(15) J. Ahlberg, E. N. Nilson, and J. L. Walsh, "The Theory of Splines and Their Applications," Academic, New York, N.Y., 1967.

(16) M. J. D. Powell, "Curve Fitting by Cubic Splines," Report T.P. 307, Theoretical Physics Division, Atomic Energy Research Establishment, Harwell, Berkshire, England, 1967.

(17) A. W. Hixson and J. H. Crowell, *Ind. Eng. Chem.*, **23**, 923 (1931).

(18) P. Veng Pedersen, *J. Pharmacokinet. Biopharm.*, **5**, 513 (1977).

(19) E. T. Whittaker and G. Robinson, "The Calculus of Observations," Blackie and Son Ltd., London, England, 1926.

(20) J. R. Rice, "The Approximation of Functions," vol. II, Addison-Wesley, Reading, Mass., 1969.

ACKNOWLEDGMENTS

Novel Deconvolution Method for Linear Pharmacokinetic Systems with Polyexponential Impulse Response

PETER VENG PEDERSEN

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:

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

A suitable method is to be derived whereby $f(t)$ can be estimated from samples of the bolus response, $c^*(t)$, and the input (absorption) response, $c(t)$.

Considering the many factors influencing the absorption process, it may be appropriate to approximate $f(t)$ with a model-independent empirical equation such as a polynomial:

$$f(t) = \sum_{i=0}^{N} x_i t^i \qquad \text{(Eq. 3)}$$

The problem is then to determine the polynomial coefficients $x_0, x_1, \ldots, x_N$. The following derivations (Eqs. 4–15) lead to an equation, Eq. 16, that is linear with respect to the coefficients. This equation can be used to calculate the coefficients by standard multiple linear regression techniques.

Inserting Eqs. 1 and 3 into Eq. 2 leads to:

$$c(t) = \frac{1}{q^*} \int_0^t \sum_{i=0}^{N} x_i (t - u)^i \sum_{j=1}^{n} a_j e^{\lambda_j t} \, du \qquad \text{(Eq. 4)}$$

The binomial expansion for $(t - u)^i$ leads to:

$$c(t) = \frac{1}{q^*} \int_0^t \sum_{i=0}^{N} x_i \sum_{j=0}^{i} \binom{i}{j} t^j (-1)^{i-j} u^{i-j} \sum_{k=1}^{n} a_k e^{\lambda_k u} \, du \qquad \text{(Eq. 5)}$$

so that:

$$c(t) = \frac{1}{q^*} \sum_{i=0}^{N} x_i \sum_{j=0}^{i} \binom{i}{j} t^j (-1)^{i-j} \sum_{k=1}^{n} a_k \int_0^t u^{i-j} e^{\lambda_k u} \, du \qquad \text{(Eq. 6)}$$

The following useful integral formula can be derived readily:

$$\int_0^t x^n e^{ax} \, dx = \frac{e^{at}}{a} \sum_{i=0}^{n} (-1)^i \frac{n!}{a^i (n-i)!} t^{n-i} - (-1)^n \frac{n!}{a^{n+1}} \qquad n \geq 0$$

$$\text{(Eq. 7)}$$

By applying this formula to the integral in Eq. 6, this equation becomes:

$$q^* c(t) = \sum_{i=0}^{N} x_i \sum_{j=0}^{i} \binom{i}{j} t^j (-1)^{i-j} \sum_{k=1}^{n} \frac{a_k}{\lambda_k} e^{\lambda_k t} \sum_{m=0}^{i-j} (-1)^m$$

$$\times \frac{(i-j)!}{\lambda_k^m (i-j-m)!} t^{i-j-m}$$

$$- \left[\sum_{i=0}^{N} x_i \sum_{j=0}^{i} \binom{i}{j} t^j (-1)^{i-j} \sum_{k=1}^{n} a_k (-1)^{i-j} \frac{(i-j)!}{\lambda_k^{i-j+1}} \right] \qquad \text{(Eq. 8)}$$

The second term in Eq. 8, indicated by a bracket, simplifies to:

$$\text{second term} = -\sum_{i=0}^{N} x_i i! \sum_{j=0}^{i} \frac{t^j}{j!} \sum_{k=1}^{n} \frac{a_k}{\lambda_k^{i-j+1}} \qquad \text{(Eq. 9)}$$

Multiplying through with t^j and rearranging terms in the first term of Eq. 8 lead to:

$$\text{first term} = \sum_{i=0}^{N} x_i i! \sum_{j=0}^{i} \frac{(-1)^{i-j}}{j!} \sum_{k=1}^{n} \frac{a_k}{\lambda_k} e^{\lambda_k t} \sum_{m=0}^{i-j} \frac{(-1)^{i-j-m}}{\lambda_k^m (i-j-m)!} t^{i-m}$$

$$\text{(Eq. 10)}$$

By changing the order of the summation and rearranging terms, this expression becomes:

$$\text{first term} = \sum_{i=0}^{N} x_i i! \sum_{k=1}^{n} \frac{a_k}{\lambda_k} e^{\lambda_k t} \sum_{j=0}^{i} \sum_{m=0}^{i-j} \frac{(-1)^{i-j+m}}{\lambda_k^m j! (i-j-m)!} t^{i-m}$$

$$\text{(Eq. 11)}$$

The coefficient to t^{i-m} resulting from the two innermost summations in Eq. 11 is:

$$\frac{1}{\lambda_k^m} \sum_{j=0}^{i-m} \frac{(-1)^{i-m-j}}{j! (i-m-j)!} = 0 \qquad m < i \qquad \text{(Eq. 12)}$$

