Detection of changes in lung tissue properties with multiple-indicator dilution

D. L. Roerig, S. H. Audi, J. H. Linehan, G. S. Krenz, S. B. Ahlf, W. Lin, and C. A. Dawson. Detection of changes in lung tissue properties with multiple-indicator dilution. J. Appl. Physiol. 86(6):1866–1880, 1999.—We evaluated the potential utility of a group of indicators, each of which targets a particular tissue property, as indicators in a multiple-indicator dilution method to detect and to identify abnormalities in lung tissue properties resulting from lung injury models. We measured the pulmonary venous outflow concentration vs. time curves of [14C]diazepam, [3H]OH, [14C]phenylethylamine, and a vascular reference indicator following their bolus injection into the pulmonary artery of isolated perfused rabbit lungs under different experimental conditions, resulting in changes in the lung tissue composition. The conditions included granulomatous inflammation, induced by the intravenous injection of complete Freund’s adjuvant (CFA), and intratracheal fluid instillation, each of which resulted in similar increases in lung wet weight. Each of these conditions resulted in a unique pattern among the concentration vs. time outflow curves of the indicators studied. The patterns were quantified by using mathematical models describing the pulmonary dispersion of each of the indicators studied. A unique model parameter vector was obtained for each condition, demonstrating the ability to detect and to identify changes in lung tissue properties by using the appropriate group of indicators in the multiple-indicator dilution method. One change that was particularly interesting was a CFA-induced change in the disposition of diazepam, suggestive of a substantial increase in peripheral-type benzodiazepine receptors in the inflamed lungs.

diazepam; phenylethylamine; mathematical modeling; lung inflammation; benzodiazepine receptors

THE MULTIPLE-INDICATOR DILUTION (MID) method is used to measure organ perfusion, cellular and chemical composition, vascular permeability, and metabolic function in a nondestructive manner in intact organs and in vivo (1–5, 7–23, 26–29, 35, 36, 38). It involves the injection of a bolus containing two or more indicators into the organ’s arterial inlet, followed by the measurement of the indicator concentration vs. time in the venous effluent. One indicator, referred to as “the vascular reference indicator,” is confined to the vascular space and does not interact with the tissue as it travels through the organ. The other indicator(s), referred to as “test indicators,” interact with the tissue in some way related to the properties of the indicator(s) and the tissue. As a result of these interactions, the concentration vs. time outflow curves of the test indicators are changed in amplitude and/or timing with respect to the reference indicator curve. Comparison of the reference and test indicator curves reveals the tissue interactions of the test indicator and provides the information necessary to evaluate aspects of the organ function that influence those interactions. MID methods applied to the lungs have the potential for discriminating among lungs having different physical and chemical properties such as occur in lung injury and disease (7, 8, 22, 23, 28, 29, 35). This is the basis for MID studies of lung water volume (8, 12, 21, 28), capillary permeability (7, 8, 14, 15, 22, 23, 36, 38), endothelial enzyme activity (8, 9, 15, 18, 29, 35), and other aspects of lung tissue function (1–5, 7, 8, 16, 19, 20).

In a previous study (16), the lipophilic amine [14C]diazepam was found to provide a measure of the perfused nonaerated lung tissue volume that was independent of the tissue water content in edematous, but otherwise normal, lungs. Thus, under the conditions of that study, the ratio of the extravascular volume accessible to [3H]OH to that accessible to [14C]diazepam provided a nondestructive index of lung wet-to-dry weight ratio (16). The present study was carried out to evaluate the potential utility of [14C]diazepam, when used in conjunction with other test indicators such as [3H]OH and [14C]phenylethylamine ([14C]PEA), for detecting and identifying abnormalities in lung tissue properties. The [3H]OH was used to trace the perfused extravascular water volume and the [14C]PEA, which is extracted by the endothelial cells (6, 18), was used as an indicator of perfused endothelial surface. The MID experiments were carried out on isolated perfused rabbit lungs to facilitate control over a number of variables (1, 2, 4, 5, 16, 28, 29). Lung tissue properties were manipulated in several ways. One was the intravenous injection of complete Freund’s adjuvant (CFA) to produce granulomatous inflammation (11, 31). This lung inflammatory stimulus induces complex changes in lung tissue composition, including a substantial increase in lung weight, which have been well defined in the rabbit (11, 31). To determine whether the chosen group of indicators could distinguish between a change in lung weight and changes in lung properties associated with the inflammatory response, the airways of otherwise normal lungs were filled with a physiological salt solution (PSS) by intratracheal instillation to increase the lung...
weight to the same extent as that caused by the inflammatory response. To manipulate the fraction of perfused tissue in some of these fluid-filled lungs, they were also embolized with enough glass beads to reduce the accessible extravascular water volume to that in the inflamed lungs. Thus several experimental groups were studied in which the lungs had different properties among groups, but some variables were also matched among groups. The differences among the outflow concentration curves in the various study groups were quantified by using mathematical models appropriate for each indicator. The results provide an example of how the MID model parameters can reveal differences in lung tissue properties.

**EXPERIMENTAL METHODS**

**Animal Preparation (Isolated Rabbit Lung)**

The experiments were performed by using an isolated rabbit lung preparation, as previously described (1, 2, 5). New Zealand White rabbits of either sex were given chlorpromazine hydrochloride (25 mg/kg im), followed by pentobarbital sodium (20–25 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 a perfusate containing a PSS (in g/l: 0.37 KCl, 0.37 CaCl2·2H2O, 0.29 MgSO4·7H2O, 0.16 KH2PO4, 6.9 NaCl, 1 glucose, and 2.1 NaHCO3) with 45 g/l of BSA (1, 2, 5). The perfusion system included a heated perfusate reservoir and a Master Flex roller pump, which pumped perfusate at a constant mean flow of 3.33 ml/s from the reservoir into the pulmonary artery, with the left atrial pressure set equal to atmospheric (pleural) pressure by adjusting the height of the venous outflow into the recirculation reservoir. Arterial and venous 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 7.17 ± 0.52 and 1.62 ± 0.54 (SD) cmH2O, respectively. The perfusate was equilibrated with the respiratory gas mixture, which maintained the pH at 7.37 ± 0.05 (SD) at 37°C. Before each of the bolus injections described below, the ventilator was stopped at end expiration for the duration of the sampling period.

To produce a bolus injection, a solenoid-operated injection loop (1, 2, 5) 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.

In the experiments involving alveolar instillation, to produce as even a distribution of the instillate as possible, the lungs were made atelectatic before perfusion. This was accomplished by ventilating the anesthetized rabbit with 100% O2 for 5 min and then clamping the trachea. Five minutes later, the chest was opened, and the lungs were cannulated and placed in the perfusion system as described above.

**MID Studies**

The 1.0-ml bolus of the perfusate solution contained 2.5 mg of FITC-labeled 40,000-mol wt dextran (FITC-Dex), and 0.5 µCi of 1H or 0.1 µCi of 14C of one or more of either 3H, [14C]diazepam, or [14C]PEA. The specific activities for 3H, [14C]diazepam, and [14C]PEA were 90 mCi/mol, 55 mCi/mmol, and 50 mCi/ml, respectively. Just before injection, the venous outflow was directed into the sample tubes of a modified (1, 2, 5) Gilson Escargot fraction collector. A total of one hundred 2-ml samples was collected, with a sampling interval of 0.6 s.

