Transit time dispersion in pulmonary and systemic circulation: effects of cardiac output and solute diffusivity

Michael Weiss,1 Tom C. Krejcie,2 and Michael J. Avram 2

1Section of Pharmacokinetics, Department of Pharmacology, Martin Luther University Halle-Wittenberg, Halle, Germany; and 2Department of Anesthesiology and the Mary Beth Donnelley Clinical Pharmacology Core Facility, Feinberg School of Medicine, Northwestern University, Chicago, Illinois

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Weiss, Michael, Tom C. Krejcie, and Michael J. Avram. Transit time dispersion in pulmonary and systemic circulation: effects of cardiac output and solute diffusivity. Am J Physiol Heart Circ Physiol 291: H861–H870, 2006. First published February 24, 2006; doi:10.1152/ajpheart.01052.2005.—We present an in vivo method for analyzing the distribution kinetics of physiological markers into their respective distribution volumes utilizing information provided by the relative dispersion of transit times. Arterial concentration-time curves of markers of the vascular space [indocyanine green (ICG)], extracellular fluid (inulin), and total body water (antipyrine) measured in awake dogs under control conditions and during phentolamine or isoproterenol infusion were analyzed by a recirculatory model to estimate the relative dispersions of transit times across the systemic and pulmonary circulation. The transit time dispersion in the systemic circulation was used to calculate the whole body distribution clearance, and an interpretation is given in terms of a lumped organ model of blood-tissue exchange. As predicted by theory, this relative dispersion increased linearly with cardiac output, with a slope that was inversely related to solute diffusivity. The relative dispersion of the flow-limited indicator antipyrine exceeded that of ICG (as a measure of intravascular mixing) only slightly and was consistent with a flow-limited indicator antipyrine exceeded that of ICG (as a measure inversely related to solute diffusivity. The relative dispersion of vascular markers has mostly been estimated by using isolated perfused organs, such as the lung (1, 6, 14), heart (4), and liver (29), whereas distributed models of blood-tissue exchange have been used for permeating indicators (3). In contrast to the wide use of both classical compartment models (8, 10, 18) and recirculatory compartmental models (11, 15, 16), only incomplete attempts have been made in the past to study whole body distribution of solutes on the basis of TTDs (25, 30, 33). Thus a qualitative understanding of the determinants of TTD of solutes in the systemic circulation is important for developing a physiologically based model of indicator distribution kinetics.

The present analyses were taken from a study of the dispositions of solutes in the systemic circulation (RDs) in vivo using arterial concentration-time curves of indicators after bolus injection; 2) provide an interpretation of the RD of particular indicators in terms of advective dispersion (intravascular mixing), permeation across the capillary wall, and extravascular diffusion in the systemic circulation; 3) derive a measure of whole body distribution dynamics on the basis of RD and RD; and 4) determine whether the inverse Gaussian distribution is a useful empirical model of TTD of markers with different diffusivity. To answer these questions, the multiple tracer dilution data from a previous study in awake dogs (16) were reanalyzed to estimate the relative TTDs of markers of the vascular space [indocyanine green (ICG)], extracellular fluid (inulin), and total body water (antipyrine) using the pulmonary and systemic circulation as subsystems in a minimal circulatory model. To investigate the effect of flow, cardiac output (Q) and its regional distribution were changed by infusing isoproterenol or phentolamine. The present study tested the hypothesis that a model previously developed to study solute distribution in the rat hindlimb (31) can be applied to whole body distribution, when the latter is treated as a lumped organ system by assessing the flow dependency of RD.

METHODS

Data and Study Protocol

The blood concentrations of the physiological markers used in the present analyses were taken from a study of the dispositions of markers of intravascular space (ICG), extracellular fluid space (inu-
lin), and total body water (antipyrine) in four awake male purpose-bred coonhounds, weighing 32–42 kg (36.6 ± 4.1 kg) (16). The dogs were studied with Northwestern University Institutional Animal Care and Use Committee approval under control conditions and in various states of altered cardiac output, peripheral cardiac output distribution, and systemic vascular resistance (SVR) produced by phenylephrine or isoproterenol infusions. The order in which these studies were conducted in each dog was randomized. Details of the preparation and conduct of the studies have been described in detail previously (16).

