parent drug and the metabolite, but the influx-efflux ratios (or cellular-plasma partition ratios) remained a constant ($k_{13}/k_{31} = k_{24}/k_{42}$; Sets 3 and 6). In these cases, the MTTs were found identical with pp and pv enzyme distributions. However, the shapes of the metabolite outflow profiles for the pp and pv cases were different (Fig. 3), resulting in differences in the relative dispersions. The latter were larger with pp enzyme distribution if the parent permeates faster than the metabolite ($k_{13}/k_{24}$; Set 3), and vice versa (Set 6).

Generally, the relations between the MTTs and enzyme zonation depended much on the relations between the influx-efflux ratios of parent drug ($k_{13}/k_{31}$) and metabolite ($k_{24}/k_{42}$). For example, when $k_{13}/k_{31} > k_{24}/k_{42}$ (Set 4), MTTs were longer with pv enzyme distribution although there was no difference in metabolite recovery ($AUC_M$). Note that in the pp case, the parent drug is converted to metabolite early on in the upstream part of the acinus, and the metabolite is delayed while traveling through the downstream part; whereas in the pv case, the parent drug is delayed in the upstream part before being transformed in the downstream part, and the metabolite then leaves the liver without being delayed. The overall mean transit time depended on the relative contributions of the parent drug and the metabolite. Because of its larger cell-plasma partition ratio, the delay for the parent drug is more pronounced than that for the metabolite, leading to a longer MTT in the pv case, when the delay of the parent drug dominates, than in the pp case. By contrast, when...

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$AUC_M^3$, recovery of metabolite; even: homogeneous acinar enzyme distribution.

Transfer coefficients were normalized by multiplying by the transit time of the whole sinusoid.

MTTs were normalized by dividing by the transit time of the whole sinusoid.

Variances were normalized by dividing by the square of the transit time of the whole sinusoid.
Metabolite Outflow Profiles for Even, pp, and pv Cases in the Presence of Metabolite Elimination ($k_{40} > 0$).

The above relationships were found to be modified with elimination of the metabolite, for example, biliary excretion. When the metabolite is eliminated ($k_{40}$), AUC$_M$ and MTT$_M$ were diminished with respect to the case where $k_{40} = 0$ (compare Sets 1 to 6 with their counterparts, Sets 1A to 6A, Table 2 and Fig. 3) and the sum of the outflow recoveries of parent drug and metabolite was <1. Metabolic zonation exerted a definitive effect; the AUC$_M$s were found to be always larger for pv than for pp regardless of the values of the transport parameters because elimination of the metabolite occurred along the entire acinus.

By contrast, the influence of $k_{40}$ on MTT$_M$ was not always consistent with a particular metabolic zonation (Table 2). When $k_{13}/k_{31} < k_{24}/k_{42}$ (Set 2), the MTT$_M$s were longer with pp than with pv enzyme distribution (Table 2).

Metabolite Outflow Profiles for Even, pp, and pv Cases in the Presence of Metabolite Elimination ($k_{40} > 0$). The above relationships were found to be modified with elimination of the metabolite, for example, biliary excretion. When the metabolite is eliminated ($k_{40} > 0$), AUC$_M$ and MTT$_M$ were diminished with respect to the case where $k_{40} = 0$ (compare Sets 1 to 6 with their counterparts, Sets 1A to 6A, Table 2 and Fig. 3) and the sum of the outflow recoveries of parent drug and metabolite was <1. Metabolic zonation exerted a definitive effect; the AUC$_M$s were found to be always larger for pv than for pp regardless of the values of the transport parameters because elimination of the metabolite occurred along the entire acinus.

By contrast, the influence of $k_{40}$ on MTT$_M$ was not always consistent with a particular metabolic zonation (Table 2). When $k_{13}/k_{31} < k_{24}/k_{42}$ (Set 2), the MTT$_M$ for pp exceeded those for pp and uniform enzyme cases. But when $k_{13}/k_{31} < k_{24}/k_{42}$ (Set 2A), the MTT$_M$ for pp was greater. Upon further exploring the influence of $k_{40}$ on MTT$_M$, the unusual pattern for Set 2A was restricted only to values of $k_{40} < 0.7 \times$ MTT$_{ref}^{-1}$. At higher $k_{40}$, this relationship was reversed (Fig. 4). Increasing the membrane permeability for the parent drug by a factor of 5 decreased the recovery of the parent drug considerably. However, the general relationships between MTT$_M$s and zonation remained the same (data not shown).

