HEPATIC CLEARANCE MODELS

Comparison of the Dispersion and Goresky Models in Outflow Profiles from Multiple Indicator Dilution Rat Liver Studies

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ABSTRACT:

The multiple indicator dilution (MID) technique is often used for investigation of the kinetic behavior of substrates and metabolites in eliminating organs. The present study was a systematic comparison of the utility of the Goresky model (GM) (a structural model) and the mixed-boundary dispersion model (DM) (a stochastic model) in the interpretation of influx, efflux, and removal (sequestration) coefficients, with data generated from rat liver-perfusion/MID studies. Although the GM and the DM are equivalent in their descriptions of membrane transport, they differ in their classifications of the dispersion of blood-borne elements. For the DM, the dispersion is an inverse Gaussian distribution of vascular transit times; for the GM, it is accounted for by the dispersion observed among noneliminated reference indicators (e.g., labeled red blood cells, albumin, sucrose, and H2O) or the derived reference. In this study, previously published rat liver-perfusion/MID data obtained for the glutathione conjugate of bromosulfophthalein and hippuric acid, compounds that exhibit saturable carrier-mediated transport, with the GM were reanalyzed with the two-compartment DM. When the fitted values for volume and transfer coefficients were compared, good correlation was found between the fitted vascular volume for the DM and the vascular volume for the reference indicator for the GM. The influx coefficients were generally similar between the models, but improved correspondence was observed when the DM was modified to include the large-vessel transit time. In contrast, the efflux and sequestration coefficients obtained for the DM did not correspond well to those from the GM. The disagreement was due, in part, to differences in the interpretation of the late-in-time component of the reference transit time distribution curve, which was not described well by the DM. Consequently, the residence time distribution and the relative dispersion were underestimated by the DM.

There has been major progress made in the past three decades in the field of modeling of the liver for the processing of drugs and metabolites. All of the models feature the physiological determinants of clearance, i.e., organ blood flow, vascular and tissue binding, membrane permeability, and enzymatic activity (Vmax) and affinity (Km) of intracellular enzyme systems and/or excretory apparatus for the removal of substrates. Several useful mathematical models that interrelate the deterministic variables have been developed to describe temporal events and, in particular, steady-state events, wherein drug and metabolite binding to liver tissue is completed and does not contribute to drug loss. Accordingly, the liver has been viewed as a well-mixed compartment (“well-mixed” model) (Rowland et al., 1973; Pang and Rowland, 1977) or as an organ receiving a series of nonsegregated parallel flows surrounded by identical single sheets of hepatocytes of uniform enzymatic activity (“parallel tube” model) (Winkler et al., 1973). These models have been construed as being too extreme and idealized: because there is either infinite (well-stirred model) or no (parallel tube model) mixing, they are unable to describe the asymmetrical outflow profiles or RTDs observed after bolus dosing. The inadequacy has been explained on the basis of heterogeneities in flow (Miller et al., 1979; Bronikowski et al., 1987) and a high degree of geometric branching within the liver, which provide an intermediate mixing or dispersion. This necessitates the reselection, refinement, or development of models that could describe the observations. The barrier-limited, distributed, capillary transit time model of Goresky (Goresky, 1963; Goresky et al., 1973) and the adaptation (Roberts and Rowland, 1985, 1986) of the DM of Perl and Chinard (1968) with closed boundary conditions (Danckwerts, 1953) are such model developments. These describe curve models that resemble the outflow dilution profile observed after pulse injection, and they recognize that the mixed-boundary dispersion is not an adequate description of the liver model.

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Abbreviations used are: RTD, residence time distribution; RBC, red blood cell; MID, multiple indicator dilution; GM, Goresky model; DM, dispersion model; BSPGSH, bromosulphophthalein glutathione conjugate; HA, hippuric acid; MTT, mean transit time; CV2, relative dispersion; CD, coefficient of determination; tL, large-vessel transit time; Vb, blood volume; CD, dispersion number; MSC, model selection criterion.
ognize that the shape and magnitude of the solute outflow concentration-time curve (the dilution profile) are complex functions of the interrelationships among blood and tissue binding, blood flow, vascular micromixing and flow heterogeneity, and cellular influx, efflux, and sequestration (metabolism or excretion). Many established principles have evolved through modeling of outflow data from experiments that encompass bolus injection of a dose containing a mixture of multiple noneliminated indicators and labeled tracer substrate; in these instances, differentiation among the injected species is enabled by the use of differential labeling and analyses (multichannel γ and β spectrometry). The kinetics of the diffusible tracer reflect those under steady-state conditions for the bulk compound, as mandated in tracer methodology. This MID technique, which was first introduced to assess the behavior of noneliminated reference substances for the understanding of liver physiology (Goresky, 1963), has become a useful experimental tool to provide mechanistic insight into the processes of transport and irreversible loss of the tracer-labeled substrate, especially in single-pass, rat liver-perfusion experiments, wherein the recirculation of solutes, which presents an added complication in vivo, is avoided (Wolkoff et al., 1979; Schwab et al., 1990; Geng et al., 1995).