Thus, only the term $1/\lambda_k^i (m = i)$ is left from the two innermost summations, which results in a substantial simplification of Eq. 11:

$$\text{first term} = \sum_{i=0}^{N} x_i i \sum_{k=1}^{n} \frac{a_k}{\lambda_k^{i+1}} e^{\lambda_k t} \qquad \text{(Eq. 13)}$$

Equation 8, according to Eqs. 9 and 13, simplifies considerably to:

$$c(t) = \frac{1}{q^*} \sum_{i=0}^{N} x_i i! \left[\sum_{k=1}^{n} \frac{a_k}{\lambda_k^{i+1}} e^{\lambda_k t} - \sum_{j=0}^{i} \frac{t^j}{j!} \sum_{k=1}^{n} \frac{a_k}{\lambda_k^{i-j+1}} \right] \qquad \text{(Eq. 14)}$$

which also can be rearranged to give:

$$c(t) = \sum_{i=0}^{N} x_i \frac{1}{q^*} i! \sum_{j=1}^{n} \frac{a_j}{\lambda_j^{i+1}} \left[e^{\lambda_j t} - \sum_{k=0}^{i} \frac{(\lambda_j t)^k}{k!} \right] \qquad \text{(Eq. 15)}$$
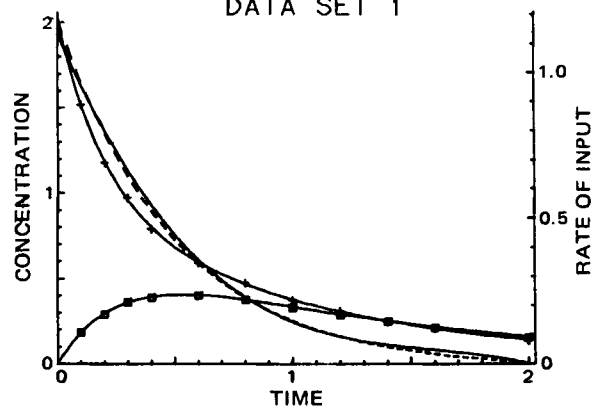

Figure 1—*Test of the new method on simulated data containing 1% random errors. Equation 1 ($n = 2$) is fitted to the impulse response data (+). The curve representing the input response data ($\square$) is calculated from Eq. 16 using the parameters $x_0, x_1, \ldots, x_N$ estimated by multiple linear regression and the parameters estimated from Eq. 1. The input curve is the input rate calculated according to Eq. 3. The broken curve is the exact input rate.*

For further simplification and to illustrate the linearity of $c(t)$ with respect to the polynomial coefficients $x_0, x_1, \ldots, x_N$, Eq. 15 can be written:

$$c(t) = \sum_{i=0}^{N} x_i \theta_i(t) \qquad \text{(Eq. 16)}$$

where:

$$\theta_i(t) = \frac{1}{q^*} i! \sum_{j=1}^{n} \frac{a_j}{\lambda_j^{i+1}} \left[e^{\lambda_j t} - \sum_{k=0}^{i} \frac{(\lambda_j t)^k}{k!} \right] \qquad \text{(Eq. 17)}$$

The expression inside the brackets in Eq. 17 is recognized as the difference between $e^{\lambda_j t}$ and a truncated Maclaurin series expansion of $e^{\lambda_j t}$. This difference becomes smaller when $\lambda_j t \to 0$ and when i increases, which may result in subtraction cancellation and loss of significant figures in the calculations. However, this result can be avoided by using alternative formulas for $\theta_i(t)$. When $|\lambda_j t|$ is small (<0.1), $\theta_i(t)$ is calculated most accurately from the tail of the Maclaurin series:

$$\theta_i(t) = \frac{1}{q^*} i! \sum_{j=1}^{n} \frac{a_j}{\lambda_j^{i+1}} \sum_{k=i+1}^{K} \frac{(\lambda_j t)^k}{k!} \qquad \text{(Eq. 18)}$$

If the computer used represents floating-point numbers to m significant decimal digits, then K in Eq. 18 should be equal to $i + m + 1$ to get the best accuracy ($|\lambda_j t| < 0.1$). (The first neglected term in the summation of the series gives a strict error estimate because the series is alternating.) In the extreme cases where i (N) is large and $|\lambda_j t|$ is not small, $\theta_i(t)$ can be accurately evaluated using the integral representation of the tail of the Maclaurin series, which is:

$$\theta_i(t) = \frac{1}{q^*} \sum_{j=1}^{n} \frac{a_j}{(-\lambda_j)^{i+1}} \int_{\lambda_j t}^{0} e^u (u - \lambda_j t)^i \, du \qquad \text{(Eq. 19)}$$

where the integral is evaluated numerically. However, in most cases, it would not be necessary to use Eq. 19 because Eq. 17 is sufficiently accurate, particularly if the calculations are done in double precision. Furthermore, the number of sampling points in the absorption phase is frequently small. To avoid "fitting to the errors in the data," it is not advisable to use a polynomial of high degree in the approximation of the input rate $f(t)$ so N will not be large.