Intermediate Enzyme Zonation. When stepwise enzyme distribution along the acinar flow path was considered ($-1 < r < 1$), the values for the moments for the drug (Fig. 5) metabolites (Fig. 6) were generally found to vary in a monotonous fashion between those for exclusively pp ($r = 1$) or pv ($r = -1$) and those for even distribution ($r = 0$). Exceptions were the values of CV$_M^2$, which in some cases (Sets 2, 2A, 4, and 4A) exhibited M-shaped patterns with distinct maxima at intermediate degrees of heterogeneity ($-1 < r < 0$ or $0 < r < +1$). Although the values of AUC, MTT, and CV$_M^2$ of the parent compound depend on the degree of heterogeneity, r, a symmetrical pattern is obtained such that no change occurs with reversal of enzyme distribution (change of the sign of r, Fig. 5). In the case of the metabolite, the dependence of AUC$_M$, MTT$_M$, and CV$_M^2$ on r shows a symmetrical pattern only for Sets 1 and 5 in which drug and metabolite show equal partitioning into cells (Fig. 6).

The ratio of these moments for positive versus negative values of r represents the change observed with reversal of flow in an experiment using the prograde-retrograde protocol. The moment ratios shown in Fig. 7 represent the change in the particular moment value with predominately pp enzyme distribution upon reversal of perfusion from prograde to retrograde. For example, a predominately pv enzyme distribution would yield the reciprocals of these ratios. The effect of flow reversal is most prominent with exclusive heterogeneity ($u_r = 1$) and diminishes with less pronounced heterogeneity. When the partition ratios of parent drug and metabolite differ largely, as in Sets 2, 2A, 4, and 4A, distinct differences in MTT$_M$ were observed also at smaller variations in enzyme heterogeneity.

By contrast, CV$_M^2$, showed a more complex dependence on the degree of heterogeneity. In particular, in Set 4A, flow reversal from prograde to retrograde would effect a decrease in CV$_M^2$ when the enzyme is distributed exclusively in the pp zone, whereas with a smaller variation in acinar heterogeneity ($0.5 < |r| < 0.9$) an increase in CV$_M^2$ would occur.
Discussion

The distinct differences in outflow dilution profiles between the pp and pv enzyme distributions revealed in the present exploration suggest that the prograde/retrograde perfusion protocol may indeed be used to assess enzyme zonation. The effect of reversal of the perfusion mode (prograde to retrograde) will be most pronounced if an enzyme is located exclusively in one of two zones, but will still be present, albeit to a lesser extent, with more uniform enzyme distributions (Fig. 5).

Theoretical considerations predict that when a barrier is present between the vasculature and the enzyme within the cell, the extraction ratio and the outflow profile of the parent drug will be altered in the presence of uneven enzyme distribution (Sato et al., 1986; Goresky et al., 1993a; Cai et al., 1995). However, they will be the same for the pp and pv cases because these are mirror images of each other. The prograde/retrograde perfusion protocol does not provide information on enzyme zonation if only the outflow profile of the parent drug is assessed (Sato et al., 1986; Goresky et al., 1993a). We have therefore extended the theoretical treatment to include analysis of metabolite data. We have included these aspects in our analysis by deriving analytical expressions for a two-zone model. Although there existed other previous exploration on the moments of metabolite profiles (Mellick et al., 1997), enzyme zonation was not considered.

For linear systems, recoveries of metabolites obtained by integration of outflow dilution profiles are equivalent to those obtained at steady state (Meier and Zierler, 1954; Lassen and Perl, 1979) and can thus be used as a means of assessing enzyme heterogeneities in the same way as in steady-state approaches (Pang and Terrell, 1981; Pang et al., 1983; St-Pierre et al., 1989). An important observation is that in the prograde mode, the recovery of a metabolite that is itself eliminated is larger with pv than with pp enzyme distribution if the elimination system for the metabolite is evenly distributed (Pang and Terrell, 1981; Pang and Stillwell, 1983). Consequently, the ratio of AUCM of prograde to retrograde flow, if smaller than unity, suggests a pp enzyme distribution, and, if greater than unity, suggests a pv enzyme distribution (Tables 2 and 3). When the metabolite is not eliminated, AUCM is the same for prograde and retrograde perfusion and will not be useful for the assessment of enzyme zonation.