The GM and the mathematical formulations have a long history, first being used for descriptions of the handling of endogenous compounds by dog liver in vivo (Goresky et al., 1973) and then being extended to analyses of xenobiotics (Schwab et al., 1990; Geng et al., 1995) and metabolic processing (Pang et al., 1994) in perfused rat liver. The approach involves the construction of a vascular reference curve based on an initial consideration of the binding and distribution properties of the diffusible solute in the vasculature and then appraisal of the outflow curve for the labeled tracer with respect to this reference (Schwab et al., 1990). In the past decade, the DM has been reintroduced and modified to describe substrate processing within the liver (Yano et al., 1989, 1990, 1991; Evans et al., 1991, 1993; Díaz-García et al., 1992; Hussein et al., 1994; Chou et al., 1993, 1995; Yasui et al., 1995a; Ohata et al., 1996; Nishimura et al., 1996; Ueda et al., 1997). The model has been extended to the two-compartment DM, with recognition that the sinusoidal membrane of the liver is a transport barrier to solutes, producing compartmentalization of the vascular and cellular compartments.

Close examination of the GM and the DM reveals both similarities and differences; the models share common mathematical descriptions of cellular events such as transmembrane transfer, cellular drug metabolism, and biliary excretion (fig. 1), as first noted by Rowlett and Harris (1976). These models typically characterize cellular events by the use of influx \( k_{in} \), efflux \( k_{out} \), and sequestration \( k_s \) coefficients (see definitions in table 1). However, the GM and the DM differ in their descriptions of the shapes of the transit time distributions of their respective vascular reference curves.

Because of the pervasive use of both models in the interpretation of MID data to provide mechanistic insight into hepatic drug processing, there is the need to fully understand the consistency of both models with the data. The intent of this report was, therefore, to compare the ramifications of the GM and the DM to gain a better understanding of the two methods and their implications for hepatic drug processing. From a practical standpoint, it is apparent that the GM is experimentally and computationally more demanding than the DM because of the requirement for noneliminated reference indicators. We therefore became interested in assessing the similarities and differences in the analysis of dilution data by the DM compared with the GM because the solutions are more readily available, with inclusion of fewer or no reference indicators. Data that had been processed by the GM with deconvolution of the sham experiments (dilution experiments without liver) were reexamined with the DM, to allow for comparison of the two models. We compared the fitted values of the common parameters (volumes and transfer coefficients) used by the GM and the DM to describe the hepatic disposition of two compounds that are bound only to albumin and that exhibited saturable sinusoidal (carrier-mediated) transport. These compounds were BSPGSH (Geng et al., 1995) and HA, the glycine conjugate of benzoate (Yoshimura et al., 1995). The solute enters the liver with flow \( Q \) and is processed in a distributed-in-space fashion. At each point, drug in plasma (concentration, \( C_p \)) exchanges with that in tissue \( C_t \). Sinusoidal membrane transfer coefficients are denoted by \( k_{in} \) and \( k_{out} \), the influx and efflux coefficients, respectively, whereas the cellular sequestration coefficient is represented by \( k_s \) (see table 1 for definitions).

### Table 1

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Coefficients for DM</th>
<th>Coefficients for GM</th>
<th>Physical Equivalents for GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influx</td>
<td>( k_{in} )</td>
<td>( f_{in} k_{in}' )</td>
<td>( P_{trans}/(1 + \gamma_{ref}) )</td>
</tr>
<tr>
<td>Efflux</td>
<td>( k_{out} )</td>
<td>( f_{out} k_{out}' )</td>
<td>( V_p/\gamma_{ref} )</td>
</tr>
<tr>
<td>Sequestration</td>
<td>( k_s )</td>
<td>( f_u k_{s} )</td>
<td>( f_{trans} C_{ref}/V_p )</td>
</tr>
</tbody>
</table>

**Coefficient**
- \( k_{in} \) and \( k_{out} \): influx and efflux coefficients, respectively.
- \( k_s \): cellular sequestration coefficient.

**Coefficients for DM**
- \( f_{in} \): influx partition coefficient.
- \( f_{out} \): efflux partition coefficient.
- \( v_{ref} \): reference space volume.

**Physical Equivalents for GM**
- \( P_{trans} \): the transport barrier to solutes, producing compartmentalization of the vascular and cellular compartments.
- \( V_p \): apparent space ratio (interstitial/vascular space).

**Transfer coefficients**
- For GM and their physical equivalents.
- \( k_{in} \): influx partition coefficient.
- \( k_{out} \): efflux partition coefficient.
- \( k_s \): cellular sequestration coefficient.

**Table Legend**
- 1: Transfer coefficients for the DM.
- 2: Transfer coefficients for the GM and their physical equivalents.
- 3: Physical equivalents for the GM.

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**Theoretical Considerations**

The GM. For the GM, a set of noneliminated reference indicators ordinarily accompanies the tracer-labeled substrate for injection into the liver. The noneliminated reference indicators include \( ^{51} \text{Cr} \)-labeled RBCs (the vascular reference, which is distributed only in the sinusoids), \( ^{125} \text{I} \)-labeled albumin and \( ^{14} \text{C} \)- or \( ^{3} \text{H} \)-labeled sucrose (which occupy the sinusoidal plasma and the interstitial or Disse space), and \( ^{12} \text{O} \) [a kinetic equivalent of water (Pang et al., 1991)] that is distributed throughout the vascular, interstitial, and accessible cellular water spaces of the organ. The outflow concentrations are normalized to the injected dose (fractional recovery per milliliter), with observations of the progressive dispersion of labeled RBCs, albumin, sucrose, and then water, based on their increasing spaces of distribution. Another
well-known property of the model is the ability of the dilution curves for the diffusible noneliminated reference indicator to be superimposed on the labeled RBC curve, after appropriate correction of the time and the concentration for the difference in distribution volumes (Goresky, 1963). The procedure revealed the existence of a transit time delay \( t_0 \), occurring in the nondispersing region or the large vessels, after correction for the transit times of the input and output catheters. The transit time profiles for albumin, sucrose, and water are superimposed on the RBC curve using the following relationship (Yoshimura et al., 1998),