EXPERIMENTAL

The method presented was tested using the same simulated and real pharmacokinetic data used previously (5) so that it could be compared to two other deconvolution methods tested on the same data (5, 6). A function subprogram was written in FORTRAN IV which evaluated the theta function $\theta_i(t)$[1]. The subprogram algorithm automatically chooses among Eqs. 17–19 so that the best accuracy is obtained. The integral in Eq. 19 was evaluated using a product-type two-point Gauss–Legendre–

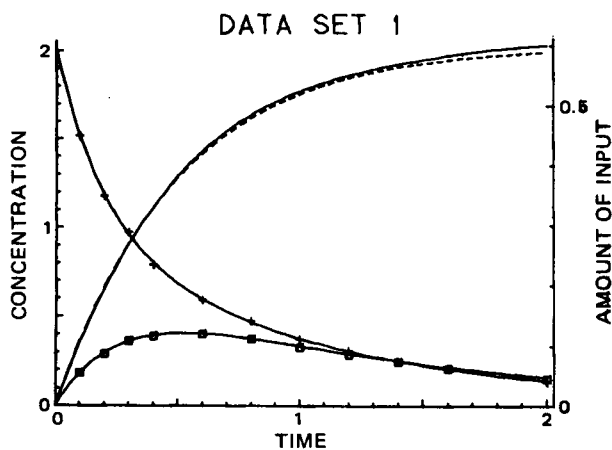[1] A listing of the subprogram is available from the author.

DATA SET 1

Figure 2—*Test of the new method on simulated data containing 1% random errors. Equation 1 (n = 2) is fitted to the impulse response data (+). The curve representing the input response data (□) is calculated from Eq. 16 using the parameters $x_0, x_1, \ldots, x_N$ estimated by multiple linear regression and the parameters estimated from Eq. 1. The increasing curve is the amount of input calculated from the integrated form of Eq. 3. The broken curve is the exact cumulative amount of input.*
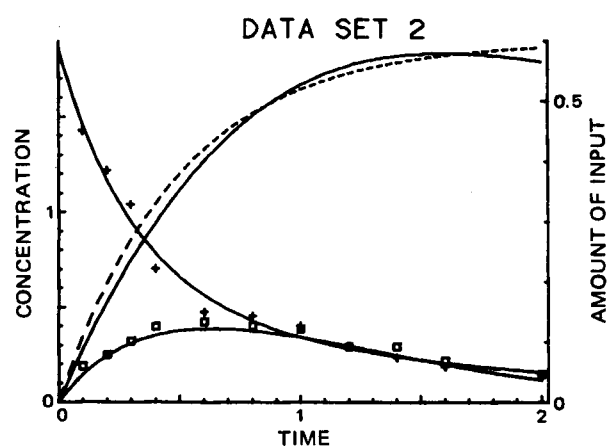
DATA SET 2

Figure 4—*Test of the new method on simulated data containing 10% random errors. Equation 1 (n = 2) is fitted to the impulse response data (+). The curve representing the input response data (□) is calculated from Eq. 16 using the parameters $x_0, x_1, \ldots, x_N$ estimated by multiple linear regression and the parameters estimated from Eq. 1. The increasing curve is the amount of input calculated from the integrated form of Eq. 3. The broken curve is the exact cumulative amount of input.*

Simpson algorithm compounded 50 times (7), which gave excellent accuracy.

The subprogram was used to generate, from the M observations c_i, t_i, $i = 1, 2, \ldots, M$, an $M \times (M + 1)$ data matrix $\{d_{ij}\}$ for input to the multiple linear regression program that calculated $x_0, x_1, \ldots, x_N$ (Eq. 3). The first data matrix column contains the M observations of the dependent variable, i.e., the observed drug concentrations $d_{i1} = c_i$, $i = 1, 2, \ldots, M$. The other data matrix columns contain the independent variables $d_{ij} = \theta_{j-2}(t_i)$, $i = 1, \ldots, M$, $j = 2, \ldots, M + 1$. The highest degree possible for the polynomial that approximates $f(t)$ is $N = M - 1$, which corresponds to a collocation approximation (zero residual sum of squares). Most of the many multiple linear regression programs available for statistical computations can be used to calculate the parameters $x_0, x_1, \ldots, x_N$ of the input function from this data matrix. The only requirement is that the regression program must have a zero-intercept option that is to be used in this regression situation (Eq. 16)[2].

The program P9R, "All Possible Subset Regression," from the

BMDP-77 biomedical computer program package (9) was used with the zero-option and TOL = 10^{-5}. The program was used to automatically find, among $x_0, x_1, \ldots, x_{M-1}$, the best subset based on Mallow's criterion (10). The parameters of the polyexponential expression, Eq. 1, fitted to the intravenous bolus data were reported previously (5) and were determined using the FUNFIT nonlinear regression program (11). The drawings (Figs. 1–9) were done by a penplotter driven by a computer[3] using a software package written by the author. The lag times used in the calculation of the pentobarbital input were those reported earlier (5).

RESULTS AND DISCUSSION

The proposed method is a least-squares deconvolution method, as is the method presented by Cutler (6). Both methods are based on a polynomial approximation of the input rate. However, the present method offers four significant advantages over Cutler's method:

1. The impulse response (bolus input response) is approximated by a polyexponential expression and not by a polynomial. A polyexponential expression does not tend to "fit to the errors" in the data as a polynomial
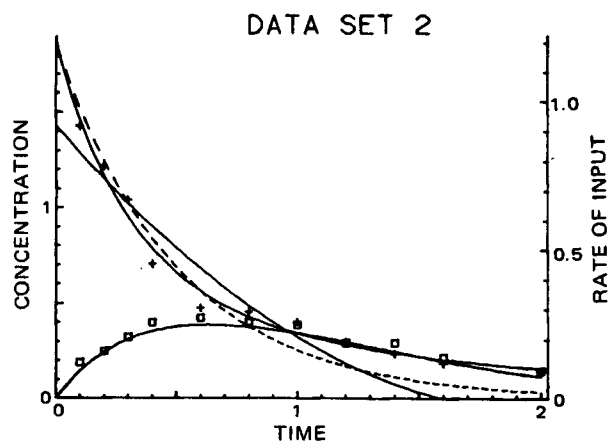
DATA SET 2

