Pulmonary disposition of lipophilic amine compounds in the isolated perfused rabbit lung


Departments of 1Biomedical Engineering and of 2Mathematics, Statistics and Computer Science, Marquette University, Milwaukee, 53201-1881; Departments of 3Physiology and of 4Anesthesiology and Pharmacology/Toxicology, Medical College of Wisconsin, Milwaukee, 53226; and 5Department of Veterans Affairs, Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin 53295

Audi, S. H., C. A. Dawson, J. H. Linehan, G. S. Krenz, S. B. Ahlf, and D. L. Roerig. Pulmonary disposition of lipophilic amine compounds in the isolated perfused rabbit lung. J. Appl. Physiol. 84(2): 516–530, 1998.—We measured the pulmonary venous concentration vs. time curves for [3H]alfentanil, [14C]lidocaine, and [3H]codeine after the bolus injection of each of these lipophilic amine compounds (LAC) and a vascular-reference indicator (fluorescein isothiocyanate-dextran) into the pulmonary artery of isolated perfused rabbit lungs. A range of flows and perfusate albumin concentrations was studied. To evaluate the information content of the data, we developed a kinetic model describing the pulmonary disposition of these LAC that was based on indicator dilution theory, and we sought a robust approach for interpreting the estimated model parameters. We found that the distribution of the kinetic model rate constants of the lipophilic amine-tissue interactions can be described by $\tilde{a}$, $H$, and $\tilde{V}$, where $\tilde{a}$ is a measure of the capacity of the rapidly equilibrating interactions between the lipophilic amine and the tissue; $H$ is a measure of the equilibrium capacity of the slowly equilibrating interactions between the lipophilic amine and the tissue; and $\tilde{V}$ is the mean sojourn time. The values of $\tilde{a}$, $H$, and $\tilde{V}$ were $0.8 \pm 0.1$ (SE), $0.6 \pm 0.1$, and $1.6 \pm 0.5$ s; $1.9 \pm 0.1$, $5.3 \pm 0.4$, and $5.6 \pm 0.5$ s; and $1.1 \pm 0.1$, $9.8 \pm 0.4$, and $4.7 \pm 0.2$ s for alfentanil, lidocaine, and codeine, respectively. These values for $\tilde{a}$, $H$, and $\tilde{V}$ reveal the relative dominance of the slowly equilibrating interactions for lidocaine and codeine in comparison with alfentanil. This approach to data analysis may have utility for the potential use of LAC to reveal and to quantify changes in lung tissue composition associated with lung disease.

codeine; alfentanil; lidocaine; multiple-indicator dilution method

VARIOUS LIPOPHILIC AMINES are rapidly and extensively extracted from the blood during passage through the pulmonary circulation (11, 14, 23, 37). The mechanism determining their distribution into the lung tissue appears to be simple diffusion followed by association with tissue components (9, 13, 14, 24, 25, 42). Depending on the physicochemical properties of the lipophilic amine, these associations can be rapidly and/or slowly equilibrating relative to the pulmonary capillary mean transit time ($t_c$) (13, 24, 38, 39). Most studies of the pulmonary disposition of lipophilic amines have been aimed at understanding the role of the normal lung in the initial pharmacokinetics of lipophilic amine drugs (14, 37, 39). However, there has also been interest in the possibility that lipophilic amines might be useful as indicators of certain aspects of lung function (2, 3, 11). For example, nonbasic lipophilic amines such as diazepam have been shown to equilibrate rapidly with the nonaqueous lipid fraction of the perfused lung tissue (2, 3, 11). After a bolus injection into the pulmonary artery (or systemic vein), their pulmonary venous (or systemic arterial) effluent concentration vs. time curves are scaled in time and concentration with respect to a reference indicator (confined to the vascular space on passage through the pulmonary circulation), in a manner consistent with the delayed-wave (flow-limited) phenomenon described by Goresky (17). Audi et al. (2, 3) exploited this phenomenon to estimate the pulmonary capillary transit time distribution. Dawson et al. (11) demonstrated how a rapidly equilibrating lipophilic amine such as diazepam and a hydrophilic indicator such as $\text{HOH}$ might be used to obtain an in vivo index of the wet-to-dry weight ratio of the perfused lung tissue. Pulmonary extraction of propranolol has been used in several studies to detect lung injury (33, 35).

Lipophilic amines with high negative log of acidic dissociation constant ($pK_a$) values, denoting basic lipophilic amines, tend to participate in more slowly equilibrating associations with lung tissue components than do nonbasic amines. These associations have cell type selectivity because of varying affinities for different subcellular constituents (4, 12, 20, 25, 39, 42, 44). After a pulmonary arterial injection, these more slowly equilibrating associations can be detected as flow-dependent changes in the shape of the pulmonary venous effluent concentration vs. time curves with respect to that of the vascular reference indicator.

The information content of the pulmonary venous effluent concentration vs. time data obtained in this manner with basic lipophilic amines, referred to as the multiple-indicator dilution (MID) method, has not been thoroughly examined. For example, in studies on the human lung, the indicator concentration data have been reduced to a single parameter, such as the percent uptake of the lipophilic indicator (39) or the integrated extraction ratio (22, 23). The limitation of this approach is exemplified by the demonstration by Roerig et al. (39) that the percent uptakes for lidocaine and sufentanil in rabbit lungs were reasonably similar (54 and 60%, respectively) but the mean transit time for sufentanil was 5.5 times that for lidocaine, suggesting a much slower dissociating component involved in the pulmonary disposition of sufentanil, and that the percent uptake did not reflect the differences in the shapes of

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the outflow concentration curves, which imply significantly different tissue interactions.

There are at least three objectives motivating these studies of the pulmonary disposition of lipophilic amine compounds. It is well established that physicochemical properties, reflected by \( pK_a \), octanol-to-water partition coefficient, and affinity for plasma proteins, are important determinants of tissue disposition of lipophilic amine compounds (13, 24, 38, 39). Thus one objective of studying the pulmonary disposition of these compounds is that correlations between parameters descriptive of the kinetics of their tissue disposition and parameters descriptive of their physicochemical properties will help predict the pulmonary (and other organ) disposition of members of this pharmacologically important class of compounds.

The pulmonary disposition of lipophilic amine compounds depends not only on their physicochemical properties but also on the physical and chemical properties of the lungs themselves (10, 22, 23, 33, 35, 36). The physical and chemical properties of the lungs are subject to substantial changes as a result of lung injury and disease (10, 22, 23, 33, 35, 36). Thus a second objective is to establish a basis for using this class of compounds as test indicators in the MID method to obtain quantitative diagnostic information regarding lung composition.

In addition, as a class of compounds, lipophilic amines have a wide range of physical and chemical properties (14, 25, 39, 42) providing a wide range in the kinetic parameters of their interactions with the lung tissue, and most are not significantly metabolized in the lungs (13, 38, 39). These characteristics make them particularly convenient for examining certain hypotheses regarding the information content of the MID data in general. Thus a third objective is to use this class of compounds to build a foundation for the interpretation of MID data. Accomplishment of this objective is important to the broader application of the MID method to nondestructive measurements of in vivo organ function, as well as to the pharmacokinetic predictions and measurement of changes in lung tissue composition indicated above, and it is the major focus of the present study.

