Impulse-response function of splanchnic circulation with model-independent constraints: theory and experimental validation

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Impulse-response function of splanchnic circulation with model-independent constraints: theory and experimental validation. Am J Physiol Gastrointest Liver Physiol 285: G671–G680, 2003. First published April 9, 2003; 10.1152/ajpgi.00054.2003.—Modeling physiological processes using tracer kinetic methods requires knowledge of the time course of the tracer concentration in blood supplying the organ. For liver studies, however, inaccessibility of the portal vein makes direct measurement of the hepatic dual-input function impossible in humans. We want to develop a method to predict the portal venous time-activity curve from measurements of an arterial time-activity curve. An impulse-response function based on a continuous distribution of washout constants is developed and validated for the gut. Experiments with simultaneous blood sampling in aorta and portal vein were made in 13 anesthetized pigs following inhalation of intravascular $[15\text{O}]\text{CO}$ or injections of diffusible 3-O-$[11\text{C}]$methylglucose (MG). The parameters of the impulse-response function have a physiological interpretation in terms of the distribution of washout constants and are mathematically equivalent to the mean transit time ($T$) and standard deviation of transit times. The results include estimates of mean transit times from the aorta to the portal vein in pigs: $T = 0.35 \pm 0.05$ min for CO and $1.7 \pm 0.1$ min for MG. The prediction of the portal venous time-activity curve benefits from constraining the regression fits by parameters estimated independently. This is strong evidence for the physiological relevance of the impulse-response function, which includes asymptotically, and thereby justifies kinetically, a useful and simple power law. Similarity between our parameter estimates in pigs and parameter estimates in normal humans suggests that the proposed model can be adapted for use in humans.

MODELING PHYSIOLOGICAL PROCESSES using tracer kinetic methods, such as blood sampling techniques or external detection by positron emission tomography (PET), requires knowledge of the time course of the tracer concentration in blood supplying the organ. PET is a unique technique for the study of regional metabolic processes, but despite the key role of the liver in metabolism, the use of PET for liver studies has been limited. The problems are due, in part, to the liver having a dual blood supply, through the hepatic artery and portal vein (PV), where inaccessibility of the PV makes direct measurement of the dual-input function impossible in humans. However, the hepatic dual-input function is a prerequisite for quantitative estimates of kinetic parameters, which has been demonstrated in the intact pig liver for the kinetics of the glucose analogs 3-O-$[11\text{C}]$methylglucose (MG) and $[18\text{F}]$fluorodeoxyglucose (FDG) using blood samples from both supply vessels (12). We therefore wished to develop a method to predict the portal venous time-activity curve (TAC) from measurements of an arterial TAC, which we validate using data obtained by simultaneous arterial and portal venous blood sampling in a pig study. It is essential to construct the mathematical forms of the requisite impulse-response functions such that they lead to agreement with the pig data and are capable of adaptation to humans by adjusting characteristic parameters. The construction and experimental validation of the splanchnic impulse-response function is of interest both for the reconstruction of the hepatic dual-input function for kinetic analysis of liver metabolism as well as for understanding of the splanchnic hemodynamics. In practice, it affects particularly the determination of kinetic parameters for which bolus injections are necessary.

The shape of the vascular bolus of PET tracer, conventionally specified by arterial sampling, is changed substantially on its way to the PV and liver by the capillary bed of the gut. The temporal evolution of bolus shapes is often described mathematically by a discrete set of washout constants $k_i$. The smallest of these, say $k_0$, determines the slowest, last exponential traditionally used for extrapolations dealing with recirculation and final recoveries of tracers (10). The deficiencies of this representation, demonstrated by Lassen and Sejrsen (11) have lead Bass et al. (4) to replace the discrete set of exponential constants $k_i$ by a continuum of $k$ values resulting in a new asymptotic form of late washout that describes the precise long-term measurements of Lassen and Sejrsen (11) on perfused muscle better than a last exponential. A further drawback of representing vascular inputs by a discrete series of exponentials appears in the numeri-
cal analysis of PET data. We have found that the exponents predicted for the PET-observed tissue curves, with exponents to be determined, interact computationally with the vascular input exponents so that the fitting of both types of exponents becomes unstable (O. L. Munk, unpublished observation). We shall therefore model the splanchnic impulse-response function, especially that of the gut, using a continuous distribution of washout constants more fully than has been done by Bass et al. (4) for muscle. Because all the moments of our impulse-response functions exist, we are able to confront the model with the mean transit time determined from our data by model-independent Bradley analysis (7) and with the variance of transit times obtained by the method of Bradley, as extended by Bass (3) and applied to the splanchnic circulation in human subjects by Winkler et al. (14). The knowledge of the mean and variance of transit times provides powerful constraints on the fitting of the parameters of our impulse-response function. The application of these Bradley-type analyses to our data is simplified by our use of the tracers [15O]CO and MG, which are not metabolized in the splanchnic domain.

MATERIALS AND METHODS

Animals

The study procedures were approved by the Danish Ethics Committee for animal research. Thirteen pigs of Danish landrace, 3 mo old, body weight 38–42 kg were used. The pigs were deprived of food for 12 h before experiments but had free access to water.

