Table IV—Average Urinary Excretion of Levodopa and Its Metabolite after Oral Administration of Levodopa to Sterilized Dogs and Control Dogs

Dogs	Total Levodopa[a]	Total Dopamine[b]	Total 3,4-Dihydroxyphenylacetic Acid[c]	Total Homovanillic Acid[d]	Total[e]
Control	0.47 ± 0.07[f]	8.9 ± 1.2	9.3 ± 1.4	30.4 ± 2.3	49.1 ± 2.4
Sterilized	0.47 ± 0.07	9.5 ± 1.6	10.1 ± 1.3	30.7 ± 2.5	50.7 ± 2.7

[a] Total levodopa = unconjugated levodopa + conjugated levodopa. [b] Total dopamine = unconjugated dopamine + conjugated dopamine. [c] Total 3,4-dihydroxyphenylacetic acid = unconjugated 3,4-dihydroxyphenylacetic acid + conjugated 3,4-dihydroxyphenylacetic acid. [d] Total homovanillic acid = unconjugated homovanillic acid + conjugated homovanillic acid. [e] Sum of total levodopa, total dopamine, total 3,4-dihydroxyphenylacetic acid, and total homovanillic acid. [f] Percent of dose excreted in 0–48-hr urine (average ± *SE*).

tabolized by intestinal microorganisms *in vitro* to *m*-hydroxyphenylacetic acid, 4-methylcatechol, and 4-methylguaiacol. Furthermore, levodopa was reported to be metabolized by intestinal microorganisms to *m*-hydroxyphenylacetic acid *in vivo* in conventional rats but not in germ-free rats (7–9). However, the small amount of metabolites formed by intestinal microorganisms reported by Bakke (6) and the fast absorption of levodopa from dogs observed in an *in situ* experiment[5] support the hypothesis that bacterial metabolism of levodopa may be insignificant.

The levodopa decarboxylase enzyme was widely distributed in the dog intestinal tract, with the greatest activity in the jejunum and the least activity in the duodenum. Taubin and Landsberg (10) suggested that catechol *O*-methyltransferase also played an important role in levodopa metabolism in the rat intestine. Administration of the levodopa inhibitor or benzerazide [*N*-*dl*-seryl-*N*-(1,2,3-trihydroxybenzyl)hydrazine], which cannot inhibit catechol *O*-methyltransferase, increased plasma levodopa levels (11–14). This finding implies that catechol *O*-methyltransferase plays a far less important role in intestinal metabolism of levodopa than levodopa decarboxylase.

The data presented here indicate that the reduced bioavailability of orally administered levodopa is due to metabolism of levodopa by levodopa decarboxylase in the intestine, with the greatest activity in the jejunum and the least activity in the duodenum.

REFERENCES

(1) K. Sasahara, T. Nitanai, T. Habara, T. Morioka, and E. Nakajima, *J. Pharm. Sci.*, **69**, 261 (1980).

(2) *Ibid.*, **69**, 1374 (1980).

(3) K. Sasahara, T. Nitanai, T. Habara, A. Ninomiya, T. Morioka, and E. Nakajima, *Ann. Rep. Sankyo Res. Lab.*, **30**, 65 (1978).

(4) P. T. Mearrick, G. G. Graham, and D. N. Wade, *J. Pharmacokinet. Biopharm.*, **3**, 13 (1975).

(5) S. Cotler, A. Holazo, H. G. Boxenbaum, and S. A. Kaplan, *J. Pharm. Sci.*, **65**, 824 (1976).

(6) O. M. Bakke, *Acta Pharmacol. Toxicol.*, **29**, 106 (1971).

(7) O. Borud, T. Midtvelt, and L. R. Gjessing, *ibid.*, **30**, 185 (1971).

(8) B. R. Goldin, M. A. Peppercorn, and P. Goldman, *J. Pharmacol. Exp. Ther.*, **186**, 160 (1971).