A useful alternative would be the observation of changes in MTTM after switching between prograde and retrograde flow, whereas changes in CVM^2 would not be interpretable because their direction may depend on the degree of heterogeneity (Fig. 7, Set 4A). The changes in MTTM are dependent on the relative magnitudes of k_{13}/k_{31}, k_{24}/k_{42}, and k_{40}. In many experimental settings, the parent drug is lipophilic and distributes readily in parenchymal cells, whereas the metabolite is hydrophilic and distributes only poorly in parenchymal cells, such that k_{13}/k_{31} > k_{24}/k_{42}. If this is the case, the ratio of the MTTM during prograde to retrograde flow, if smaller than unity, suggests a pp enzyme distribution, and, if larger than unity, suggests a pv enzyme distribution (Table 3). When k_{13}/k_{31} < k_{24}/k_{42} interpretation of the observed prograde/retrograde ratio of MTTMs becomes less defined because the ratio of the MTTM further depends on the value of k_{40} (Fig. 4, Set 2A).

Fig. 7. Effect of flow reversal on moments for the metabolite at various degrees of enzyme heterogeneity.

Each moment ratio is the ratio of AUCM (continuous lines), MTTM (long-dashed lines), or CVM^2 (short-dashed lines) at a positive value of r (r = +|r|) to the corresponding moment at the negative value of r with the same absolute value |r| (r = −|r|). These ratios represent the change in moment with predominantly pp enzyme distribution upon reversal of perfusion from prograde to retrograde; predominantly pv enzyme distribution would yield the reciprocals of these ratios. A stepwise increase or decrease of enzyme concentration along the flow path according to eqs. 21 and 22 was effected by varying the value of the heterogeneity parameter |r| between even (|r| = 0) and exclusively pp or pv (|r| = ±1).
TABLE 3

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<th>pp Enzyme Distribution</th>
<th>pv Enzyme Distribution</th>
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<td>( k_{40} &gt; 0 )</td>
<td>Prograde &lt; Retrograde</td>
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<td>high ( k_{40} )</td>
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Another example is on the deacetylation of acetylsalicylic acid in perfused liver (Mellick and Roberts, 1996). In this study, the sum of the recoveries for the parent drug (acetylsalicylic acid) and the metabolite (salicylate) was complete and hepatic metabolism of salicylate could be viewed as insignificant. The MTT of the preformed salicylate was much larger than that of the parent drug, and the observation is reasonable with the absence of elimination of salicylate (\( k_{40} = 0 \)). Although the observed difference in MTTM between progradely and retrogradely perfused livers was insignificant, a shorter MTTM resulted in four of five experiments with retrograde perfusion upon subtraction of the MTTs of sucrose, the reference indicator. There was, however, no report on \( k_{11}/k_{31} \) or \( k_{22}/k_{42} \) although these could have been deduced from the MID data. The lack of these essential data precludes the proper interpretation of enzyme zonation.

A third study is on 4-methylumbelliferyl sulfate (4MUS), which furnished similar recoveries of the desulfated metabolite 4-methylumbelliferone (4MU) during steady state with prograde and retrograde flows (Chiba et al., 1998). Unfortunately, the recoveries from the outflow dilution profiles of labeled 4MU were too low to define the MTTM properly. The influx-efflux ratio for 4MUS (\( k_{42}/k_{31} \)), though predicted to be less than that for 4MU (\( k_{42}/k_{31} \)), becomes irrelevant in the interpretation of the data because the desulfation activity of 4MUS is evenly distributed (Anundi et al., 1986; El Mouelhi and Kauffman, 1986; Chiba et al., 1998).

In summary, the present study has successfully extended the theory on outflow dilution profiles of a parent drug to its metabolite(s) generated after pulse dosing of the parent to the perfused rat liver preparation in MID experiments. It was found that zonal metabolic activity for formation of the metabolite is an important determinant of metabolic outflow profiles, and of its MTT and relative dispersion in MID studies. When coupled with prograde and retrograde flow, the MID experiments augment our ability in the assessment of zonal metabolic heterogeneity in the liver, as outlined in the changes in AUCM and MTTM of the metabolite predicted with the presence of enzyme zonation, if the cell-plasma partitioning characteristics of the parent and metabolite and elimination of the metabolite are considered. The usefulness of the method, however, needs to be re-assessed when drug removal is complicated by the presence of multiple metabolic pathways.

References


El Mouelhi M and Kauffman FC (1986) Subcellular distribution of transferases and hydrolases...


Glossary

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<th>Symbol</th>
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