Figure 3—*Test of the new method on simulated data containing 10% random errors. Equation 1 (n = 2) is fitted to the impulse response data (+). The curve representing the input response data (□) is calculated from Eq. 16 using the parameters $x_0, x_1, \ldots, x_N$ estimated by multiple linear regression and the parameters estimated from Eq. 1. The input curve is the input rate calculated according to Eq. 3. The broken curve is the exact input rate.*

DATA SET 3

Figure 5—*Test of the new method on simulated data containing 1% random errors. Equation 1 (n = 2) is fitted to the impulse response data (+). The curve representing the input response data (□) is calculated from Eq. 16 using the parameters $x_0, x_1, \ldots, x_N$ estimated by multiple linear regression and the parameters estimated from Eq. 1. The input curve is the input rate calculated according to Eq. 3. The broken curve is the exact input rate.*

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[2] The two most widely used statistical software packages SPSS (8) and BMDP (9) contain several multiple linear regression programs. However, only the BMDP programs have the zero-intercept option. These programs allow the data to be weighted.
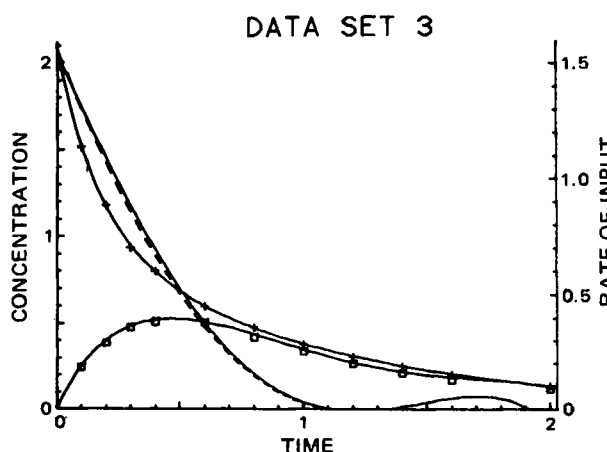
[3] IBM 370.

Table I—Rate and Extent of Input Calculated from Data Set 1

Time	Exact Rate	Rate	Percent Difference[a]	Exact Amount	Amount	Percent Difference[b]
0.1	0.9825	0.9712	−0.94	0.1088	0.1058	−0.49
0.2	0.8044	0.8146	0.86	0.1978	0.1950	−0.48
0.3	0.6586	0.6780	1.62	0.2707	0.2694	−0.21
0.4	0.5392	0.5598	1.72	0.3304	0.3312	0.13
0.6	0.3614	0.3732	0.98	0.4193	0.4234	0.69
0.8	0.2423	0.2434	0.10	0.4789	0.4842	0.90
1.0	0.1624	0.1590	−0.29	0.5188	0.5238	0.84
1.2	0.1089	0.1084	−0.04	0.5456	0.5501	0.75
1.4	0.0730	0.0803	0.61	0.5635	0.5687	0.86
1.6	0.0489	0.0631	1.18	0.5755	0.5829	1.23
2.0	0.0220	0.0160	−0.50	0.5890	0.6003	1.89
Mean[c]			0.80			0.77
SD[c]			0.56			0.49

[a] Calculated as 100 × (calculated rate − exact rate)/1.2, where 1.2 is the initial exact input rate. [b] Calculated as 100 × (calculated amount − exact amount)/0.6, where 0.6 is the exact total amount of input ($t = \infty$). [c] The mean and standard deviation of the absolute values of the percent difference.

Table II—Rate and Extent of Input Calculated from Data Set 2

Time	Exact Rate	Rate	Percent Difference[a]	Exact Amount	Amount	Percent Difference[b]
0.1	0.9825	0.8303	−12.68	0.1088	0.0876	−3.53
0.2	0.8044	0.7426	−5.15	0.1978	0.1662	−5.27
0.3	0.6586	0.6595	0.08	0.2707	0.2363	−5.74
0.4	0.5392	0.5810	3.48	0.3304	0.2983	−5.36
0.6	0.3614	0.4378	6.36	0.4193	0.3998	−3.24
0.8	0.2423	0.3129	5.89	0.4789	0.4746	−0.71
1.0	0.1624	0.2065	3.67	0.5188	0.5262	1.24
1.2	0.1089	0.1184	0.79	0.5456	0.5584	2.14
1.4	0.0730	0.0486	−2.03	0.5635	0.5748	1.88
1.6	0.0489	−0.0027	−4.30	0.5755	0.5791	0.59
2.0	0.0220	−0.0503	−6.02	0.5890	0.5660	−3.83
Mean[c]			4.59			3.05
SD[c]			3.41			1.88

[a] Calculated as 100 × (calculated rate − exact rate)/1.2, where 1.2 is the initial exact input rate. [b] Calculated as 100 × (calculated amount − exact amount)/0.6, where 0.6 is the exact total amount of input ($t = \infty$). [c] The mean and standard deviation of the absolute values of the percent difference.

does. It is smoother and does not oscillate between data points as a polynomial often does. It is also superior to a polynomial with respect to the extrapolation to time zero, which plays an important role in the accuracy of any deconvolution method. Polyexponential expressions have been used successfully and extensively in pharmacokinetics to describe intravenous bolus data, and many application programs are available for this curve-fitting procedure (8, 11, 12).

2. The rate and extent of input are given in a simple, explicit mathematical form by the new method. Cutler (6) expressed the input rate as

a sum of polynomial functions which, through a linear transformation, gives a set of orthogonal functions. Although a back-transformation and summation of terms may provide a single polynomial expression, this approach substantially increases the complexity of the method.

