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Model-Independent Method of Analyzing Input in Linear Pharmacokinetic Systems Having Polyexponential Impulse Response II: Numerical Evaluation

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Abstract □ The investigated method is based on an exact mathematical solution to the deconvolution problem of linear pharmacokinetic systems with a polyexponential impulse response. The accuracy of the method is determined only by how well the curves fitted to the intravenous and absorption data represent the true drug level. Consequently, the method enables objective evaluation of the input. It permits the user to assess whether discrepancies in a calculated input are due to an improper data representation, as judged from the fitted curves, to the inherent nature of the data, or to a violation of the pharmacokinetic assumptions. The method is compared to another method using simulated data containing various degrees of random noise. The accuracy of the two methods was not significantly different and was of the same magnitude as the noise level of the data. The theoretical properties of the two methods and their expected performance in various pharmacokinetic situations are discussed. The method is applied to pentobarbital data from oral and intravenous administrations.

Keyphrases □ Pharmacokinetics—linear systems, polyexponential impulse response, analysis of input, model-independent method □ Model-independent analysis—linear pharmacokinetic systems, polyexponential impulse response, analysis of input □ Drug input—linear pharmacokinetic systems, polyexponential impulse response, analysis by model-independent method

A previous article (1) presented the theoretical derivation and analysis of a novel input analysis method. The method allows a drug input to be evaluated in the presence or absence of any combination of intravenous bolus input and infusion input. Three approaches were discussed: evaluation of the input rate based on a separate intravenous bolus experiment and an input (absorption) experiment, evaluation based on a merged intravenous bolus and input experiment, and evaluation based on a merged infusion and input experiment.

The present work is confined to the first approach and is based on an exact mathematical solution to the deconvolution problem of linear pharmacokinetic systems with a polyexponential impulse response. Several methods for deconvolution have been presented (2–5). The Wagner–Nelson method usually is limited to one-compartment systems (6), and the methods investigated by Benet and Chiang (2) were shown to be very sensitive to errors in the data. The numerical deconvolution method presented by Gamel *et al.* (3) did not provide satisfactory results, possibly due to numerical ill conditioning. Cutler (5) improved Gamel's approach by using orthogonal polynomials to avoid the problem of ill conditioning. The improved method seems to be the most accurate method for numerical deconvolution. It appears to be superior to the many model-dependent methods (6–10) because it is based on fewer assumptions and, therefore, is more likely to result in a meaningful evaluation of the drug input.

This work compares the new method with Cutler's approach using Cutler's simulated test data that contain various degrees of random noise (4, 5).

THEORY

Let the concentration of drug in the blood, $c^*(t)$, following an initial intravenous bolus dose, q^*, be described by a multiexponential relationship (1):

$$c^*(t) = \sum_{i=1}^{n} a_i e^{\lambda_i t} \qquad \text{(Eq. 1)}$$

Let $c(t)$ denote the drug level resulting from an unknown input of a drug that, in the same subject with the same linear relationship between input and response, results in the blood level $c^*(t)$ (Eq. 1) when an intravenous bolus dose, q^*, is given. It has been shown (1) that the rate of input of the drug then is given by:

$$f(t) = q^* \left[-\frac{\sum_{i=1}^{n} a_i \lambda_i}{\left[\sum_{i=1}^{n} a_i\right]^2} c(t) + \frac{c'(t)}{\sum_{i=1}^{n} a_i} + \sum_{i=1}^{n-1} b_i e^{\beta_i t} \int_0^t c(u) e^{-\beta_i u}\, du \right] \qquad \text{(Eq. 2)}$$

where β_i, $i = 1, 2, \ldots, n-1$ are the roots of the $(n-1)$th-degree polynomial:

$$Q(x) = \sum_{i=1}^{n} a_i \prod_{\substack{j=1 \\ \neq i}}^{n} (x - \lambda_j) \qquad \text{(Eq. 3)}$$

and b_ν, $\nu = 1, 2, \ldots, n-1$ are obtained from:

$$b_\nu = \left[\sum_{i=1}^{n} \frac{a_i}{\beta_\nu - \lambda_i} \sum_{\substack{j=1 \\ \neq i}}^{n} \frac{1}{\beta_\nu - \lambda_j} \right]^{-1} \qquad \text{(Eq. 4)}$$

Integration of $f(t)$ from time zero to time t yields the cumulative amount of input:

$$A(t) = q^* \left[-\left\{ \frac{\sum_{i=1}^{n} a_i \lambda_i}{\left(\sum_{i=1}^{n} a_i\right)^2} + \sum_{i=1}^{n-1} \frac{b_i}{\beta_i} \right\} \int_0^t c(t)\, dt + \frac{c(t)}{\sum_{i=1}^{n} a_i} + \sum_{i=1}^{n-1} \frac{b_i}{\beta_i} e^{\beta_i t} \int_0^t c(u) e^{-\beta_i u}\, du \right] \qquad \text{(Eq. 5)}$$

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It may be more convenient to describe the amount as a percentage of the dose, q, given, *i.e.*:

$$\mathrm{PCT}(t) = 100\frac{q^*}{q}\left[-\left\{\frac{\sum_{i=1}^{n} a_i\lambda_i}{\left(\sum_{i=1}^{n} a_i\right)^2} + \sum_{i=1}^{n-1}\frac{b_i}{\beta_i}\right\}\int_0^t c(t)dt + \frac{c(t)}{\sum_{i=1}^{n} a_i} + \sum_{i=1}^{n-1}\frac{b_i}{\beta_i}e^{\beta_i t}\int_0^t c(u)e^{-\beta_i u}\,du\right] \quad \text{(Eq. 6)}$$