Briefly stated, at time \( t = 0 \) min, 5 mg of ICG in 1 ml of ICG diluent, 30 \( \mu \)Ci of \([1^4]\)C]ulin in 1.5 ml of ICG diluent, and 25 mg of antipyrine in 1 ml of ICG diluent were flushed into the right atrium within 4 s using 10 ml of a 0.9% saline solution. Arterial blood samples were collected via an indwelling iliac artery catheter every 0.03 min for the first 0.48 min and every 0.06 min for the next 0.54 min using a roller pump and fraction collector. Subsequently, 35-3 ml arterial blood samples were drawn manually at 0.2-min intervals to 2 min; at 0.5-min intervals to 4 min; at 5 and 6 min; every 2 min to 20 min; at 25 and 30 min; every 10 min to 60 min; every 15 min to 120 min; and every 30 min to 360 min. Plasma ICG and antipyrine concentrations were measured by high-performance liquid chromatography, whereas plasma \([1^4]\)C]ulin concentrations were measured by liquid scintillation counting (16). Plasma ICG and inulin concentrations were converted to blood concentrations by multiplying them by one minus the hematocrit, as neither ICG nor inulin partitions into erythrocytes. Plasma antipyrine concentrations were converted to blood concentrations by using an in vivo technique that corrects for antipyrine partitioning into erythrocytes by calculating its apparent blood flow for MTT

\[ D_v \frac{\hat{f}_i(s)}{Q} \left( 1 - (1 - E)^{1 - \left( \frac{s}{2MTT_p} \right)} \right) \frac{Q}{2piRD_p^2} \exp \left[ \left( 2piRD_p^2 \right) \left( s + \left( \frac{1}{2(V/Q)RD_p^2} \right) \right)^{1/2} \right] \]
increases with decreasing capillary permeability $P_s$, of different permeability-limited markers. After rearrangement of Eq. 4, $P_s$ can be calculated from the estimated relative dispersions, distribution volumes, and cardiac output:

$$P_s = \frac{2Q}{1 + v} \frac{1}{2Q/3 + (1 + v)^2}$$

Extravascular marker: antipyrine. On the other hand, for flow-limited distribution of lipophilic drugs, for which antipyrine is a prototypical marker, the second term in Eq. 4 approaches zero because $Q/PS_1 \ll 1$. The resulting $RD_s^2$ vs. $Q$ relationship has an intercept of $RD_s^2$, and a small slope is expected because $2v \sigma C(1 + v)^2 \approx 0.05$ in Eq. 4 for a marker of whole body water. The relaxation time constant ($d_0$) of the equilibration process (radial mixing by extravascular diffusion) can be calculated:

$$d_0 = (RD^2_0 - RD_s^2)^3 \frac{3V_s(1 + v)^2}{2Qv_s}$$

Vascular marker: ICG. That advective dispersion leads to mixing in a circulatory system becomes obvious in the hypothetical case of no dispersion (plug flow), where the injected impulse of a vascular marker would circulate. In contrast to organs, including the pulmonary circulation, where $RD_s^2$ is determined by advective transport through the random capillary network, the transit time dispersion across the systemic circulation $RD_s^2$, is determined by the heterogeneity of perfusion ($Q$) and vascular volumes ($V_s$) of organs as well as mixing within individual organs ($RD_s^2$) assumed to be arranged in parallel ($26, 28$):

$$RD^2_s = \sum_{i=1}^{N} \frac{Q}{Q_i} \left( \frac{V_{i,s}}{V_{i,B}} \right)^2 (RD^2_{i,B} + 1) - 1$$

where the subscript $i$ denotes the $i$th organ or subsystem with blood flow $Q_i$ and vascular volume $V_i$; $Q = \sum_{i=1}^{N} Q_i$ denotes cardiac output and $V_{i,B} = \sum_{i=1}^{N} V_{i,B}$ is the vascular volume of the systemic circulation. It is obvious from Eq. 7 that the TTD dispersion of a vascular marker in the systemic circulation, $RD_s^2$, may be affected by hemodynamic changes that lead to a redistribution of organ blood flow among the $N$ organs or organ groups.

Total distribution clearance. A mixing (or distribution) clearance $CL_M$ can be defined as a function of the relative dispersion of indicator transit time through the circulation as a model-independent measure of indicator distribution kinetics in the body (see Appendix B):

$$CL_M = \frac{2Q}{RD^2_0 - 1}$$

where $RD^2_0$ is the relative dispersion of circulation times, which is mainly determined by $RD^2_0$ (Eq. A2). An interpretation of $CL_M$ in terms of a mammillary compartmental model has been given elsewhere (32).