\[
h_{\text{Ab}}(t) = \frac{1}{1 + \gamma} h_{\text{RBC}} \left( \frac{t - t_0}{1 + \gamma} + t_0 \right)
\]

where \( h(t) \) is the transfer function that describes the RTD of the reference indicator after deconvolution of the distortion caused by the inflow and outflow catheters and the injection device. The superposition procedure, when carried out by a fitting procedure, provides the optimized fitted values of \( t_0 \) and \( \gamma \), a value denoting the space ratios [stationary to moving spaces, \( i.e. \) Disse space/sinusoidal plasma for albumin and sucrose and (cellular water + Disse space)/(sinusoidal plasma water + RBC water) for D_2O; see table 1 for definition].

The basic premise of the GM for the study of propagation of tracers is the use of vascular indicators for development of an appropriate vascular reference curve. By definition, the appropriate reference curve is the hypothetical outflow profile for a given solute that would result if the solute were neither taken up nor eliminated by the cells. Depending on the experimentally determined distribution of the solute between RBCs and plasma and on plasma protein binding, the reference curve is constructed from the combined behaviors of the labeled RBC, albumin, and sucrose curves, to illustrate the vascular distribution of the labeled tracer. For example, a tracer substrate that is neither distributed into RBCs nor bound to plasma albumin is expected to behave identically to the observed sucrose curve, in terms of the shape and the estimated vascular distribution volume. In contrast, for a drug that is very highly bound to plasma protein and is not distributed into RBCs, the vascular distribution of the reference compound is similar to that for labeled albumin. By extension, a drug that is rapidly and reversibly bound to albumin but is not bound to RBCs would have a vascular reference curve that defines a distribution space intermediate between those for sucrose and albumin. In such a situation, the reference requires the construction of the following relationship (Geng et al., 1995),

\[
\gamma_{\text{ref}} = f_s \gamma_{\text{Suc}} + (1 - f_s) \gamma_{\text{Ab}}
\]

where \( \gamma_{\text{ref}} \) is the apparent distribution space (interstitial/vascular space) ratio for the tracer and \( f_s \) is the unbound plasma fraction, which is defined by conventional methods such as equilibrium dialysis or ultrafiltration. Another relationship is also used when the reference is described in relation to sucrose, which is used for fitting,

\[
\gamma_{\text{rel}} = \frac{1 + \gamma_{\text{ref}}}{1 + \gamma_{\text{Suc}}} - 1
\]

where \( \gamma_{\text{rel}} \) is the factor that describes the appropriate interstitial space reference for the drug. The apparent volume for the reference curve (\( V_{\text{ref}} \)) is thus described with respect to that for sucrose, as

\[
V_{\text{ref}} = V_{\text{Suc}}(1 + \gamma_{\text{rel}})
\]

where the total volume for sucrose, \( V_{\text{Suc}} \), can be obtained by moment analysis of the sucrose curve (catheter-corrected mean transit of sucrose × plasma flow rate).

For barrier-limited tracers, the theoretical reference transfer function may then be appropriately related to that for sucrose in describing the extracellular behavior of the labeled tracer. The organ transport function for diffusible labeled tracer can be calculated with the following equation (Yoshimura et al., 1998),

\[
h_{\text{Suc}}(t) = \sum_{n=1}^{\infty} \frac{\left( f_s k_1 \delta' \right)_{n} \left( t - t_0 \right) t^{-n+1}}{n!(n-1)!} d\tau
\]

where \( \theta \) is the ratio of the cellular water space (\( V_{\text{cell}} \)) to the sinusoidal plasma space (\( V'_p \) and \( \theta' = \theta_1 + \gamma_{\text{ref}} \)), with parameters describing the rate coefficients for influx (\( f_s k_1 \delta' \)), efflux (\( k_{-1} \)), and sequestration (\( k'_\text{seq} \)) (see definitions in table 1). The fitting procedure furnishes the estimates of \( \gamma_{\text{rel}} \) and the rate coefficients. These, in turn, provide the influx and efflux rate constants \((k_1, k_{-1})\), as defined by Goresky and co-workers (Goresky et al., 1973; Geng et al., 1995; Yoshimura et al., 1998), and the permeability surface area products for influx and efflux across the hepatocyte membrane (table 1). The first term of eq. 5 represents the throughput component or material that propagates through the system, \( i.e. \) the portion of the tracer that passes through the liver without entering parenchymal cells; this throughput component is obtained from eq. 5 by setting \( k_{-1} \to 0 \). The second term represents material that enters the liver cell and later returns to the vascular compartment, or the returning component; this is given by the difference between the total outflow profile and the throughput component.

The DM. The DM conceptualizes that the blood flowing through a labyrinth of ramified interconnecting sinuses is nonideal and causes a dispersion of noneliminated and eliminated substances (Roberts and Rowland, 1985, 1986). There are two parameters that characterize the model, \( i.e. \) \( D_{\text{sc}} \) or the inverse of the Peclet number, which describes the degree of mixing or dispersion resulting from heterogeneous blood flow through the microvasculature, and the efficiency number, \( R_N \), or the term for removal

\[
R_N = \frac{f_s C_{\text{clus}} \rho}{Q}
\]

where \( f_s \) is the unbound fraction, \( C_{\text{clus}} \) is the intrinsic clearance or the inherent ability for removal of the unbound substrate, and \( \rho \), as defined in earlier publications (Roberts and Rowland, 1985, 1986), is given by \( P/(P + C_{\text{clus}}) \), where \( P \) is the permeability. The use of \( \rho = 1 \) is justified when permeabilities far exceed the intrinsic clearance.