After each experiment, the lungs were removed from the perfusion system, and an additional bolus containing FITC-Dex was made, with the arterial and venous canulas connected directly together. The data from this injection were used to obtain the moment for the passage of the bolus through the tubing from injection to fraction collector in the absence of the lungs. In one of these experiments, all three test indicators were also included in a bolus to ensure that no separation of the test indicators and the FITC-Dex occurred within the tubing alone.

The concentration of the FITC-Dex in the outflow samples was measured spectrophotometrically (494 nm). The 14C and/or 3H activities were measured by liquid scintillation 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 the calculation of indicator concentrations. The fractions of injected indicators recovered in the collected samples, calculated based on these standards, are given in Table 1.

**Conditions Studied**

Control conditions. The MID studies described above were carried out on lungs from seven normal rabbits.

Granulomatous inflammation. Eight rabbits were each given a 1-ml ear vein injection of CFA (8.5 ml Bayol F, 1.5 ml Arlacial, and 5 mg Myco. Butyricum) (11, 31). After 13.8 ± 7.9 (SD) days, these rabbits were anesthetized, and the MID studies were carried out on the lungs.

Alveolar instillation. In contrast to the complex changes in lung tissue composition resulting from the inflammatory response induced by CFA, well-defined changes in lung wet weight and tissue composition were induced by instilling 35 ml of a solution that had the same composition as the perfusate (PSS containing 4.5% BSA (PSS + BSA)) into the alveolar space of the isolated lungs from normal rabbits. The volume of fluid instilled was chosen to result in similar lung weight as that obtained in lungs treated with CFA. In 6 of the 13 lungs, the instilled solution was 35 ml of PSS with no BSA. For these lungs, the instilled PSS solution included 4.5% dextran (70,000 mol wt) to match the oncotic pressure of the PSS + BSA. The MID studies were carried out before (atelectasis) and after the alveolar instillation of the fluid.

Embolism. In a subset of the fluid instillation groups (six filled with PSS + BSA and five filled with PSS), following the

<table>
<thead>
<tr>
<th>Condition</th>
<th>FITC-Dex, %recovery</th>
<th>[14C]Diazepam, %recovery</th>
<th>[14C]PEA, %recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>99.8 ± 0.3</td>
<td>94.1 ± 0.9</td>
<td>96.1 ± 0.9</td>
</tr>
<tr>
<td>CFA</td>
<td>97.6 ± 1.3</td>
<td>60.3 ± 3.2*</td>
<td>95.4 ± 0.6</td>
</tr>
<tr>
<td>Atelectasis</td>
<td>99.2 ± 0.7</td>
<td>96.1 ± 0.9</td>
<td>99.0 ± 1.9</td>
</tr>
<tr>
<td>PSS</td>
<td>99.2 ± 1.2</td>
<td>98.4 ± 1.6</td>
<td>100.1 ± 3.2</td>
</tr>
<tr>
<td>PSS + BSA</td>
<td>97.7 ± 0.7</td>
<td>97.0 ± 1.4</td>
<td>100.1 ± 3.2</td>
</tr>
<tr>
<td>PSS + embolism</td>
<td>97.6 ± 1.0</td>
<td>97.0 ± 1.4</td>
<td>100.1 ± 3.2</td>
</tr>
<tr>
<td>BSA + BSA + embolism</td>
<td>96.3 ± 0.9</td>
<td>91.9 ± 1.4</td>
<td>98.0 ± 1.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. FITC-Dex, FITC-labeled dextran; [14C]PEA, [14C]diazepam injected as phenylethylamine; CFA, complete Freund’s adjuvant; PSS, physiological salt solution. *Significantly different from control (P < 0.05).
last bolus injection, 240 mg of glass beads (2.6 × 10^4 beads, 194 μm in diameter) were slowly introduced into the pulmonary artery to occlude a portion of the vascular bed. The number of beads was chosen, based on previous studies (17), to reduce the 3H2O-accessible extravascular water volume of these fluid-filled lungs to approximately that accessible in lungs with granulomatous inflammation. The MID studies were then repeated.

PEA metabolites. PEA is metabolized within the pulmonary endothelial cells to phenylethylacetic acid (PAA) (6, 18). Thus, as time progresses during bolus passage, a fraction of the [14C] injected as [14C]PEA returns to the perfusate as PEA and PAA. A maximum of two peaks of [14C]PAA was detectable, which corresponded to PEA and PAA. A [14C]PAA peak was not detectable until samples collected after the peak of the FITC-Dex concentration vs. time curve. The analysis described below is based only on the [14C]PEA concentration in samples obtained up to the peak of the FITC-Dex curve.

After each experiment, the lungs, except those involving alveolar instillation, were weighed and lyophilized to a constant weight. For the seven normal lungs, the wet and dry lung weights were 9.4 ± 0.4 and 1.59 ± 0.1 g, respectively, and the wet-to-dry ratio (total lung wet weight to lung dry weight) was 5.8 ± 0.04 (SE). For the eight CFA-treated lungs, the wet lung dry lung weights were 44.8 ± 0.1 and 8.8 ± 0.9 g, respectively, and the wet-to-dry ratio (total lung wet weight to lung dry weight) was 5.3 ± 0.1. For comparison with the other groups, estimates of the wet weight of the fluid-filled lungs shown in Table 2 were obtained by adding the weight of the water in the instilled solution; i.e., the weight of the instillate (35 ml × 1.02 g/ml) minus the weight of non-water constituents of the instillate (and glass beads if they were injected) to the average wet weight of control lungs. The dry weight was assumed equal to the average dry weight of control lungs.

The body weights and arterial and venous pressures for each group studied are given in Table 2. The airway pressure during the bolus passage was 1.6 ± 0.5 (SD) cmH2O for air-filled lungs and atmospheric at the trachea for the fluid-filled lungs.

**EXPERIMENTAL RESULTS**

Figure 1 shows an example of the measured venous effluent concentration vs. time curves for FITC-Dex, 3H2O, [14C]diazepam, and [14C]PEA from isolated rabbit lungs under each of the conditions studied. Each condition provided a unique pattern among the four indicator curves. Both CFA treatment and alveolar

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**Table 2. Measured physiological variables**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Body Weight, kg</th>
<th>Wet Weight, g</th>
<th>Dry Weight, g</th>
<th>(Wet – Dry), g</th>
<th>Qw/(Wet – Dry), %</th>
<th>Venous Pressure, cmH2O</th>
<th>Arterial Pressure, cmH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.83 ± 0.11</td>
<td>9.4 ± 0.4</td>
<td>1.59 ± 0.10</td>
<td>7.56 ± 0.39</td>
<td>82.0</td>
<td>2.29 ± 0.29</td>
<td>8.36 ± 0.32</td>
</tr>
<tr>
<td>CFA</td>
<td>2.62 ± 0.08</td>
<td>44.8 ± 0.1</td>
<td>8.76 ± 0.89</td>
<td>37.1 ± 3.7</td>
<td>45.0</td>
<td>1.63 ± 0.62</td>
<td>10.69 ± 0.68</td>
</tr>
<tr>
<td>Atelectasis</td>
<td>2.75 ± 0.06</td>
<td>9.4</td>
<td>1.59</td>
<td>7.56</td>
<td>79.6</td>
<td>2.01 ± 0.37</td>
<td>9.63 ± 0.28</td>
</tr>
<tr>
<td>PSS</td>
<td>2.72 ± 0.10</td>
<td>43.0</td>
<td>1.59</td>
<td>41.44</td>
<td>79.4</td>
<td>3.06 ± 0.44</td>
<td>8.63 ± 0.28</td>
</tr>
<tr>
<td>PSS + BSA</td>
<td>2.78 ± 0.08</td>
<td>43.0</td>
<td>1.59</td>
<td>41.44</td>
<td>79.4</td>
<td>2.94 ± 0.53</td>
<td>8.36 ± 0.29</td>
</tr>
<tr>
<td>PSS + embolism</td>
<td>2.79 ± 0.08</td>
<td>43.0</td>
<td>1.59</td>
<td>41.44</td>
<td>79.4</td>
<td>1.68 ± 0.58</td>
<td>11.57 ± 0.58</td>
</tr>
<tr>
<td>PSS + BSA + embolism</td>
<td>2.72 ± 0.06</td>
<td>43.0</td>
<td>1.59</td>
<td>41.44</td>
<td>79.4</td>
<td>1.44 ± 0.43</td>
<td>10.38 ± 0.41</td>
</tr>
</tbody>
</table>