Consider as an example the case where the drug level after an intravenous bolus dose, q^*, is described by a two-exponential expression:

$$c^*(t) = a_1e^{\lambda_1 t} + a_2e^{\lambda_2 t} \quad \text{(Eq. 7)}$$

The auxiliary parameters β_1 and b_1, according to Eqs. 3 and 4, are given by:

$$\beta_1 = \frac{a_1\lambda_2 + a_2\lambda_1}{a_1 + a_2} \quad \text{(Eq. 8)}$$

and:

$$b_1 = -\frac{a_1a_2(\lambda_1 - \lambda_2)^2}{(a_1 + a_2)^3} \quad \text{(Eq. 9)}$$

so that the rate of input becomes:

$$f(t) = q^*\left[-\frac{a_1\lambda_1 + a_2\lambda_2}{(a_1 + a_2)^2}c(t) + \frac{c'(t)}{a_1 + a_2} - \frac{a_1a_2(\lambda_1 - \lambda_2)^2}{(a_1 + a_2)^3} \times \exp\left(\frac{a_1\lambda_2 + a_2\lambda_1}{a_1 + a_2}t\right)\int_0^t c(u)\exp\left(-\frac{a_1\lambda_2 + a_2\lambda_1}{a_1 + a_2}u\right)du\right] \quad \text{(Eq. 10)}$$

and the cumulative amount of input expressed as a percentage of the dose, q, becomes:

$$\mathrm{PCT}(t) = 100\frac{q^*}{q}\left[-\left\{\frac{a_1\lambda_2 + a_2\lambda_1}{(a_1 + a_2)^2} - \frac{a_1a_2(\lambda_1 - \lambda_2)^2}{(a_1\lambda_2 + a_2\lambda_1)(a_1 + a_2)^2}\right\} \times \int_0^t c(t)dt + \frac{c(t)}{a_1 + a_2} - \frac{a_1a_2(\lambda_1 - \lambda_2)^2}{(a_1\lambda_2 + a_2\lambda_1)(a_1 + a_2)^2}\exp\left(\frac{a_1\lambda_2 + a_2\lambda_1}{a_1 + a_2}t\right) \times \int_0^t c(u)\exp\left(-\frac{a_1\lambda_2 + a_2\lambda_1}{a_1 + a_2}u\right)du\right] \quad \text{(Eq. 11)}$$

which simplifies to:

$$\mathrm{PCT}(t) = 100\frac{q^*}{q}\left[-\frac{\lambda_1\lambda_2}{a_1\lambda_2 + a_2\lambda_1}\int_0^t c(t)dt + \frac{c(t)}{a_1 + a_2} - \frac{a_1a_2(\lambda_1 - \lambda_2)^2}{(a_1\lambda_2 + a_2\lambda_1)(a_1 + a_2)^2}\exp\left(\frac{a_1\lambda_2 + a_2\lambda_1}{a_1 + a_2}t\right) \times \int_0^t c(u)\exp\left(-\frac{a_1\lambda_2 + a_2\lambda_1}{a_1 + a_2}u\right)du\right] \quad \text{(Eq. 12)}$$

The procedure for evaluating the input is as follows. The two-exponential expression (Eq. 7) is fitted by nonlinear regression to the intravenous bolus data to estimate a_1, a_2, λ_1, and λ_2. A suitable empirical function[1], $s(t)$, is fitted to the absorption data to estimate the true drug level, $c(t)$. The rate and extent of absorption then are estimated according to Eqs. 10 and 12 using the estimates obtained for a_1, a_2, λ_1, λ_2, and $c(t)$.

The function $s(t)$ chosen to estimate $c(t)$ is an adaptive least-squares cubic spline function (12)[2]. This function is fitted to the data by minimizing the following expression:

$$E = \sum_{i=1}^{m}[w_i|c_i - s(t_i)|]^2 + \sum_{i=2}^{N-1}[\theta_i d_i]^2 \quad \text{(Eq. 13)}$$

where w_i are weights and (c_i, t_i), $i = 1, 2, \ldots, m$ are the absorption data. This expression consists of the usual least-squares term (the first term) and a smoothing term that is determined automatically using statistical tests on errors (16). The function $s(t)$ is a cubic spline with knots $\xi_1, \xi_2, \xi_3, \ldots, \xi_N$ and d_i represents the discontinuity in $s'''(t)$ at ξ_i, *i.e.*:

$$d_i = s'''(\xi_i + 0) - s'''(\xi_i - 0) \quad \text{(Eq. 14)}$$

The θ_i values are weight factors used for smoothing $s(t)$. In the fitting procedure, the number of knots, N, their position, and the smoothing weight factors, θ_i, are optimized automatically so that the fitted spline function attempts to follow trends in the data but ignores random errors (16). The rate of input (Eq. 2) and the extent of input (Eq. 5 or 6) can be evaluated accurately without numerical integration errors[3] when a cubic spline function, $s(t)$, is used to estimate $c(t)$.

This evaluation leads to the following expressions:

$$f(t) = q^*\left[-\frac{\sum_{i=1}^{n} a_i\lambda_i}{\left(\sum_{i=1}^{n} a_i\right)^2}s(t) + \frac{s'(t)}{\sum_{i=1}^{n} a_i} + \sum_{i=1}^{n-1} b_i\left\{-\sum_{k=0}^{3}\frac{s^{(k)}(t)}{\beta_i^{k+1}} + e^{\beta_i t}\sum_{k=0}^{3}\frac{s^{(k)}(0)}{\beta_i^{k+1}} + \beta_i^{-4}\sum_{k=2}^{j} e^{\beta_i(t-\xi_k)}d_k\right\}\right] \quad \text{(Eq. 15)}$$

where j is the highest integer for which $t \le \xi_j$ is satisfied, *i.e.*:

$$j = \overset{\sup}{i}\,(t \le \xi_i) \quad \text{(Eq. 16)}$$

and:

$$\mathrm{PCT}(t) = 100\frac{q^*}{q}\left[-\left(\frac{\sum_{i=1}^{n} a_i\lambda_i}{\left[\sum_{i=1}^{n} a_i\right]^2} + \sum_{i=1}^{n-1}\frac{b_i}{\beta_i}\right)\int_0^t s(t)dt + \frac{s(t)}{\sum_{i=1}^{n} a_i} + \sum_{i=1}^{n-1}\frac{b_i}{\beta_i}\left\{-\sum_{k=0}^{3}\frac{s^{(k)}(t)}{\beta_i^{k+1}} + e^{\beta_i t}\sum_{k=0}^{3}\frac{s^{(k)}(0)}{\beta_i^{k+1}} + \beta_i^{-4}\sum_{k=2}^{j} e^{\beta_i(t-\xi_k)}d_k\right\}\right] \quad \text{(Eq. 17)}$$

EXPERIMENTAL

The simulated data used to test the new method consist of four sets of data presented by Cutler (4) and denoted as Data Sets 1–4. Each set consists of a set of 11 simulated unit impulse response data with random noise added and a set of 11 simulated input response data also containing noise from the same linear system. The method of analysis is model independent. Therefore, the models used to simulate data from a linear system to test the method can be chosen arbitrarily.