The dispersion observed in the outflow dilution profile for a given solute is modeled with a second-order partial differential equation that can be described in Laplace transformations after delineation of the boundary conditions. In general, the mixed-boundary DM (Roberts et al., 1988) is preferred over the closed-boundary DM of Perl and Chinard (1968) (Danckwerts, 1953; Roberts et al., 1988) because of the ease of calculation in the former. The frequency output of a solute can be presented in the Laplace domain (Evans et al., 1991),

\[
f(s) = w(s)_{\text{h1}} \cdot w(s)_{\text{h1}}
\]

where \( s \) is the Laplace operator and \( w(s)_{\text{h1}} \) and \( w(s)_{\text{h2}} \) are the transfer functions that describe the spread of solute by passage through the hepatic and nonhepatic (inflow and outflow catheters, tubing, and devices) regions, respectively. The transfer function of the frequency...
output, $f(t)$ or $QC(t)/dose$, in the absence of the liver can be described by (Evans et al., 1991)

$$w(s)_{NH} = \exp \left( 1 - \sqrt{1 + 4D_{N,NH}MTT_{NH}} \right)$$

where $D_{N,NH}$ is the nonhepatic $D_N$ and $MTT_{NH}$ is the MTT of the reference in the sham experiment (volume of nonhepatic portion/flow or $V_{NH}/Q$).

The $w(s)_{HI}$ value, or hepatic transport function of the solute, may be calculated for the one- or two-compartment DMs. The one-compartment DM is used to describe the processing of solutes that establish rapid equilibration between the vascular and the cells. For the noneliminated reference, which is not removed, the weighting function is

$$w(s)_{HI} = \exp \left( 1 - \sqrt{1 + 4D_N V_{hi}/Q} \right)$$

where $V_H$ is commonly considered as the volume of the distribution for the noneliminated reference compound in the central compartment and is the sum of the vascular volume and the Disse space for albumin or sucrose and $Q$ is the perfusate flow rate (Evans et al., 1991). For a solute that is removed in the one-compartment model (Roberts and Rowland, 1986), the following weighting function applies,

$$w(s)_{HI} = \exp \left( 1 - \sqrt{1 + (4D_N/Q)(V_{hi}^2 + p_h/CLe_{in})} \right)$$

where $V_{hi}$ is the extracellular volume (combined sinusoidal and Disse spaces) and $CLe_{in}$ is the intrinsic clearance for removal of unbound substrate.

However, with recognition that the hepatocyte membrane is a barrier that facilitates or retards the entry of solutes, producing different concentrations in the vasculature and cells, the two-compartment model has been developed for the description (Yano et al., 1989). Elimination from the vascular (Yano et al., 1990, 1991) and/or peripheral (Yano et al., 1989; Evans et al., 1991, 1993) compartments has been considered. With the assumption that the vascular and cellular compartments are physiological spaces that are equivalent to the central and peripheral compartments, respectively, which are separated by the hepatocyte membrane, cellular removal likely occurs in the peripheral compartment. Under these circumstances, the weighting function for a solute that is distributed between the extra- and cellular regions in liver is described as follows (Evans et al., 1991),

$$w(s)_{HI} = \exp \left[ 1 - \sqrt{1 + 4D_N V_{hi}(s + k_{12} - k_{21})/(s + k_{12} + k_s)} \right]$$

where $V_{hi}$ is commonly considered the extracellular volume (combined vascular and Disse spaces) and $k_{12}$, $k_{21}$, and $k_s$ are the influx, efflux, and elimination coefficients, respectively (fig. 1 and table 1).

For the DM, the curve form of the vascular reference is a modified inverse Gaussian distribution, and the same function identifies the curve profile for the vascular reference. The curve form differs from that for the GM, which is constructed from the noneliminated references. For the DM, the $V_{hi}$, the combined volume of the extracellular spaces (vascular volume + Disse space), is equivalent to the vascular volume of distribution for the reference in the GM. For the DM, the rate coefficients have been defined with respect to the compartment wherein the flux originates and have not been corrected for binding; for the GM, all of the rate constants are defined with respect to the accessible cellular water space ($V_{cell}$), and the permeability surface area products have been corrected for protein binding (table 1). As in the GM, the throughput component for the DM can be obtained by setting $k_{21}$ to 0, and the corresponding hypothetical reference curve can be determined by setting the influx parameter, $k_{12}$, to 0 (Evans et al., 1993). Again, the difference between the total outflow profile and the throughput component yields the returning component. For the DM, the transit time of the noncapillary vessels ($t_0$) has not been previously considered. The absence of this delay factor in the DM might simply have been an oversight, or the factor might have been viewed as being unimportant in the distortion of the impulse. In contrast, the GM describes the large vessels as a nonexchanging and nondispersing region characterized by its transit time, $t_0$.