Values are means ± SE. Italics denote estimates of wet weights of atelectatic and fluid-filled lungs, obtained by adding weight of water in the instilled solution to the average wet weight of control lungs. Dry weights were assumed equal to the average dry weight of control lungs (see text). Qw, perfused extravascular lung water volume accessible to 3H2O.
fluid instillation resulted in a reduction in the peak and a prolongation of the $^3$H$_{OH}$ concentration curves relative to control, reflecting the increases in the lung water volume under these conditions.

Filling the alveolar space with PSS resulted in almost no change in the $^{14}$C diazepam curves, whereas both PSS + BSA instillation and CFA treatment resulted in marked changes in diazepam curves. When the lungs were filled with PSS + BSA, the changes in the diazepam curves were qualitatively similar to the changes in the water curves. This reflects the rapidly equilibrating associations of the diazepam with BSA (2, 5, 16). In CFA-treated lungs, there was a large reduction in the recovery of diazepam relative to that under the other conditions (Table 1), and the peak of the diazepam curve shifted to the left (Fig. 1), resulting in a very different shape from that in the other conditions.

The $^{14}$C PEA curve was hardly affected by either CFA treatment or alveolar fluid instillation, although the tail of the $^{14}$C curve in the CFA-treated lungs tended to be depressed. Figure 1, E and F, shows that reduction in the fraction of perfused tissue by embolizing fluid-filled lungs with glass beads shifted the curves of all indicators upward and to the left while the relationships among the curves were essentially maintained.

The data analysis described below is an attempt to provide a quantitative basis for comparison among these patterns.
equilibrating associations in Q, and Qt,

\[ Q_S = \Psi_k \]

Virtual volume reflective of the capacity of slowly equilibrating classes of association (ml)

\[ Q_t \]

Tissue volume

\[ Q_v \]

Pulmonary vascular volume = \( F \times (t_R - t_f) \)

\[ Q_w \]

Perfused extravascular water volume accessible to \(^3\)HOH = \( F \times t_e \)

\[ [R](x, t) \]

Vascular concentration of the reference indicator at distance x from the capillary inlet and time t

\[ RD_v = \sqrt{\sigma^2 - \sigma^2_t} / (t_R - t_f) \]

Vascular relative dispersion

\[ SSD \]

Sum of squares differences

\[ t \]

Time

\[ t_m \]

Mean transit time (first moment)

\[ t_e = t_f - t_R \]

Extravascular mean transit time of \(^3\)HOH

\[ t_R, t_f, t_e, \text{ and } t_t \]

Mean transit times of CR(t), CF(t), hC(t), and CT(t), respectively

\[ V_F \]

Average linear flow velocity

\[ W \]

Distance from the capillary inlet (x = 0)

\[ z \]

z score = \( \left\{ \text{parameter for unknown - mean of control group} \right\} / \text{(SD of control group)} \)

\[ z_i \]

Mean z score for the ith parameter for the jth experimental group

\[ \beta = 1 + \sum_{i=0}^{N} [b_i]K_i \]

\[ \sigma^2 \]

Variance (second central moment)

\[ \sigma^2_R, \sigma^2_F, \sigma^2, \text{ and } \sigma^2 \]

Variances of CR(t), CF(t), hC(t), and CT(t), respectively

\[ \sigma^2_t \]

Variance of hC(t)

\[ \epsilon = K_p / ([P] + K_p) \]

Sojourn time distribution

\[ \psi(t) = k_{-1} e^{-k_{-1} t} \]

Mean sojourn time (first moment) of \( \psi(t) \)

\[ \Psi = 1/k_{-1} \]

FITC-Dex

The mean transit times (t) and the second (\( \sigma^2 \)) and third (\( \alpha^2 \)) central moments of the outflow curves of FITC-Dex, \(^3\)HOH, and the tubing outflow curve [C_T(t)] were obtained by fitting each to a shifted random walk function, the functional form of which can be specified by its first three moments (1, 2, 10).

The vascular volume (Q_v) was estimated as the product of the flow (F) and the difference between the mean transit times of the outflow curves of the vascular reference indicator FITC-Dex, which are denoted by C_R(t) and C_T(t) from

\[ Q_v = F \times (t_R - t_f) \]

where \( t_R \) and \( t_f \) are the mean transit times of C_R(t) and C_T(t), respectively.

The vascular relative dispersion (RD_v) was estimated from the moments of C_R(t) and C_T(t) from

\[ RD_v = \sqrt{\sigma^2 - \sigma^2_t} / (t_R - t_f) \]

where \( \sigma^2_R \) and \( \sigma^2_t \) are the second central moments of C_R(t) and C_T(t), respectively.

\(^3\)HOH

The perfused extravascular water volume (Q_w) was estimated as the product of the flow F and the difference between the mean transit times of the outflow curves of \(^3\)HOH, CF(t), and of FITC-Dex, CT(t), from

\[ Q_w = F \times t_e \]

[\(^{14}\)C]Diazepam Concentration vs. Time-Outflow Curves

In previous studies in which \(^{14}\)C-diazepam was used (16), the analysis has been similar to that for \(^3\)HOH indicated above. However, in CFA-treated lungs, the \(^{14}\)C-diazepam behavior was clearly more complex (Fig. 1). Therefore, in this study, data analysis was carried out by using the more general model we have developed previously (1) as follows.

Single-capillary model. A single-capillary element of the general model we have developed (1) is composed of a capillary volume (Q_c) and a tissue volume (Q_t). The model assumes rapid equilibration between the free and protein-bound diazepam in Q_c (1) and that the free form of the diazepam is the species having diffusional access to Q_t. Within Q_t, the various diazepam-tissue associations can have a range of rate constants and are represented by N classes of associations with different dissociation rate constants (1). If one visualizes the associations as analogous to binding to a particular molecular species, [b_i], i = 1, ..., N, would be then the concentration of ith binding species and [D_b] the concentration of diazepam bound to that species, with association and dissociation rate constants k_i and k_i, respectively. Physically, these associations or interactions could be the dissolution of diazepam in membrane lipid or other types of interactions with the various chemical and cellular constituents of the tissue (1).