The present study was carried out by using the MID method in isolated perfused rabbit lungs to begin to evaluate the information content of the pulmonary effluent concentration curves for three lipophilic amines: alfentanil, lidocaine, and codeine. These three lipophilic amines encompass a range of physicochemical properties (1–3, 7, 11, 19, 39) (reflected by the \( pK_a \), octanol-to-water partition coefficient, and affinity for plasma protein given in Table 1) and interact with lung tissue sufficiently rapidly, on the time course of the pulmonary transit time, that the interactions are detectable and quantifiable from a single pass through the lung. The pulmonary dispositions of the three amines (also referred to subsequently as test indicators) were studied over a range of flows and perfusate albumin concentrations. A kinetic model was developed to help interpret the pulmonary venous concentration profiles in terms of the test indicator-tissue interactions and to

### Table 1. Descriptors of the properties of lipophilic amine compounds alfentanil, lidocaine, and codeine thought to be important in determining their lung disposition (see Refs. 1–3, 7, 11, 19, 40)

<table>
<thead>
<tr>
<th>Compound</th>
<th>( pK_a )</th>
<th>Octanol-to-Water Partition Coefficient</th>
<th>%Bound in 4.5% BSA Solution</th>
<th>( K_a ) (per %BSA)</th>
<th>Mol Wt, g/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfentanil</td>
<td>6.5</td>
<td>126</td>
<td>92</td>
<td>2.50</td>
<td>419</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>7.9</td>
<td>21</td>
<td>78</td>
<td>0.77</td>
<td>234</td>
</tr>
<tr>
<td>Codeine</td>
<td>8.2</td>
<td>2.2</td>
<td>14</td>
<td>0.04</td>
<td>299</td>
</tr>
</tbody>
</table>

BSA, bovine serum albumin. \( K_a \), BSA equilibrium dissociation constant; \( g \) BSA\(^{-1}\)100 ml perfusate\(^{-1}\). \( pK_a \), negative log of acidic dissociation constant.
Vascular mean transit
Capillary mean transit

\( k_{i} \) Dissociation rate constant of test indicator to the \( i \)th class of associations within \( Q_t \)

\( K_i = k_{i}/k_i \) Equilibrium dissociation constant of the \( i \)th class of associations

\( k_f = \sum_{i=1}^{M} k_i[b_i] \) Association rate of the \( M \) classes of slowly equilibrating associations

\( k_i \) with \( M = M_i \)

\( K_p = k_i/k_a \) Plasma protein equilibrium dissociation constant

\( M \) Number of classes of slowly equilibrating associations

\( \tilde{M} \) Number of classes of slowly equilibrating associations resolvable from the data

\( m_c^2 \) 3rd central moment of \( h_c(t) \)

\( N \) Number of classes of associations having different dissociation rate constants

\( [P] \) Plasma protein concentration

\( P_a \) Pulmonary arterial pressure

\( P_v \) Pulmonary venous pressure

\( q \) Mass of the injected indicator

\( Q_c \) Capillary volume

\( Q_e = Q_c/b_e \) Virtual extravascular volume including \( Q_t \) and the effects of rapidly equilibrating associations in \( Q_t \) and \( Q_c \)

\( Q_t \) Tissue volume

\( [R](x,t) \) Vascular concentration of the reference indicator at distance \( x \) from the capillary inlet and time \( t \)

\( \text{RD} \) Relative dispersion of vascular reference indicator (FITC-dex) outflow curve

\( \text{RWF}(t) \) Shifted random walk function

\( \alpha \) Distance from the capillary inlet (\( x = 0 \))

\( \alpha = Q_e/Q_c \) Measure of the capacity of test indicator's rapidly equilibrating interactions within \( Q_t \), in comparison with \( Q_c \)

\( \tilde{\alpha} \) Estimated value of \( \alpha \) from experimental data

\( \beta = 1 + \sum_{i=(M+1)}^{N} \frac{[b_i]}{K_i} \) Fraction of test indicator in the vascular space that is not bound to plasma protein

\( \theta_i = \frac{k_i}{[P] + K_p} \) Sojourn time distribution

\( \epsilon = \frac{K_p}{[P] + K_p} \) Mean sojourn time [first moment of \( \Psi(t) \)]

\( \Psi(t) = \sum_{i=1}^{M} \frac{k_i[b_i]}{\beta} e^{-k_{-i}t} \) Variance or 2nd central moment of \( h_c(t) \)

\( [\Psi] = \Psi \) with \( M = \tilde{M} \) \( \sigma_c^2 \)

EXPERIMENTAL METHODS

The experiments were performed by using an isolated rabbit lung preparation previously described (1, 3). New Zealand White rabbits [2.62 ± 0.27 (SD) kg, n = 25] of either sex were given chlorpromazine hydrochloride (25 mg/kg im) followed by pentobarbital sodium (15–20 mg/kg) via an ear vein and then were heparinized (1,200 IU/kg) and exsanguinated via a carotid artery catheter. The pulmonary artery, vein, and trachea were cannulated, and a ligature was secured around the ventricles. The lungs were removed from the chest and attached to the perfusion system primed with Krebs-Ringer bicarbonate solution containing 4.5% bovine serum albumin (BSA) and 5.5 mM glucose. The perfusion system included a heated perfusate reservoir and a Master Flex roller pump, which pumped perfusate at a constant mean flow (F) from the reservoir into the lobar artery. Initially, the lung was perfused at a flow of 3.33 ml/s, with the left atrial pressure set equal to atmospheric (pleural) pressure by adjusting the height of the venous outflow into the recirculation reservoir. Pulmonary arterial (Pa) and venous (Pv) pressures were referenced to the level of the left atrium. The lung was ventilated with 95% O2-5% CO2 at 10 breaths/min under positive pressure with the use of a solenoid respirator with end-inspiratory and end-expiratory airway pressures of −7.4 ± 0.26 (SD) and 1.5 ± 0.27 cmH2O,
respectively. The perfusate was equilibrated with the respiratory gas mixture, which maintained the pH at 7.36 ± 0.09 (SD) at 37°C. Before each of the bolus injections described below, the ventilator was stopped at end expiration.

To produce a bolus injection, an injector loop (1, 3) was situated in the inflow tubing so that a 1.0-ml bolus could be rapidly introduced into the inflow stream without changing the flow or pressure. The injector consisted of a Y tube on the outflow side of the pump, which followed perfusate to flow through either of two parallel segments of tubing, each containing ~2 ml volume. A double solenoid pinch valve permitted flow through only one segment at a time. Thus a bolus injection could be made by injecting the indicators into the stagnant segment and then activating the solenoid pinch valve, so that the flow was directed through the bolus-containing segment. The 1.0-ml bolus contained 2.5 mg of FITC-dex and one of the test indicators was injected with the bolus through either of two parallel segments of tubing, each outflow side of the pump, which allowed perfusate to flow rapidly introduced into the inflow stream without changing the flow rate for the evaluation of the kinetics of the pulmonary disposition of these test indicators, as indicated below.

The concentration of the dye in the outflow samples was measured spectrophotometrically (494 nm). The [14C] and/or [3H] were calculated by liquid-liquid extraction counting. Measured quantities of the solution used as the injectate were added to sample tubes collected before the emergence of the indicators. These samples served as internal standards for calculation of indicator concentrations. At 4.5% BSA perfusate solution, the fractions of injected indicators recovered in the collected samples and calculated based on these standards were 98 ± 4.3 (SD)% for the FITC-dex, 102 ± 3.9% for [3H]alfentanil, 94 ± 3.4% for [14C]lidocaine, and 95 ± 8.5% for [3H]codeine.