Animal Preparation

The pig was anesthetized by injection of 20 ml Dormicum (midazolam 5 mg/ml) and 12 ml Ketalar (ketamin 50 mg/ml) followed by intravenous infusion of a mixture of 10 ml/h Dormicum, 10 ml/h Ketalar, and 30 ml/h isotope saline. Every fourth hour, the pig received an analgesic injection of Dormicum, 10 ml/h Ketalar, and 30 ml/h isotone saline. Oxygen saturation and pH in arterial blood samples were measured every hour and were adjusted toward >98% and 7.45, respectively, by changing the amount of air delivered from the respirator. Arterial blood glucose levels were measured every 2 h after tracer administration.

Blood Sampling Experiments

Blood sampling experiments were performed in seven pigs following 500-MBq [15O]CO 10-s inhalations. In addition, blood sampling experiments were performed in six pigs following 500-MBq 3-O-[11C]MG 15-s intravenous injections. After CO inhalations, arterial and PV blood samples of each 1 ml were collected manually for 310 s starting at injection time in the following intervals: 8 × 5, 4 × 15, 3 × 30, and 2 × 60 s. After MG injection, blood was sampled for 8 min in the following intervals: 18 × 5, 3 × 10, and 2 × 30 s and 1 × 2 and 1 × 3 min. Blood radioactivity concentrations were measured using a well counter (Packard Instruments, Meriden, CT), which was calibrated using a 68Ge/68Ga solution with known activity concentration. All measurements in the blood samples were decay corrected to the start of the tracer administration. Blood flows in hepatic artery and PV were measured continuously throughout the experiment.

Modeling

Model independent analysis. If the concentrations of blood input [Ci(t)] and blood output [Co(t)] of an organ are known, the mean transit time can be calculated by Bradley’s method (7)

$$\bar{T} = \int_{0}^{\infty} \left[ C_i(t) - C_o(t) \right] dt$$

where $C_o$ is the tracer concentration in the final equilibrium state $C_i = C_o = C_\infty$ and $\bar{T}$ is the mean transit time. It has been shown by Bass (3) that the variance $\sigma^2$ of the transit times is given by

$$\sigma^2 = 2 \int_{0}^{\infty} t \left[ C_i(t) - \bar{T} - C_o(t) \right] dt$$

Together, the extended Bradley analysis (Eqs. 1 and 2) yields information of the distribution of transit times without requiring knowledge of the underlying impulse-response function.

Formulation of the impulse-response function. We assume the impulse-response function $h(t)$ of the gut in the form

$$h(t) = A e^{-G(k) t}$$

where $A$ is a normalization constant and $G(k)$ is a non-negative, normalized density function that may be interpreted as the probability distribution of $k$ values pertaining to sites in the tissue that bind tracer molecules for various time intervals before allowing them to return to the blood independently of each other (11). The form of $G(k)$ should have three salient features. There should be a smallest $k$, denoted $k_0$, corresponding to the “last exponential” (4, 11). There should be a distribution around a most frequent $k$. The function $G(k)$ should tend toward zero as $k$ increases, reflecting the finite rates of transport in a capillary bed. We choose

$$G(k) = \begin{cases} 0 & 0 < k \leq k_0 \\ B(k - k_0) e^{-\beta(k - k_0)} & k > k_0 \end{cases}$$

as shown in Fig. 1, where $B$ is a normalization constant, and $1/\beta$ is the most frequent value of $(k - k_0)$. The complete expression of $h(t)$ is obtained by inserting the normalization constants $A$ and $B$ into $G(k)$ and $h(t)$ (see APPENDIX I).
and the mean transit time $\bar{T}$ and mean square transit time $\bar{T}^2$, i.e. the first and second moment of $h(t)$ are (see APPENDIX I)

$$T = \frac{\beta e^{\beta k_0}}{1 - \beta k_0 e^{\beta k_0}} [E_1(\beta k_0) - E_2(\beta k_0)]$$

and

$$\bar{T}^2 = \frac{\beta}{k_0} \{1 - \beta k_0 e^{\beta k_0} [2E_1(\beta k_0) - E_2(\beta k_0)]\}$$

Comparing Eqs. 6 with 7, we see that

$$\bar{T}^2 = \frac{\beta}{k_0} \{1 - k \bar{T}\}$$

and using the definition of variance $\sigma^2 = \bar{T}^2 - \bar{T}^2$

$$\sigma^2 + \bar{T}^2 = \frac{1}{\beta} \frac{T}{k_0}$$

We recall that $h(t)$ involves only two parameters, $\beta$ and $k_0$, which determine $\bar{T}$ and $\sigma$ by Eqs. 6 and 9. Conversely, a Bradley-type determination of $\bar{T}$ and $\sigma$ (3, 7, 14) determines $h(t)$ and hence predicts the complete relationship between measured arterial concentrations $C_i(t)$ and the measured portal venous concentrations $C_o(t)$ for all times

$$C_i(t) = \int_0^{\infty} h(t - \tau)C_i(\tau)d\tau$$

Asymptotic form of $h(t)$. For data that are not sufficiently extensive to allow determination of the full impulse-response function $h(t)$, an asymptotic form can be used. If $k_0 \ll 1$, then on a time interval for which $k_0 t \ll 1$, we find asymptotically from Eq. 5

$$h(t) = \frac{\beta}{(t + \beta)^2}$$

In this asymptotic form, $h(t)$ is a power function, which does not contain $k_0$. This one-parametric asymptotic form of $h(t)$ allows a fit to the data despite lacking physiological interpretation: all of its moments including $\bar{T}$ and $\sigma$ are infinite.