Parameter Estimation

To fit Eq. 1 to the data, one needs a nonlinear regression software program by which $C(t)$ can be calculated by numerical inverse Laplace transformation, $C(t) = \mathcal{L}^{-1}[C(s)]$. We used SCIENTIST (MicroMath Scientific Software, Salt Lake City, UT) in which such a procedure is implemented. For each subject, first the ICG data were fitted to estimate the vascular volumes $V_{i,B}$ and relative dispersions $RD_{i,B}$ of the pulmonary and systemic subsystems ($i = p,s$) together with cardiac output $Q$ and the systemic extraction ratio of ICG, $E_{SCG}$. The estimate of $Q$ was then held fixed in fitting the inulin and antipyrine disposition data. Data were weighted according to $1/C^2$ to achieve a satisfactory fit of the tail part of the curve. Since this distorts fitting of the early mixing phase (up to $\sim 2$ min), we used an iterative procedure based on the fact that this first phase of the curve mainly contains information on TTD of the pulmonary circulation. The “goodness of fit” was judged by the model selection criterion (MSC) provided by SCIENTIST (a modified Akaike Information Criterion normalized to the number and scaling of data points). The most appropriate model, from a statistical point of view, is that with the largest MSC. Additionally, the $R^2$ value of the fits was reported. The reliability of parameter estimation was assessed by the asymptotic coefficients of variation of individual parameter estimates. Having estimated the parameters $Q, V_{p,B}, RD_{p,B}, V_{s,B}, RD_{s,B}$, and $V_p, RD_p, V_s, RD_s$ from the disposition data of the intravascular (i.e., blood) marker (ICG) and extravascular markers (inulin and antipyrine), respectively, the $RD^2$ estimates can be interpreted by Eq. 4 in terms of intravascular mixing and blood-tissue exchange. The total volume of distribution and the total body clearance are obtained as $V_{ss} = V_p + V_s$ and $CL = E \times Q$, respectively. The results are expressed as means (SD) of the parameters estimated in four dogs. For comparisons of treatments with control, a one-way repeated-measures ANOVA followed by Bonferroni’s multiple comparison tests was performed. $P$ values of $<0.05$ were considered statistically significant.

Sensitivity Analysis

Sensitivity analysis provides useful information regarding the influence of each parameter on the concentration-time profile $C(t)$. Thus the temporal variability in sensitivities affects parameter uncertainty. Information about a model parameter $p$ may be most accurately gained at time points with a high sensitivity to the parameter $p$. The sensitivity function

$$S_p(t) = \frac{p}{C(t)} \frac{dC(t)}{dp} = \frac{p}{C(t)} T(t) - 1 \left( \frac{dC(t)}{dp} \right)$$

determines the relative change in $C(t)$ caused by a small relative change in the model parameter $p$. Because $S_p$ is nondimensional, it allows a comparison of results obtained for different parameters and indicators. With the use of Eq. 1, the sensitivity functions were calculated with the help of Maple 5 (Waterloo Maple, Waterloo, Ontario, Canada) after implementing a numerical method of inverse Laplace transformation (19).

RESULTS

The fitting of the model (Eq. 1) to indicator disposition data under the various conditions studied is exemplified in Fig. 2. Satisfactory fits were achieved, with the exception of systematic deviations in the early mixing phase (i.e., immediately after the first-pass peak). The mean $R^2$ values, which are a measure of the goodness of fit of the model, were 0.993, 0.989, and 0.993 for ICG, inulin, and antipyrine, respectively, under control conditions. Similarly, the mean MSC values were 4.1, 5.9, and 5.5 for ICG, inulin, and antipyrine, respectively. No significant changes in these goodness-of-fit parameters were observed for concentration versus time data obtained during an infusion of isoproterenol or phenylephrine. The adjustable parameters $Q, V_{p,B}, RD_{p,B}, V_{s,B}, RD_{s,B}$, and $E$ for ICG and $V_p, RD_p, V_s, RD_s$, and $E$ for the extravascular indicators inulin and antipyrine, could be estimated with reasonable precision. The average model parameters and estimation errors (as coefficients of variation) are listed in Table 1. Parameters were estimated with relatively low coefficients of variation. The sensitivity functions shown in Fig. 3 indicate how the relative dispersions influence the disposition curve in the investigated
time range. We have limited sensitivity analysis to the relative
dispersions because it is also obvious from previous work (16)
that the other (model independent) parameters could be esti-
mated without problems. For inulin and antipyrine, the model
output, \( C(t) \), was sensitive to parameter \( \text{RD}_s^2 \) in the first 15 min,
whereas from 50 to 200 min, the sensitivity was higher for
inulin. As expected, \( C(t) \) was almost insensitive to variation in
\( \text{RD}_{p}^2 \), except for the time around the initial and recirculation
peak concentration. For the vascular marker ICG, the sensitiv-
ity for \( \text{RDB}_{p}^2 \) was narrowed to an interval of \( \approx 0.1 \) min after
bolus injection. The requirement of high sampling rate in
regions with high sensitivity was fulfilled for the relative
dispersion by the experimental design of this study.