(9) P. Goldman, M. A. Peppercorn, and B. P. Goldin, *Am. J. Clin. Nutr.*, **27**, 1368 (1974).

(10) H. L. Taubin and L. Landsberg, *Clin. Res.*, **20**, 872 (1972).

(11) A. Pletscher and G. Bartholini, *Clin. Res. Ther.*, **12**, 344 (1971).

(12) W. Birkimayer and E. Neumayer, *Z. Neurol.*, **202**, 257 (1972).

(13) A. Barbeau, L. Gillo-Joffray, and H. Mars, *Clin. Res. Ther.*, **12**, 353 (1972).

(14) E. M. Miller and L. Wiener, *Neurology*, **5**, 482 (1972).

[5] Part V of this series, to be published.

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Computational Problems of Compartment Models with Michaelis–Menten-Type Elimination

CARL M. METZLER *x and D. D. M. TONG ‡

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Abstract □ The Michaelis–Menten equation has been applied successfully in the study of enzyme kinetics. It usually is used to estimate v_{max} and k_m from observations of the initial rate of reaction, v, at various substrate concentrations, C_s. A variation of this expression recently was used in pharmacokinetics, where it was assumed that the elimination rate of drug from some compartment is $VC(t)/[K + C(t)]$, where $C(t)$ is the drug concentration. The meaning of V and K in this context is not clear. Attempts were made to estimate V, K, and other model parameters by fitting the model to observed drug concentrations at sampling times after dosing. This paper discusses the ill-conditioning of the estimation of parameters of a differential equation that includes the so-called Michaelis–Menten output. The solution of the equation is bound by the solutions to two first-order differential equations. Parameter values in an infinite region of the parameter space are shown to have solutions also lying within these two bounds. Simulations show that a minor change in the data (observations) or in the initial estimate of the parameters may cause a large change in the final estimates. In many cases, estimation and comparison of parameter values are meaningless.

Keyphrases □ Models, mathematical—compartment models with Michaelis–Menten-type elimination, computational problems, parameter estimation □ Michaelis–Menten equation—computational problems of compartment models, parameter estimation □ Compartment models—computational problems of models with Michaelis–Menten-type elimination, parameter estimation □ Pharmacokinetics—analysis, computational problems of compartment models with Michaelis–Menten-type elimination, parameter estimation

Linear compartment models have been used successfully in pharmacokinetics for the past 40 years. Like all mathematical models, they are an abstraction from the real biological system to a mathematical system and thus are

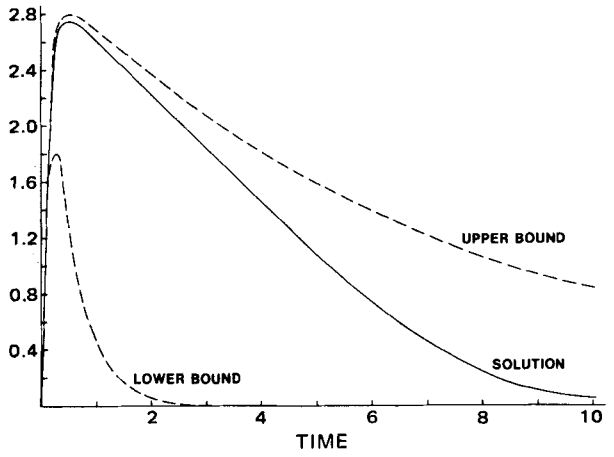

Figure 1—*Solution and bounds for Michaelis–Menten elimination model with* D = *3,* K_A = *8,* V = *0.418, and* K = *0.182.*

approximations. Nevertheless, their successful use indicates that they are a good approximation with many drugs. For other drugs, they are not a good approximation, and attempts have been made to find nonlinear compartment models that are better.