Constraining the impulse-response function $h(t)$. We can use $\bar{T}$ and $\sigma$, obtained by model-independent Bradley-type analysis of the data, to constrain the nonlinear regression fits of $h(t)$. This gives rise to four distinct methods; 1) $\bar{T}$ and $\sigma$ are estimated by Bradley analysis and used to determine $h(t)$ directly by determining $\beta$ and $k_0$ from Eqs. 6 and 9; 2) $\bar{T}$ is estimated by Bradley analysis and is used to constrain the determination of $\beta$ and $k_0$ in $h(t)$; 3) both $\beta$ and $k_0$ are determined by regression without constraints; and 4) only $\beta$ is determined without constraints in the asymptotic form of $h(t)$ given by Eq. 11. When $\bar{T}$ or $\sigma$ is not used as a constraint, it can be calculated from $h(t)$ using Eqs. A1.6 and A1.7.

Parameter estimation and statistical criteria. When estimating the impulse-response functions (Eq. 10), a time shift $\Delta t$ was included in the fitting for describing the difference in the sampling catheter volumes, the volume of the PV catheter (0.7 ml) being slightly larger than that of the arterial catheter (0.7 ml), and possible differences in the manual blood withdrawal speed. Parameters were estimated by minimizing the residual sum of squares (RSS). Plots of the weighted residuals against time were examined for systematic errors. The best model fitted to the data is not necessarily the model producing the smallest RSS, because adding more parameters in general decreases the RSS. Therefore, we identified the statistically favorable model based on a criterion that included penalty functions proportional to the number of parameters in the model. The Akaike Information Criterion (AIC) (2) was used to measure the quality of each fit

$$AIC = N \ln (RSS) + 2P$$

where $P$ is the number of parameters in the model and $N$ is the number of data points. Small values of AIC should be preferred. Results are given as means ± SE.

RESULTS AND DISCUSSION

The PV is the outlet from the prehepatic splanchnic organs, including gut and spleen, as well as one of the two inlets into the liver, the other being the hepatic artery. As discussed, methods to predict the portal venous TAC are of interest both for the understanding of the splanchnic hemodynamics as well as for being able to reconstruct the portal venous TAC needed for reconstruction of the hepatic dual-input function. Consequently, we need to predict the portal venous TAC so that a sufficiently simple impulse-response function $h(t)$, valid in pigs, might be adapted to humans by adjusting only a few parameters. To achieve this, the passage of a bolus through the gut should be described by only a few adjustable parameters with a clear physiological interpretation. The impulse-response function, proposed in this paper, is described by a pair of parameters, $\beta$ and $k_0$, which have a clear interpretation in relationship to the distribution of washout constants $G(k)$ as well as being mathematically equivalent to the mean transit time $\bar{T}$ and standard deviation of transit times $\sigma$. Apart from the minor shift of the time axes of the data, $C_i(t)$ and $C_o(t)$, either pair determines the impulse-response function completely. In trying to reduce the number of free parameters, we combined conventional regression with model-independent determination of the mean transit time $\bar{T}$ and standard deviation of transit times $\sigma$, which leads to the four methods of parameter estimation used in this paper. The methods were validated for an intravascular (CO) and a diffusible (MG) tracer.

Simulating impulse-response function $h(t)$. Figure 1 shows the distribution of washout constants $G(k)$ and the corresponding impulse-response function $h(t)$ for a realistic set of $\beta$ and $k_0$. Figure 2 shows simulations of the $h(t)$ by variation of its two parameters, $\beta$ and $k_0$. Small values of $\beta$ result in $h(t)$ with pronounced peaks, whereas larger $\beta$s flatten $h(t)$ and thereby introduce more dispersion and a considerable tail (Fig. 2A). By comparing Fig. 2, A and B, it is seen that the impulse-response function is more sensitive to $\beta$ than to $k_0$. However, small values of $k_0$ tend to flatten $h(t)$, produce more dispersion, and increase the importance of the tail, and as $k_0 \rightarrow 0$/min, $h(t)$ approaches the asymptotic form (Eq. 11).
Measured TACs. A typical set of blood sampling TACs following C\textsuperscript{15}O inhalation is shown in Fig. 3A, and a set of blood sampling TACs following an MG injection is shown in Fig. 3B. For both tracers, the arterial TAC had a rapid phase with a narrow peak, whereas the portal venous TAC was delayed and dispersed during the passage through the intestines. The difference between the arterial and portal venous TACs was most pronounced around the peak, immediately after the bolus injection. After some time, the TACs get less dynamic, and dispersion and delay through the intestines become less important. Eventually, the two blood TACs reached a quasi-steady state, where they were practically identical. The time point at which the two blood TACs coincide is important for the model-independent Bradley-type analysis (Eq. 1–2) as discussed in APPENDIX II for estimates of the mean transit time. For the intravascular tracer (CO), the quasi-steady state was reached after 2–3 min, whereas the TAC of the diffusible tracer (MG) was more dispersed due to a larger distribution volume and reached quasi-steady state after 5–8 min.