The models employed by Cutler to generate the test data (Table I) were chosen because of their resemblance to models commonly employed in drug release and drug absorption analyses. They can be considered, in the classical linear pharmacokinetic sense, as a two-compartment model with first-order input (Data Sets 1 and 2) and as a two-compartment model with dissolution rate-limited release from an intramuscular injection, where the drug release follows the well-known cube-root relationship (17) (Data Sets 3 and 4). The results shown for Cutler's method (Tables II–V) were chosen as the best results reported (5).

Data Treatment—Polyexponential expressions of increasing degree n (Eq. 1) were fitted to the unit impulse response data with equal weights using the nonlinear regression program FUNFIT (18). A two-exponential expression fit the data adequately (Table VI). The cubic spline function, $s(t)$, was fitted to the input response data using the subroutine VCO3A from the Harwell subroutine library (12). A subroutine was written that calls VCO3A and calculates, according to Eqs. 15 and 17, the rate and extent of input at the observation times and, optionally, at any number of equally spaced time points from time zero to the last observation time for graphical representation of the input[4]. The pentobarbital data used were those reported by Smith *et al.* (11).

[1] The term empirical function is used here to denote a function that describes empirical data in a low information situation where no specific mathematical model is assumed.

[2] References 13–15 give an introduction to the theory and applications of spline functions in data analysis.

[3] This is the case because analytical expressions exist for $s'(t)$, $s''(t)$, and $s'''(t)$ and because $\int_0^t s(t)dt$ (Eq. 17) leads to a polynomial expression that can be evaluated exactly.

[4] Plotted using a Tektronix 4662 penplotter using a FORTRAN IV graphical software package written by the author.

Table I—Origin of the Simulated Data in Data Sets 1–4 [a]

Data Set	Rate of Input	Amount of Input, $f(t)$	Random Noise Level[b], %	Unit Impulse Response Data	Input Response Data
1	$f(0)e^{-kt}$	$\frac{f(0)}{k}(1-e^{-kt})$	1	$a_1e^{\lambda_1 t}+a_2e^{\lambda_2 t}+\epsilon$	$c^*(t)*f(t)+\epsilon$
2	$f(0)e^{-kt}$	$\frac{f(0)}{k}(1-e^{-kt})$	10	$a_1e^{\lambda_1 t}+a_2e^{\lambda_2 t}+\epsilon$	$c^*(t)*f(t)+\epsilon$
3	$\frac{3D}{t_d}\left(1-\frac{t}{t_d}\right)_+^2$	$D\left[1-\left(1-\frac{t}{t_d}\right)_+^3\right]$	1	$a_1e^{\lambda_1 t}+a_2e^{\lambda_2 t}+\epsilon$	$c^*(t)*f(t)+\epsilon$
4	$\frac{3D}{t_d}\left(1-\frac{t}{t_d}\right)_+^2$	$D\left[1-\left(1-\frac{t}{t_d}\right)_+^3\right]$	10	$a_1e^{\lambda_1 t}+a_2e^{\lambda_2 t}+\epsilon$	$c^*(t)*f(t)+\epsilon$

[a] The following notations are used: $(1-x)_+ = \max(0, 1-x)$; and $c^*(t)*f(t)$ is the convolution of $c^*(t)$ and $f(t)$, where $c^*(t) = a_1\exp(\lambda_1 t) + a_2\exp(\lambda_2 t)$. The parameters used in the simulations are: $a_1 = a_2 = 1$, $\lambda_1 = -5$, $\lambda_2 = -1$, $k = 2$, $D = 0.6$, $t_d = 1.15$, and $f(0) = 1.2$. [b] See Ref. 4 for details.

Table II—Input Rates Calculated from Data Set 1 Using the New Method and the Cutler Method

Time	Exact Rate	New Method	Percent Difference[a]	Cutler Method	Percent Difference[a]
0.1	0.9825	0.9666	−1.32	0.967	−1.29
0.2	0.8044	0.8366	2.68	0.810	0.467
0.3	0.6586	0.6884	2.48	0.674	1.28
0.4	0.5392	0.5500	0.901	0.556	1.40
0.6	0.3614	0.3658	0.364	0.370	0.717
0.8	0.2423	0.2339	−0.701	0.240	−0.192
1.0	0.1624	0.1555	−0.577	0.156	−0.533
1.2	0.1089	0.1160	0.598	0.105	−0.325
1.4	0.0730	0.0790	0.506	0.076	0.250
1.6	0.0489	0.0404	−0.712	0.057	0.675
2.0	0.0220	0.0230	0.083	0.003	−1.583
Mean[b]			0.99		0.79
SD[b]			0.84		0.50

[a] Calculated as 100 × (calculated rate − exact rate)/1.2, where 1.2 is the initial exact input rate. [b] The mean and the standard deviation of the absolute values of the percent difference.

Table III—Input Rates Calculated from Data Set 2 Using the New Method and the Cutler Method

Time	Exact Rate	New Method	Percent Difference[a]	Cutler Method	Percent Difference[a]
0.1	0.9825	0.8784	−8.67	0.904	−6.54
0.2	0.8044	0.8087	0.36	0.813	0.72
0.3	0.6586	0.7242	5.47	0.724	5.45
0.4	0.5392	0.6358	8.05	0.639	8.32
0.6	0.3614	0.4771	9.64	0.479	9.80
0.8	0.2423	0.3308	7.37	0.338	7.98
1.0	0.1624	0.2199	4.79	0.219	4.72
1.2	0.1089	0.1409	2.67	0.125	1.34
1.4	0.0730	0.0708	−0.18	0.058	−1.25
1.6	0.0489	0.0129	−3.00	0.020	−2.41
2.0	0.0220	0.0091	−1.08	0.027	0.42
Mean[b]			4.65		4.41
SD[b]			3.46		3.45

[a] Calculated as 100 × (calculated rate − exact rate)/1.2, where 1.2 is the initial exact input rate. [b] The mean and the standard deviation of the absolute values of the percent difference.

The data treatment of the pentobarbital data (Table VII) was identical to the treatment of the simulated data with the exception of the additional determination of the absorption lag time. The lag time was determined by linear extrapolation using the line that agreed with the fitted spline function and its slope at the first observation time. The appropriate time transformation then was performed before the rate and extent of input were calculated. Although the intravenously administered pentobarbital was given by a 5-min infusion, it was considered computationally as a bolus input because of the short infusion time relative to the absorption time and the rate of drug elimination.

RESULTS AND DISCUSSION

The analysis method does not require the input of a particular linear or nonlinear form, nor does it assume a specific linear pharmacokinetic model. The method only assumes a time-invariant linearity between input and response and that the impulse response (intravenous bolus input) can be approximated adequately by a polyexponential expression.