Assuming that no radial concentration gradients of the free diazepam exist within Q_c or Q_t (3–5), the
spatial and temporal variations in the concentrations of the reference indicator and diazepam are described by the following species balance equations. In the capillary volume

$$\frac{\partial [R]}{\partial t} + W \frac{\partial [R]}{\partial x} = 0 \quad (4)$$

$$\frac{\partial [D]}{\partial t} + W \left( \frac{Q_c}{Q_c + Q_R} \right) \frac{\partial [D]}{\partial x} = \left( \frac{Q_R}{Q_c + Q_R} \right) \left[ \sum_{i=1}^{M} \left( k_{-i}[D_{a_i}] - k_{b_i} \beta [D] \right) \right] \quad (5)$$

In the tissue volume

$$\frac{\partial [D_{a_i}]}{\partial t} = \frac{k_{b_i}}{\beta} [D] - k_{-i}[D_{a_i}] \quad i = 1, \ldots, M \quad (6)$$

where $M (M < N)$ is the number of classes with slowly equilibrating associations and $(N - M)$ is the number of classes with rapidly equilibrating associations (1). $[R](x, t)$ and $[D](x, t)$ are the vascular concentrations of the reference indicator and the free diazepam at a distance $x$ from the capillary inlet and time $t$, respectively. $Q_{cR} = (Q_{aR} \beta) e$ is a virtual volume including $Q_{aR}$ and the effects of rapidly equilibrating associations in $Q_c$ and $Q_R$. $[D_{a_i}(x, t) = [D_{b_i}(x, t)] \beta$ is the concentration of diazepam in $Q_{aR}$ bound to the binding species with association and dissociation rate constants $k_i$ and $k_{-i}$, respectively. The $e = K_p/(K_p + [P])$ is the fraction of the diazepam in the vascular space that is not bound to plasma protein, where $[P]$ is the plasma protein concentration, $K_p = k_0/[P]$ is the plasma protein dissociation constant, and $k_0$ and $k_1$ are the association and dissociation rate constants of the diazepam to plasma protein, respectively. The

$$\beta = 1 + \sum_{i=(M+1)}^{N} N[b_i]/K_i$$

is a factor scaling $Q_{a_i}$, which results from the $(N - M)$ rapidly equilibrating classes of associations. $K_i = k_{-i}/k_i$ is the equilibrium dissociation constant of the $i$th class of associations, where $k_i$ and $k_{-i}$ are the association and dissociation rate constants for the $i$th class of associations, respectively. $W$ is the average linear flow velocity within $Q_c$ equal to the $F$ divided by the capillary cross-sectional area. The model parameters are $Q_R$ (ml), $k_i/[b_i] \beta$ (s$^{-1}$), and $k_{-i}$ (s$^{-1}$), $i = 1, \ldots, M$.

Previously (1), we showed that the resolution of the MID data limits the identifiability of the kinetic parameters for each of the potentially large number of classes of associations $M$. In addition, we showed that $M = 2$ is the number of classes of associations ($M$, $M < M$, is the largest number of classes of slowly equilibrating associations resolvable from the data) to fit the data over a wide range of flows and a wide spectrum of physicochemical properties, which reduces the number of model parameters from $2M + 1$ to five, namely, $Q_R$ (ml), $k_1/[b_1] \beta$ (s$^{-1}$), $k_2/[b_2] \beta$ (s$^{-1}$), $k_{-1}$ (s$^{-1}$), and $k_{-2}$ (s$^{-1}$).

To account for the fact that in CFA-treated lungs a significant fraction of diazepam was not recovered within the MID sampling time, the dissociation rate constant for one of these two classes, $k_{-2}$, was set equal to zero. Hence, Eqs. 5 and 6 reduce to

$$\frac{\partial [D]}{\partial t} + W \left( \frac{Q_c}{Q_c + Q_R} \right) \frac{\partial [D]}{\partial x} = \left( \frac{Q_R}{Q_c + Q_R} \right) \left[ k_{-1}[D_{a_1}] - k_{b_1} \beta [D] \right] \quad (7)$$

and the number of model parameters reduces to four, namely, $Q_R$ (ml), $k_1/[b_1] \beta$ (s$^{-1}$), $k_{-1}$ (s$^{-1}$) and $k_2/[b_2] \beta$ (s$^{-1}$).

To model a bolus injection, the solution to Eqs. 4, 7 and 8 is constrained by the initial ($t = 0$) conditions, $[D](x, 0) = [D_{a_1}(x, 0) = [D_{b_1}(x, 0)] \beta$, and boundary ($x = 0$) conditions $[D_{a_1}(0,t) = 0, [D](0,t) = C_{a_0}(t)$, and $[R](0,t) = C_{a_0}(t)$, where $C_{a_0}(t)$ is the capillary input function.

The above deterministic model provides a conceptual basis for the evolution of the data, but the model parameters can involve several terms that are not separately identifiable. In addition, there is no obvious reason to expect that the ith kinetic parameter characterizes the same physicochemical phenomenon for more than one set of experimental conditions (1). Therefore, for making comparisons, it is convenient to express the model parameters as stochastic parameters as follows (1). Integrating Eq. 8 in time results in

$$[D_{a_1}(x, t)) = k_1 \int_0^t e^{-k_{-1}t-\tau}[D](x, \tau) d\tau \quad (9)$$

where $k_1 = Q_R(k_1/[b_1]) \beta$ (ml/s) is the effective association rate of diazepam with the binding species, with association and dissociation rate constants $k_1$ and $k_{-1}$. Substituting Eq. 9 into Eq. 7 reduces Eqs. 7 and 8 into the following

$$\frac{\partial [D]}{\partial t} + W \left( \frac{Q_c}{Q_c + Q_R} \right) \frac{\partial [D]}{\partial x} = - \left( \frac{1}{Q_c + Q_R} \right) k_1[D]$$

$$+ \left( \frac{1}{Q_c + Q_R} \right) k_f \int_0^t \Psi(t-\tau)[D](x, \tau) d\tau$$

$$- \left( \frac{1}{Q_c + Q_R} \right) k_{seq}[D] \quad (10)$$

where $k_{seq} = Q_R(k_2/[b_2]) \beta$ (ml/s) is the sequestration rate of diazepam within $Q_{aR}$ and $\Psi(t) = k_{-1}e^{-k_{-1}t}$ is the
sojourn time distribution (for the slowly equilibrating classes of interactions) (1). The mean sojourn time is the first moment, \( \Psi \) (1), of \( \Psi(t) \)

\[
\Psi = \frac{1}{k_{-1}}
\]

(11)

The terms on the right-hand side of Eq. 10 represent three possible classes of diazepam-tissue interactions. Physically, \( Q_R \) (ml) and \( Q_S = \overline{V} k_e \) (ml) represent virtual volumes that are reflective of the capacities of two classes of associations referred to as rapidly and slowly equilibrating classes, respectively (1). The rapidly (relative to the capillary mean transit time) equilibrating associations of diazepam within \( Q_R \) and \( Q_c \), which are not mathematically distinguishable from each other, are all represented by \( Q_T \) (ml). The slowly equilibrating associations are quantified by \( Q_S \) (ml) and by the mean sojourn time \( \Psi \) (s). A third class of diazepam-tissue interactions with dissociation rate constants that are so small that there is virtually no return to the perfusate within the sampling period is described by the sequestration rate \( k_{eq} \) (ml/s).

\[ [14C]PEA \]

The model used to interpret the uptake of PEA by the pulmonary endothelial cells was developed in Ref. 2. Again, each capillary element includes a vascular volume \( Q_c \). The PEA also has access to a flow-limited volume \( Q_S \), within which it can participate in rapidly equilibrating associations with the tissue or it can be transported into the endothelial cells via passive diffusion (18) or some other linear transport mechanism (6) having a permeability-surface area product (PS). This transport is assumed to be unidirectional, as discussed below. The transit of PEA through such a capillary element can be described by the following equation

\[
\frac{\partial[D_p]}{\partial t} + W \left[ \frac{Q_c}{Q_c + Q_F \left( 1 + \frac{[n_e]}{K_e} \right)} \right] \frac{\partial[D_p]}{\partial x} = -PS \left[ \frac{Q_c + Q_F \left( 1 + \frac{[n_e]}{K_e} \right)}{Q_c + Q_F \left( 1 + \frac{[n_e]}{K_e} \right)} \right] [D_p] \]

(12)

where \( [D_p] \) of \( x, t \) is the vascular concentration of PEA at distance \( x \) from the capillary inlet and time \( t \); \( [n_e] \) represents the concentration of the rapidly equilibrating association sites within \( Q_c \), having association and dissociation rate constants \( k_e \) and \( k_{-e} \), respectively, such that \( K_e = k_{-e}/k_e \) is the equilibrium dissociation rate constant. The model parameters are PS (ml/s) and \( V_F = Q_F \left( 1 + \frac{[n_e]}{K_e} \right) \) (ml).