Because our aim is to reconstruct the hepatic dual-input function in cases where portal venous samples are unavailable, we need to model the portal venous TAC during the dynamic phase. Therefore, we model the CO data for 5 min and the MG data for 8 min. Afterwards, the dual-input TAC is well approximated using an arterial TAC (Fig. 3).

Modeling [\textsuperscript{15}O]CO data. For intravascular CO data, the time course of the fits were similar for all methods. An example of a fit using the unconstrained form (method 3) is shown in Fig. 4A and a corresponding fit using the asymptotic form (method 4) is shown in Fig. 4B. Fits using the constrained forms (methods 1 and 2) were visually identical and therefore not shown. Table 1 shows the parameter estimates of the four methods for CO inhalations in all seven pigs using data sampled for 5 min. All methods were performed similarly as seen from the statistical criteria. Method 2 is the best (although not statistically significant), and for this method, the mean values of the parameters are $\beta = 0.14$ min and $k_0 = 0.19$/min, which correspond to a mean transit time $\bar{T} = 0.35$ min and standard deviation of transit times $\sigma = 0.86$ min. With the use of the measured portal venous flow for the CO pigs, $F = 0.56 \pm 0.21$ l/min, we can calculate the splanchic blood volume in pigs: $V_{co} = F\bar{T} = 0.21$ l.

CO is a purely intravascular substrate and therefore washed relatively fast through the intestines having a relatively large final exponential $k_0$. Consequently, one would expect the asymptotic form of $h(t)$ for $k_0 \to 0$ (method 4, Eq. 11) to perform worse than the other...
methods. Judged by the goodness-of-fit criterion, it does perform slightly worse than the other methods, but the difference between the AIC scores of the four methods was not statistically significant. A statistical criterion such as AIC can be useful but does not contain information about physiological interpretability of the model parameters. We should compare statistical criteria only when comparing methods that we found equally good based on their physiological predictions. Therefore, methods 1–3 are clearly to be preferred to method 4, because $h(t)$ has finite moments and therefore allows calculation of mean transit time $T$ and standard deviation of transit times $\sigma$. When comparing methods 1–3, we focus on the parameter estimates of the models. The SE of parameter estimates has contributions from the different physiological states of the individual animals and from possible deficiencies of the model. The SE can never be less than the physiological variation between animals, but it is reasonable to prefer a method that contributes only little to the total SE without causing damaging bias, such as would have occurred if we had constrained $h(t)$ by values of $T$ and $\sigma$ derived from a population of fits. We avoid such a bias because we constrain $h(t)$ by model-independent measures of $T$ or $T$ and $\sigma$ obtained from each individual data set. Method 3 provides sets of $\beta$ and $k_0$, which imply $T$ in agreement with observed $T$, but implies too large a $\sigma$ when compared with $\sigma$ from extended Bradley analysis. This is caused by the low sensitivity to $k_0$, which is barely identifiable from our data sets by method 3 (see Fig. 2). In addition, the estimates fluctuate as seen by large SEs, which suggests a benefit of constraining the model. Method 2 is constrained by $T$ and yields sets of $\beta$ and $k_0$ that, by subsequent calculation of $\sigma$, are in agreement with the extended Bradley analysis. Furthermore, the estimates of $\beta$ and $k_0$ are more stable, judged by the smaller SE. On the basis of these observations, method 2 performs better than method 3. Method 1 puts our proposed $h(t)$ to an uncommonly severe test: the question is whether the physiological contents of the model are sufficiently detailed to allow for all parameters of $h(t)$ to be independently determined while maintaining an accurate prediction of the portal venous data. With the use of method 1, there is no free parameter except for the time shift used to account for different catheter volumes. The shape of $h(t)$ is completely determined by extended Bradley analysis, and as seen from Table 1, method 1 fits the data equally well as the other methods. This is strong evidence of the physiological relevance of the proposed impulse-response function.

### Modeling $3\O{-[11C]}$MG data

Examples of fits using all four methods are shown in Fig. 5, A–D, and Table 2 shows parameter estimates of the four methods for MG

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**Fig. 3.** Typical blood time-activity curves from an artery (○) and portal vein (PV; ●). The blood-sampling protocols were sufficiently dense to measure the dynamic phase of both the arterial and portal venous time-activity curves. A: intravascular tracer: $^{13}$O inhalation in pig 7. B: diffusible tracer: MG injection in pig 5. Lines are linear interpolations between data points. The time scales of the 2 plots are different to emphasize the time points at which arterial and portal venous measurements coincide within experimental error.