### Materials and Methods

#### Data Set

Data from single-pass, rat liver-perfusion/MID experiments were used in the present analysis. The data had been previously analyzed using the GM. In the first set of studies ($N = 12$), a bolus injection of $[^3H]$BSPGSH was introduced simultaneously with the reference indicators ($[^51]Cr$-labeled RBCs, $[^123]I$-albumin, $[^14C]$sucrose, and $D_2O$) (Geng et al., 1995), under steady-state conditions, with various concentrations of BSPGSH (20–214 μM). Similarly, in another set of studies ($N = 19$), $[^1H]$HA was injected, together with the set of noneliminated reference indicators, with a background concentration of HA (1–930 μM), under steady-state conditions, with or without the presence of lactate (20 mM) or benzoate (10–873 μM), compounds that were found to inhibit HA uptake (Yoshimura et al., 1998). The outflow of $[^3H]$BSPGSH or $[^1H]$HA was expressed as the fraction of the administered dose eluting per second ($C(t)/dose$) or as the frequency output [$C(t)/dose$].

#### Data Analysis

The frequency output was analyzed according to the DM (Evans et al., 1991). Data from sham experiments, in which labeled RBCs were injected into the inflow and outflow catheters, were used to evaluate $w(s)_{NH}$ (eq. 8). Preliminary fits indicated that eq. 8 was unable to describe the observed exponentially multiphasic decline in the sham experiments. Therefore, $w(s)_{HI}$ was empirically fitted to the two-compartment DM for noneliminated references where there is no elimination

$$w(s)_{NH} = \exp \left[ 1 - \sqrt{1 + (4D_{N,NH}/Q)(s + k_{12} - k_{21})/(s + k_{12} + k_s)} \right]$$

where $D_{N,NH}$ is the nonhepatic $D_N$, $V_{NH}$ is the volume of the nonhepatic experimental system, $Q$ is the perfusate flow rate, and $k_{12}$ and $k_s$ are the parameters accounting for the biphasic decline. Least-squares fitting of the sham experiment data to eq. 12 yielded estimates of 0.0163, 1.04 ml, 0.105 sec$^{-1}$, and 0.388 sec$^{-1}$ for $D_{N,NH}$, $V_{NH}$, $k_{12}$, and $k_s$, respectively.

Because we had previously demonstrated that both BSPGSH and HA exhibit barrier limitation (carrier-mediated saturable uptake processes), the hepatic transfer function, $w(s)_{HI}$, for the two-compartment DM (eq. 11) was used. Since only hepatic uptake and efflux occur for HA, the hepatic transport function in eq. 11 was modified by setting $k_s = 0$.

Further analysis of the outflow data was performed, in which the DM included a lag time, $t_0$, that characterizes the hepatic large-vessel transit time of the GM. The $t_0$ was obtained either from the GM or from the fitting procedure. In these analyses, the frequency output was described by the following equation in the Laplace domain,

$$f(s) = w(s)_{HI} \cdot \exp(-t_0 \cdot s) \cdot w(s)_{NH}$$

The MTT and the CV$^2$ of the diffusible tracers were estimated for the DM according to the equations provided by Yano et al. (1989). The MTTs and variances from the GM were obtained by integration of the model functions with 1000 points using the trapezoid rule, with or without monoeponential extrapolation. The number of points was large enough that there should be no problem with accuracy. From these, correction of the MTTs and CV$^2$ for the catheter was made.
The dashed lines were established by linear regression (equations shown, with correlation coefficients, R), whereas the solid lines are the lines of identity.

**Fitting.** The frequency outputs were fitted to eq. 7 or 13 with the use of a weighted least-squares minimization procedure, using a Levenberg-Marquardt algorithm, found in the program SCIENTIST (Micromath Scientific Software, Salt Lake City, UT). Numerical inversion of the Laplace transforms was accomplished using the Piessens-Huysmans algorithm used by the program. All parameters (\(D_m\), \(V_B\), the transfer coefficients, and, where appropriate, \(t_0\)) were estimated during the fitting procedure with the weighting chosen at 1/observed value. For each experiment, the frequency output data were modeled with the two-compartment DM without inclusion of \(t_0\) (set A), with \(t_0\) obtained from GM (set B), and with fitted \(t_0\) (set C).

**Statistical Analysis.** All data are presented as mean ± SD. The paired \(t\) statistic was used for the comparison of paired data of sets A or C of the DM with those of the GM. Data of set A were compared with those of set C, because these are data fits of the DM with and without \(t_0\), respectively. The MSC (SCIENTIST for Windows manual; Micromath Scientific Software), a modified Akaike information criterion, was used to compare the goodness of fit to the model; a larger value for MSC suggests a superior model.

**Results**

**BSPGSH Data.** Good correlation was obtained between the estimated apparent \(V_B\) from sets A, B, and C in the DM and the vascular volume for the reference in the GM (0.90) (fig. 2 and table 2). However, these values (approximately 3–3.4 ml) were significantly greater than the volume for the vascular reference according to the GM (2.7–3 ml). Similarly, an excellent correlation (\(R > 0.91\)) was found between the influx coefficients determined by the DM (\(k_{12}\)) and those determined by the GM (\(f_j k_i \theta_j\)) (fig. 3). Paired analysis of the \(k_{12}\) values estimated in sets A, B, and C failed to show differences between the DM and the GM (\(p > 0.05\)) (table 2), although clearly the correlation improved with inclusion of \(t_0\) (fig. 3). In contrast, values for \(k_{21}\) and \(k_e\) obtained for the DM did not correspond well to the respective efflux and sequestration coefficients obtained from the GM (figs. 4 and 5 and table 2). The DM values for \(k_{21}\) and \(k_e\) were higher than those from the GM (set C compared with set A, \(p < 0.01\)) (table 2). The addition of \(t_0\) to the DM improved the correlation between the respective parameters \(k_{21}\) and \(k_e\) (compare fig. 4A with fig. 4, B and C, and compare fig. 5A with fig. 5, B and C) and increased \(D_N\) significantly (almost 3-fold) (table 2).