To model a bolus injection, the solution to Eq. 12 is constrained by the initial \( (t = 0) \) condition, \( [D_p] \) \( (x, 0) = 0 \), and the boundary condition \( [D_p] \) \( (0, t) = C_{in}(t) \), where \( C_{in}(t) \) is the capillary input function.

**Organ Models**

Equations 4, 7, 8, and 12 are for single capillary elements. To construct an organ model, the distribution of pulmonary capillary transit times, \( h_t(t) \), needs to be taken into account (1–5, 7, 8). Previously (4), we demonstrated that the effect of \( h_t(t) \) on the estimated kinetic model parameters for test indicator-tissue interactions can be accounted for by a function \( h_t(t) \) the mean transit time and first two central moments of which can be specified from the moments of the concentrations vs. time curves of a flow-limited indicator such as \( ^3HOH \), \( C_{in}(t) \), and a vascular reference indicator \( C_{R}(t) \), by using Eq. 13. a–c, which relates the mean transit time \( t_e \), the variance (second central moment) \( \sigma_e^2 \), and the third central moment \( \mu_3 \) of \( h_t(t) \) to those of \( C_{F}(t) \), \( C_{R}(t) \), and \( C_{T}(t) \)

\[
\begin{align}
\xi_e &= \frac{\xi_{\nu}}{\sqrt{\frac{\sigma_{e}^{2} - \sigma_{F}^{2}}{\sigma_{R}^{2} - \sigma_{T}^{2}}}} - 1 \\
\sigma_e^2 &= \left[ \frac{\sigma_{e}^{2} - \sigma_{R}^{2}}{1 + \frac{1}{t_{e}} - \frac{1}{t_{c}}} \right] \\
\mu_3 &= \left[ \frac{m_{F}^{3} - m_{R}^{3}}{1 + \frac{1}{t_{e}} - \frac{1}{t_{c}}} \right] - 1
\end{align}
\]

(13 a-c)

where \( \xi_e = \xi_{\nu} - \xi_{F} \) and the subscripts \( F, R, \) and \( T \) refer to \( h_t(t) \), \( C_{F}(t) \), \( C_{R}(t) \), and \( C_{T}(t) \), respectively; \( \xi_{\nu} \) is the extravascular part of \( ^3HOH \) mean transit time; \( \sigma_e^2 \) is the variance of the measured tubing concentration vs. time outflow curve \( C_{T}(t) \); and \( h_t(t) \) was represented by a shifted random walk function, the functional form of which can be specified by its first three moments (1, 2, 4, 10).

The \( h_t(t) \), which accounts for the system dispersion outside of the capillaries (i.e., in arteries, veins, connecting tubing, and the injection system) is related to \( h_t(t) \) and \( C_{R}(t) \), the organ reference indicator outflow curve, by \( C_{R}(t) = (q/F) h_t(t) \ast h_c(t) \), where \( \ast \) is the convolution operator, \( q \) is the mass of the injected indicator, and \( F \) is the total flow through the organ. As described previously (1, 2, 4, 10), \( h_t(t) \) was also represented by a shifted random walk function the parameters of which were specified by iteratively convolving \( C_{in}(t) = (q/F) h_t(t) \) with \( h_c(t) \), until the optimal least square fit to \( C_{R}(t) \) 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 \( C_{in}(t) \) nor the anatomic sequence of dispersing components of the system needs to be specifically considered (1–5, 10).

For given initial and boundary conditions, Eqs. 4, 7, 8, and 12 or Eqs. 4 and 12 were solved numerically by using the finite-difference method (1, 4). The solution is for a single-capillary element with \( C_{in}(t) \) as the capillary input concentration curve. As previously described (1, 4), 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, 2, 4). To provide the whole organ output for vascular reference indicator $C_R(t)$ and test indicator $C_D(t)$ for diazepam or $C_P(t)$ for PEA, the outputs for all transit times are summed, each weighted according to $h_t(t)$ (1, 2, 4).

Parameter Estimation

For each of the conditions studied, the first three moments of $h_t(t)$ were estimated from the moments of the outflow curves of $^3$HOH, FITC-Dex, and the tubing concentration vs. time outflow curve $C_{T1}(t)$ by using Eq. 13, a and b.

Given $h_t(t)$ and $h_n(t)$ for each of the conditions studied, the kinetic model parameters descriptive of diazepam-tissue interactions were obtained by fitting Eqs. 7 and 8 to the outflow curve of diazepam. The number of model parameters identifiable from the diazepam data was determined by using the F ratio for nested models (25, 32). The concentration vs. time outflow curve of diazepam was first fitted to the model with one parameter, namely, $Q_R$, with the other three parameters set to zero. The number of model parameters was then increased stepwise, and the superiority of the sequential fits was evaluated by using the F-test for nested models. To minimize the instability due to the high correlation between $Q_R$ and the class of slowly equilibrating associations, the values of $k_2/b_2$ and $k_1/b_1$ are very large, an upper bound of $2F_R$ was placed on $k_2 (s^{-1})$ and $k_1/b_1$ (s$^{-1}$), above which they are considered rapidly equilibrating.

For each of the conditions studied, given $h_t(t)$, the kinetic model parameters descriptive of PEA-tissue interactions were obtained by fitting Eq. 12 to the outflow curve of $[^14C]$PEA, up to the peak of the FITC-Dex concentration curve.

Statistical Analysis

Parameter values are given as means ± SE. For each parameter, statistically significant differences among the different groups studied were determined by using one-way analysis of variance, followed by the Dunnett’s method for multiple comparisons vs. the control group. P < 0.05 was considered statistically significant.

MODEL RESULTS

Table 3 shows the kinetic model parameter estimates from the model fits to the diazepam outflow curves and the measures of precision of these estimates under the conditions studied (1, 25). The stochastic parameters for diazepam and the other MID parameters given in Table 4 reflect the condition-specific patterns in the concentration curves for the experimental groups studied.

FITC-Dex

Atelectasis and embolism reduced $Q_w$, whereas neither alveolar fluid instillation nor CFA treatment had a significant effect on $Q_v$. The vascular relative dispersion $RD_v$ (Eq. 2) increased in atelectatic lungs in CFA-treated lungs and in fluid-filled lungs after embolism.

$^{3}$HOH

$Q_w$ was significantly increased in CFA-treated and in fluid-filled lungs, and it returned toward normal values after embolization of the fluid-filled lungs.

$[^14C]$Diazepam

Figure 2 exemplifies the model fits to the $[^14C]$diazepam data under each of the conditions studied. The coefficients of variation for the model fits were on average 9.7 ± 0.5 (SE) %. The example sensitivity functions plotted in Fig. 3 provide a graphic representation of the sensitivities of the kinetic model parameters, and the correlation matrix shown in Table 3 quantifies the correlation between these parameters (1, 25, 32).