Experiments performed. To determine the effect of flow (or residence time) on the measured concentration vs. time curves of each of the test indicators, a bolus containing FITC-dex and one of the test indicators was injected with the flow set at 400, 200, 100, and 50 ml/min, and the outflow was sampled at a rate of 3.33, 1.67, 0.83, and 0.42 samples/minute, respectively. The mean pulmonary vascular pressure ([P a + Pu]/2) was set equal to that at 400 ml/min for all flows by adjusting the height of the venous outflow. After each bolus injection at a specific flow, the flow was returned to 200 ml/min while preparations were made for the next bolus injection at a different flow. The flow sequence was randomized. This protocol was repeated in six, five, and six lungs with [3H]alfentanil, [14C]lidocaine, and [3H]codeine as the test indicator, respectively. The flow range was selected to encompass the normal resting cardiac output in rabbits [0.5–18 s] were extracted in ethanol and spotted on thin-layer chromatography plates, which were developed in a solvent system consisting of CHCl3-methanol-concentration of NH4OH (45:7.5:0.05). In all 30 samples, only one peak of 3H was found, which had the same retention factor (RF) (~0.5) as the injected codeine. This is consistent with the observation that compounds like codeine are not appreciably metabolized in the lung in the time course encompassed by the MID method (13, 38, 39).

EXPERIMENTAL RESULTS

Variations in perfusate flow F. Figure 1 shows examples of the measured venous effluent concentration vs. time curves for the FITC-dex reference indicator and for [14C]lidocaine (Fig. 1A), [14C]lidocaine (Fig. 1B), and [3H]codeine (Fig. 1C) at each flow. The normalized venous effluent concentration vs.
time curves for the three test indicators were separated in amplitude and time from that for the vascular reference indicator, revealing interactions with the tissue during passage through the lungs. For alfentanil, there was little change in the shape of the outflow concentration curves when the perfusate flow was changed. This behavior indicates that, within the flow range of 50–400 ml/min, the dominant alfentanil perfusate-tissue interactions approached equilibrium on a time scale that was rapid relative to the pulmonary capillary transit times. In contrast, for lidocaine and codeine, when the perfusate flow was increased, pulmonary extraction concomitantly decreased, as indicated by the rise in the peak of the outflow concentration curves. This behavior reflects interactions with the tissue constituents that equilibrate relatively slowly with respect to pulmonary capillary transit times. There were also qualitative and quantitative differences between the lidocaine and codeine outflow curves, reflecting differences in their interactions with lung tissue.

The mean pressure \( \frac{(P_a + P_v)}{2} \), vascular volume, \( F \times \) vascular mean transit tissue \( \bar{t} \), and the FITC-dex relative dispersion (RD) varied little over the eightfold range of flows at which the alfentanil, codeine, and lidocaine injections were carried out as shown in Fig. 2, A and B. The \( \bar{t} \), was calculated as the difference between the mean transit times of the FITC-dex outflow curve and tubing curve obtained with the lungs removed from the perfusion system. The RD was calculated as the ratio of the square root of the difference between the second central moments of the FITC-dex outflow curve with the lungs in the system and with the lungs removed, divided by \( \bar{t} \).

Fig. 1. Venous effluent concentration vs. time for fluorescein isothiocyanate-dextran (FITC-dex) and \([^{3}H]\) alfentanil (A), \([^{14}C]\) lidocaine (B), or \([^{3}H]\) codeine (C) after bolus injection of these indicators into pulmonary artery of an isolated rabbit lung (separate lung for each test indicator) perfused at 4 flows indicated. Concentrations on this and subsequent graphs are normalized to amount of injected indicator and are thus the fraction of injected dose per milliliter of effluent perfusate. Solid lines superimposed on data in A–C are the result of fitting Eqs. 2–3 (no. of classes of slowly equilibrating associations resolvable from data \( \hat{M} = 1 \) for alfentanil, \( \hat{M} = 2 \) for codeine and lidocaine) to outflow curves of either alfentanil, lidocaine, or codeine for all 4 flows simultaneously.

Fig. 2. A: pulmonary arterial (\( P_a \)), average [pulmonary capillary (\( P_c \)) = \( (P_a + P_v)/2 \)], and venous (\( P_v \)) pressures. B: vascular volume and relative dispersion (RD) vs. flow for bolus injections. \( n \), no. of lungs.
Variations in perfusate BSA concentration. Figure 3, A and B, shows the measured venous effluent FITC-dex and [14C]lidocaine and [3H]codeine concentrations vs. time data from two isolated rabbit lungs. Each symbol represents data obtained with a different perfusate BSA concentration as indicated. The perfusate BSA concentration had a systematic effect on the lidocaine outflow concentration curves but little effect on the codeine curves.

Figure 4 shows the measured venous effluent FITC-dex and [14C]lidocaine concentration vs. time data at two different flows with the perfusate BSA concentrations at 4.5% (top) and 2% (bottom). Both flow and perfusate BSA concentration had systematic effects on the lidocaine outflow concentration curves.

**MODEL AND THEORY**

Single capillary model. A schematic representation of the kinetic model for the pulmonary disposition of a lipophilic amine in a single capillary element is presented in Fig. 5. The capillary element is composed of a capillary volume (Qc) and a tissue volume (Qt). The model development assumes that no radial concentration gradients of the free test indicator exist within either Qc or Qt. In Qt, the test indicator associations are assumed to have a range of rate constants. Physically, these associations or interactions could be the dissolution of the lipophilic test indicator in membrane lipid or other types of interactions with the various chemical and cellular constituents of the tissue (9, 13, 14, 24, 25, 39, 42). The volume Qt is a space containing the various molecular interactions, and, as such, it need not be the same for each kind of interaction. However, as will be indicated below, the Qc for any specific interaction is not separately identifiable from the kinetic terms containing Qc and the concentrations of interacting tissue components. Thus it is parsimonious to represent the potential Qc values in the conceptual model by a single symbol as in Fig. 5. Assuming that the unbound form of the test indicator is the species having diffusional access to tissue, then plasma protein binding affects the pulmonary disposition of the test indicator and is, therefore, included in Qc (Fig. 5). Because of the low metabolic activity for lipophilic amine compounds in the lung (13, 38, 39) and the short time course of MID studies, metabolic alteration of the test indicators is not specifically included in the model.

Assuming rapid equilibration between the free and protein-bound test indicator in Qc (2, 3, 11), the spatial and temporal variations in the concentrations of the reference and test indicators are described by the
PULMONARY DISPOSITION OF LIPOPHILIC AMINES

Fig. 5. Schematic diagram of a single capillary element depicting pulmonary disposition of a lipophilic aminetest indicator in capillary (Qc) and tissue (Qt) volumes. [D](xt), vascular concentration of free test indicator at distance x from the capillary inlet at time t; [P] and F, plasma albumin concentration and flow, respectively; [DB](xt), concentration of plasma protein-bound fraction of test indicator in Qc at distance x and time t, respectively; [b], concentrations of ith (i = 1, ..., N) classes of associations within Qc; [Db], concentration of ith-bound association site; k1 and k−1, association and dissociation rate constants of the test indicator to plasma protein, respectively; L, capillary length.