**Fig. 4.** Example of 2 fits using data sampled for 5 min following a CO inhalation (pig 7). Black circles are PV data points. The lines are the fitted model solutions. A: Method 3: fit using the unconstrained form. The fitted parameters were $\beta = 0.16$ min and $k_0 = 0.11/min$, which correspond to $T = 26$ s and $\sigma = 64$ s. B: Method 4: fit using the asymptotic form. The fitted parameter was $\beta = 0.13$ min.
injections in all six pigs using data sampled for 8 min. The fit using the fully constrained method 1 is inferior to methods 2–4, which is evident from Fig. 5 as well as judged by the statistical criteria in Table 2. Methods 2 and 4 provide the best methods based on statistical criteria (statistically significant). For method 2, the mean values of the parameters are \( \beta = 0.57 \text{ min} \) and \( k_0 = 0.024/\text{min} \), which correspond to a mean transit time \( \bar{T} \) of 1.7 min and a standard deviation of transit times \( \sigma \) of 4.6 min. With the use of the measured portal venous flow for the MG pigs, \( F = 0.78 \pm 0.12 \text{ l/min} \), we can calculate the splanchnic distribution volume of MG in pigs: \( V_{MG} = F \bar{T} = 1.3 \text{ l} \), which is around six times the blood volume.

MG is a diffusible glucose analog that can cross the membrane of intestinal cells but is not metabolized. The relatively large distribution volume causes MG to be washed out more slowly than CO. Therefore, the last exponential \( k_0 \) is small, suggesting that the asymptotic form (method 4) can provide good fits, which is supported by the AIC scores in Table 2. Method 2 is better

### Table 1. Parameter estimates and statistical criterion using C\(^{15}\)O inhalation in pigs

<table>
<thead>
<tr>
<th>Method</th>
<th>Parameters of ( h(t) )</th>
<th>Distribution of Transit Times</th>
<th>Statistical Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta ), min</td>
<td>( \bar{T} ), min</td>
<td>AIC</td>
</tr>
<tr>
<td></td>
<td>( k_0 ), per min</td>
<td>( \sigma ), min</td>
<td></td>
</tr>
<tr>
<td>Method 1</td>
<td>0.11 ± 0.02</td>
<td>0.35 ± 0.05</td>
<td>157 ± 8</td>
</tr>
<tr>
<td>Method 2</td>
<td>0.14 ± 0.02</td>
<td>0.35 ± 0.05</td>
<td>154 ± 9</td>
</tr>
<tr>
<td>Method 3</td>
<td>1.0 ± 0.4</td>
<td>0.33 ± 0.07</td>
<td>157 ± 6</td>
</tr>
<tr>
<td>Method 4</td>
<td>0.11 ± 0.02</td>
<td>0.86 ± 0.13</td>
<td>160 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 7). *Parameter obtained by model-independent Bradley-type analysis and used to constrain the regression fits of impulse-response function \( [h(t)] \). AIC, Akaike Information Criterion; \( \bar{T} \), mean transit time; \( \sigma \), standard deviation of transit times; \( k_0 \), smallest exponential constant \( \beta \), most frequent \( k - k_0 \).

### Table 2. Parameter estimates and statistical criterion using \([11C]\)MG injection in pigs

<table>
<thead>
<tr>
<th>Method</th>
<th>Parameters of ( h(t) )</th>
<th>Distribution of Transit Times</th>
<th>Statistical Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta ), min</td>
<td>( \bar{T} ), min</td>
<td>AIC</td>
</tr>
<tr>
<td></td>
<td>( k_0 ), per min</td>
<td>( \sigma ), min</td>
<td></td>
</tr>
<tr>
<td>Method 1</td>
<td>694 ± 373</td>
<td>1.7 ± 0.1(^*)</td>
<td>246 ± 11</td>
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<tr>
<td>Method 2</td>
<td>57 ± 0.07</td>
<td>1.7 ± 0.1(^*)</td>
<td>138 ± 11</td>
</tr>
<tr>
<td>Method 3</td>
<td>42 ± 0.07</td>
<td>2.8 ± 0.6</td>
<td>184 ± 29</td>
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<tr>
<td>Method 4</td>
<td>0.42 ± 0.05</td>
<td>4.6 ± 0.4</td>
<td>136 ± 11</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6). *Parameter obtained by model-independent Bradley-type analysis and used to constrain the regression fits of \( h(t) \). MG, methylglucose.

![Fig. 5. Example fits using data sampled for 8 min following a methylglucose injection (pig 5). Black circles are PV data points, and the lines are the fitted model solutions.](http://ajpgi.physiology.org/ Downloaded from)

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than the unconstrained method 3 and provides the best parameter estimates with small SEs and better AIC. For completeness, we included in Table 2 the results obtained by method 1. As shown in Appendix II, an extended Bradley analysis on data sampled for relatively short time can be problematic when used to estimate parameters for diffusible substances, which are washed out slowly. For MG, the model-independent parameters are obtained using data sampled for 8 min, and whereas this is enough for reliable estimates of $T$, it is too short for estimating $\sigma$ by the model-independent analysis (see Appendix II). Consequently, the abnormal results using method 1 are due to constraining $h(t)$ with an inaccurate $\sigma$ rather than to deficiencies in the splanchnic model. The physiological realism of our splanchnic model is confirmed by consistencies between model-independent measures and parameter estimates using methods 1 and 2 for CO and similarly method 2 for MG. For MG, however, the unconstrained method 3 was unable to predict $T$ and $\sigma$. Estimates of $k_0$ are uncertain because it is small and not as sensitive as $\beta$. However, method 2 worked well for MG yielding estimates of $k_0$ that were about 10 times lower than for CO. Substantially lower $k_0$ values (and higher $\sigma$ values) are to be expected when comparing a diffusible tracer with an intravascular tracer. To transfer the impulse function to the study of human livers by diffusible tracer, we recommend using the asymptotic solution (without $k_0$). This will be sufficient for calculating the liver kinetics, but it will not provide the characteristics of the GI tract.