3. The present method requires very little programming on a computer. Only a short program is needed that, according to Eq. 17 or 18 (or Eq. 19 in rare cases), generates the data matrix for input to a standard multiple linear regression program. This programming can be done even using an ordinary programmable pocket calculator. The input function is calculated directly by the linear regression program in terms of the polynomial coefficients. Implementation of Cutler's method requires the
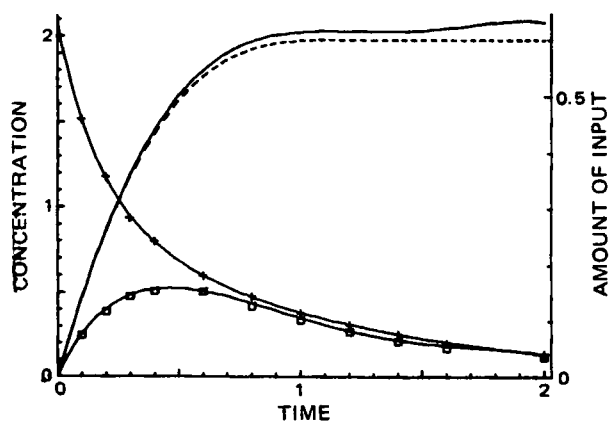

DATA SET 3

Figure 6—*Test of the new method on simulated data containing 1% random errors. Equation 1 (n = 2) is fitted to the impulse response data (+). The curve representing the input response data (□) is calculated from Eq. 16 using the parameters $x_0, x_1, \ldots, x_N$ estimated by multiple linear regression and the parameters estimated from Eq. 1. The increasing curve is the amount of input calculated from the integrated form of Eq. 3. The broken curve is the exact cumulative amount of input.*
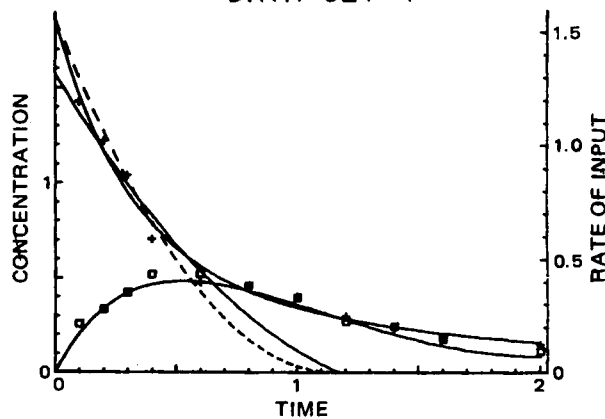

DATA SET 4

Figure 7—*Test of the new method on simulated data containing 10% random errors. Equation 1 (n = 2) is fitted to the impulse response data (+). The curve representing the input response data (□) is calculated from Eq. 16 using the parameters $x_0, x_1, \ldots, x_N$ estimated by multiple linear regression and the parameters estimated from Eq. 1. The input curve is the input rate calculated according to Eq. 3. The broken curve is the exact input rate.*

Table III—Rate and Extent of Input Calculated from Data Set 3

Time	Exact Rate	Rate	Percent Difference[a]	Exact Amount	Amount	Percent Difference[b]
0.1	1.3048	1.3133	0.54	0.1433	0.1427	−0.10
0.2	1.0681	1.0924	1.55	0.2618	0.2629	0.18
0.3	0.8551	0.8844	1.87	0.3577	0.3616	0.65
0.4	0.6657	0.6936	1.78	0.4336	0.4404	1.13
0.6	0.3580	0.3758	1.14	0.5344	0.5458	1.90
0.8	0.1450	0.1543	0.59	0.5831	0.5972	2.35
1.0	0.0266	0.0305	0.25	0.5987	0.6141	2.57
1.2	0	−0.0082	−0.52	0.6000	0.6151	2.52
1.4	0	0.0112	0.72	0.6000	0.6147	2.45
1.6	0	0.0478	3.05	0.6000	0.6207	3.45
2.0	0	−0.0625	−3.99	0.6000	0.6321	5.35
Mean[c]			1.45			2.06
SD[c]			1.18			1.54

[a] Calculated as 100 × (calculated rate − exact rate)/(1.8/1.15), where 1.8/1.15 is the exact initial input rate. [b] Calculated as 100 × (calculated amount − exact amount)/0.6, where 0.6 is the exact total amount of input ($t = \infty$). [c] The mean and standard deviation of the absolute values of the percent difference.

Table IV—Rate and Extent of Input Calculated from Data Set 4

Time	Exact Rate	Rate	Percent Difference[a]	Exact Amount	Amount	Percent Difference[b]
0.1	1.3048	1.1487	−9.97	0.1433	0.1235	−3.30
0.2	1.0681	0.9863	−5.23	0.2618	0.2301	−5.28
0.3	0.8551	0.8353	−1.27	0.3577	0.3211	−6.10
0.4	0.6657	0.6956	1.91	0.4336	0.3976	−6.00
0.6	0.3580	0.4504	5.90	0.5344	0.5114	−3.83
0.8	0.1450	0.2507	6.75	0.5831	0.5808	−0.38
1.0	0.0266	0.0964	4.46	0.5987	0.6147	2.67
1.2	0	−0.0125	−0.80	0.6000	0.6223	3.72
1.4	0	−0.0759	−4.85	0.6000	0.6127	2.12
1.6	0	−0.0938	−5.99	0.6000	0.5950	−0.83
2.0	0	0.0067	0.43	0.6000	0.5715	−4.75
Mean[c]			4.32			3.54
SD			2.95			1.93

[a] Calculated as 100 × (calculated rate − exact rate)/(1.8/1.15), where 1.8/1.15 is the exact initial input rate. [b] Calculated as 100 × (calculated amount − exact amount)/0.6, where 0.6 is the exact total amount of input ($t = \infty$). [c] The mean and standard deviation of the absolute values of the percent difference.

development of a fairly extensive and specialized program. Moreover, the user would not have many of the valuable statistical options that are supplied by the standard multiple linear regression programs (9).