The fit of the BSPGSH outflow curve according to the DM was further explored by simulation of the reference curves (fig. 6A) with the fitted parameters for \(D_A\) and \(V_B\) for set C (example, at a BSPGSH concentration of 214 \(\mu M\)) and was compared with that of the GM. For the GM, the reference curve fell between that of labeled sucrose and labeled albumin, because there is substantial binding of BSPGSH to albumin (unbound fraction = 0.1); the reference curve decayed slightly faster than the sucrose curve. However, for the DM, the downslope of the reference curve decayed much more rapidly. The predicted throughput component for the DM was much smaller than that for the GM; consequently, the returning component was greater (fig. 6B). All of these discrepancies existed even though the fit of the data to the GM was only marginally better than that for the DM.

**HA Data.** Fitting was successful for all experimental data in sets A and C. However, convergence was obtained for only 14 of the 19 experiments in set B, because of the unexpectedly large \(t_0\) obtained with the GM. Hence, data from only the 14 experiments were reported among all data sets. The estimates of \(V_B\) for sets A, B, and C correlated well with the volume for the reference determined by the GM (3.0 ± 0.5 ml) (fig. 7 and table 2). The \(k_{12}\) of the DM was moderately correlated with the influx coefficient of the GM in sets A and C (fig. 8). Inclusion of \(t_0\) in the DM did not improve the correspondence for \(k_{12}\) between the DM and GM estimates (compare fig. 8A with fig. 8, B and C). However, improved correspondence in the efflux parameter (\(k_{21}\)) was observed for sets B and C with GM values when \(t_0\) was included in the DM (compare fig. 9A with fig. 9, B and C). Again, a significant increase of the \(D_N\) value was observed with the inclusion of \(t_0\) (table 2).

A representative fit of the indicator dilution profile for \([\text{H}]\text{HA}\) (930 \(\mu M\)) is shown in fig. 10. For the GM, the reference curve closely followed that for sucrose (fig. 10A), owing to the modest unbound fraction of HA in plasma (unbound fraction = 0.54). The reference curve for the DM lay close to the sucrose curve at early time points and then decayed in a monoexponential fashion. The model fit to the \([\text{H}]\text{HA}\) data was similar for the GM and the DM, although the GM tended to describe the late-in-time data better (fig. 10B). For \([\text{H}]\text{HA}\), the DM estimated a greater throughput and thus a smaller returning component, compared with the GM.

The MTT for BSPGSH estimated with the DM was significantly smaller than that from the GM; however, similar values were found for HA (table 3). Values for CV\(^2\) were generally lower for the DM than for the GM; however, significance was found only for HA and not BSPGSH.

**Discussion**

In this report, we compared the fitted values for the common parameters that characterize the hepatic disposition of two solutes
with saturable uptake (BSPGSH and HA) in the GM and the DM. From the various parameter estimates of the two models, several conclusions can be drawn. The first regards the similarities between the volume for the appropriate reference in the GM and the apparent volume of distribution in the DM and among the influx constants; the second, the greater than predicted dispersion of the vascular reference curves; and the third, the influence of the large-vessel transit time delay on $D_N$ and the rate coefficients.

### Table 2

Summary of fitted parameters from the DM (set A, DM without $t_0$; set B, DM with GM $t_0$; set C, DM with fitted $t_0$) and the GM

<table>
<thead>
<tr>
<th>Solute</th>
<th>$D_N$</th>
<th>$V_B$</th>
<th>$t_0$</th>
<th>$k_{12}$</th>
<th>$k_{21}$</th>
<th>$k_e$</th>
<th>$C_D$</th>
<th>$M_SC$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BSPGSH (13 ± 2 g liver)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM</td>
<td>NA</td>
<td></td>
<td></td>
<td>2.7 ± 0.8</td>
<td>21 ± 6</td>
<td>4.1 ± 1.1</td>
<td>0.08 ± 0.05</td>
<td>0.005 ± 0.004</td>
</tr>
<tr>
<td>DM set A</td>
<td>0.04 ± 0.01</td>
<td>3.0 ± 0.8a</td>
<td>23 ± 7b</td>
<td>0</td>
<td>0.08 ± 0.03</td>
<td>0.033 ± 0.019b</td>
<td>0.055 ± 0.009b</td>
<td>0.98 ± 0.01</td>
</tr>
<tr>
<td>DM set B</td>
<td>0.19 ± 0.06b</td>
<td>3.0 ± 0.9b</td>
<td>23 ± 7b</td>
<td>4.1 ± 1.1</td>
<td>0.08 ± 0.05</td>
<td>0.013 ± 0.009c</td>
<td>0.041 ± 0.010c</td>
<td>NA</td>
</tr>
<tr>
<td>DM set C</td>
<td>0.16 ± 0.08d</td>
<td>3.0 ± 0.9b</td>
<td>23 ± 7b</td>
<td>3.6 ± 1.2</td>
<td>0.08 ± 0.04</td>
<td>0.013 ± 0.009d</td>
<td>0.041 ± 0.008d</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td><strong>HA (11 ± 1.3 g liver)</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>GM</td>
<td>NA</td>
<td>3.0 ± 0.5</td>
<td>27 ± 4</td>
<td>4.3 ± 1.1</td>
<td>0.063 ± 0.023</td>
<td>0.091 ± 0.026</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>DM set A</td>
<td>0.08 ± 0.05</td>
<td>2.9 ± 0.5</td>
<td>26 ± 5</td>
<td>0</td>
<td>0.069 ± 0.024</td>
<td>0.073 ± 0.016c</td>
<td>0</td>
<td>0.98 ± 0.01</td>
</tr>
<tr>
<td>DM set B</td>
<td>0.43 ± 0.16c</td>
<td>3.4 ± 0.7bc</td>
<td>30 ± 5bc</td>
<td>4.3 ± 1.1</td>
<td>0.066 ± 0.069</td>
<td>0.107 ± 0.048c</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>DM set C</td>
<td>0.18 ± 0.09d</td>
<td>3.0 ± 0.5</td>
<td>27 ± 4</td>
<td>2.2 ± 1.2b</td>
<td>0.056 ± 0.018d</td>
<td>0.073 ± 0.022b</td>
<td>0</td>
<td>0.99 ± 0.01</td>
</tr>
</tbody>
</table>