THEORETICAL

One class of nonlinear models assumes that elimination of drug from the system, rather than being a first-order process, is a Michaelis–Menten process. The well-known Michaelis–Menten equation has been used widely and successfully in enzyme kinetic studies despite difficulties in the theoretical derivation (1, 2). The Michaelis–Menten equation:

$$v = v_{max}C_s/(k_m + C_s) \qquad \text{(Eq. 1)}$$

is used to estimate v_{max} and k_m from observations of the initial reaction rate, v, at various substrate concentrations, C_s. It was adapted to pharmacokinetic models by assuming that the elimination rate from a compartment has a form similar to the right side of Eq. 1. Thus, if $C(t)$ is the drug concentration in a compartment, then the rate of change of $C(t)$ is:

$$\frac{dC(t)}{dt} = f(t, \theta) - VC(t)/[K + C(t)] \qquad \text{(Eq. 2)}$$

where $f(t, \theta)$ is a function determined by the input into the compartment. In the usual pharmacokinetic application of Eq. 2, attempts are made to estimate V and K along with the other model parameters, θ, by fitting the model to observed drug concentrations at n sampling times after dosing. Although Michaelis–Menten-type elimination models had been used previously, a detailed description and analysis of the simplest model incorporating Michaelis–Menten-type elimination—the one-compartment model with bolus intravenous input—were published in 1973 (3). For that model, $f(t, \theta)$ of Eq. 2 is zero; the model is expressed as:

$$\frac{dC(t)}{dt} = -VC(t)/[K + C(t)] \qquad \text{(Eq. 3)}$$

As Wagner (3) pointed out, for this model there is no solution that expresses $C(t)$ as an explicit function of t; thus, the solution is an implicit function. For more complex models, *i.e.*, a one-compartment model with first-order input, for which $f(t, \theta)$ in Eq. 2 is an exponential function, a solution in closed form does not exist.

It is possible, however, to fit these models to observed data by numerically integrating Eq. 2 to obtain points $[t, C(t)]$ predicted by the model. Many recent pharmacokinetic studies obtained parameter estimates by combining a nonlinear regression algorithm with numerical integration. Numerical integration requires more computation than fitting data to the solutions of the differential equations and can be expensive in terms of computer time. When combined with nonlinear regression algorithms that require many evaluations of the solution, the expense of fitting models in this way can be prohibitive. Furthermore, these methods may be unsatisfactory in terms of convergence and final fit.

These problems motivated a search for an approximate solution to Eq. 2 that might make estimation of the parameters with this model easier. During this search, mathematical properties of compartment models with Michaelis–Menten-type elimination were derived. The implications of these properties for computation and parameter estimation will be discussed. Finally, the results of computer simulation reveal the statistical properties of the parameter estimates obtained by fitting the two simplest Michaelis–Menten-type models to data.

RESULTS

Mathematical Development—Tong and Metzler (4) derived the mathematical properties of solutions to Eq. 2. The mathematical details are given elsewhere (4), and only the two most important results for parameter estimation will be discussed here. The conditions on the model of Eq. 2 are that $f(t, \theta)$ be nonnegative and bounded, with $C(0) = 0$; *i.e.*, there must exist two positive constants, C_0 and s, such that:

$$0 \leqq f(t, \theta) \leqq C_0 \exp(-st) \qquad \text{for } t \geqq 0 \qquad \text{(Eq. 4)}$$

Given these mild restrictions, the function $F(t, A)$ is a lower bound for $C(t)$ with $A = V/K$ and an upper bound for $C(t)$ with $A = V/(K + B)$, where B is the maximum of $C(t)$, and was defined as (4):

$$F(t, A) = \exp(-At) \int_0^t f(\tau) \exp(A\tau)\, d\tau$$

$$= \begin{cases} \dfrac{C_0}{A - s}[\exp(-st) - \exp(-At)] & A \neq s \\ C_0 t \exp(-At) & A = s \end{cases} \qquad \text{(Eq. 5)}$$