Inverse Circe transformation. For humans, where portal venous samplings are unavailable, the time course of tissue activity concentrations as seen by PET may be used for fitting kinetic parameters of hepatic metabolism together with the parameters of the splanchnic impulse-response function. The pig was chosen as our experimental animal due to its similarity to human splanchnic and liver circulation. To adapt the impulse-response function from pigs to humans, we need to relate our parameter estimates in pigs to similar measurements performed in humans.

To our knowledge, no other estimate of the transit time of MG in the gut is reported in the literature, so only the parameter estimates from our CO experiments are compared with other studies. Our estimate of mean transit time of blood from aorta to the PV in pigs (Table 1; $\overline{T} = 0.35 \pm 0.05$ min) is in close agreement with experiments in humans, $\overline{T} = 0.37 \pm 0.05$ min, reported by Fine et al (8). The mean total hepatic blood flow ($F$) and mean hepatic blood volume ($V_h$) of pigs, $F = 1.02 \text{ ml.min}^{-1}.\text{ml}^{-1}$ and $V_h = 0.25 \text{ ml/ml}$, respectively, have been reported (12) from which the hepatic mean transit time $\overline{T}_{\text{Liver}} = 0.25 \text{ min}$ can be derived. This value is also in accordance with estimates from human $\overline{T}_{\text{Liver}} = 0.22 \pm 0.03$ (8) and 0.21 min (calculated using volume = 0.31 and volumetric flow = 1.45 l/min) (9).

Furthermore, the similarity of the splanchnic circulation between pig and human is supported by measurements of the distribution of flow between hepatic artery and PV: PV flow fraction = 0.8 in pigs (12) and PV fraction = 0.75 in humans (8) and PV fraction = 0.76 in humans (9). The model-independent determination of $\overline{T}$ and $\sigma$, which we used to constrain our impulse-response function (Eq. 1–2), has been applied successfully in normal human subjects as well as in patients with cirrhosis and with end-to-side portocaval shunts using labeled erythrocytes and blood sampling in the abdominal aorta as well as in a liver vein (14). Their estimate of the splanchnic mean transit time including the liver was 0.61 min in normals, which compares with our values in pigs for the mean transit time from artery to PV $\overline{T} = 0.35 \text{ min}$ added to the estimate of hepatic mean transit time $\overline{T}_{\text{Liver}} = 0.25 \text{ min}$.

In addition to the similarity between pig and human in the splanchnic domain, this agreement can be due to the cancellation of factors relating flow rate and blood volume between the species in the relationship $\overline{T} = V_h/F$. To relate the two species more closely, we may assume tentatively that the ratio of the smallest $k$ to the most frequent $k$, $k_d(1/\beta) = \beta k_0$, is approximately the same for both species for any indicator. Then, Eq. 4 implies that $\overline{T}/\beta$ is also the same in both species, so that both $k_0$ and $\beta$ (and so the entire impulse-response function) in humans would be determined from $\beta$ and $k_0$ in pig if also $\overline{T}$ in humans was determined for the prehepatic splanchnic domain. But this $\overline{T}$ was shown above to be close to that in pig for labeled erythrocytes. These tentative suggestions must be examined further by involving parameters of hepatic kinetics inferred by PET data in the best fit of the portal input.

Power-law limit of tracer washout. Extrapolation of tracer TACs beyond the experimental data acquisition is necessary for calculation of the area under the curve that can be used for determination of mean transit time as well as derived quantities such as organ blood flow (10). It has been a general belief that washout processes were exponential by nature, which is a point of view that agrees with a compartmental scheme, because the washout from a well-mixed compartment is monoexponential. Therefore monoexponential extrapolations of venous tracer TACs have been used for decades in experimental and clinical work (10, 11). However, correct extrapolation of tracer time activity is crucial for accurate determination of the physiological parameters, and the monoexponential extrapolation has been shown to be inadequate, leading to some underestimation of the mean transit time and thereby overestimation of blood flow (4, 5, 11).

In this paper, we described the venous outflow from an organ, especially the gut, by an impulse-response function based on a continuous distribution of washout constants (Eq. 4). The mathematical form of our impulse-response function (Eq. 5) contains a power-law term, which becomes dominant in the limit of a slow
final washout constant: as \( k_0 \to 0 \), \( h(t) \) approaches asymptotically the form of a power law (Eq. 11).