4. The present method is computationally efficient and fast. With Cutler's method, a convolution integral (Ref. 6, p. 249) is evaluated numerically several times during the computations. The integration may introduce significant errors and may add significant cost to the computations if the results are to be presented graphically.

Simulation Results—The present results and comparisons are related

DATA SET 4

Figure 8—*Test of the new method on simulated data containing 10% random errors. Equation 1 (n = 2) is fitted to the impulse response data (+). The curve representing the input response data (□) is calculated from Eq. 16 using the parameters $x_0, x_1, \ldots, x_N$ estimated by multiple linear regression and the parameters estimated from Eq. 1. The increasing curve is the amount of input calculated from the integrated form of Eq. 3. The broken curve is the exact cumulative amount of input.*

to a similar investigation recently published (5). Comparing Tables I–IV with Tables II–V in Ref. 5 shows that the present method and Cutler's method do not differ significantly in the accuracy of estimating the input for Data Sets 1, 2, and 4 (Tables I, II, and IV). However, the present method appears to be significantly more accurate for Data Set 3 (Table III). Both methods perform well, considering that the average relative error in all estimations is of the same magnitude as the noise added to the simulated data.

Each method tends consistently to underestimate the initial input rate. This trend is probably related to the use of a polynomial in the input approximation. It may be explained theoretically ["Runge's phenomenon" (13)]. A special sampling procedure may eliminate or reduce this trend and give an overall better estimation. This procedure, based on the min–max principle using a Tschebyscheff sampling (13), is as follows. Let it be known from preliminary investigations that the absorption of the drug is completed (e.g., 95%) in approximately T hr after the lag time and that the lag time is approximately t_{lag} hr. If the experimental protocol allows m samples to be drawn, then these samples should be taken at the following times:

$$t_i = t_{lag} + \frac{T}{2}\left[1 + \cos\frac{(2i - 1)\pi}{2m}\right] \qquad i = 1, 2, \ldots, m \quad \text{(Eq. 20)}$$

A few additional samples are required after $T + t_{lag}$ hr to check for a change in the disposition kinetics between the intravenous and the absorption experiments. However, these points should not be included in the actual calculation of the input because they may interact unfavorably with the curve fitting in the absorption region.

Calculation of Pentobarbital Input—It is of interest to compare the present method with the other deconvolution method presented by the author (Ref. 5, Fig. 9). The pentobarbital data are rather difficult to treat, because the absorption phase is defined by only three or four data points and the data contain rather large errors. However, it is surprising how similarly the two methods perform in the absorption region. The total percent absorbed, measured by the maximum of the percent absorbed curve, does not differ much between the methods. The times for the absorption are also rather close.

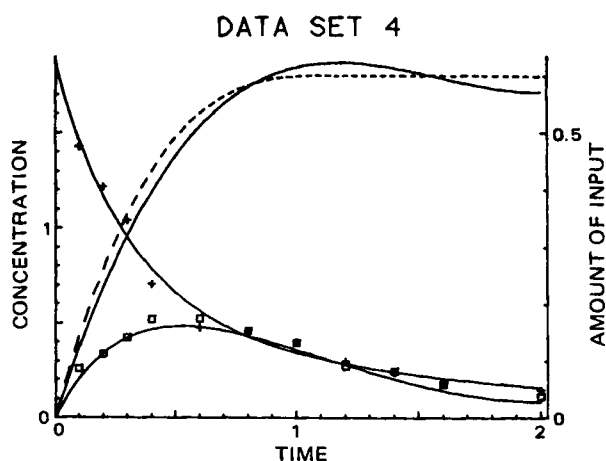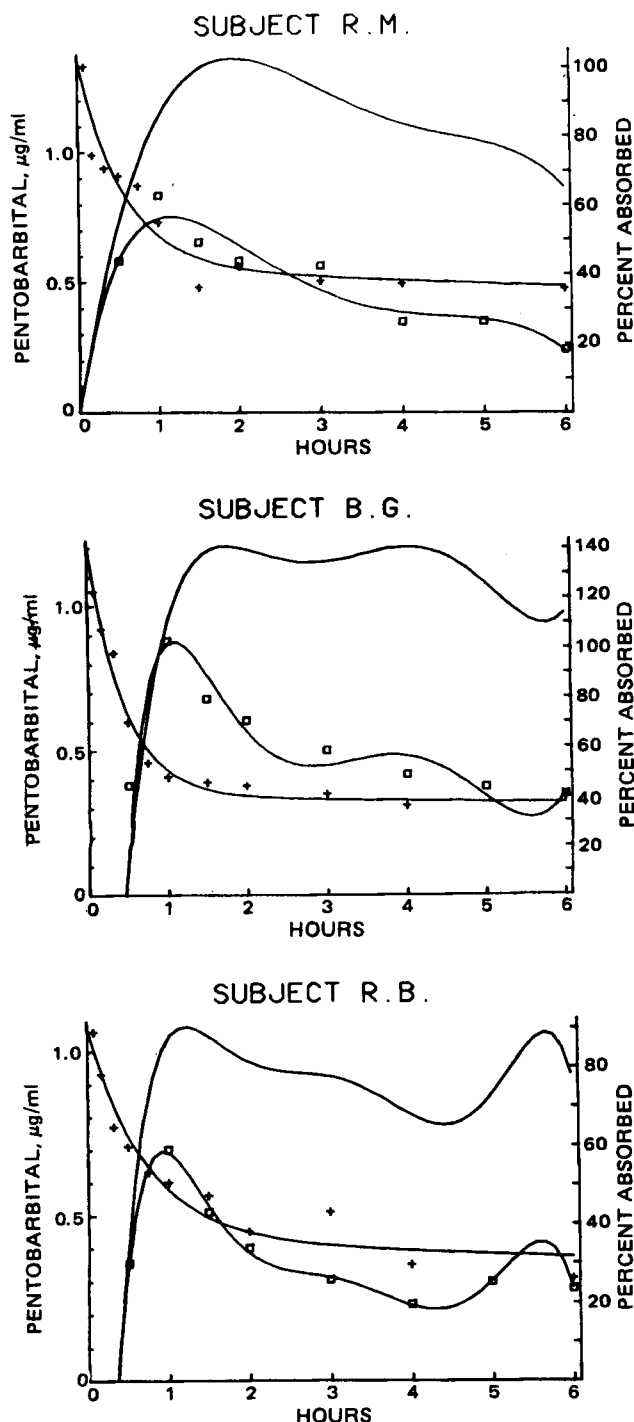However, the present method does not perform as well with respect