Following species balance equations. In the capillary volume

\[
\frac{d[R]}{dt} + W \frac{d[R]}{dx} = 0
\]

\[
\frac{d[D]}{dt} + W \left( \frac{Q_c}{Q_c + Q_t} \right) \frac{d[D]}{dx} = \left( \frac{Q_c}{Q_c + Q_t} \right) \left( \sum_{i=1}^{N} k_i \frac{[Db]}{\beta} - \frac{[b]}{\beta} [D] \right)
\]

where N is the number of classes of associations having different dissociation rate constants. M (M < N) is the number of classes with slowly equilibrating associations, and (N − M) is the number of classes with rapidly equilibrating associations. [R](x,t) and [D](x,t) are the vascular concentrations of the reference indicator and the free test indicator at distance x from the capillary inlet and time t, respectively. Qc = (Qfβε) is a virtual volume including Qf and the effects of rapidly equilibrating associations in Qc and Qt. The ε = Kp/(P + Kp) is the fraction of the test indicator in the vascular space that is not bound to plasma protein, where [P] is the plasma protein concentration, Kp is the plasma protein equilibrium dissociation constant, and k1 and k−1 are the association and dissociation rate constants of the test indicator to plasma protein, respectively. The

\[
\beta = 1 + \sum_{i=[M+1]}^{N} [b]_i
\]
is a factor scaling Qc, which results from the (N − M) rapidly equilibrating classes of associations. K1 = k1/k−1 is the equilibrium dissociation constant of the ith class of associations, where k1 and k−1 are the association and dissociation rate constants for the ith class of associations, respectively. W is the average linear flow velocity within Qc, equal to the flow F divided by the capillary cross-sectional area. With visualization of the associations as analogous to binding to a particular molecular species, [b]i would be the concentration of ith binding species and [Db]i the concentration of the test indicator bound to that species. In the tissue volume

\[
\frac{d}{dt} \left( \frac{[Db]}{\beta} \right) = \frac{k_i [b]}{\beta} [D] - \frac{k}{\beta} [Db]_i i = 1, ..., M
\]

To model bolus injections, the solution to Eqs. 1–3 is constrained by the initial (t = 0) conditions, [D](x,0) = [Db](x,0) = [R](x,0) = 0, and boundary (x = 0) conditions [Db](0,t) = 0, and [D](0,t) = [R](0,t) = C0(t), where C0(t) is the capillary input function. The model parameters are Qc (ml), k1/β (s−1), and k−1 (s−1), i = 1, ..., M.

Organ model. Equations 1–3 are for a single capillary element. To construct an organ model, the distribution of pulmonary capillary transit times [h(t)] needs to be taken into account (1–3). Previously, we (3) have estimated that for normal rabbit lungs perfused in this perfusion system the T was ~44% of the total T, the RD of h(t), RD = σ2t0, was ~0.9, and the skewness coefficient of h(t), k3/σ3, was ~2, where σ2 and σ3 are the third central moment and SD of h(t), respectively. Table 2 shows at each of the four flows F studied the measured T and calculated T. In this study, the functional form of h(t) was approximated by using a shifted random walk function, RWF(t) (1, 29), which is a probability density function whose functional values are determined by its first three moments (1, 41).

The organ reference indicator outflow curve

\[
C_R(t) = (q/F) h_0(t) = h_0(t)
\]

where * is the convolution operator, q is the mass of the injected indicator, F is the total flow through the organ, and h0(t) is the noncapillary (arteries, veins, connecting tubing, and the injection system) transit time distribution. As described previously (1), h0(t) was also represented by a shifted random walk function, the parameters of which were specified by iteratively convolving it with h0(t) until the optimal fit to CR(t), in the least-square sense, was obtained. Because tracer concentrations were used for all test indicators, all kinetic processes are first order, and neither the actual magnitude of the organ input concentration curve [C0(t)] = (q/

Table 2. Measured total vascular and calculated capillary mean transit times at each of the 4 flows studied

<table>
<thead>
<tr>
<th>F, ml/s</th>
<th>t, s</th>
<th>t0, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.82 ± 0.007</td>
<td>11.84 ± 0.31</td>
<td>5.21 ± 0.14</td>
</tr>
<tr>
<td>1.63 ± 0.010</td>
<td>5.99 ± 0.16</td>
<td>2.64 ± 0.07</td>
</tr>
<tr>
<td>3.30 ± 0.013</td>
<td>2.92 ± 0.06</td>
<td>1.28 ± 0.03</td>
</tr>
<tr>
<td>6.65 ± 0.037</td>
<td>1.38 ± 0.03</td>
<td>0.61 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. F, flow; T, total vascular mean transit time; t0, capillary mean transit time.
F[h_i(t)] nor the fact that the total noncapillary dispersion, which includes contributions both upstream and downstream from the capillaries, needs to be specifically considered (1–3).

For given initial and boundary conditions, Eqs. 1–3 were solved numerically by using the finite-difference method (15). The solution is for a single capillary element with C_{in}(t) as the capillary input concentration curve. By virtue of the form of Eqs. 1–3, the model solution for a single capillary having the maximum capillary transit time also provides the output for all capillary transit times between the minimum and maximum capillary transit times (1, 29). To provide the whole organ output for vascular reference indicator C_{R}(t) and test indicator C_{T}(t), the outputs for all transit times are summed, each weighted according to s(t_i)

Estimation of model parameters. An objective of the mathematical modeling is to determine what can be learned about the kinetics of the lung disposition of each of the three test indicators studied by using the MID method. However, the resolution of the indicator dilution data and its impact on sensitivity to each of the M classes of associations limits the identifiability and estimability of the kinetic parameters for each of these classes of associations. Therefore, the approach was to represent the potentially large number of classes of associations M by the smallest number (M) required to fit the data over the range of flow rates studied. We used the F-test for nested models to determine M, the number of different classes of associations resolvable from the data (34).

RESULTS OF MODEL ANALYSIS

Variations in perfusate flow F. In the analysis of the data for each of the three test indicators, access of the test indicators to the sites of association is assumed to be flow independent (1). Thus, for each test indicator, the kinetic-model parameters were estimated by simultaneously fitting the model to the test indicator outflow curves obtained at all flows with one set of parameters. Parameter optimization was carried out by using the IMSL subroutine DBCLSF, which solves the nonlinear least squares problem by using a modified Levenberg-Marquardt algorithm with finite-difference Jacobian (32). The optimization procedure was carried out to estimate one set of parameter values for which the model (Eqs. 2–3) best fits the test indicator venous effluent concentration-time data from all four flows simultaneously. The optimization was first carried out with M = 0. The value of M was increased successively until there was no significant improvement, as indicated by the F-ratio (34). According to this criterion, Eqs. 2–3 with M = 1 provided the best fit for alfentanil, and Eqs. 2–3 with M = 2 provided the best fit for lidocaine and codeine. The solid lines superimposed on the data in Fig. 1 are the model fit to the alfentanil, lidocaine, or codeine data. The model parameter estimates from the model fits and different measures of precision of these estimates (27) are given in Table 3.