For the capillary permeability-limited solute inulin, \( \text{RD}_s^2 \)
increased linearly with cardiac output (Fig. 4), in accordance
with theory because \( \frac{Q}{PS_s} > 1 \) and \( \frac{d_s}{MTT_{s}} \ll 1 \) (Eq. 4). At
the opposite extreme, the \( \text{RD}_s^2 \) of the flow-limited indicator
antipyrine (\( \frac{Q}{PS_s} \ll 1 \)) showed no significant dependency on
cardiac output (Fig. 5), but the intercept of the regression line,
3.3 ± 0.5, was similar to the \( \text{RD}_{p}^2 \) of the vascular marker,
3.0 ± 0.3 (Table 1), indicating that there was no significant
contribution from tissue diffusion to \( \text{RD}_s^2 \) (the third term in Eq.
4). However, this linear relationship held only for the control
and isoproterenol treatments, whereas the phenylephrine treat-
ment resulted in higher \( \text{RD}_s^2 \) than the other treatments, despite
producing a lower cardiac output (Fig. 5). The latter result
cannot be explained by Eq. 4 (vide infra). Note that the slope
of the \( \text{RD}_s^2 \) versus Q relationship estimated for inulin (Fig. 4) of
4.1 min/l leads to a permeability surface product \( PS_s \) of 0.11
l/min (substituting the estimated average \( v_s = 0.46 \) into Eq. 4).

Fig. 2. Representative fits of circulatory model to arterial indicator concentrations after bolus injection (dog 2) for indocyanine green (ICG) (A), inulin (B), and antipyrine (C) without vasoactive drug (Con, control), during phenylephrine (Phe) infusion, and during isoproterenol (Iso) infusion. Inset: disposition curve up to limit of detection.

If we alternatively calculate \( PS_s \) in each dog (Eq. 5) using the
estimates of relative dispersion of inulin (\( \text{RD}_s^2 \)) and ICG
(\( \text{RDB}_{s}^2 \)), we get values of 0.54 ± 0.26, 0.45 ± 0.19, and 0.37 ±
0.05 for the control, phenylephrine, and isoproterenol treat-
ments, respectively. Although the slope of the \( \text{RD}_s^2 \) versus Q
relationship was not significantly different from zero for anti-
pyrine (Fig. 5), \( \text{RD}_s^2 \) was significantly higher than \( \text{RDB}_{s}^2 \) in the
control group; thus Eq. 6 could be used to calculate the equilibration time constant \( d_s \) of 10.1 ± 4.6 min that charac-
terizes whole body distribution of antipyrine due to extravas-
cular diffusion.

There were no significant changes in \( \text{RDB}_{s}^2 \) and \( \text{RDB}_{p}^2 \) of ICG
under control conditions and during infusion of the vasoactive
drugs, indicating that a change in cardiac output did not alter
the heterogeneity of capillary transit times. However, treating
the estimates for ICG and antipyrine as one group, a significant
increase in the relative dispersion through the pulmonary
circulation, \( \text{RD}_{p}^2 \), during phenylephrine infusion was observed.