SUBJECT R.M.

SUBJECT B.G.

SUBJECT R.B.

Figure 9—*Application of the new method to calculate the cumulative amount of pentobarbital input in human subjects. Equation 1 (n = 2) is fitted to the plasma drug level data (+) from intravenous administration. The curve representing oral absorption data (□) is calculated from Eq. 16 using the parameters $x_0, x_1, \ldots, x_N$ estimated by multiple linear regression and the parameters estimated from Eq. 1. The input is calculated according to the integrated form of Eq. 3.*

to the curve fitting after the absorption phase. It demonstrates some of the undesirable effects originating from the polynomial approximation of the input rate; the absorption curve for Subject R.B. passes too close to all of the points, probably resulting in a poor filtering of the errors in the data. The curve for Subject B.G. shows excessive oscillations. However, the curve for Subject R.M. seems satisfactory overall. The same discrepancies between the intravenous and the absorption data reported previously (5) are also observed using the present method.

It is important to note that the calculation of the rate and extent of drug input at a given time, $t = t_0$, depends only on the fitted curves up to that

Table V—Calculated Polynomials that Describe the Rate of Input [a]

Data Set	Rate of Input
1	$f(t) = 1.149 - 1.891t + 1.139t^2 - 0.2385t^3$
2	$f(t) = 0.9225 - 0.9457t + 0.2297t^2$
3	$f(t) = 1.542 - 2.311t + 0.1076t^2 + 1.059t^3 - 0.3680t^4$
4	$f(t) = 1.322 - 1.794t + 0.5682t^2$

[a] $0.1 \leq t \leq 2.0$.

time, t_0, according to the fundamental convolution integral equation (Eq. 2). Thus, the accuracy of the calculated input depends only on the goodness of the fit in the absorption phase. The goodness of the fit in the postabsorptive phase has no influence on the calculations. A fitting in this region is not required, but it provides a means of detecting discrepancies between the intravenous and absorption data (5). The fits in the absorption phase (Fig. 9) appear satisfactory considering the sparseness of the data in this region.

Assumptions and Limitations of Deconvolution Methods of Analysis of Drug Input into Blood—The deconvolution methods are based on the assumption of a time-invariant linearity between input and response in the sampleable response environment (the blood). A necessary condition for an environment to be linear is that the principle of superposition applies; *i.e.*, the sum or superposition of any two inputs, $f_1(t) + f_2(t)$, results in a response that is the superposition, $c_1(t) + c_2(t)$, of the individual responses (14). Corollaries to the superposition principle are:

1. No input affects the response of other inputs.
2. There is no interaction among the responses caused by different inputs.
3. The total response of several inputs into the environment can be determined by determining separately the response of the environment to each individual input and then superimposing the responses to get the total response.

These principles are well known from linear system theory (14). However, the real problem in applying these principles to pharmacokinetics apparently has not been discussed, namely, the effect of the input path on the response. The deconvolution methods can be used only if the response to a well-defined input is determined. The input is only well defined if it is direct intravenous (bolus and/or infusion). Consequently, the information about the system's input–response relationship is based only on a direct intravenous input. However, the inputs that are of interest are not direct intravenous. Therefore, the validity of the deconvolution approach is directly related to Corollary 1.

The crucial question is: Does the drug in its indirect path to the sampleable response environment affect the change in the concentration of the drug already in the environment so that the concentration change is different from that predicted had the input been direct? This question is identical to the question posed previously in dealing with the evaluation of input into linear compartmental systems (4): Is the input interacting?

Deconvolution methods can only be used to evaluate a noninteracting, primary input (4). Therefore, to verify the validity of the deconvolution approach, the system must be tested not only with respect to superposition but also with respect to interaction. A superposition test requires a minimum of two different direct intravenous bolus or intravenous infusion inputs. A test for interaction requires two experiments, one involving only a direct intravenous input and the other involving the indirect and direct inputs combined, where the drug in either the indirect or the direct input must be labeled in order to evaluate a possible interaction between the two inputs.

In spite of the crucial role that these aspects play in drug input evaluation, there does not seem to have been any work done in this fundamental area.