The equations for the rate (Eq. 2) and the extent (Eqs. 5 and 6) of input are mathematically exact expressions. The accuracy with which the input is evaluated is determined solely by the accuracy with which $c^*(t)$ (as described by a_i, λ_i, $i = 1, 2, \ldots, n$) and $c(t)$ are estimated. Therefore, the accuracy of the estimation depends on the qualities of two curve fittings. The functional form of $c^*(t)$ has been established as Eq. 1 (1). The method estimates $c^*(t)$ by least-squares regression which, according to the central limit theorem and the Gauss–Markov theorem, can be considered to be statistically the best estimation when no prior information is available about the statistical properties of the errors. Appropriate weighting can be applied if the variances of the errors can be estimated (18).

The estimation of $c(t)$ is the most difficult step. Its functional form is unknown in any model-independent approach. The main problem is to find an empirical function that can be fitted so that it follows the trend and the intrinsic values of the data but ignores the errors. A proper choice of function must be based on assumptions about the properties of the errors and the real behavior of $c(t)$. The function chosen must be able, when fitted, to reflect and demonstrate these properties properly. The present method generally assumes random errors. Although it is difficult to make assumptions about the real behavior of $c(t)$, it seems important

Table IV—Input Rates Calculated from Data Set 3 Using the New Method and the Cutler Method

Time	Exact Rate	New Method	Percent Difference[a]	Cutler Method	Percent Difference[a]
0.1	1.3048	1.2780	−1.71	1.256	−3.11
0.2	1.0681	1.1080	2.55	1.060	−0.51
0.3	0.8551	0.8959	2.61	0.893	2.42
0.4	0.6657	0.6839	1.16	0.715	3.15
0.6	0.3580	0.3609	0.19	0.356	−0.13
0.8	0.1450	0.1312	−0.88	0.115	−1.92
1.0	0.0266	0.0202	−0.41	0.026	−0.04
1.2	0	0.0011	0.07	0.004	0.26
1.4	0	0.0010	0.06	−0.010	−0.64
1.6	0	0.0008	0.05	0.048	3.07
2.0	0	0.0007	0.04	−0.526	33.61
Mean[b]			0.88		4.44
SD[b]			1.00		9.76

[a] Calculated as 100 × (calculated rate − exact rate)/(1.8/1.15), where 1.8/1.15 is the exact initial input rate. [b] The mean and the standard deviation of the absolute values of the percent difference.

Table V—Input Rates Calculated from Data Set 4 Using the New Method and the Cutler Method

Time	Exact Rate	New Method	Percent Difference[a]	Cutler Method	Percent Difference[a]
0.1	1.3048	1.1761	−8.22	1.235	−4.46
0.2	1.0681	1.0614	−0.43	1.058	−0.65
0.3	0.8551	0.9224	4.30	0.895	2.55
0.4	0.6657	0.7740	6.92	0.744	5.00
0.6	0.3580	0.4934	8.65	0.481	7.86
0.8	0.1450	0.2480	6.58	0.270	7.99
1.0	0.0266	0.0859	3.79	0.109	5.26
1.2	0	0.0057	0.36	0.000	0.00
1.4	0	0.0008	0.05	−0.059	−3.77
1.6	0	0.0000	0.00	−0.066	−4.22
2.0	0	0.0000	0.00	0.074	4.73
Mean[b]			3.57		4.23
SD[b]			3.55		2.51

[a] Calculated as 100 × (calculated rate − exact rate)/(1.8/1.15), where 1.8/1.15 is the exact initial input rate. [b] The mean and the standard deviation of the absolute values of the percent difference.

to consider the concept of smoothness. It seems unrealistic to assume $c(t)$ to be as smooth as the functions fitted in model-dependent approaches, considering the natural oscillations in the many body functions affecting absorption. It is more realistic to consider $c(t)$ to have a certain degree of fluctuation.

The adaptive least-squares cubic spline function was chosen to estimate $c(t)$ because it has the following properties. It is flexible enough to follow whatever shape $c(t)$ may have (15). Due to the smoothing term (the second term of Eq. 13), it is not so floppy that it fits to the errors in the data, *i.e.*, by going through all of the data points. The fit of the spline function in one region bears little relationship to its behavior in another region (15). Polynomials and other continuous mathematical functions that are not defined in a piecemeal fashion have just the opposite property. Namely, their behavior in any region determines their behavior everywhere. This unique and important property of the spline function enables it to describe the local behavior of $c(t)$ with no interference from nonlocal data points. The spline function has a smoothness that seems realistic in relation to the response it attempts to describe. It is well recognized in numerical analysis that spline functions are excellent tools for differentiation and integration. Therefore, a spline function representation should provide good estimates for the rate and the extent of drug input, considering the algebraic form of Eqs. 2, 5, and 6. Spline functions, when fitted to data by the least-squares method, conserve the first two moments of the data (19). This statistical property makes spline functions suitable for the analysis of observations with random errors because estimates of the mean, variance, and confidence interval of the true response can be obtained.

The theoretical basis for the described properties of spline functions

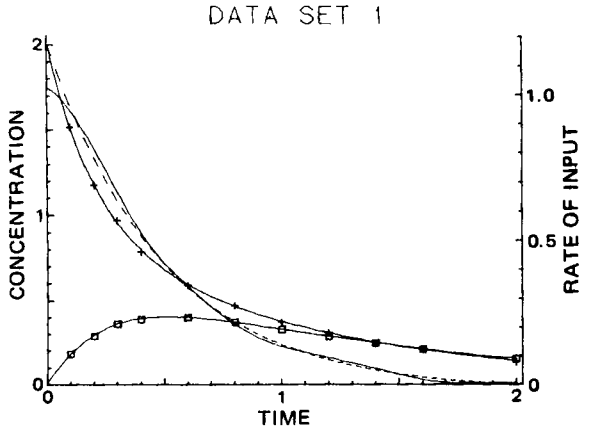

Figure 1—*Test of the new method on simulated data containing 1% random error. Equation 1* (n = 2) *is fitted to the impulse response data* (+). *An adaptive least-squares cubic spline function,* s(t), *is fitted to the input response data* (□). *The slightly sigmoid-shaped curve is the rate of input calculated from* a_i, λ_i, i = *1, 2 and* s(t) *according to Eq. 15. The broken curve is the exact rate of input.*