*NA, not applicable.

**Significantly different from data from the GM ($p < 0.05$, paired $t$ test).

**Set B significantly different from set A ($p < 0.05$, paired $t$ test).

**Set C significantly different from set A ($p < 0.05$, paired $t$ test).

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**Fig. 3.** Relationship between influx coefficients ($k_{12}$) determined by the DM and the GM for BSPGSH (data from Geng et al., 1995).

The dashed lines were established by linear regression (equations shown, with correlation coefficients, R), whereas the solid lines are the lines of identity.

**Fig. 4.** Relationship between efflux coefficients ($k_{21}$) determined by the DM and the GM for BSPGSH (data from Geng et al., 1995).
GM \[ V_{sr}(1 + \gamma_{so}) \]. The closeness of the estimates suggests that the fitted value derived from the DM provides a sound estimate of the apparent volume of distribution for the solute in the vasculature (defined as “the reference” in the GM). This volume is not the combined volumes of the sinusoidal blood and the Disse space, as originally conceived. However, the difference is expected to be small, because the apparent volume of distribution for the solute approximates the total albumin space or the total sucrose space for drugs that are totally bound or unbound to albumin, respectively. Moreover, the \textit{a priori} assignment of \( V_B \) as 15% of the wet liver mass, as adopted in many reports (Evans \textit{et al.}, 1991, 1993), underestimates the volume for the reference in the DM and leads to poor fits (data not shown).

The influx coefficient \( (k_{12}) \) of the DM was found to be highly correlated with that \( (f_{k1}) \) of the GM (figs. 3 and 8). The observation was the result of the similarities of the respective reference curves at the early times (upswing portions of figs. 6 and 10), such that the predictions of the two models coalesced, as noted by Rowlett and Harris (1976). However, the respective reference curves eventually deviated, mostly for the later-in-time segments or the “tails” of the dilution profiles, such that the efflux and sequestration coefficients that characterize the returning component for the labeled tracer were also dissimilar. For this reason, the fit to the GM is superior (compare CD values in table 2) to that to the DM, although the fit to the DM is generally quite good, especially for the early data.

To investigate whether the large-vessel transit time could account for these differences, we included the lag time parameter \( t_0 \) in the DM, either as an assigned parameter (value of \( t_0 \) from the GM, set B) or as a fitted parameter (set C). Inclusion of \( t_0 \) in the DM (sets B and C) slightly improved the correspondence of influx, efflux, and sequestration coefficients between the DM and the GM. The most significant change occurred with \( D_N \), which increased approximately 2–3-fold as a direct result of the incorporation of \( t_0 \) (table 2). This was also found in an analysis of the DM with closed- or mixed-boundary conditions, with or without \( t_0 \) as a fitted parameter, in rat liver-perfusion/MID studies conducted with catheters of varying lengths, in which different \( D_N \) estimates were observed for the noneliminated reference indicators in the absence of \( t_0 \) (Schwab \textit{et al.}, 1998). The inclusion of \( t_0 \) in the DM provided a common \( D_N \) (\( \sim 0.22 \)) for the noneliminated indicators (labeled RBCs, albumin, sucrose, and water), as expected with the dispersive capability of the liver. The universality of this value provides full justification for including the time delay. The finding suggests that, after the initial delay, the same extent of dispersion exists for the noneliminated references, with a common \( D_N \) (varying from 0.16 to 0.31 among preparations), regardless of the distribution volumes or catheter sizes (Schwab \textit{et al.}, 1998). Interestingly, the goodness of fit was also improved when \( t_0 \) was considered in the DM in the present analysis (higher values for the CD and MSC).
The hypothesis that the large nonexchanging vessels would not add to the dispersion of solutes in the liver thus appears valid. Further support for the use of $t_0$ is provided by the excellent superposition of the curves for the diffusible noneliminated indicators onto the labeled RBC curve (scaling the time and concentration terms by the ratio of the distribution volumes) when the elapsed time is corrected for $t_0$ (Goresky, 1963). Audi et al. (1994, 1995) have demonstrated that, in the lung, virtually all of the dispersion occurs in the capillary beds and only an undetectable amount of dispersion exists in the arterial and venous trees. Taking these findings together, we conclude that the addition of $t_0$ for fitting is an appropriate refinement of the DM for hepatic modeling. However, there appears to be no consistent relationship for the $t_0$ values between the DM and the GM (fig. 11): the $t_0$ values for BSPGSH were quite similar for the DM and the GM, but the fitted $t_0$ values for the DM were consistently lower than those obtained by the superposition procedure of Goresky.