This derivation of the power law explains why the portal venous outflow for both intravascular (CO) and diffusible (MG) tracers (Tables 1 and 2) can be described accurately by a one-parametric function, which, taken out of the limiting context, would carry the unacceptable implication that the mean transit time, the variance of transit times, and all higher moments must necessarily be infinite. The asymptotic character of the power law supports and helps to clarify recent evidence for power-law behavior of tracer washout from isolated perfused rabbit hearts (5) and in plasma clearance curves for whole body kinetic studies (13). Although we derived the power law as a limit of our continuous set of washout constants, other processes such as flow heterogeneity or fractal washout have also been shown to give rise to power-law behavior (6). The limiting process \( k_0 \to 0 \) also raises a question concerning the accuracy of the Bradley analyses, which we address in APPENDIX II.

In conclusion, an impulse-response function based on a continuous distribution of washout constants was developed and validated for the gut in pig experiments with simultaneous blood sampling in aorta and PV. The prediction of the portal venous TAC was shown to benefit from constraining the model by model-independent estimates of the mean transit time (and the standard deviation of transit times), which is strong evidence for the physiological relevance of the model. Our impulse-response function includes asymptotically, and thereby justifies kinetically, a useful and simple power law. Similarity between our parameter estimates in pigs and in normal humans suggest that the proposed model can be adapted for use in humans.

APPENDIX I

Normalization of \( G(k) \)

The probability distribution of \( k \) values \( G(k) \), given by Eq. 4, is normalized by the constant \( B \)

\[
1 = \int_{0}^{\infty} G(k) dk = B \int_{k_0}^{\infty} (k - k_0)e^{-\beta k - k_0} dk = \frac{B}{B^2} \int_{0}^{\infty} e^{-u} du = \frac{B}{B^2}
\]

by substituting \( \beta(k - k_0) = u \) and \( \beta dk = du \), and thereby

\[
B = \frac{1}{\beta^2}
\] (A1.1)

Normalization of \( h(t) \)

The impulse-response function \( h(t) \), given by Eq. 5, is normalized by the constant \( A \)

\[
1 = \int_{0}^{\infty} h(t) dt = A \int_{k_0}^{\infty} \left[ -\frac{1}{k} e^{-kt} \right]_0^{\infty} G(k) dk = A \int_{0}^{\infty} \frac{G(k)}{k} dk
\]

so that

\[
\frac{1}{A} = \frac{1}{\beta^2} \int_{k_0}^{\infty} \frac{k - k_0}{k} e^{-\beta(k - k_0)} dk = \frac{1}{\beta^2} \int_{0}^{\infty} e^{-u} du = \frac{1}{\beta^2}
\]

\[
= \beta \int_{0}^{\infty} e^{-u} du - \beta^2 k_0 e^{\beta k_0} \frac{1}{\beta k} d(\beta k)
\]

then

\[
\frac{1}{A} = \beta \left[ 1 - \frac{\beta k_0 e^{\beta k_0}}{\beta k} E_1(\beta k_0) \right] (A1.2)
\]

where

\[
E_1(x) = \int_{x}^{\infty} \frac{e^{-u}}{u} du, \quad x > 0
\] (A1.3)

\[
E_2(x) = x \int_{x}^{\infty} \frac{e^{-u}}{u^2} du = e^{-x} - x E_1(x), \quad x > 0
\] (A1.4)

are exponential integrals (1) illustrated in Fig. 6.

Derivation of \( h(t) \)

The complete impulse-response function \( h(t) \) is derived by substituting Eqs. A1.1–2 into Eqs. 3–4.

\[
h(t) = \frac{A \beta^2}{B^2} \int_{k_0}^{\infty} e^{\beta k} (k - k_0)e^{-\beta k - k_0} dk
\]

\[
= \frac{A \beta^2}{B^2} \int_{k_0}^{\infty} e^{-(\beta + \beta)(k - k_0)} dk
\]

\[
= \frac{A \beta^2}{B^2} e^{\beta k_0} \int_{k_0}^{\infty} \frac{e^{-\mu \beta}}{\beta^2} d\mu
\]

by substituting \( t + \beta(k - k_0) = \mu \). So

\[
h(t) = \frac{\beta}{1 - \beta k_0 e^{\beta k_0}} E_1(\beta k_0) \left( t + \beta \right)^2
\] (A1.5)

is fully specified by the two parameters \( \beta \) and \( k_0 \).

![Fig. 6. Illustration of the exponential integrals \( E_1(x) \) and \( E_2(x) \).](http://ajpgi.org)
**Derivation of the First and Second Moments of h(t)**

Here, the mean transit time and the mean squared transit time, i.e. the first and second moments of h(t), are evaluated. The first moment is

\[ \tilde{T} = \int_0^\infty t h(t) dt = A \beta^2 \int_0^\infty t e^{-\beta t} \frac{e^{-\beta t}}{(t + \beta)^2} dt \]

\[ = A \beta^2 e^{-\beta \beta} \int_0^\infty \left[ e^{-\beta t} + \frac{\beta e^{-\beta t}}{t + \beta} \right] dt \]

\[ - A \beta^2 e^{-\beta \beta} \int_0^\infty \left\{ \frac{e^{-\beta t}}{t + \beta} - \beta \frac{e^{-\beta t}}{u^2} \right\} du \]

by substituting \( k_0(t + \beta) = u \). So

\[ \tilde{T} = \frac{\beta e^{\beta k_0} [E_1(k_0) - E_2(k_0)]}{1 - \beta k_0 e^{\beta k_0} E_1(k_0)} \]  