Interpretation of model parameters. The expectation that a particular model parameter will provide a robust representation of a particular kinetic process is not high, since the test indicator-tissue interactions are probably better thought of as involving many types of molecular interactions, with a range of kinetics reflective of the complexity of the tissue composition, as indicated in the model development. In addition, there is no obvious reason to expect that the ith kinetic parameter characterizes the same physicochemical phenomenon for more than one test indicator or set of experimental conditions. Thus, in what follows, the model parameters are viewed as representing the continuous distribution of the test indicator-tissue interactions by a parsimonious number of classes of interactions (the smallest number M needed to fit the data for a given test indicator over a given range of flows) for which parameter estimability can be shown. The question then becomes, What can be learned about this distribution that can be quantified from the kinetic parameters of the M classes of associations?

As shown in the APPENDIX, substituting the solution of Eq. 3 into Eq. 2 reduces Eqs. 2 and 3 into the following

\[
\frac{\partial[D]}{\partial t} + W\left(1 + \frac{1}{1 + \alpha}\right)\frac{\partial[D]}{\partial x} = -\left(1 + \alpha \right) k_1[D] \left(1 + \alpha \right) k_1[D] + \left(1 + \alpha \right) k_1 \int_0^t \Psi(t-\tau)[D]d\tau
\]

where

\[
\Psi(t) = \sum_{i=1}^M \left( k_i \frac{k_i[b_i]}{\beta} \right) e^{-k_i t} \left( \sum_{i=1}^M k_i \frac{k_i[b_i]}{\beta} \right)
\]

The \( \Psi(t) \), referred to as the sojourn time distribution, has the same form as Eq. 7 in Gitterman and Weiss (16) and \( f(t) \) (Eq. 24, as \( d \to 0 \)) in Weiss and Roberts (43). One useful descriptor of \( \Psi(t) \) is the mean sojourn time \( \Psi \) [the first moment of \( \Psi(t) \)]

\[
\Psi = \sum_{i=1}^M \frac{k_i[k_i]}{k_i \beta} \frac{k_i}{k_i \beta}
\]

Thus, for a given test indicator, the rapidly (relative to the \( t_e \)) equilibrating associations of the test indicator within \( Q_i \) and \( Q_o \) are not mathematically distinguishable from each other and are all represented by \( \alpha \). The descriptors of the M classes of slowly equilibrating associations are described by the sum of association rates \( k_i \) (s\(^{-1}\)) and by the \( \Psi \) (s).

Physically \( \alpha \) and

\[
H = (\alpha k_i \Psi) = \sum_{i=1}^M \frac{k_i}{k_i}
\]
Table 3. Kinetic parameters and measures of precision in the estimates of their values obtained by fitting Eqs. 2–3 to the outflow curves of alfentanil, lidocaine, and codeine from all 4 flows simultaneously

<table>
<thead>
<tr>
<th>Parameter Model Parameters</th>
<th>Alfentanil</th>
<th>Lidocaine</th>
<th>Codeine</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_i ) ( \beta ) ( S^{-1} )</td>
<td>0.54 0.17 0.082 ± 0.049 20.9 ± 21.8 0.16 ± 0.09 = 6 1.0</td>
<td>0.42 0.04 0.023 ± 0.0063 5.5 ± 1.90 0.044 ± 0.133 = 6 1.0</td>
<td>0.058 0.006 0.0071 ± 0.0029 12.8 ± 6.62 0.014 ± 0.0056 -0.14 ± 0.15 -0.18 ± 0.017 0.61 ± 0.056 0.88 ± 0.032 1.0</td>
</tr>
<tr>
<td>( Q_c ) ml</td>
<td>3.30 0.39 0.15 ± 0.076 4.4 ± 1.49 0.22 ± 0.098 -0.78 ± 0.093 1.0</td>
<td>0.080 0.020 0.014 ± 0.0033 20.6 ± 7.62 0.028 ± 0.0056 -0.14 ± 0.27 -0.29 ± 0.016 0.83 ± 0.029 1.0</td>
<td>0.058 0.006 0.0071 ± 0.0029 12.8 ± 6.62 0.014 ± 0.0056 -0.14 ± 0.15 -0.18 ± 0.017 0.61 ± 0.056 0.88 ± 0.032 1.0</td>
</tr>
<tr>
<td>( k_{1-s} ) S (^{-1} )</td>
<td>0.721 0.135 0.14 ± 0.16 17.3 ± 15.19 0.20 ± 0.18 0.88 ± 0.037 -0.59 ± 0.081 1.0</td>
<td>0.080 0.020 0.014 ± 0.0033 20.6 ± 7.62 0.028 ± 0.0056 -0.14 ± 0.27 -0.29 ± 0.016 0.83 ± 0.029 1.0</td>
<td>0.058 0.006 0.0071 ± 0.0029 12.8 ± 6.62 0.014 ± 0.0056 -0.14 ± 0.15 -0.18 ± 0.017 0.61 ± 0.056 0.88 ± 0.032 1.0</td>
</tr>
</tbody>
</table>

Values for kinetic parameters are means ± SE; \( n \) no. of lungs. \( Q_c \), virtual volume including tissue volume (\( Q_t \)) and effects of the rapidly equilibrating associations of the test indicator in \( Q_t \) and capillary volume (\( Q_c \), \( k_i \), Dissociation rate constant of the \( i \)th class of slowly equilibrating associations. \( (k_i/b_i)\beta \), Association rate constant of the \( i \)th class of slowly equilibrating associations scaled by the effect of rapidly equilibrating test indicator-tissue interactions. Second column is mean of the parameter estimates for alfentanil (\( n = 6 \)), lidocaine (\( n = 5 \)), and codeine (\( n = 6 \)) lungs studied. Third column, \( SE \) of mean of model parameter estimates for \( n \) lungs studied. CV, coefficient of variation, between data and the simultaneous model fit to outflow curves from all 4 flows, which was 11.7 ± 0.9 for alfentanil, 9.7 ± 0.7 for lidocaine, and 14.2 ± 1.4 for codeine. Measures of precision of the parameter estimates from data for each lung are given in remaining columns. For \( i \)th parameter, SD is the \( i \)th diagonal element of covariance matrix. CV is for a parameter estimate, CV = 100 × SD/estimated value of model parameter. The \( \pm \) for last 7 columns is standard deviation among \( n \) lungs studied.

where \( \theta_i = \alpha_i/b_i/\beta \) are partition coefficients reflecting the ratio of the amount of the test indicator within the \( Q_t \) to the amount in the \( Q_c \). Thus, \( \alpha_i \) is a measure of the capacity of the test indicator’s rapidly equilibrating interactions within \( Q_t \) in comparison with \( Q_c \), and \( H \) is a measure of the equilibrium capacity of the slowly equilibrating associations within \( Q_t \) in comparison with \( Q_c \), that are accessible to the test indicator within the range of capillary transit times represented in the data.

For a given test indicator, \( \tilde{\alpha}, \tilde{k}_i, \tilde{\nu}, \) and \( \tilde{H} \) are the estimates of \( \alpha, k_i, \nu, \) and \( H \), respectively, using Eqs. 5 and 6 with \( M = M \). Table 4 gives the mean values of \( \tilde{\alpha}, \tilde{k}_i, \tilde{\nu}, \) and \( \tilde{H} \) for alfentanil, lidocaine, and codeine estimated from the values of the kinetic parameters summarized in Table 3 by using Eqs. 5 and 6.