With the assumed restrictions, Eq. 2 includes many compartment models with Michaelis–Menten-type elimination from one compartment. Equation 5 shows that the time–concentration curves of these models are bounded by functions of the same form as the model equations for a one-compartment model with first-order input and first-order output (5). This is in agreement with the statement by Wagner (3) and others (5) that, for the simplest model, the solution to Eq. 2 approaches the linear solution as the concentration increases. The distance between the bounds given by Eq. 5 depends on V, K, and the maximum of $C(t)$. As an example, the one-compartment model with first-order input (absorption rate = K_A) and Michaelis–Menten-type elimination will be considered. For this model, $f(t, \theta)$ is given by:

$$f(t, K_A, D) = K_a D \exp(-K_a t) \qquad \text{(Eq. 6)}$$

where D is a dilution factor, roughly equal to the dose divided by a volume of distribution. By Eq. 5, the lower and upper bounds of the solution are:

$$\frac{K_A D}{K_A - V/K}[\exp(-Vt/K) - \exp(-K_A t)]$$

$$\frac{K_A D}{K_A - Q}[\exp(-Qt) - \exp(-K_A t)]$$

where Q is the only solution of the equation:

$$Q + \frac{K_A D}{K}\left(\frac{Q}{K_A}\right)^{K_A/(K_A - Q)} - \frac{V}{K} = 0 \qquad \text{(Eq. 7)}$$

The bounds for the case in which $D = 3$, $K_A = 8$, $V = 0.418$, and $K = 0.182$ are shown in Fig. 1. For this case, the bounds are not restrictive. For the case in which $D = 3$, $K_A = 8$, $V = 1$, and $K = 2$, the bounds are shown in Fig. 2; for this case, the solution is bound rather closely by functions that are solutions to the linear compartment model. For larger V and K values, the bounds are so close together that the bounds and the solution appear as one line when plotted.

The other result of Tong and Metzler (4) is illustrated in Fig. 3. If (K_0, V_0) is a point in the (K, V) parameter space, then there is an infinite, open-ended wedge such that for any other (K, V) in this wedge, the solution to Eq. 2 with parameters V and K lies within the bounds of the solution to Eq. 2 with parameters V_0 and K_0. Also, there is a line through (K_0, V_0) within this wedge, such that any (K, V) on this line determines a solution to Eq. 2 that is nearly identical to the solution of Eq. 2 with the parameters (K_0, V_0). In other words, there is an infinite set of (K, V) values that all generate the same time–concentration curve (to a finite accuracy). This observation will be illustrated further.

The parameters of Eq. 1 often are estimated by applying one of several linearizing transformations to the equation (1, 2). The parameters also may be estimated directly (and more correctly) from Eq. 1 by using a nonlinear estimation technique. In any case, the estimation of v_{max} and k_m will suffer from a high correlation of the estimates since the param-

Table I—Specifications of Models and Simulations

Case	Model (Input)	Parameters	Parameter Values	Error Structure	n	N
1	Bolus intravenous	C_0, V, K	2.0, 0.22, 0.11	Additive	11	200
2	Bolus intravenous	C_0, V, K	2.0, 0.22, 0.11	Proportional	11	200
3	First order	K_a, V, K	1.5, 2.0, 5.0	Additive	11	200
4	First order	K_A, V, K	1.5, 2.0, 5.0	Proportional	11	200
5	First order	K_A, V, K, D	1.5, 2.0, 5.0, 10.0	Additive	11	200
6	First order	K_A, V, K, D	1.5, 2.0, 5.0, 10.0	Proportional	11	200
7	First order (three functions)	K_A, V, K, D_1, D_2, D_3	1.5, 2.0, 5.0, 10.0, 20.0, 40.0	Proportional	33	100
8	First order	K_A, V, K, D	1.5, 2.0, 5.0, 10.0	Proportional	33	100
9	First order	K_A, V, K, D	1.5, 2.0, 5.0, 40.0	Proportional	33	100

eters appear in a ratio in the equation and a change in the estimate of v_{max} may be compensated for by a change in k_m. This problem is accentuated when, as in Eq. 2, the ratio of parameters does not appear in the function itself but rather in the derivative of the function being fit.