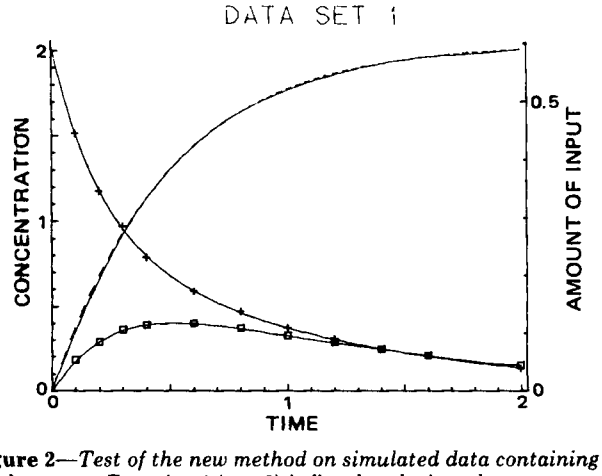

Figure 2—*Test of the new method on simulated data containing 1% random error. Equation 1* (n = 2) *is fitted to the impulse response data* (+). *An adaptive least-squares cubic spline function,* s(t), *is fitted to the input response data* (□). *The continuously increasing curve is the amount of input calculated from* a_i, λ_i, i = *1, 2 and* s(t) *according to Eq. 17.*

Table VI—Least-Squares Unit Impulse Response Parameters Used in the Calculation of the Input [a]

Data Set	a_1	a_2	λ_1	λ_2	Sum of Squares	Correlation Coefficient, r	Figure
1	1.0427	0.97617	−5.0526	−0.97567	4.54×10^{-4}	0.9998	1 and 2
2	1.3377	0.52381	−3.1851	−0.61065	3.14×10^{-2}	0.9919	3 and 4
3	1.0308	1.0487	−5.9131	−1.0262	2.18×10^{-4}	0.9999	5 and 6
4	1.3377	0.52381	−3.1851	−0.61065	3.14×10^{-2}	0.9919	7 and 8

[a] Determined with the FUNFIT program (18).

Table VII—Least-Squares Parameters Obtained from Plasma Pentobarbital Data from Intravenous Administration and Used in the Calculation of the Input [a]

Subject	a_1, μg/ml	a_2, μg/ml	λ_1, hr^{-1}	λ_2, hr^{-1}	Sum of Squares, $(\mu g/ml)^2$	Correlation Coefficient, r	Figure
R.M.	0.8014	0.5515	−1.7518	-2.391×10^{-2}	7.442×10^{-2}	0.9777	9
B.G.	0.8654	0.3396	−2.203	-9.940×10^{-3}	1.704×10^{-2}	0.9918	9
R.B.	0.6493	0.4222	−1.375	-1.984×10^{-2}	3.34×10^{-2}	0.9850	9

[a] Determined with the FUNFIT program (18).

and their relationship to their application in empirical data analysis have been discussed extensively (13–15, 19).

Numerical Results—The two linear systems from which the simulated data were generated will be denoted as Systems 1 and 2, corresponding to Data Sets 1 and 2 and 3 and 4, respectively.

Both the rate and the extent of input were evaluated from the four data sets, giving a total of eight determinations (Figs. 1–8 and Tables II–V).

System 1, Data Set 1 (Noise Level 1%)—There was no significant difference in the results obtained by the new method and Cutler's method (t test, $p = 0.95$). The pattern of the errors was similar for the two methods. The average percent relative error was on the same order of magnitude as the percent error added to the data. The methods appeared to be least accurate in the initial stage of the input (Table II and Figs. 1 and 2).

System 1, Data Set 2 (Noise Level 10%)—Raising the error level from 1 to 10% did not differentiate between the two methods. The pattern of the errors again was similar, and the average percent error was of the same order of magnitude as the noise added to the data (Table III and Figs. 3 and 4).

System 2, Data Set 3 (Noise Level 1%)—Although the Cutler method appeared to perform better in the initial stage of the input, it did not estimate the input as accurately as did the new method at later sampling times. The difference was particularly great at the last sampling point. The new method seemed to give an overall better estimate of the input (Table IV and Figs. 5 and 6).

System 2, Data Set 4 (Noise Level 10%)—The two methods did not seem to be significantly different in their overall accuracy when the error level was raised from 1 to 10%. However, the new method appeared to determine the input rate more accurately at the later sampling times. The average percent error for the two methods was on the same order of magnitude as the noise level and came close to the averages obtained for System 1 where the same degree of noise was added to the data (Table V and Figs. 7 and 8).

It is not possible to differentiate between the two methods on the basis of the test data. They both performed well considering that the average relative error in all estimations was of the same magnitude as the noise added to the simulated data. However, both methods appeared to underestimate the initial release rate consistently. Cutler's test problems could not discriminate between the two methods because the test data simulate rather ideal, somewhat synthetic cases. In practice, it is uncommon to have so many points sampled in the absorption phase, and the sampling is rarely taken so evenly and at the same times after intravenous and oral administrations. Also, it is uncommon not to have a lag time in the absorption phase.

An excessive number of simulation tests is required to establish experimentally, using a variety of linear systems and sampling schemes, the relative performance of the two methods. However, the following theoretical considerations should give an assessment of the methods and how they may perform in various situations.

Both methods use empirical functions. The Cutler method uses polynomials of various degrees to represent the input function and the unit impulse response. The new method uses an adaptive least-squares cubic spline function to represent the input response and a polyexponential expression (Eq. 1) to represent the impulse response. It is important to recognize the properties of polynomials relative to the properties of a

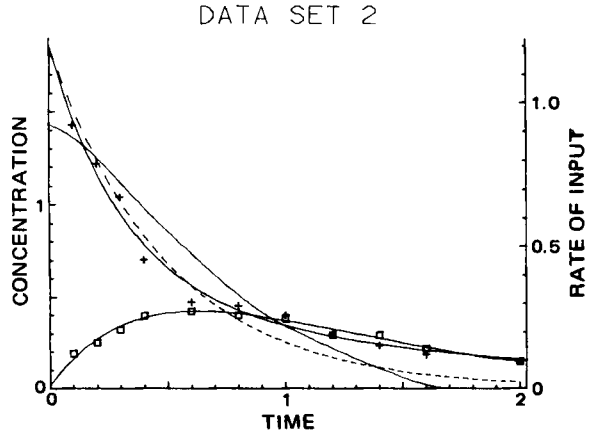

Figure 3—*Test of the new method on simulated data containing 10% random error. Equation 1 (n = 2) is fitted to the impulse response data (+). An adaptive least-squares cubic spline function, s(t), is fitted to the input response data (□). The slightly sigmoid-shaped curve is the rate of input calculated from a_i, λ_i, i = 1, 2 and s(t) according to Eq. 15. The broken curve is the exact rate of input.*