The estimates of \( \text{RD}_s^2 \) and \( \text{RD}_{p}^2 \) were used to calculate \( CL_M \)
(Eq. 8) as a measure of whole body distribution kinetics. The
distribution clearance, \( CL_M \), of antipyrine significantly in-
creased and decreased during isoproterenol and phenylephrine
infusion, respectively, and exceeded significantly that of inulin,
except during the phenylephrine infusion (Fig. 6). Like \( \text{RD}_s^2 \),
the \( CL_M \) of antipyrine during the phenylephrine infusion was
inconsistent with the linear relationship between \( CL_M \) and Q
observed during control and isoproterenol infusion conditions
(Fig. 7). Hemodynamic changes had no significant influence on
the \( CL_M \) of inulin (Fig. 6).
Table 1. *Estimated and derived parameters of ICG, inulin, and antipyrine disposition in four dogs under control conditions as well as during phenylephrine and isoproterenol infusions*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cardiac Output</th>
<th>Relative Dispersions</th>
<th>Distribution Volumes</th>
<th>Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Q, l/min</td>
<td>RD2</td>
<td>RD2</td>
<td>Vss, liters</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICG</td>
<td>Mean (SD)</td>
<td>4.81 (0.62)</td>
<td>3.04 (0.26)</td>
<td>0.039 (0.007)</td>
</tr>
<tr>
<td></td>
<td>CV (SD)</td>
<td>1.4 (0.9)</td>
<td>15.8 (11.4)</td>
<td>1.2 (2.1)</td>
</tr>
<tr>
<td>Inulin</td>
<td>Mean (SD)</td>
<td>17.81 (5.46)</td>
<td>0.069 (0.001)</td>
<td>12.36 (1.6)</td>
</tr>
<tr>
<td></td>
<td>CV (SD)</td>
<td>7.9 (3.2)</td>
<td>15.3 (10.2)</td>
<td>3.0 (1.1)</td>
</tr>
<tr>
<td>Antipyrine</td>
<td>Mean (SD)</td>
<td>3.7 (0.54)</td>
<td>0.045 (0.007)</td>
<td>27.13 (4.66)</td>
</tr>
<tr>
<td></td>
<td>CV (SD)</td>
<td>6.2 (1.69)</td>
<td>4.1 (3.1)</td>
<td>2.7 (0.8)</td>
</tr>
<tr>
<td><strong>Phenylephrine</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ICG</td>
<td>Mean (SD)</td>
<td>2.91 (0.72)</td>
<td>3.09 (0.77)</td>
<td>0.055 (0.006)</td>
</tr>
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<td></td>
<td>CV (SD)</td>
<td>2.3 (0.4)</td>
<td>21.3 (1.8)</td>
<td>3.3 (2.5)</td>
</tr>
<tr>
<td>Inulin</td>
<td>Mean (SD)</td>
<td>13.34 (1.79)</td>
<td>0.074 (0.018)</td>
<td>15.98 (1.99)</td>
</tr>
<tr>
<td></td>
<td>CV (SD)</td>
<td>16.0 (11.3)</td>
<td>14.0 (8.9)</td>
<td>9.1 (9.5)</td>
</tr>
<tr>
<td>Antipyrine</td>
<td>Mean (SD)</td>
<td>9.69* (2.78)</td>
<td>0.062 (0.01)</td>
<td>29.32 (5.87)</td>
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<tr>
<td></td>
<td>CV (SD)</td>
<td>10.8 (3.2)</td>
<td>0.8 (2.1)</td>
<td>6.4 (2.6)</td>
</tr>
<tr>
<td><strong>Isoproterenol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICG</td>
<td>Mean (SD)</td>
<td>11.24 (1.78)</td>
<td>3.19 (0.66)</td>
<td>0.048 (0.004)</td>
</tr>
<tr>
<td></td>
<td>CV (SD)</td>
<td>1.3 (0.2)</td>
<td>10.0 (2.9)</td>
<td>1.2 (2.1)</td>
</tr>
<tr>
<td>Inulin</td>
<td>Mean (SD)</td>
<td>48.30* (8.07)</td>
<td>0.069 (0.014)</td>
<td>12.62 (1.51)</td>
</tr>
<tr>
<td></td>
<td>CV (SD)</td>
<td>9.4 (4.4)</td>
<td>11.6 (7.6)</td>
<td>3.9 (1.6)</td>
</tr>
<tr>
<td>Antipyrine</td>
<td>Mean (SD)</td>
<td>4.42 (0.33)</td>
<td>0.041 (0.006)</td>
<td>27.66 (2.92)</td>
</tr>
<tr>
<td></td>
<td>CV (SD)</td>
<td>11.1 (5.0)</td>
<td>5.8 (3.5)</td>
<td>3.8 (2.4)</td>
</tr>
</tbody>
</table>

Values are means (SD) or coefficient of variation (SD). Subscripts s and p denote systemic and pulmonary circulation, respectively. †Vss = Vs + Vp; ‡CL = E × Q. Asymptotic coefficient of variation (CV%) is a measure of imprecision of individual parameter estimates. ICG, indocyanine green. *P < 0.05 vs. control.

DISCUSSION

The results of this study show that 1) the inverse Gaussian distribution can be used as TTD for inulin and antipyrine, extravascular indicators with opposite extreme diffusivity properties, as well as for the intravascular markers (ICG), to estimate the relative dispersions; 2) the relative dispersion in the systemic circulation (RDs) is a linearly increasing function of cardiac output with a slope that is inversely related to solute diffusivity (Figs. 4 and 5), in accordance with the theoretical prediction when no flow redistribution occurs; 3) the total distribution clearance decreases with increasing RDs (Eq. 8 and Fig. 6) and increases with cardiac output for highly diffusible solutes (Fig. 7); and 4) the concomitant estimation of relative dispersion of ICG as a measure of intravascular mixing kinetics (multiple indicator dilution approach) allows the calculation of overall capillary permeability or diffusional equilibration time of extravascular markers. Taken together, these results suggest that RDs is a useful model-independent measure of whole body distribution kinetics that can be interpreted in terms of the underlying physiological mechanisms. The approach avoids the assumption of well-mixed homogeneous compartments underlying previous models of whole body distribution of solutes (8, 10, 18). Apart from the possibility that this simplification may produce biased parameter estimates due to model misspecification, these models cannot predict, for example, the dependency of solute distribution on cardiac output.