Model fit to individual flows. To help further reveal the motivation for casting the kinetic parameters in terms of \( \tilde{\alpha}, \tilde{k}_i, \tilde{\nu}, \) and \( \tilde{H} \), we fit the model to the data from each individual injection independently. As with the simultaneous fits to all flows, this was done by successively increasing \( M \) (0, to 1, to 2) until there was no significant improvement in the F-ratio. The result was that the model with \( M = 0 \) or 1 fit the alfentanil data, and the model with \( M = 1 \) fit all the lidocaine and codeine data. The resulting model parameters as a
function of flow are shown in Fig. 6. The overall coefficients of variation for the model fits to the data were 6.8 ± 0.44, 7.1 ± 0.43, and 13.4 ± 0.5% for alfentanil, lidocaine, and codeine, respectively, which were smaller than the values for the global fit (Table 3). However, for all three test indicators, the estimated values of the model parameters and the resulting $\tilde{a}$, $\tilde{k}_f$, and $\tilde{C}$ were flow dependent. This is a reflection of the flow-dependent correlations between model parameters, as is discussed below.

Variations in perfusate BSA concentration $[P]$. In Eqs. 2–4, $\alpha$ is the model parameter that is dependent on the perfusate albumin concentration $[P]$ by the following relationship

$$\frac{1}{\alpha} = \frac{Q_c}{Q_t} + \frac{Q_c[P]}{Q_t\beta K_P}$$

(7)

Equation 7 predicts a linear relationship between $1/\alpha$ and $[P]$ such that $K_P$ is equal to the intercept-to-slope ratio.

To examine the model prediction of the influence of plasma protein binding, Eqs. 2–3 with $M = 2$ were fit to each of the lidocaine outflow curves obtained during perfusion with a particular perfusate BSA concentra-

| Table 5. Values of $\tilde{a}$ at 5 different perfusate albumin concentrations estimated by fitting Eqs. 2–3 to data obtained at 5 perfusate BSA concentrations with only $Q_e$ as a free parameter |
|---|---|---|---|---|---|
| BSA | $\tilde{a}$ | CVm |
| 6% | 1.60 ± 0.36 | 14.7 ± 1.3 |
| 4.5% | 1.99 ± 0.44 | 11.6 ± 1.0 |
| 3.5% | 2.39 ± 0.43 | 10.6 ± 1.9 |
| 2.5% | 2.98 ± 0.48 | 9.2 ± 1.5 |
| 1.5% | 3.95 ± 0.68 | 9.8 ± 1.0 |

Values are means ± SE; $n = 4$ lungs. CVm, coefficient of variation between the data and the model fit to the outflow curves of lidocaine at each of 5 perfusate bovine serum albumin (BSA) concentrations.

Fig. 6. Effect of flow on estimated kinetic parameters (A–C) for alfentanil, lidocaine, and codeine obtained by separately fitting Eqs. 2–3 ($M = 1$ or 0 for alfentanil, and $M = 1$ for lidocaine and codeine) to the data from each flow. $n$, No. of lungs.

Fig. 7. Example of relationship between the estimated values of $1/\tilde{a}$ for lidocaine (●) and plasma BSA concentration ([P]) from 1 experiment. Solid line is the linear regression line based on Eq. 7.
4.5% perfusate BSA concentration from the same lung. The solid lines in Fig. 4 are an example of the model fit to lidocaine outflow curves. The estimated values of $\tilde{\alpha}$, $\tilde{k}_i$, and $\tilde{\Psi}$ from the lidocaine outflow curves at 400 and 100 ml/min with 4.5% perfusate BSA concentration (Table 6) are consistent with those in Table 3 estimated from the outflow curves at 400, 200, 100, and 50 ml/min. In addition, the predicted value of $K_p$ based on the flow data at 4.5 and 2% BSA concentration was $\sim 1.04 \pm 0.32\%$ BSA, which is also not far from the equilibrium value. For alfentanil, a similar analysis was carried out in a previous study (3).

The solid line superimposed on the codeine data in Fig. 3B is a simulated outflow curve generated by using Eqs. 2–3 with the average parameter values in Table 3 for codeine. The lack of a detectable systematic effect of albumin concentration on codeine concentration vs. time outflow curves vitiated the use of Eqs. 7 for codeine.

**DISCUSSION AND CONCLUSIONS**

Differences in lung disposition of alfentanil, lidocaine, and codeine are directly observable in the data shown in Fig. 1. A major objective of this study has been to provide a quantitative kinetic description useful for making comparisons between test indicators having different physical and chemical properties or between experimental conditions for a given test indicator. The kinetic model described by Eqs. 2–3 represents a standard approach to data analysis (1, 2, 7, 16, 18, 29, 30). However, the kinetic parameter estimates obtained by fitting such a model to a particular set of data are equivocal for making these kinds of comparisons. The results demonstrate that the estimates of the model parameters depend strongly on the range of capillary transit times (flows) encompassed by the data, which reinforces the view that there is no obvious reason to expect that the kinetic parameter characterizes the same physicochemical phenomenon for more than one test indicator or set of experimental conditions. The approach we have taken to address this problem is to consider the vector of estimated kinetic parameters as reflecting the distribution of the kinetic parameters of the classes of interactions between test indicator and the perfusate and lung tissue components that are quantitatively significant in determining the disposition of the indicator within the range of transit times encompassed by the data. The descriptors of the overall kinetics, $\tilde{\alpha}$, $\tilde{k}_i$, and $\tilde{\Psi}$, are the means for making the desired comparisons. To help put the mathematical issues and their solution developed herein into perspective, we generated simulated indicator concentration vs. time outflow curves over the experimental flow range, 50–400 ml/min, by using model Eqs. 2–3 with a distribution of six classes of slowly equilibrating indicator-tissue associations. Table 7 summarizes the distribution of the association and dissociation rate constants among the six $(M = 6)$ classes of associations. The parameter descriptive of the rapidly equilibrating associations, $Q_w$, was set at 5.0 ml. The simulated concentration vs. time outflow curves, generated by using Table 7 values and $Q_w = 5$ ml for each of the four flows, are shown in Fig. 8 in the same format as the experimental data in Fig. 1. Comparison of Table 8 with Table 4 shows that the calculated descriptors of the $(M = 6)$ distribution, $\tilde{\alpha}$, $\tilde{k}_i$, and $\tilde{\Psi}$, are similar to $\alpha$, $k_i$, and $\Psi$, respectively, estimated for lidocaine and codeine (Table 4). Treating the model simulations in Fig. 8 as data, the model Eqs. 2–3 with $M = 1$ and $M = 2$ were each fit to the simulated outflow curves for all four flows simultaneously. The results are given in Table 8 and demonstrate two particularly relevant points. First, Eqs. 2–3 with $M = 2$ yield a very good fit to the outflow curves in Fig. 8 simulated by using Eqs. 2–3 with $M = 6$. The coefficient of variation was <1%, which means, from the experimental point of view, that the model fit with $M = 2$ was indistinguishable from the actual $M = 6$ model simulation output. The model fit with $M = 1$ was not quite as good (coefficient of variation = 14.5%) and would probably be distinguishable experimentally. The second point is that the ability to fit the $M = 6$ simulations with a $M < 6$ model, in which the parameters have no obvious correspondence with the "actual" $M = 6$ simulated parameters, reveals that the estimability of the model parameters is not sufficient to equate those individual parameters with any specific interaction of the test indicator within the tissue. On the other hand, Table 8 shows that, once the model had a sufficient number of parameters to fit the data, the values of $\tilde{\alpha}$, $\tilde{k}_i$, and $\tilde{\Psi}$ approximate $\alpha$, $k_i$, and $\Psi$, respectively, quite well. Thus, $\tilde{\alpha}$, $\tilde{k}_i$, and $\tilde{\Psi}$ can provide a relatively robust description of the interactions of the test indicator with lung tissue.