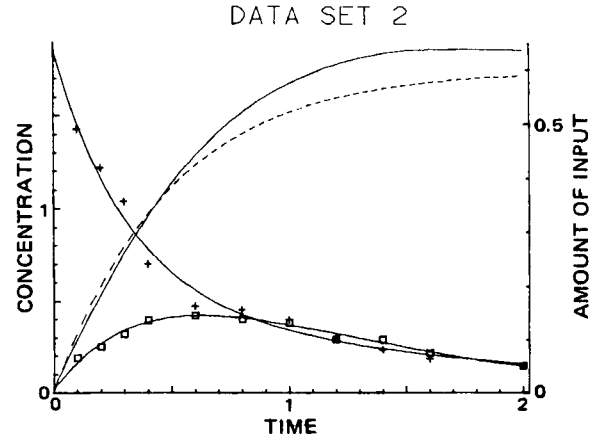

Figure 4—*Test of the new method on simulated data containing 10% random error. Equation 1 (n = 2) is fitted to the impulse response data (+). An adaptive least-squares cubic spline function, s(t), is fitted to the input response data (□). The continuously increasing curve is the amount of input calculated from a_i, λ_i, i = 1, 2 and s(t) according to Eq. 17.*

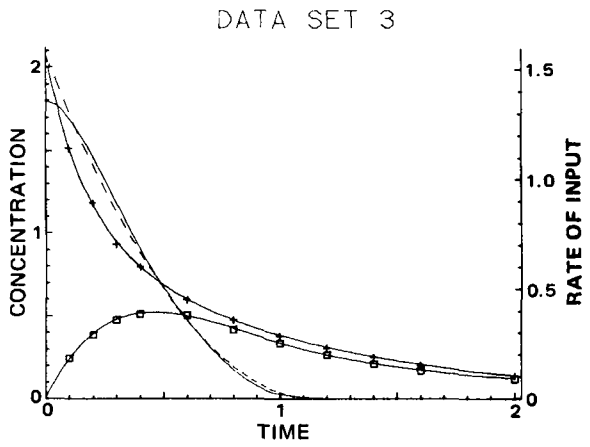

Figure 5—*Test of the new method on simulated data containing 1% random error. Equation 1* (n = 2) *is fitted to the impulse response data* (+). *An adaptive least-squares cubic spline function,* s(t), *is fitted to the input response data* (□). *The slightly sigmoid-shaped curve is the rate of input calculated from* a_i, λ_i, i = *1, 2 and* s(t) *according to Eq. 15. The broken curve is the exact rate of input.*

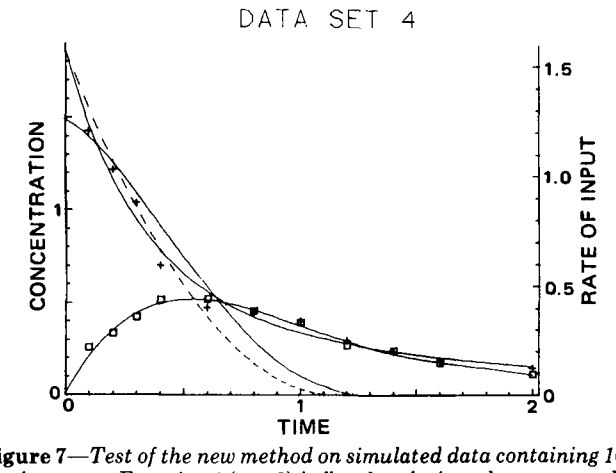

Figure 7—*Test of the new method on simulated data containing 10% random error. Equation 1* (n = 2) *is fitted to the impulse response data* (+). *An adaptive least-squares cubic spline function,* s(t), *is fitted to the input response data* (□). *The slightly sigmoid-shaped curve is the rate of input calculated from* a_i, λ_i, i = *1, 2 and* s(t) *according to Eq. 15. The broken curve is the exact rate of input.*

least-squares cubic spline function and a polyexponential expression since the differences may explain and predict how the two methods perform under various conditions. Least-squares polynomials usually give a good data representation when the data points are fairly equally spaced and do not change too rapidly or contain excessive errors as in the described simulation study. They then interpolate well but perform poorly when used for extrapolation beyond the end-points. The extrapolation error frequently increases rapidly with the distance from the end-point. Cutler used a polynomial to represent the impulse response (the intravenous bolus response). The polynomial is extrapolated from the first sampling time to time zero to calculate the convolution integral (p. 249, Ref. 5) that plays an essential role in his method. The chance of introducing a substantial error in this way becomes particularly significant if early intravenous data are lacking.

It sometimes is desirable to evaluate the absorption of a drug that has been sampled longer than its intravenous bolus response. Inaccurate results may be expected using Cutler's method in such cases because it requires a polynomial extrapolation beyond the last intravenous bolus data point to calculate the convolution integral. It is well known that polynomials may interpolate badly and show large oscillations between some data points if the data are not spaced fairly equally or contain one or more large gaps, due to missing data for example. The Cutler method is expected to be deficient when applied to such data. It is well known in empirical data fittings that cubic spline functions are superior to polynomials with respect to interpolation and extrapolation (20). It also is evident that the polyexponential expression, Eq. 1, is superior to a polynomial for interpolation and especially for extrapolation of the intravenous bolus response, considering the well-documented and successful use of Eq. 1 in pharmacokinetics. Therefore, the new method is expected to be more accurate with respect to the interpolations and the extrapolations that greatly influence the accuracy of the two methods.

Cutler's method should perform at its best when the sampling times from intravenous and nonintravenous administrations coincide as in the test examples. This result occurs because the polynomial representation of the input function is best defined under this condition, due to the local emphasis inherent in the sum of squares expression being minimized. The accuracy of the new method does not depend on whether the sampling times coincide because of the different approach used. Most absorption data in pharmacokinetics contain a lag time. Cutler did not discuss how his method should be applied to take this factor into account.

It seems apparent from the discussion that the test data used tend to give an optimistically biased evaluation of the performance of Cutler's method. A differentiation of the two methods may result if more realistic simulation studies are performed.