Relative Dispersion in Pulmonary Circulation

Although the primary aim of this study was the estimation and interpretation of relative dispersion in the systemic circulation, our estimate of vascular pulmonary dispersion (RDp = 0.039, i.e., RDp = 0.2) obtained by analyzing the whole body disposition data is in the same order of magnitude as the values of 0.46 and 0.38 estimated in the anesthetized dog (14) and perfused dog lung lobes (6), respectively. The phenylephrine-induced increase in RDp by 41% and decrease of Vp by 17% is comparable to the changes of 75 and 15%, respectively, reported after hypoxic pulmonary vasoconstriction (7). Because Audi et al. (1) have shown that a flow change per se does not change RDp, the increase in relative dispersion by phenylephrine may be attributed to an increased heterogeneity of flow distribution. Because of the small interstitial space of the lungs (v0.02) (20), whole body methods are not sensitive enough to detect differences in the pulmonary distribution volumes and relative dispersion of indicators.
Relative Dispersion in Systemic Circulation

The relative dispersion of an intravascular marker in the systemic circulation, $\text{RD}_{s,2}^2$, is due to the advective dispersion in the vascular networks. In the present lumped organ model, the main determinant of $\text{RD}_{s,2}^2$ is the heterogeneity of mean transit times $(\text{MTT}_i = \frac{V_i}{Q_i})$ through the organs arranged in parallel (Eq. 7). Benowitz et al. (5) used a microsphere technique to measure blood flow distribution in monkeys under control conditions and during an isoproterenol infusion. With the use of their data, but neglecting the influence of very slowly equilibrating tissues and assuming nearly well-mixed tissue systems $(\text{RD}_{i,2}^2 = 1.4)$ (29), Eq. 7 gives $\text{RD}_{s,2}^2$ values of 2.0 and 3.4 for control and isoproterenol, respectively. With consideration of the different experimental conditions and species, these predictions are in reasonable agreement with our estimates of 3.0 $\pm$ 0.3 and 3.2 $\pm$ 0.7, respectively. If we use in Eq. 7 the volume and flow estimates previously obtained in the same dogs for the intravascular marker ICG (16) by using a recirculatory model in which the systemic circulation consists of only two compartments, the splanchnic and nonsplanchnic circulation (11), we obtain $\text{RD}_{s,2}^2$ values of 2.0 and 2.7, respectively. Thus neither phenylephrine nor isoproterenol infusion led to a significant change in $\text{RD}_{s,2}^2$. It should be also pointed out that the estimated relative dispersion of circulation times, $\text{RD}_{i,2}^2$ (see Eq. A2), of 1.3 $\pm$ 0.1 is surprisingly near to the value of 1.4, characterizing an optimal circulatory mixing process for an inverse Gaussian TTD (29). This verifies the practical usefulness of the “initial mixing volume” approximation underlying Eq. 8.
The linear increase of RD\textsuperscript{2} of inulin with cardiac output (Fig. 4) was predicted by theory (Eq. 4) and suggests that, for permeability-limited solutes, the systemic TTD becomes more heterogeneous when cardiac output increases. This relationship allows the calculation of whole body average of PS\textsubscript{S}, (0.11 l/min) that is in accordance with the value of 0.1 l/min measured in human forearm muscle (13). As expected for capillary permeability-limited distribution, where the influence of extravascular diffusion (third term in Eq. 4) is negligible (4), no significant change of PS\textsubscript{S} was observed during phenylephrine and isoproterenol infusions. The latter is in accordance with the finding that an increase in coronary flow by isoproterenol had no effect on PS\textsubscript{S} of inulin in the perfused rabbit heart (36). Note, furthermore, that the relative dispersion of ICG is the main determinant of RD\textsubscript{S} during the phenylephrine infusion, where, due to the concomitant increase in RD\textsuperscript{2} discussed above, the cardiac output reduction leads to a more pronounced decrease in CL\textsubscript{M} (Eq. 8) to a value near that observed for inulin. Thus both the decrease in cardiac output and its redistribution may affect mixing of antipyrine into its distribution volume (16). In contrast, the distribution clearance of the permeability-limited indicator inulin was not influenced by hemodynamic changes (Fig. 6). This becomes clear if we note that Q practically cancels in Eq. 8 after substitution of RD\textsuperscript{2} \sim Q/PS\textsubscript{S} (for Q/PS\textsubscript{S} \gg 1, Eq. 4 and Fig. 4). For antipyrine, at the other extreme, RD\textsuperscript{2} becomes practically independent of Q (Q/PS\textsubscript{S} \ll 1, Eq. 4 and Fig. 5), with the consequence that CL\textsubscript{M} increases proportional to Q (Fig. 7). Thus, for other solutes with an intermediate diffusivity, one would expect a slope of the CL\textsubscript{M} \sim Q relationship between the values found here for inulin and antipyrine.