The calculated stochastic parameters given in Table 4 show that CFA treatment resulted in an increase in $R_{seq}$ and $Q_S$, whereas simply increasing the water content of the lungs by alveolar instillation of PSS resulted in no significant changes in any of the diazepam parameters. Instillation of the PSS + BSA increased $Q_R$. Embolization of fluid-filled lungs resulted in a return toward normal values in $Q_w/(Q_R + Q_S)$.

PEA

The PS for PEA was not significantly affected by CFA treatment or by alveolar fluid instillation but was decreased when the lungs were embolized. It was also decreased in atelectatic lungs.

DISCUSSION

Each of the indicators used in this study was chosen to target a particular tissue property. However, that does not imply that the lung disposition of each indicator is expected to be affected only by the targeted property. For example, the extravascular water volume accessible to $^3$HOH, $Q_w$, is affected not only by the lung extravascular water volume but also by the fraction of the lung tissue that it can reach by diffusion. This is revealed by the experiments wherein the tissue water volume and the fraction of perfused lung tissue were manipulated by filling the lungs with saline solution and by embolizing these lungs, respectively. Disappearance of PEA from the perfusate is via uptake into the endothelial cells (6, 18). Its uptake is expected to be sensitive to changes in the number of endothelial cells exposed to flowing perfusate and, possibly, to alterations in the endothelial cell PEA permeability mechanism (6, 18). Diazepam was chosen because it accesses the lipoid fraction of the lung tissue relatively independently of the water volume (3, 5, 16). It is expected to also be affected by the fraction of the lung tissue that it can reach by diffusion. An unexpected finding was that the diazepam disposition was also altered qualitatively in the CFA-inflamed lungs. This is revealed by the marked changes in the shape of the effluent concentration vs. time outflow curve of diazepam and by the large
## Kinetic parameters and measures of precision in the estimates of their values obtained by fitting Eqs. 7 and 8 to the outflow curves of diazepam for each experimental condition studied

<table>
<thead>
<tr>
<th>Estimated Model Parameters</th>
<th>Measures of Precision of Model Parameter Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Normal (n = 7)</td>
<td></td>
</tr>
<tr>
<td>$k_1[b_1]/\beta$, S$^{-1}$ × 10$^{-1}$</td>
<td>1.30 ± 0.24</td>
</tr>
<tr>
<td>$Q_n$, ml</td>
<td>12.78 ± 0.74</td>
</tr>
<tr>
<td>$k_1$, S$^{-1}$ × 10$^{-1}$</td>
<td>2.10 ± 0.73</td>
</tr>
<tr>
<td>$k_2[b_1]/\beta$, S$^{-1}$ × 10$^{-2}$</td>
<td>2.20 ± 0.63</td>
</tr>
<tr>
<td>CFA (n = 8)</td>
<td></td>
</tr>
<tr>
<td>$k_1[b_1]/\beta$, S$^{-1}$ × 10$^{-1}$</td>
<td>4.01 ± 0.46</td>
</tr>
<tr>
<td>$Q_n$, ml</td>
<td>15.97 ± 1.31</td>
</tr>
<tr>
<td>$k_1$, S$^{-1}$ × 10$^{-1}$</td>
<td>1.60 ± 0.06</td>
</tr>
<tr>
<td>$k_2[b_1]/\beta$, S$^{-1}$ × 10$^{-2}$</td>
<td>19.00 ± 3.50</td>
</tr>
<tr>
<td>PSS + BSA (n = 7)</td>
<td></td>
</tr>
<tr>
<td>$k_1[b_1]/\beta$, S$^{-1}$ × 10$^{-1}$</td>
<td>1.90 ± 1.00</td>
</tr>
<tr>
<td>$Q_n$, ml</td>
<td>41.90 ± 4.97</td>
</tr>
<tr>
<td>$k_1$, S$^{-1}$ × 10$^{-1}$</td>
<td>2.70 ± 1.30</td>
</tr>
<tr>
<td>$k_2[b_1]/\beta$, S$^{-1}$ × 10$^{-2}$</td>
<td>1.40 ± 0.40</td>
</tr>
<tr>
<td>PSS + BSA + embolism (n = 6)</td>
<td></td>
</tr>
<tr>
<td>$k_1[b_1]/\beta$, S$^{-1}$ × 10$^{-1}$</td>
<td>3.60 ± 1.20</td>
</tr>
<tr>
<td>$Q_n$, ml</td>
<td>15.24 ± 4.29</td>
</tr>
<tr>
<td>$k_1$, S$^{-1}$ × 10$^{-1}$</td>
<td>4.90 ± 1.80</td>
</tr>
<tr>
<td>$k_2[b_1]/\beta$, S$^{-1}$ × 10$^{-2}$</td>
<td>3.20 ± 0.92</td>
</tr>
<tr>
<td>PSS (n = 6)</td>
<td></td>
</tr>
<tr>
<td>$k_1[b_1]/\beta$, S$^{-1}$ × 10$^{-1}$</td>
<td>0.15 ± 0.10</td>
</tr>
<tr>
<td>$Q_n$, ml</td>
<td>19.57 ± 1.76</td>
</tr>
<tr>
<td>$k_1$, S$^{-1}$ × 10$^{-1}$</td>
<td>0.26 ± 0.17</td>
</tr>
<tr>
<td>$k_2[b_1]/\beta$, S$^{-1}$ × 10$^{-2}$</td>
<td>0.34 ± 0.34</td>
</tr>
<tr>
<td>PSS + embolism (n = 5)</td>
<td></td>
</tr>
<tr>
<td>$k_1[b_1]/\beta$, S$^{-1}$ × 10$^{-1}$</td>
<td>0.21 ± 0.21</td>
</tr>
<tr>
<td>$Q_n$, ml</td>
<td>8.18 ± 0.92</td>
</tr>
<tr>
<td>$k_1$, S$^{-1}$ × 10$^{-1}$</td>
<td>0.24 ± 0.24</td>
</tr>
<tr>
<td>$k_2[b_1]/\beta$, S$^{-1}$ × 10$^{-2}$</td>
<td>2.60 ± 0.98</td>
</tr>
<tr>
<td>Atelectasis (n = 13)</td>
<td></td>
</tr>
<tr>
<td>$k_1[b_1]/\beta$, S$^{-1}$ × 10$^{-1}$</td>
<td>3.70 ± 0.47</td>
</tr>
<tr>
<td>$Q_n$, ml</td>
<td>8.28 ± 0.54</td>
</tr>
<tr>
<td>$k_1$, S$^{-1}$ × 10$^{-1}$</td>
<td>3.00 ± 0.73</td>
</tr>
<tr>
<td>$k_2[b_1]/\beta$, S$^{-1}$ × 10$^{-2}$</td>
<td>4.50 ± 0.80</td>
</tr>
</tbody>
</table>

Values in the last 5 columns are means ± SD among n lungs studied. n is no. of lungs; n is no. of lungs for which the inclusion of the corresponding parameter was significant by F-test. Measures of precision of the parameter estimates from data from each lung include 95% confidence intervals and correlation matrix. The i,jth entry of correlation matrix is the correlation coefficient between the ith and jth model parameters. See text for further explanation and Glossary for symbols.
fraction of the diazepam that did not return to the perfusate within the 60-s sampling period. The mechanism(s) responsible for these changes in the lung disposition of diazepam are not certain. However, it appears likely that the lung tissue level of “peripheral” or “mitochondrial” benzodiazepine receptors (24) was increased in the inflamed lungs. Whatever the mechanism, the sequestration phenomenon may reflect invasion by monocytes or some other cell type or a change in the function of the resident cells, and it may be worthwhile to determine whether it has specificity for inflammatory responses of different types.