The user of Cutler's method is faced with several different estimations of the input corresponding to the various degrees of the polynomial used in the approximations of the input rate. The problem of picking the "best" results is complicated by possible multiple minima of the residual mean square function (5). If a polynomial of too high a degree is used, it will fit to the errors in the data and produce unreliable results. In favor-

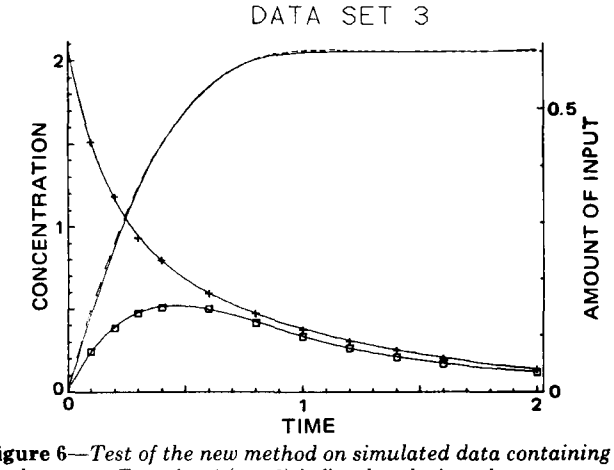

Figure 6—*Test of the new method on simulated data containing 1% random error. Equation 1* (n = 2) *is fitted to the impulse response data* (+). *An adaptive least-squares cubic spline function,* s(t), *is fitted to the input response data* (□). *The continuously increasing curve is the amount of input calculated from* a_i, λ_i, i = *1, 2 and* s(t) *according to Eq. 17.*

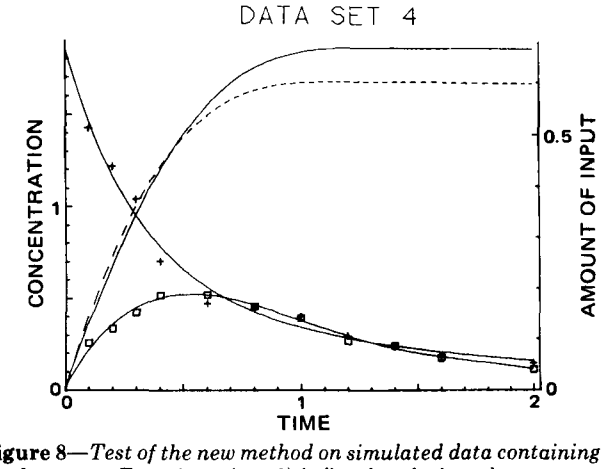

Figure 8—*Test of the new method on simulated data containing 10% random error. Equation 1* (n = 2) *is fitted to the impulse response data* (+). *An adaptive least-squares cubic spline function,* s(t), *is fitted to the input response data* (□). *The continuously increasing curve is the amount of input calculated from* a_i, λ_i, i = *1, 2 and* s(t) *according to Eq. 17.*

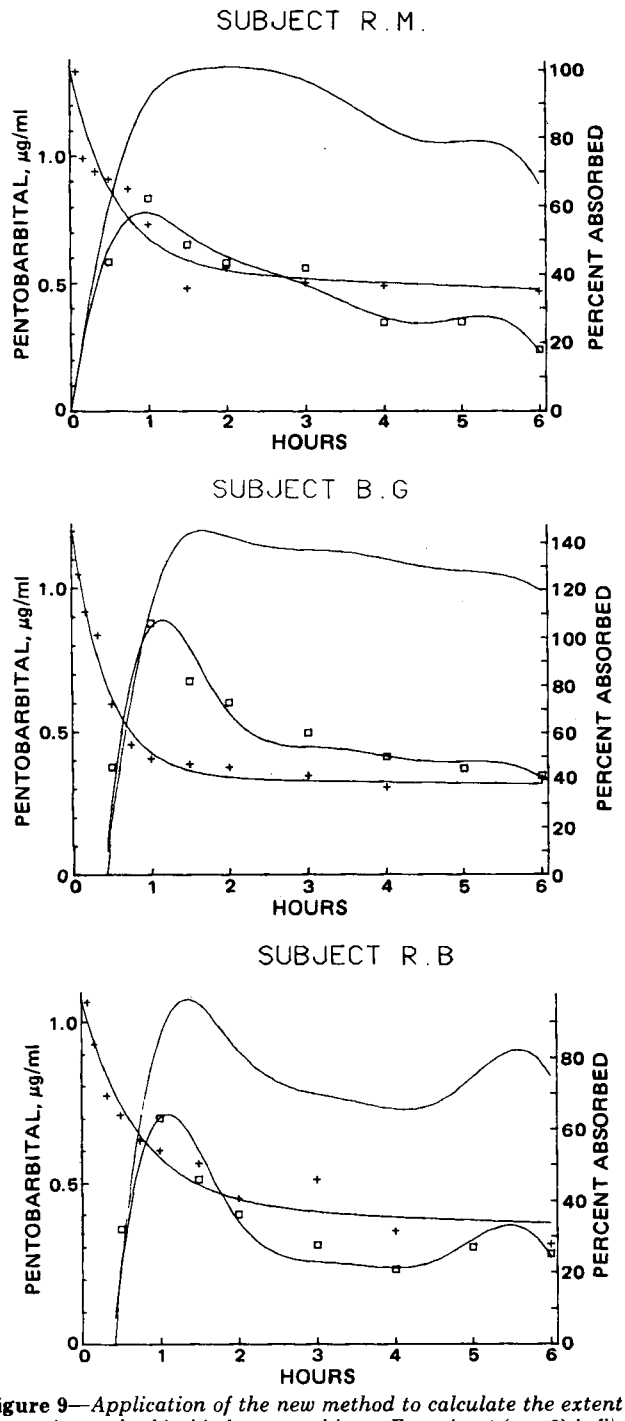

Figure 9—*Application of the new method to calculate the extent of input of pentobarbital in human subjects. Equation 1* (n = 2) *is fitted to the plasma drug level data* (+) *from intravenous administration. An adaptive least-squares cubic spline function,* s(t), *is fitted to the oral absorption data* (□). *The drug input (upper curves) is calculated from* a_i, λ_i, i = *1, 2 and* s(t) *according to Eq. 17.*

able cases, a suitable, well-defined minimum may exist, but the problem is expected to be particularly difficult in more realistic situations. The additional problem of choosing the proper polynomial to represent the impulse response was not discussed by Cutler. The number of multiple solutions is multiplied for each possible polynomial, thereby making a choice even more difficult.