**Table 6.** $\tilde{\alpha}$, $\tilde{k}_i$, and $\tilde{\Psi}$ calculated by using Eqs. 5–6 from kinetic parameters estimated by simultaneously fitting Eqs. 2–3 to the outflow curves of lidocaine from 2 flows (100 and 400 ml/min) at either 2 or 4.5% perfusate BSA concentration

<table>
<thead>
<tr>
<th>BSA (ml)</th>
<th>$\tilde{\alpha}$</th>
<th>$\tilde{k}_i$</th>
<th>$\tilde{\Psi}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2%</td>
<td>$3.53 \pm 0.12$</td>
<td>$1.92 \pm 0.085$</td>
<td>$0.57 \pm 0.043$</td>
</tr>
<tr>
<td>4.5%</td>
<td>$5.71 \pm 0.35$</td>
<td>$5.12 \pm 0.64$</td>
<td>$5.71 \pm 0.35$</td>
</tr>
</tbody>
</table>

Values are means $\pm SE$; $n$, no. of lungs. CV, values between the data and the model fits to lidocaine outflow curves at either 2 or 4.5% perfusate BSA concentration were $13.6 \pm 0.4$ and $8.9 \pm 0.7\%$, respectively. See text for details.

**Table 7.** Values of the association, $k_i(b_i)/\beta$, and dissociation, $k_{-i}$, $(i = 1, \ldots, 6)$ rate constants of 6 classes $(M = 6)$ of slowly equilibrating associations used to generate simulated concentration vs. time outflow curves in Fig. 8

<table>
<thead>
<tr>
<th>$k_i(b_i)$, s$^{-1}$</th>
<th>$k_{-i}$, s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.72</td>
</tr>
<tr>
<td>0.65</td>
<td>0.57</td>
</tr>
<tr>
<td>0.25</td>
<td>0.37</td>
</tr>
<tr>
<td>0.10</td>
<td>0.20</td>
</tr>
<tr>
<td>0.15</td>
<td>0.058</td>
</tr>
<tr>
<td>0.08</td>
<td>0.048</td>
</tr>
</tbody>
</table>
without requiring specific knowledge of the number or types of interactions involved.

When the above approach is used, differences in the lung disposition of alfentanil, lidocaine, and codeine, observable in Fig. 1, can be appreciated from Table 4. The sum of $\alpha$ and $H = (\tilde{\alpha} k_f C)$ represents the equilibrium partition coefficient involving both the rapid and the slow interactions that have a quantitative impact within the range of transit times represented in the data. For alfentanil, this sum was smaller than for lidocaine and codeine, as reflected in the relatively small separation of alfentanil outflow curve from FITC-dex outflow curve seen on Fig. 1. In addition, for alfentanil, the mean sojourn time $\bar{\tau}$ for the slowly equilibrating associations was only $\sim 1.6$ s, which is within the range of $t_c$ (see $t_c$ in Table 2) studied. This is consistent with the nearly flow-limited behavior of alfentanil; that is, with the fact that there was little change in the shape of its venous effluent concentration curves on a normalized time basis with flow in that range. Lidocaine and codeine had more extensive uptake during passage through the lungs, as reflected by the larger values of $\tilde{\alpha}$ and, particularly, $\hat{H}$. Their $\bar{\tau}$ values were close to the $t_c$ at the lowest flow studied (at 50 ml/min), reflecting the greater influence of the slowly equilibrating interactions.

Effect of flow on model parameters. The model simulations in Fig. 8 also reveal another aspect of the problem with parameter interpretation, namely, the flow, or transit time, dependence of the calculated parameters when the model was fit to the data from each flow separately. Figure 9 shows similar plots for the parameters of Eqs. 2–3 ($M = 1$) fit to the simulated data ($M = 6$) for each flow separately. This flow dependency is a manifestation of the flow dependency of the correlation between parameters revealed by the sensitivity analysis discussed below. This result is an important demonstration of the difficulty inherent in attempting to use the MID method for examining hypotheses regarding the effect of flow on the kinetics of blood-tissue interactions. For example, an increase in an extensive kinetic parameter (e.g., $H$) with flow is not sufficient evidence that an actual extensive property of the lung such as perfused surface area (i.e., vascular recruitment providing access to a larger $Q_t$) was in-

to the data from each flow separately, the parsimonious $M$ decreased from 2 to 1. The model fits to the data were quite good, but the parameter estimates were flow dependent. Figure 9 shows similar plots for the parameters of Eqs. 2–3 ($M = 1$) fit to the simulated data ($M = 6$) for each flow separately. This flow dependency is a manifestation of the flow dependency of the correlation between parameters revealed by the sensitivity analysis discussed below. This result is an important demonstration of the difficulty inherent in attempting to use the MID method for examining hypotheses regarding the effect of flow on the kinetics of blood-tissue interactions. For example, an increase in an extensive kinetic parameter (e.g., $H$) with flow is not sufficient evidence that an actual extensive property of the lung such as perfused surface area (i.e., vascular recruitment providing access to a larger $Q_t$) was in-

Table 8. $\alpha$, $k_t$, and $\bar{\tau}$ for $M = 6$ simulations; $\tilde{\alpha}$, $\tilde{k}_t$, and $\tilde{\bar{\tau}}$ estimated by fitting simulated data with $M = 1$, and $M = 2$

<table>
<thead>
<tr>
<th>Flow (ml/min)</th>
<th>Exact model</th>
<th>$\alpha$, $k_t$ s$^{-1}$</th>
<th>$\bar{\tau}$, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 ml/min</td>
<td>1.25</td>
<td>1.48</td>
<td>4.67</td>
</tr>
<tr>
<td>100 ml/min</td>
<td>1.27</td>
<td>1.42</td>
<td>4.84</td>
</tr>
<tr>
<td>200 ml/min</td>
<td>1.53</td>
<td>0.84</td>
<td>1.11</td>
</tr>
<tr>
<td>400 ml/min</td>
<td>1.14</td>
<td>1.11</td>
<td>14.5</td>
</tr>
</tbody>
</table>

$CV_m$ is between simulated data and simultaneous fit of Eqs. 2–3 to outflow the coapplication of variation curves of the hypothetical test indicator from all 4 flows.

Fig. 8. Simulated venous effluent concentration vs. time outflow curves for a vascular reference indicator $[C_R(t)]$ and a hypothetical test indicator $[C_D(t)]$ at 4 flows. Simulation model has the 6 ($M = 6$) slowly equilibrating classes of associations indicated in Table 7.

Fig. 9. Kinetic parameters (A–C) estimated by fitting Eqs. 2–3 ($M = 1$) to simulated hypothetical test indicator venous effluent concentration vs. time curve from each flow separately.
creased (1, 30). Therefore, interpretation of a parameter change associated with a change in flow for in vivo study could be ambiguous.