Previous work (4) showed that, for most (K, V) values, the solution to Eq. 2 is bounded by a function of two exponentials; in the previous example, these functions have one less parameter than the solution. (Approximately, K_E is replaced by V/K.) Thus, if the data can be fit to Eq. 5, trying to fit the data to Eq. 2 by nonlinear regression methods will result in a situation analogous to singularity in the case of linear regression.

The result expressed in Fig. 3 implies that if (V, K) are estimated by least-squares techniques, the sum of squares surface will have a long valley with steep sides but little change along the floor. This situation results in difficult estimation problems (6). If an estimation algorithm can find a minimum along this narrow valley, the estimates of V and K will be highly correlated since they can move together along the valley floor without much change in the sum of squared residuals.

Thus, there are at least two sources of computational difficulties in fitting Eq. 2 to data:

1. Since there is no closed form solution, numerical integration must be used to obtain predicted values of $C(t)$ for a given model and t.
2. Parameter estimation is hampered by a high correlation between estimates of V and K.

To understand the implications of the second difficulty, Monte Carlo simulations were performed.

Computer Simulation—The statistical properties of parameter estimates of nonlinear models are known only asymptotically, and it generally is not known how well these asymptotic properties approximate the results for small samples. Thus, Monte Carlo simulations were needed to determine the statistical characteristics of the estimates for a given model and experiment (7, 8). In view of the mathematical properties derived for Michaelis–Menten-type elimination models, a simulation study of the statistical properties for some simple models seemed necessary.

One shortcoming of simulation studies is that only a small part of the problem can be simulated. For this report, some representative cases (Table I) were chosen with the hope that they would yield insight into the general problem. All models were one-compartment ones with either bolus input or first-order absorption and Michaelis–Menten-type elimination. Two types of error structure were considered: (*a*) additive error in which each simulated "observation" was the model-predicted value plus a normal random number from the distribution $N(0, 0.09)$, and (*b*) proportional error in which each observation was the model-predicted value plus the product of the model value and an $N(0, 0.0025)$ random variable.

The proportional error structure is equivalent to a 5% error. This error structure violates one assumption of least-squares estimation, and either a transformation should be done or variable weights should be used. The usual least-squares estimation was carried out, however, in the belief that it reflects common practice where no error analysis is done and the error structure is not known.

The model values were obtained by numerically integrating either Eq. 3 or Eqs. 2 and 6 with the parameter values given in Table I. Each data set had n observations. The values of t were 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, and 10 for the intravenous bolus model and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, and 18 for the first-order input models. Each model was simulated N times; that is, N sets of observations were generated; for each set of n observations, the parameters were estimated by the computer program NONLIN (9), a nonlinear regression program. This program also estimated the standard deviation for each estimate and the correlation matrix of the estimates.

Table II summarizes the simulation results of Case 5 (Table I). Similar tables were prepared for all cases in Table I; for space reasons, only this table is presented in full. However, the conclusions and discussion are based on all of the tables[1].

In Table II, the first column identifies the parameters used to generate the $N = 200$ sets of observations, the standard deviations of the estimates, the largest eigenvalue ($E1$) of the estimated correlation matrix, and the correlations of the estimates. The second column gives either the values of the parameters or, in parentheses, the sample values of the 200 estimates. Thus, column 2 of the row labeled $s(V)$ contains the standard deviation of the 200 estimates of V; column 2 of the row labeled $C13$ contains the correlation of the 200 pairs of estimates of the first and third parameters (K_A and V). The remaining columns of Table II give the sample statistics of the 200 estimates: minimum, maximum, 10th and 90th percentiles, mean, and median. Thus, in the row labeled $s(V)$, the first column gives the standard deviation of the 200 estimates of V; the following columns give the sample value followed by the statistics of the 200 estimates of $s(V)$ computed by the estimation algorithm from each