Table VI—Rate of Input of Pentobarbital

Subject	Rate[a], mg/hr	Lag Time, hr
B.G.	$f(t) = 159 - 266T + 150T^2 - 34.7T^3 + 2.77T^4$	0.46
R.M.	$f(t) = 69.9 - 64.7T + 17.8T^2 - 1.54T^3$	0.0
R.B.	$f(t) = 149 - 365T + 313T^2 - 122T^3 + 22.1T^4 - 1.49T^5$	0.36

[a] $T = \text{time} - \text{lag time}$.

The method presented, when applied with care, should be a valuable tool in drug absorption investigations. It seems to have some distinct advantages over the method proposed by Cutler (6). The simulation data generated by Cutler do not differentiate between the two approaches. However, these data represent rather ideal, somewhat unrealistic cases. More realistic tests might result in a definite distinction of the methods. The relative accuracy of the two approaches in practice is closely related to the issue of whether the impulse response data (i.e., the intravenous bolus data) are best approximated by a polynomial or a polyexponential expression. It seems quite evident that the latter is the case, so the present method should be generally more accurate. Although this method seems promising, it appears from previous theoretical considerations (4) that the other deconvolution method presented by the author (5) is superior. However, the new method has the advantage that the rate and the amount (cumulative) of input are summarized in a simple polynomial form (Tables V and VI).

REFERENCES

(1) V. F. Smolen, J. Pharmacokinet. Biopharm., 4, 337 (1976).
(2) P. Veng Pedersen, J. Pharm. Sci., 67, 187 (1978).
(3) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence Publications, Hamilton, Ill., 1975.
(4) P. Veng Pedersen, J. Pharm. Sci., 69, 298 (1980).
(5) Ibid., 69, 305 (1980).
(6) D. J. Cutler, J. Pharmacokinet. Biopharm., 6, 243 (1978).
(7) W. R. Boland and C. S. Duris, BIT, 11, 139 (1971).
(8) N. H. Nie, C. H. Hull, J. G. Jenkins, K. Steinbrenner, and D. H. Bent, "SPSS, Statistical Package for the Social Sciences," McGraw-Hill, New York, N.Y., 1975.
(9) W. J. Dixon and M. B. Brown, "BMDP Biomedical Computer Programs," University of California Press, Berkeley, Calif., 1977.
(10) C. Daniel and F. S. Wood, "Fitting Equations to Data," Wiley, New York, N.Y., 1971, p. 86.
(11) P. Veng Pedersen, J. Pharmacokinet. Biopharm., 5, 513 (1977).
(12) C. M. Metzler, "A Users Manual for NONLIN," Tech. Rept. 7293/7292/005, Upjohn Co., Kalamazoo, Mich., 1969.
(13) G. Dahlquist and Å. Björk, "Numerical Methods," Prentice-Hall, Englewood Cliffs, N.J., 1974.
(14) B. M. Brown, "The Mathematical Theory of Linear Systems," Chapman and Hall, London, England, 1965.

Novel Approach to Bioavailability Testing: Statistical Method for Comparing Drug Input Calculated by a Least-Squares Deconvolution Technique

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Abstract □ A novel approach to bioavailability testing is presented. The approach is model independent because it does not assume a specific pharmacokinetic model and does not use absorption, distribution, or elimination rate constants or a volume term. The method, which requires intravenous administration, is compared to classical bioavailability evaluation methods. Evaluation of drug input is based on the same assumptions required for using the area under the curve. No extrapolation beyond the last data point is required. Two statistics are derived that enable a comparison of the rate and the cumulative amount of input of two inputs for various times. A differential confidence profile is calculated that allows a more detailed and intrinsic bioavailability comparison than previous methods. The approach is demonstrated on simulated data containing random noise and shows satisfactory performance.

Keyphrases □ Bioavailability—testing, drug input, least-squares deconvolution technique □ Deconvolution—least-squares technique, bioavailability testing, drug input □ Drug availability—testing, least-squares deconvolution technique

The quality of a drug product as a drug delivery system is determined by the rate and extent of delivery of the active form to the biological environment responsible for the pharmacological effect. In most cases, this environment is neither known nor may be sampled for the drug. However, a close relationship usually exists between the drug concentration in a sampled environment (e.g., blood) and its pharmacological response. Therefore, this environment may be a useful indicator of the drug input into the response environment.

Drug delivery usually is characterized in bioavailability terms with the blood as the sampleable environment. Bioavailability commonly is defined by the rate and the extent of drug input into the systemic circulation (1). Bioavailability comparisons usually are based on three parameters from a single-dose blood level curve: (a) the area under the curve, AUC (extrapolated); (b) the time of the peak concentration, t_{max}; and (c) the peak concentration, C_{max} (2).

These parameters are associated conceptually with the extent (AUC) and the rate (t_{max} and C_{max}) of input. This association is related to linear pharmacokinetic assumptions. For example, AUC is a proper measure of the total input only if the response (concentration) is linear with respect to the input (3, 4). If the system is not linear, a larger AUC does not guarantee greater input. Comparisons of the drug input on the basis of AUC may be inaccurate for several reasons:

1. The tail area must be estimated by extrapolation. Consequently, this area can be determined only by a model-dependent approach that assumes a certain functional form for the tail or the total curve.

2. The tail area frequently is estimated from the last observations (e.g., log-linear extrapolation), based on the assumption that these points predict the behavior in the tail. The tail area usually is determined poorly in this way due to low information density of the terminal set of points. This problem is complicated further by constraints in time and the number of samples when dealing with human subjects. The experimenter must decide whether more samples should be taken in the terminal phase, where little or no input takes place, to predict the tail area better or whether these samples would be more valuable in the input phase where the real information about the input is present and where C_{max} and t_{max} are to be estimated.

3. The problem is complicated by the fact that the tail