The role of CL\textsubscript{M} in whole body distribution kinetics is illustrated in Fig. 8, which shows the corresponding normalized equilibration curves, i.e., concentration-time profiles in a hypothetical noneliminating system. The area under the mixing curve, AUC\textsubscript{M}, of antipyrine increases and decreases, respectively, during phenylephrine and isoproterenol infusions (Eq. A6). Note that the mixing curves for inulin superimpose on that of antipyrine during phenylephrine infusion. Thus, while neither phenylephrine nor isoproterenol influence the rate of inulin mixing into the interstitial fluid, the much higher rate of antipyrine distribution into total body water is further increased by isoproterenol and reduced by phenylephrine to the value observed for capillary permeability-limited distribution. Finally, these results provide an explanation for the observation made by Small and Homer (25) that solute mixing in extravascular fluid occurs more rapidly if the variance of circulatory transit time is small.

Comparison of Models

A condition that limits the complexity of models of solute distribution in the body is that they must be theoretically and...
practically identifiable while remaining consistent with known physiology. For the previously applied multicompartment circulatory model (15, 16), this necessary simplification consists of the assumption of well-mixed compartments arranged in parallel in the systemic circulation. In this work, we take an alternative strategy that lumps the parallel tissue systems into one heterogeneous subsystem, without assuming well-mixed distribution volumes. Thus each of the two modeling approaches has pros and cons. Reflecting the fundamental structure of the circulatory system, the current model extracts information about circulatory mixing, permeation kinetics, and extravascular diffusion of indicators without providing information on the role of parallel distribution channels in the systemic circulation. The multicompartment model provides information on distribution of indicators across distributive and nondistributive parallel pathways in the systemic circulation, without distinguishing between advective and diffusive transport processes. In quantifying the contribution of circulatory mixing to RD2 as given by the RD1, estimates of ICG, the marker ICG was mainly determined by the heterogeneity of muscular uptake clearance (and RD2) parameters like distribution volumes and systemic clearance (extraction ratio), correspond well with the estimates previously reported and discussed using a multicompartment recirculatory model (16). Thus our discussion in this study is focused on distribution kinetics.

Recall that the task here was to test both the usability of the inverse Gaussian distribution as an empirical model to estimate the relative dispersion of systemic transit times and its physiological interpretability by means of a lumped organ model. The parameter CLM was proposed as a measure of overall rate of solute mixing into their distribution volumes. The next step is to split the systemic circulation into two subsystems, skeletal muscle and the rest, and to identify this more complicated system by using a priori information (Bayesian estimation technique). With provision of bridge between the alternative approaches discussed above, this extended model would allow the estimation of muscular uptake clearance (and RD2) of, for example, insulin, which has shown to distribute like inulin (24).

We conclude that the estimation of transit time dispersions on the basis of a recirculatory model assuming inverse Gaussian TTDs is a useful method to study the distribution kinetics of solutes in whole body multiple indicator dilution experiments. The systemic transit time dispersion of the vascular marker ICG was mainly determined by the heterogeneity of organ mean transit times. For the extravascular solutes inulin and antipyrine, the dependency of RD2 on drug-induced changes in cardiac output was consistent with the prediction of a lumped organ model if the influence of flow redistribution among organs can be neglected. Apart from providing a better quantitative understanding of the determinants of whole body distribution of solutes, this approach allows to study the effect of disease states not only on physiological distribution volumes but also on the underlying distribution kinetics of the respective indicators. The results obtained here for the indicators inulin and antipyrine also provide information on distribution kinetics of, for example, insulin (24) and ammonia (35), thus suggesting that the uptake rate of insulin into skeletal muscle (which mainly determines CLM) is independent of flow, whereas the distribution clearance of ammonia is proportional to cardiac output. Given the information provided by RD2, the model may find application in other fields, such as analysis of noninvasively measured cardiopulmonary TTDs (23).