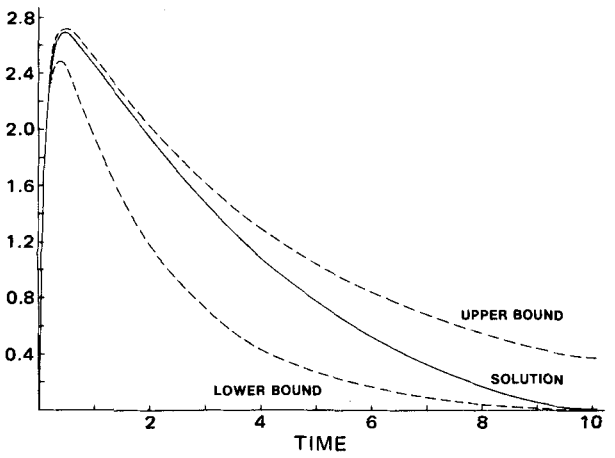

Figure 2—*Solution and bounds for Michaelis–Menten elimination model with* D = 3, K_A = 8, V = 1, *and* K = 2.

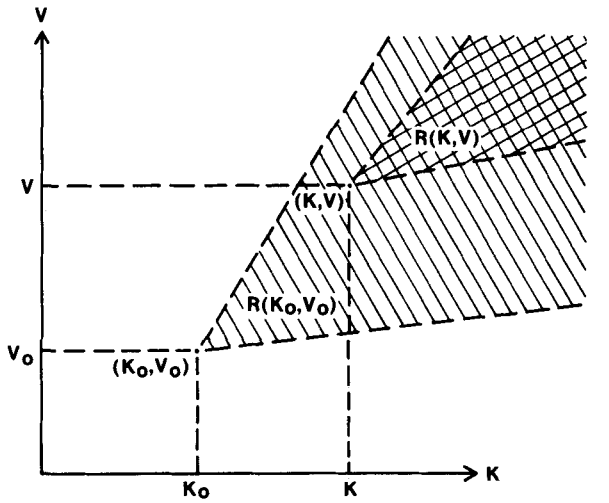

Figure 3—*Relation of solutions in the* V–K *parameter space.*

[1] Copies of the full set of tables may be obtained from the authors.

Table II—Summary of Simulation of Case 5, Table I

Parameter	Parameter or Sample Value	Sample Statistics					
		Minimum	P_{10}	Mean	Median	P_{90}	Maximum
K_A	1.50	0.88	1.24	1.48	1.48	1.74	2.33
V	2.00	0.84	1.18	52.69	2.30	18.67	1561.0
K	5.00	0.59	1.51	215.74	6.17	89.67	5875.0
D	10.00	8.30	9.33	10.18	10.09	11.10	13.66
$s(K_A)$	(0.205)	0.065	0.118	0.183	0.177	0.252	0.329
$s(V)$	(201.62)	0.098	0.296	3596.0	1.254	113.87	∞
$s(K)$	(803.12)	0.380	1.211	14,596.0	5.427	556.14	∞
$s(D)$	(0.738)	0.170	0.334	0.691	0.598	1.144	3.565
$E1$	—	3.088	3.328	3.506	3.518	3.677	3.860
$C12$	(−0.762)	−0.921	−0.841	−0.776	−0.776	−0.712	−0.599
$C13$	(−0.715)	−0.920	−0.821	−0.718	−0.727	−0.610	−0.480
$C14$	(−0.908)	−0.992	−0.960	−0.914	−0.920	−0.866	−0.765
$C23$	(0.992)	0.940	0.971	0.988	0.993	1.000	1.000
$C24$	(0.826)	0.763	0.809	0.841	0.840	0.877	0.944
$C34$	(0.767)	0.556	0.679	0.771	0.778	0.860	0.943

data set.

Table II displays a number of points important to understanding the deficiency of Michaelis–Menten-type models for pharmacokinetic analysis. The distributions of the V and K estimates are skewed strongly to the right with some very large estimates. This skewness inflates the means so that they are ~25–45 times larger than the parameter values used to generate the data. The medians also are shifted to the right, particularly the median of the K estimates.