Sensitivity, identifiability, and estimability analysis. To put the flow dependence of the parameter estimates in perspective, a sensitivity analysis is useful. For a given model where $\lambda$ is the vector of $N$ parameters, let $C_0(t)$ be the time-dependent venous effluent concentration function predicted by the model for given parameter values. For the model output, the sensitivity function ($S$) with respect to the $j$th parameter, $\lambda_j$ ($j = 1, \ldots, N$), is defined as

$$S_j(t) = \frac{\partial C_0(t)}{\partial \lambda_j} = \frac{\Delta C_0(t)}{\Delta \lambda_j}$$  \hspace{1cm} (8)

A parameter is said to be sensible if its sensitivity function is different from zero (5). In this study, $S_j(t)$ is approximated as the change in $C_0(t)$, $\Delta C_0(t)$, resulting from changing the $j$th parameter by 1%, divided by the change in parameter value $\Delta \lambda_j$.

A parameter may be sensible, but not identifiable, since “identifiability is concerned with the question of theoretical uniqueness of solutions for a given model and experiment” (21). Two sensible parameters are dependent and, hence, not independently identifiable if their corresponding sensitivity functions are identical, linearly dependent, or are linear combinations of each other (1, 5).

Because estimability is concerned with the question of practical uniqueness of solutions in the presence of experimental noise or error (21), two parameters can be identifiable but not independently estimable if they are highly correlated. Therefore, the estimability of a given model parameter is directly proportional to its sensitivity function and inversely proportional to its correlation with other model parameters. The sensitivity functions plotted in Fig. 10 provide a graphic representation revealing the sensitivities of the kinetic parameters, correlations between kinetic parameters, and how the sensitivities and correlations depend on the range of transit times (flows) encompassed by the data. The values of the sensitivity function for a given rate parameter are flow dependent. At high flow rates, sensitivities were low because there was little time for test indicator association during the short transit time. As flow decreased, the values of the sensitivity function increased but plateaued at levels above which further reduction in the flow had little effect. Figure 10B, which plots the sensitivity function for $k_2$ at 400 and 50 ml/min, shows how reduction in the flow increased the sensitivity for $k_2$. The correlations between model parameters were also flow dependent. Figure 10, C and D, shows the sensitivity functions for $(k_1/b_1)\beta$ and $Q_e$ at 400 and 50 ml/min, respectively. At 400 ml/min, the two parameters have different effects on the shape of the test indicator outflow curve and, hence, they have a low correlation coefficient; whereas at 50 ml/min the effects of the sensitivity functions for the two parameters are almost indistinguishable and, hence, have a high correlation coefficient.

Thus, as discussed previously (1), simultaneous fitting of the test indicator outflow curves over a sufficiently wide range of flows can improve the estimability of model parameters over that for data obtained at a single flow by reducing the overall correlation between model parameters and/or by increasing parameter sensitivity.

Correlation between model parameters and physical and chemical properties of test indicators. The test indicators used in this study have a range of physicochemical properties, which are assumed to be important in determining the kinetics of their interactions with the lungs. Comparisons between test indicators in Tables 1 and 6 show that correlations exist between the descriptors of the overall test indicator-tissue interactions ($a$, $k_i$, $\bar{V}$, and $\bar{H}$) and the physical and chemical properties of the three test indicators studied. As stated earlier, one potential application of the approach outlined in this study is the use of such correlations for predicting the pulmonary (and other organ) disposition of members of this pharmacologically important class of compounds. Thus the results from this study are consistent with that possibility, but studies with additional test indicators having various combinations of physicochemical properties will be required before this potential can be fully evaluated.
One prediction of the model hypotheses, which is consistent with the results, is that the association of lidocaine with the BSA was rapidly equilibrating, as indicated in Fig. 6. Thus, at a given BSA concentration, the equilibrium binding was predicted from the M1D data using Eq. 7. This result is similar to that obtained previously with alfentanil (3).

Although not explicitly stated in the modeling section, it is the unbound, nonionized form of the test indicator that is assumed to diffuse into the lung tissue rapidly enough to be significant on the time course of these experiments (13, 24, 38, 39). Under the conditions relevant to the present experiments (constant pH), and with the assumption of rapidly equilibrating ionization, this assumption could be included in the models by a simple notational change. The resulting species balance equations will have the same form as Eqs. 2-3, but the definition of $\epsilon$ would include a scaling factor $(1 + [H^+] / K_i)$, where $[H^+]$ is the hydrogen ion concentration in the perfusate, and $K_i$ is the ionization constant. Within the tissue volume, the interactions of the ionized and nonionized forms of the test indicator are not mathematically distinguishable under the assumption of rapid ionization. Hence, the N types of associations in Fig. 5 could involve interactions between the lung tissue and either the test indicator ionized form, nonionized form, or both. This might be further evaluated by future experiments in which the pH is varied. It is anticipated that the complexity of such studies will be greater than simply changing perfusate pH, due to possible effects of pH on BSA binding and unknown $[H^+]$ equilibrium times between tissue and perfusate. Therefore, the pH effect was considered to be outside the scope of the present study but potentially interesting for future study.

Another potentially important area for future studies is the impact of formed elements in the blood. If the interactions of the test indicator with blood cells are rapid, relative to the tissue, then they will be represented in the model the same way as BSA binding, except that the differences in capillary velocities of cells and plasma will need to be included (6). If there are nonequilibrium interactions within the blood cell, analogous to those with the tissue, they could be evaluated by varying the time during which the indicators are in contact with the blood before reaching the lungs, as previously described (31).

In conclusion, in this study, we have developed a kinetic model for characterizing the pulmonary disposition of lipophilic amine compounds by using the M1D method and a robust interpretation of the estimated kinetic model parameters. The ability of the model to fit the outflow curves of lipophilic amines with a range of physicochemical properties over a range of flow and perfusate composition is encouraging with respect to the potential use of estimated model parameters for predicting pulmonary disposition of drugs and, perhaps, for detecting and quantifying changes in lung tissue composition when using a combination of these or other lipophilic amine compounds (9, 11, 36).

**APPENDIX**

Integrating Eq. 3 in time results in

$$[D_b](x, t) = \sum_{i=1}^{M} \frac{k_i}{\beta} \int_0^t e^{-k_i \tau} [D](x, \tau) d\tau$$  \hspace{1cm} (A1)

Substituting Eq. A1 into Eq. 2 reduces Eqs. 2-3 to

$$\frac{\partial [D]}{\partial t} + W \frac{1}{1+\alpha} \frac{\partial [D]}{\partial x} = -\left( \frac{\alpha}{1+\alpha} \right) k_i [D]$$  \hspace{1cm} (A2)

Let

$$k_i = \sum_{i=1}^{M} \frac{k_i}{\beta}$$

and $\alpha = Q_o / Q_c$, then Eq. A2 becomes

$$\frac{\partial [D]}{\partial t} + W \frac{1}{1+\alpha} \frac{\partial [D]}{\partial x} = -\left( \frac{\alpha}{1+\alpha} \right) k_i [D]$$  \hspace{1cm} (A3)

After changing the order of the integration and the summation, Eq. A3 reduces to

$$\frac{\partial [D]}{\partial t} + W \frac{1}{1+\alpha} \frac{\partial [D]}{\partial x} = -\left( \frac{\alpha}{1+\alpha} \right) k_i [D]$$  \hspace{1cm} (A4)

where

$$\psi(t) = \sum_{i=1}^{M} \int_0^t e^{-k_i \tau} \frac{k_i}{\beta} e^{-k_i \tau} \sum_{i=1}^{M} \frac{k_i}{\beta}$$

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**REFERENCES**