Previously (3, 5, 16), we found that under control conditions diazepam was nearly flow limited by the criteria that, over a wide range of flows, its venous effluent concentration curves were nearly congruent on a time scale normalized to the lung mean transit time. In the present study, we discovered that in inflamed lungs the behavior of diazepam was clearly different than in normal lungs. To compare the kinetics of tissue disposition of diazepam under all conditions studied, we used the general model, of which the flow-limited model is a nested version, and the same fitting procedure for all conditions. When this was done, in several cases, multiple parameters turned out to be identifiable for diazepam even under control conditions. The reasons for this apparent incongruity and for the large SE values in the estimated values of some diazepam parameters in Table 3 and the stochastic parameters in Table 4 are developed in the APPENDIX.

The fact that the tissue disposition of the injected indicators is affected by multiple factors helps to complicate interpretation. However, the concept proposed herein and discussed previously (1, 7, 8, 16, 23, 29) is that with a sufficient number of indicators, each having at least one unique or relatively selective interaction, enough of the ambiguity can be eliminated to distinguish among lungs having different properties. For example, Harris et al. (23) demonstrated how the ratio of permeability surface area products for hydrophilic and amphipathic indicators could be used to distinguish changes in the capillary permeability from

<table>
<thead>
<tr>
<th>Condition</th>
<th>FITC-Dex</th>
<th>²HOH</th>
<th>¹⁴C-diazepam</th>
<th>¹⁴C-PEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Qₜ, ml</td>
<td>Qₜ, %</td>
<td>Qₜ, ml</td>
<td>Qₜ, ml</td>
</tr>
<tr>
<td>Control</td>
<td>9.51 ± 0.45</td>
<td>38.0 ± 1.6</td>
<td>6.20 ± 0.29</td>
<td>12.78 ± 0.74</td>
</tr>
<tr>
<td>Atelectasis</td>
<td>10.43 ± 0.42</td>
<td>45.0 ± 1.6</td>
<td>11.61 ± 0.97</td>
<td>15.97 ± 1.31</td>
</tr>
<tr>
<td>PSS</td>
<td>9.82 ± 0.49</td>
<td>36.0 ± 1.0</td>
<td>13.06 ± 1.05</td>
<td>19.57 ± 1.76</td>
</tr>
<tr>
<td>PSS + BSA</td>
<td>10.09 ± 0.50</td>
<td>37.0 ± 1.0</td>
<td>13.00 ± 0.84</td>
<td>41.16 ± 4.97</td>
</tr>
<tr>
<td>PSS + embolism</td>
<td>13.07 ± 0.50</td>
<td>37.0 ± 2.2</td>
<td>12.80 ± 1.37</td>
<td>8.18 ± 0.92</td>
</tr>
</tbody>
</table>

Values are means ± SE. A | or a | in a table cell indicates a significantly larger or smaller value, respectively, than control (P < 0.05). Qₜ, RD, and Qw, vascular volume, vascular relative dispersion, and perfused extravascular water volume accessible to ²HOH, respectively; Qₜ and Qw, virtual volumes that are reflective of the capacities of 2 classes of associations referred to as rapidly and slowly equilibrating classes, respectively; kₑₜ, sequestration rate of diazepam within tissue volume and the mean sojourn time, respectively; PS and Vₑ, permeability-surface area product and a scaled flow-limited virtual volume accessible to PEA, respectively (see text).
changes in perfused surface area. Merker and Gillis (29) used the lipophilic indicator propranolol to separate the effects of changing surface area and endothelial cell metabolism on endothelial serotonin uptake in injured lungs. We had demonstrated how an index of wet-to-dry weight ratio could be obtained by using the ratio of the volumes accessible to $^3$HOH and a lipophilic indicator such as diazepam, i.e., $Q_w/(Q_{R} + Q_{S})$ (16). In the present study, that ratio is modified to $Q_{w}/Q_{R}$ (16). In the context of the present study, a key observation was that its uptake was not substantially affected when the alveolar fluid instillation was carried out on atelectatic lungs to obtain as even filling as possible (16). Atelectasis allows for more even filling by eliminating the effects of surface tension at the distributed air-liquid interface and by eliminating any mixture of air- and liquid-filled alveoli. MID injections were also made into the atelectatic lungs before fluid filling to make sure that the atelectasis alone did not have some unforeseen effects. The effects of atelectasis on vascular volume, PS, and RD$_v$ are predictable from the known effects of atelectasis on the pulmonary vascular bed (33).

The ratio of the extravascular water accessible to $^3$HOH, $Q_w$, to the water volume of the normal lungs, measured as the difference between the lung wet and dry weights, was 82%, as shown in Table 2. Part of the difference was no doubt due to the fact that we made no attempt to account for the perfusate trapped in the vascular space, which contributes to an overestimation of the lung wet weight (12). Issues regarding the fraction of the extravascular water volume recovered by indicator dilution methods have been discussed extensively by others (12, 13) and are not of major concern in the present study. On the other hand, the fact that this fraction was hardly affected by filling the lungs with fluid indicates that the instilled alveolar fluid was accessible to about the same extent as the normal cellular and interstitial water. Thus the longer diffusion distances that resulted from alveolar filling did not have a substantial impact on the accessibility of the extravascular water volume to the $^3$HOH bolus. Table 2 shows that the result was different for CFA-treated lungs, wherein only ~45% of the lung water volume measured as the lung wet − dry weight was detected by $^3$HOH, even though these injured lungs had almost as large a gravimetrically detectable water volume as the fluid-filled lungs. This may imply a significantly more heterogeneous access to the extravascular water volume in lungs with granulomatous inflammation. Such an effect may be mimicked by embolization which, as Table 2 shows, resulted in an even lower $Q_{w}$/to-lung wet minus dry weight ratio, presumably because the water in some areas wherein the perfusion was obstructed was too far from perfused vessels to be effectively traced by the $^3$HOH.

In lungs filled with fluid having the same composition as the perfusate (PSS + BSA), assuming that all of the instillate is accessible via diffusion, the model would predict that $Q_w$ would have increased by the volume of the instillate. The increases in $Q_w$ on average ~32 ml in comparison to the 35 ml instilled, which suggests that the alveolar fluid was in fairly rapid diffusional communication with the vascular perfusate (16).

PEA was chosen as the endothelial surface indicator because its extraction on passage through the lungs has been found to be mainly through the endothelial uptake (6, 18). In contrast to the lipophilic amine diazepam, PEA is a hydrophilic amine, and, in the context of the present study, a key observation was that its uptake was not substantially affected when the water volume of the lungs was changed by fluid instillation or by CFA treatment. This is consistent with the dominance of the endothelial barrier over the tissue water volume in determining PEA extraction. A potential advantage of PEA instead of, or in addition to, some hydrophilic indicators that have been used in the lungs, such as urea or Na (2, 22, 38), is that its extraction is relatively high, providing more sensitivity for detecting decreases in extraction. Its high uptake is probably partly the result of the fact that it is metabolized within the cells, thus lessening the impact of cellular accumulation (backdiffusion) on net uptake. A disadvantage of metabolism is that the $^{14}$C in the effluent can be contaminated by the metabolite. In the case of PEA, its
metabolite PAA is relatively cell impermeant, which may also help retard the backdiffusion of $^{14}$C. A common approach to the problem of backdiffusion and metabolite contamination has been to assume that unidirectional uptake dominates the net extraction during the rising portion of the effluent concentration curves (26). We took this approach in the present study to avoid having to measure $[^{14}$C]PAA concentration in the several hundred samples collected. However, it is possible that, in future studies, the useful information content of the data would be increased by measuring the metabolite concentration curves as well.