The new method seems more appealing from the user's point of view because it gives clear, unambiguous results. It has the advantage that, contrary to Cutler's approximation method, it is based on an exact mathematical solution to the deconvolution problem of linear compartmental pharmacokinetics. The method also provides, as a by-product of the least-squares spline fitting to the absorption data, estimates of the important model-independent quantities, *i.e.*, time to the peak, maximum response, and area under the curve (truncated).

Pentobarbital Input—The pentobarbital data are difficult to treat because the absorption phase is defined by only three or four data points and because the absorption lag time is not well defined due to the lack of early sampling points (Fig. 9). Although the lag times for the absorption by Subjects R.B. and B.G. (0.36 and 0.46 hr, respectively) came close, it was not possible to detect a significant lag time for the absorption by Subject R.M. due to the low information density of the data in the absorption phase. It is satisfactory that the extent of absorption for R.M. and R.B. both came close to the label claim (100 and 96%, respectively) judged from the maximum of the percent absorbed curves (Fig. 9). However, the excessive value for Subject B.G. (144%) and the significant decline of the percent absorbed curves for all subjects indicate a pronounced violation of the assumption of time-invariant linearity between input and response.

The observed discrepancy was not caused by the method of analysis or the numerical treatment. The rate of decline of the absorption data after the absorption phase was significantly larger than the rate for the intravenous data, indicating apparently faster elimination after the oral administration. Under the given assumptions, this effect will computationally result in a negative input, *i.e.*, a decline in the percent absorbed curve after the absorption phase as shown due to an elimination rate that is faster than that expected judging from the intravenous data.

Several explanations may be investigated to explain this discrepancy between the oral and intravenous data. One possibility is that the linearity between input and response is only of a relatively short duration so that it changes significantly between administrations but not within the duration of a single administration. The merged intravenous bolus and input approach or the merged infusion and input approach (1) can be applied in such cases. These methods do not require a washout period between the intravenous and nonintravenous administrations. Therefore, the two administrations can be brought close together to minimize errors arising from a time-dependent input–response linearity. This ability should result in a more reliable drug input evaluation than is possible with other model-independent methods because these methods are based on a complete washout of drug between the administrations.

The new method appears to be a potentially powerful tool for the evaluation of drug input and bioavailability. It is based on an exact mathematical solution to the deconvolution problem of linear compartmental pharmacokinetics. The accuracy of the method is determined by how well the fitted curves represent the true response. The superimposed graphical representation of the estimated impulse and input responses and the calculated input, provided by the method (Fig. 9), consequently is of significant conceptual value. It enables the user to evaluate visually whether discrepancies or peculiarities in a calculated input are due to an improper data representation, judged from the fitted curves, or to the inherent nature of the impulse and input response data. The method is not used as a "black box" but enables the user to be critical about the results obtained and the assumptions made about the pharmacokinetics. Hopefully, this work will lead to a less assumptive and more objective approach in bioavailability testing.

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Novel Deconvolution Method for Linear Pharmacokinetic Systems with Polyexponential Impulse Response

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Abstract □ A novel least-squares deconvolution method for estimating the rate and the extent of drug input into the systemic circulation is presented. The method is based on a polyexponential approximation of the impulse response and a polynomial approximation of the input rate. The method, which is readily implemented on a computer using any multiple linear regression program with a zero-intercept option, is compared to two other deconvolution methods using simulated data with various degrees of random noise added. It appears to have several significant advantages. The method is applied to plasma pentobarbital level data from oral and intravenous administration. The assumptions and limitations of deconvolution methods for analyzing drug input into the blood are discussed.

Keyphrases □ Pharmacokinetics—deconvolution method, linear pharmacokinetics, polyexponential impulse response, drug input into blood □ Blood—drug input, deconvolution method for pharmacokinetic estimation □ Deconvolutions—pharmacokinetic estimation of drug input into blood □ Drug bioavailability—blood, deconvolution method for pharmacokinetic estimation

Drug input analysis is of utmost importance in biopharmaceutics because of its fundamental role in drug design, evaluation, and administration. Accurate quantitation of a drug's input–response relationship in a subject or patient population is, therefore, important. The relevant response is usually the drug concentration in the blood, in a certain tissue, or in an organ, but it may be a pharmacological or toxicological response.

The response environment is the destination for the input to be quantitated. There are two kinds of response environments: sampleable, *i.e.*, environments that can be quantitatively sampled for the drug, and nonsampleable. The blood, a tissue, or an organ are sampleable environments. Drug receptors and other biochemical structures responsible for a drug's pharmacological–toxicological response are usually nonsampleable environments. A drug's input can be experimentally verified only for sampleable environments. The evaluation of a drug's input into a nonsampleable environment would be based on some hypothesis about the quantitative relationship between the drug concentration in the nonsampleable response environment and the sampleable response. Therefore, it is not possible to quantitate drug input from pharmacological measurements if the input is defined with respect to a nonsampleable response environment, *e.g.*, drug receptors or "the biophase" (1).

For only a few drugs is it possible to establish experimentally a quantitative functional relationship between the drug concentration in a sampleable environment (*e.g.*, the blood) and the pharmacological response. In such cases, the pharmacological response may be used to evaluate the input into the sampleable environment. The possibility of noninvasive, nonanalytical techniques to quantitate drug input from pharmacological response measurements is exciting (1). However, the sources of errors are enormous and generally result in very inaccurate results. The blood is usually not the "site of action" for a drug and, therefore, may not represent the ultimate destination for the drug input. However, the transfer of a drug to the site of action from the blood is often direct, or the barriers involved are often insignificant compared to the physical, chemical, and biological barriers the drug encounters to get into the blood. Thus, it is adequate in most cases to evaluate the drug input with the blood as the response environment, as in the present approach.

The treatment presented is limited to linear pharmacokinetic systems, *i.e.*, systems where the input–response relationship follows the linear superposition principle. The classical linear compartmental systems (2, 3) belong to the family of linear systems. The method presented can be characterized in the classical (linear compartmental) pharmacokinetic sense as model independent. The various published approaches for evaluating drug input were discussed previously (4, 5).

THEORY

The drug level in the blood, $c^*(t)$, after an intravenous bolus dose, q^*, can often be well described by a polyexponential expression:

$$c^*(t) = \sum_{i=1}^{n} a_i e^{\lambda_i t} \qquad \text{(Eq. 1)}$$

If the blood drug level behaves linearly with respect to input into the blood, then the unit impulse response is $c^*(t)/q^*$, and the response to an arbitrary input rate, $f(t)$, is given by the general expression:

$$c(t) = \frac{1}{q^*} \int_0^t f(t-u)c^*(u)du \qquad \text{(Eq. 2)}$$

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