**APPENDIX A**

**Circulatory Minimal Model of Indicator Disposition**

The assumption of a well-mixed (sampling) compartment made in classical compartmental modeling is in contrast to noninstantaneous circulatory mixing after intravenous bolus injection. The use of TTDs, i.e., TTD functions f(t) of compounds across organs or subsystems of the body, avoids such an assumption and provides a more general framework for the evaluation of solute distribution kinetics. In the Laplace domain, we can take advantage of the simple rule to derive the TTD of two subsystems connected in series, f(s) = f1(s)f2(s), to analyze the minimal circulatory system (Fig. 1). The input to the pulmonary circulation is given by C\text{pul,in} = C(s) f(s)(1 - E) + D\text{Q/Q} (output of the systemic circulation plus contribution of bolus dose), and because C(s) = C\text{pul,out} = C\text{pul,in}(s)f(s), we obtain Eq. 1 by solving the resulting equation for C(s). Note that the factor (1 - E) is the probability that a molecule with a systemic extraction ratio E = CL/Q will pass through the systemic circulation. Because
the TTD of the two subsystems in series, $f_{xuc}(s) = f_x(s) f_i(s)$ is the TTD of circulation times, with mean circulation time given by $MCT = MTT_x + MTT_i$. The extent of distribution in the $i$th noneliminating subsystem is then determined by the mean transit time (MTTi) across the subsystem and the flow rate Q. $V_t = Q MTT_i,$ where $V_t$ is the apparent (steady state) distribution volume of subsystem $i$ and $MTT_i = \int_0^\infty f_i(t)dt$. Information on the dynamics of distribution is given by the relative dispersion of transit times

$$RD_t^2 = \frac{VRT}{MTT^2} = \int_0^\infty \frac{(t - MTT)^2 f_i(t)}{MTT^2} dt$$

(A1)

Because of the additivity of variances, the variance of circulation times $VCT = VTT_x + VTT_i$ (where $VTT_i$ is the variance of transit times across subsystem $i$), the relative dispersion of circulation times is given by

$$RD_t^2 = \frac{RD_x^2 MTT_x^2 + RD_i^2 MTT_i^2}{(MTT_x + MTT_i)^2}$$

(A2)

**APPENDIX B**

**Total Distribution Clearance**

The concentration-time curve $C_M(t)$ observed after bolus injection of an extravascular indicator into a hypothetical noneliminating system can be referred to as a “mixing curve” because it is solely determined by mixing of indicators into their particular distribution volumes. This concept has been introduced as a tool for a model-independent characterization of distribution kinetics assuming a monotonically decreasing disposition curve (30). [Note that $C_M(s)$ is described by Eq. 1 when the extraction ratio E is set equal to zero.] The area under the mixing curve $AUC_M$, i.e., the area located between $C_M(t)$ and the concentration level reached at equilibrium $C_M(\infty) = D_i/V_{trans}$, quantifies the transient departure of the system from equilibrium distribution since this area disappears in the case of a well-mixed system in which equilibrium distribution is established immediately. This measure is given by (30)

$$AUC_M = \frac{1}{2} \frac{D_n}{Q} (RD_t^2 - 1)$$

(A3)

Because of the disposition curves are regarded as decreasing, the initial period of about two min after bolus input is neglected. Note that for a well-stirred system characterized by an exponential TTD ($RD_t^2 = 1$) the equilibrium concentration $C_M(\infty)$ is achieved instantaneously at $t = 0$ and $AUC_M = 0$ as predicted by Eq. A3, whereas for $RD_t^2 > 1$, equilibrium distribution is reached asymptotically. Eq. A3 can be written in a dose-independent form as

$$\frac{AUC_M}{C_M} = \frac{1}{2} \frac{V_t}{Q} (RD_t^2 - 1)$$

(A4)

By analogy to the elimination clearance, one can define a “mixing clearance” $CL_M$ as a global measure of the rate of indicator distribution in the body. In a noneliminating system, $CL_M$ governs the distribution of indicator (amount, $D_n$) out of the initial mixing space $V_0$ until equilibrium in the rest of the body is attained, according to

$$\frac{dC(t)}{dt} = - CL_M (C(t) - C(\infty))$$

(A5)

With integration of Eq. A5 and the assumption of $C(\infty) V_0 \ll D_n$, we obtain the following relationship after substituting $AUC_M = \int_0^\infty [C(t) - C(\infty)]dt$ and $C(0) V_0 = D_n$:

$$CL_M = \frac{D_n}{AUC_M} = \frac{2Q}{RD_t - 1}$$

(A6)

Although this approach is based on the simplifying assumption that indicator dose $D_n$ mixes quasi-instantaneously within $V_n$, no further assumptions on the structure of the body are made.

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