Pattern Recognition

Although each indicator was chosen to target a particular property, the results can also be evaluated nonmechanistically in terms of the parameter patterns generated. The numbers of indicators and variations in lung properties in this study were small in comparison to the large number of possible indicators selective for various tissue properties and the wide range of tissue properties affected by different lung diseases. Even with these relatively small numbers of MID parameters and experimental conditions, the tabular representations such as Table 4 are compiled enough to make it difficult to quickly discern the distinguishing features of the pattern for each experimental group by perusing the numbers in Table 4. On the other hand, in this limited example, a trivial pattern-recognition scheme distinguishes among experimental groups. For a given group, a $\uparrow$ or a $\downarrow$ entry in Table 4 indicates a significant increase or a significant decrease in the corresponding parameter, compared with that under control conditions, respectively. Each group can be uniquely identified by simply counting the number of $\uparrow$ and $\downarrow$ in a row of Table 4, without even addressing which parameters deviate from normal. To provide an example of the inverse problem, that of identifying the end result is that each lung can be characterized by a set of MID parameters for each experimental condition studied. Based on its set of MID parameters, the lung is classified as a member of the experimental group with the minimum SSD. Using this criterion, of the 52 lungs in the experimental groups indicated in Table 4, 50 were correctly identified. The fact that this approach worked to the extent that it did on this limited sample is not of very profound significance, since there is a fairly high expectation that most of the lungs contributing to a particular average pattern in Fig. 4 should have similar patterns. However, it demonstrates the concept that, with a sufficient number of indicators having different relative specificities for different tissue properties, the parameter vector itself may be a phenotype useful for detecting and identifying a diseased or injured lung.

Fig. 4. Mean z score (2) patterns representing a normalized change in estimated model parameters for each of experimental conditions studied. Ratio = $Q_w/(Q_R + Q_S)$, where $Q_w$ and $Q_R$ represent virtual volumes that are reflective of the capacities of 2 classes of diazepam-tissue associations, referred to as rapidly and slowly equilibrating classes, respectively; $Q_R$ and $Q_S$ are vascular volume, vascular relative dispersion, and perfused extravascular water volume accessible to $^{3}$HOH, respectively; $k_{seq}$ and $\Psi$ are sequestration rate of diazepam within tissue volume and mean sojourn time, respectively; and $PS$ and $VF$ are permeability-surface area product and a scaled flow-limited volume accessible to $^{14}$C[PEA, respectively (see text).
be able to take advantage of the many different types of tissue interactions that various lipophilic amines can participate in (1, 30, 34) to characterize lung tissue properties.

APPENDIX

Previously, we (3–5) developed a method for estimating the pulmonary capillary transit time distribution, \( h_c(t) \), based on the use of "flow-limited" indicators. In that study, diazepam was found to be nearly flow limited by the criterion that, over a wide range of flows, its venous effluent concentration curves were nearly congruent on a time scale normalized to the lung mean transit time. In the present study, we discovered that, in inflamed lungs, the behavior of diazepam was clearly different than in normal lungs. The shape of the concentration curve was different, and a substantial fraction of the injected diazepam was not recovered within the sampling period. Hence, diazepam could not be used to estimate \( h_c(t) \) in inflamed lungs in which the required flow-limited behavior no longer exists. Because in the present study we have at least one condition wherein diazepam is not flow limited, to compare the tissue disposition of diazepam under all conditions studied, we used the general model, of which the flow-limited model is a nested version, and the same model-fitting procedure for all conditions. When this was done, additional parameters were commonly identifiable (as revealed by the sensitivity analysis exemplified in Fig. 3) and significant (as indicated by the F-test) for diazepam, even under control conditions. At least two related factors contribute to this result and to the large SEs in the estimated values of some diazepam kinetic model parameters in Table 3 and in the stochastic parameters in Table 4.

One is that the F-test is an objective means of demonstrating whether adding parameters improves the fit, but it has virtually nothing to do with the robustness of the parameters that it adds to the model. Very small improvements in the fit can pass the F-test when the concentration curve, such as for diazepam in normal and fluid-filled lungs, approaches that of a flow-limited indicator. For instance, Fig. 5 shows two model fits to the diazepam concentration curve from a normal lung. One fit was obtained with the full model, i.e., with all four
parameters free (dashed line). The other was obtained with the flow-limited model, i.e., with $Q_a$ as the only free parameter (solid line) and the other three parameters set equal to zero. Based on the F-test for nested models, the fit to the diazepam outflow curve with the full model is superior to the fit with the flow-limited model, even though the superiority is difficult to discern by visual observation. One result is that the number of parameters varies from lung to lung, contributing to the large SEs in the estimates of some of the diazepam model parameters under conditions wherein diazepam is nearly flow limited.

The second related factor is the sensitivity of the model fit to very small variations in the capillary transport function as the pattern of the outflow curve approaches that of a flow-limited indicator. This sensitivity to $h(t)$ is, in fact, one of the reasons that the flow-limited indicators can be used to estimate $h(t)$ (3–5). In our previous work (4), we demonstrated that the effect of $h(t)$ on the estimated kinetic model parameters for test indicator-tissue interactions from MID data can be accounted for by a function $h(t)$ the mean transit time and first two central moments of which can be specified from the moments of a flow-limited indicator and a vascular reference indicator by using Eq. 13, a–c. In the present study, the outflow curve of $^3$HOOH was chosen to estimate $h(t)$, since it does not participate in any slowly equilibrating tissue binding under any experimental condition. As discussed previously (4), the sensitivity of the estimates of the kinetic model parameters to small errors in $h(t)$ decreases as the behavior of the test indicator deviates from that for a flow-limited indicator. This aspect of the sensitivity of the model to $h(t)$ is revealed in Fig. 6, which shows the model fit to diazepam concentration vs. time outflow curve from a normal lung with two different $h(t)$ values obtained by using the moments of the outflow curve of either diazepam (i.e., assuming that diazepam is flow limited under normal conditions) or $^3$HOOH in Eq. 13, a–c. Although the two estimated $h(t)$ values shown in Fig. 6 are very different, they resulted not only in different values for the model parameters but also in different numbers of identifiable parameters. With the use of the F-test, only two parameters were identifiable with the $h(t)$ obtained by using the moments of the diazepam outflow curve in Eq. 13, a–c, whereas four parameters were identifiable with the $h(t)$ obtained by using the moments of the $^3$HOOH outflow curve in Eq. 13, a–c. For the CFA comparison, it could not be assumed that diazepam could give a reasonable approximation to $h(t)$. Therefore, to simulate the impact of choosing the wrong $h(t)$ in the CFA case, we used the $h(t)$ estimated by using the moments of $^3$HOOH in Eq. 13, a–c and an $h(t)$ that was presumed to deviate from the moments of that obtained by using the moments of $^3$HOOH by the same ratio as for diazepam in the normal lung. In the resulting simulation, similar differences between the two $h(t)$ values had no effect on the number of kinetic model parameters for diazepam from CFA-treated lungs and little effect on the estimated values of these parameters, and the fits were indistinguishable on the scale of the figures. The key point is that the ability of the general model to fit the diazepam data from normal lungs better than the flow-limited model in some cases does not imply that diazepam is not virtually flow limited under these conditions; rather, it is a reflection of the high degree of sensitivity to small variations in $h(t)$.

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REFERENCES