Because the large-vessel transit time could not explain the major differences in the estimates for the transfer coefficients obtained with the GM and the DM, differences in the vascular reference curves for BSPGSH or HA might be the reason for the disparities. For the DM, the reference curve is an inverse Gaussian distribution of transit times and is described by a rapid upslope followed by a monoexponential decay; for the GM, the reference curve is molded by the shapes of curves for the appropriate reference indicators, i.e., albumin and sucrose and their dispersions within the liver, because of the partial binding of the solute to albumin (figs. 6 and 10). It was readily apparent that the reference curves for the DM and the GM were similar at early time points but diverged after 2 or 3 MTTs. The small difference in the early reference curves reflected the similarities in estimates for $k_{12}$ determined by the two models. Whereas the reference curve for the DM declined monoexponentially, the corresponding profile for the GM decayed in a fashion that closely resembled that of sucrose, with a slightly protracted tail. This difference in the downslopes of the reference curves brought about uncertainties in the throughput and returning components. There was lack of a definitive pattern observed for BSPGSH and HA with regard to the throughput and returning components (figs. 6 and 10). The throughput component is highly correlated with the magnitude of $k_{12}$ and the shape and
downslope of the reference curve. When the estimated $k_{12}$ for the DM is greater than that for GM, the throughput component for the DM is greater than that for the GM and, conversely, the returning component is lower. A greater disparity exists for the returning component, which is obtained as a difference curve, especially when it is small. This is manifested as highly variable estimates for $k_{21}$ and $k_e$ with the DM and the GM.

It must be reemphasized that, for the GM, the reference curve is constructed from the full complement of noneliminated reference indicators, and its basis should be firmly established. Comparison of the outflow profile for the diffusible labeled tracer with that for the reference would, in essence, correct for the heterogeneities in capillary transit time and account for micromixing or geometric tortuosity, as claimed by some investigators of liver physiology (King et al., 1996; Weiss, 1997). The suitability of the model is apparent when the late-in-time data are well fitted and displayed in semilogarithmic plots (high CD values) (table 2). This was seldom performed with the DM for noneliminated reference indicators and the eliminated tracer solute. However, in some instances, the tailing profile for tracers such as enalaprilat (Schwab et al., 1990) and BSPGSH (in Eisai hyperbilirubinemic mutant rats) (Geng et al., 1998) could not be explained by the present DM and GM but could be modeled by the presence of a deep intracellular pool (equivalent to three-compartment DM fitting; fits not shown). In other instances, the influence of tight intracellular binding or slow diffusion (Luxon and Weisiger, 1993; Yasui et al.,

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>BSPGSH</th>
<th></th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MTT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CV&lt;sup&gt;b&lt;/sup&gt;</td>
<td>MTT&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GM</td>
<td>17.4 ± 5.4</td>
<td>1.10 ± 0.27</td>
<td>26.1 ± 3.0</td>
</tr>
<tr>
<td>DM&lt;sup&gt;e&lt;/sup&gt;</td>
<td>15.8 ± 4.3</td>
<td>0.88 ± 0.42</td>
<td>25.9 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

<sup>a</sup> MTT corrected for inflow and outflow catheters and large-vessel transit times.

<sup>b</sup> Relative variance.

<sup>c</sup> Two-compartment DM with fitted $t_0$ (set C).

<sup>d</sup> Paired $t$ test.
identical to the quantities of the named drug or its metabolites. These recommendations ensure the proper use of indicator dilution profiles in providing mechanistic insight into drug uptake processes.

References


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FIG. 11. Plots of the fitted t0 for the DM vs. the t0 for the GM, obtained with the superposition procedure, for BSPGSH (A) and HA (B).

1995b) could explain the unexpected RTDs of these solutes. For the DM, it should also be recognized that parameter identification was overestimated for efflux and removal, especially when the presence of a deep intracellular pool was evoked (fits not shown). Moreover, one must recognize the strong interrelationships between $V_H$ and $k_{12}$, in addition to $V_H$ and $D_{OA}$, with the fitting procedure.

The comparison, however, reveals the utility of the DM. To reiterate a few points, DM is simpler with respect to computation and experimental strategy than the GM. The volume for the reference and the influx coefficient (and therefore uptake clearance) are well estimated, and this should provide insight into the uptake mechanism for solutes. Also, the exact amount of the dose can be verified independently using the area of the reference curve, thus circumventing experimental errors in its volumetric assessment. For optimization of the DM, however, model fitting with $t_0$ is necessary to provide improved estimates of $V_H$ and $k_{12}$. Furthermore, proper application of tracer methodology necessitates the use of tracers in the injection dose under steady-state conditions for the bulk unlabeled compound. The appropriateness of the curve function for the sham experiment (without liver) must be demonstrated, because it is of paramount importance in the deconvolution procedure for the tracer outflow profiles. If the simple transfer function with monoeponential decay (eq. 8) were used instead of the biphasic decay curve model (eq. 12) in the analysis of the sham experiment data for the DM in the present study, $D_{OA}$, $V_H$, and $t_0$ would be overestimated, whereas $k_{12}$, $k_{21}$, and $k_3$ would be underestimated in the labeled tracer data (comparison not shown).

Experimentally, it is necessary that data collection be extended beyond 3 MTTs, and it is imperative that the radiolabels quantified are...