(A1.6)

Next, the second moment is evaluated

\[ \tilde{T}^2 = \int_0^\infty t^2 h(t) dt = A \beta^2 \int_0^\infty t^2 e^{-\beta t} \frac{e^{-\beta t}}{(t + \beta)^2} dt \]

\[ = A \beta^2 \left\{ \int_0^\infty e^{-\beta t} dt - \int_0^\infty \frac{2 \beta e^{-\beta t}}{t + \beta} dt + \int_0^\infty \frac{\beta^2 e^{-\beta t}}{(t + \beta)^2} dt \right\} \]

\[ = A \beta^2 \frac{1}{k_0} \left\{ 2 \beta k_0 e^{\beta k_0} E_1(k_0) + \beta^2 k_0 e^{\beta k_0} E_2(k_0) \right\} \]

So

\[ \tilde{T}^2 = \frac{\beta}{k_0} \frac{\{ 1 - \beta k_0 e^{\beta k_0} [2 E_1(k_0) - E_2(k_0)] \}}{1 - \beta k_0 e^{\beta k_0} E_1(k_0)} \]  

(A1.7)

**APPENDIX II**

**Bradley Asymptotics: Cut-Off Error of the Mean Transit Time**

In the closed recirculating system of the body, an organ of interest has influx \( F_{in} \), efflux \( F_{out} \), and a volume of distribution of tracer \( V_a \). Bradley’s mean transit time (7) involves integration over data to infinite time \( C_0(t) = C_a(t) = C_a \), thus

\[ \tilde{T} = \frac{V_a}{F} \int_0^\infty \left( C_0(t) - C_a(t) \right) dt \]  

(A2.1)

and similarly for the \( \sigma \) (3, 14). In practice, the integration is to some finite time \( t_B \), conventionally chosen at a time when \( C_a \) approaches \( C_a \) to within experimental error (7, 14). However, when such \( t_B \) is much less than the time scale 1/\( k_0 \) of the last exponential (Fig. 1; see also Refs. 10 and 11), it seems possible that an important contribution (positive or negative) to the true \( \tilde{T} \) accumulates within the experimental error during the late time \( t > t_B \).

Here, we quantify this idea by taking into consideration the circulation time \( T_c \) outside the organ of interest, which does not appear in Eq. A2.1 or in the determination of \( \sigma \) (3, 14). However, \( T_c \) is implicit in the choice of \( t_B \); the bolus of tracer must recirculate several times before the dispersion of transit times, within the organ and outside it, equilibrates the tracer activity concentration throughout the body.

We show that as 1/\( k_0 \) becomes large compared with \( T_c \), its influence on the true \( \tilde{T} \) vanishes. It will suffice to construct and examine a coarse but transparent upper limit on the correction of \( \tilde{T} \) from \( t > t_B \).

From Eq. A2.1 we write

\[ C_a(\tilde{T}) = \int_0^\infty [C_0(t) - C_a(t)] dt = \int_0^\infty \left( \frac{C_0(t) - C_a(t)}{C_a} \right) \]  

(A2.2)

We overestimate the magnitude of the remainder integral, from \( t_B \) to \( \infty \), by 1) putting a group of \( k_0 \) on the \( k_0 \) value, so that there would be a delta function at \( k_0 \) in Fig. 1 and 2) by neglecting dispersion outside the organ of interest, where we assume to be only a time delay by the circulation time \( T_c \)

\[ C_a(t) = C_a(t + T_c), \ t > t_B \]  

(A2.3)

From \( t_B \) onward, we extrapolate by the final exponential

\[ C_a(t) = C_a + [C_0(t_B) - C_a] e^{-k_0 t_B} \]  

(A2.4)

\[ C_a(t) = C_a + [C_a(t_B) - C_a] e^{-k_0 (t_B + T_c)} \]  

(A2.5)

We define the error \( \delta \tilde{T} \) in \( \tilde{T} \) due to events at times \( t > t_B \) as

\[ \delta \tilde{T} = \int_{t_B}^\infty \left( \frac{C_a(t) - C_a(t)}{C_a} \right) dt \]  

(A2.6)

With the use of Eqs. A2.4–5 to evaluate the integral in Eq. A2.6 and writing

\[ \delta C_a = C_a - C_a(t_B) \]  

(A2.7)

for the uncertainty in the true \( C_a \), we find

\[ \delta \tilde{T} = T_c \frac{\delta C_a}{C_a} \]  

(A2.8)

Because the last fraction in Eq. A2.8 cannot exceed unity, the magnitude of \( \delta \tilde{T} \) has the upper limit

\[ |\delta \tilde{T}| < T_c \frac{|\delta C_a|}{C_a} \]  

(A2.9)

independent of \( k_0 \).

Expansion of the last fraction in Eq. A2.8 in powers of \( k_0 T_c \) shows that a very small \( k_0 (k_0 T_c << 1) \) drops out of Eq. A2.8 entirely. If \( D \) is the dose of the tracer and \( V \) is its volume of distribution in the body, then \( D/V \) is an independent estimate of \( C_a \) against which the observed \( C_a(t_B) \) can be checked, whereby \( \delta C_a \) is limited.

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**DISCLOSURES**

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