It may be thought that when extreme values are estimated for V or K, the plots of the fitted curves indicate that something is wrong. However, Fig. 4 shows that very extreme values (V = 1561 and K = 5165) generate curves that are not much different from those generated when V = 2 and K = 5. Certainly, they could not be distinguished if each were represented by 11 observations containing 5% or more noise.

Table II also shows the high correlation between estimates of V and K; for these 200 estimates, the correlation (Spearman) is 0.992. The estimate of the correlation computed by NONLIN for each data set indicates this finding; the mean estimated correlation is 0.988, the median is 0.993, and the minimum of the 200 is 0.940. The eigenvalues of the estimated correlation matrix sum to the number of parameters estimated (in this case, four) and the size of the largest eigenvalue ($E1$) indicate the dimensionality of the parameter estimation space. The average of the largest eigenvalue is ~3.5, indicating that some of the remaining three eigenvalues are close to zero. Thus, the dimensionality of the parameter space is less than four, a condition of singularity. Figure 5 is a plot of the estimates of V and K. The top 10% was censored so that the other estimates can be shown in a reasonable scale. The estimates all lie close to a straight line.

Because the distributions of the V and K estimates are extremely skewed, the standard deviations are quite large [$s(V)$ = 201.6 and $s(K)$ = 803.1]. However, the 90th percentile indicates that, of the standard deviation estimates made with each data set, 90% of the $s(V)$ estimates are <114 and 90% of the $s(K)$ estimates are <557.

The main points made by Table II are:

1. Estimates of V and K are highly skewed to the right, with many of the estimates being many times larger than the true parameter values.
2. Estimates of V and K are very highly correlated so that the estimate of one almost determines the estimate of the other.
3. Estimates of V and K have very high variances, although the estimated variances as computed by NONLIN from individual data sets underestimate the variability.

Although not shown in Table II, the correlations of estimates of V and K with their respective standard deviation estimates also are high. For these 200 data sets, the correlation between estimates of V and $s(V)$ is 0.977; the correlation between estimates of K and $s(K)$ is 0.980.

Thus, the estimated variances of the V and K estimates, as computed by a nonlinear regression program, do not give a true picture of the uncertainty in the estimates.

As a related point, Table II shows that the linear parameters, K_A and D, are estimated well in that the distributions of their estimates are symmetric with small bias and small standard deviations. The correlation of their estimates, while smaller than that of the V and K estimates, is still large (−0.762 for this set of 200 estimates).

The tables of the simulations of the other cases show similar results. Proportional error with a standard deviation of 5% gives much the same result as a constant error with a standard deviation of 0.05. For Cases 3 and 4 (Table I), it was assumed that D = 10 was known; only three parameters were estimated. This procedure improved the estimation, but the distributions still were skewed to the right for Case 3, with means of 2.58 and 8.04 for V and K, respectively, and 90th percentiles of 3.80 and 14.02. The correlation of the V and K estimates was 0.993.

Estimation was best for the simple model (Cases 1 and 2 of Table I). However, even for these cases, the distributions were skewed to the right with some very extreme values, and the correlations were high (0.934 for Case 1 and 0.974 for Case 2).

For all of these simulations, the initial estimates for the iterative nonlinear regression program were the true values of the parameters used to generate the data. A limited amount of simulation with other starting values indicated that, for a particular data set, the initial values affected the final estimates but, averaged over 200 simulations, initial estimates within 30% of the true values did not make much difference. Initial estimates that differ by more than 30% often converge to values far removed from the true parameters.

A Michaelis–Menten-type model may be well approximated by a linear model for a single drug exposure, but observations at more than one dose level will expose the nonlinearity of the system. Thus, an attempt to evaluate the effect of simultaneous fitting of observations at three dose levels was made (Case 7, Table I). For Case 7, it was assumed that V and

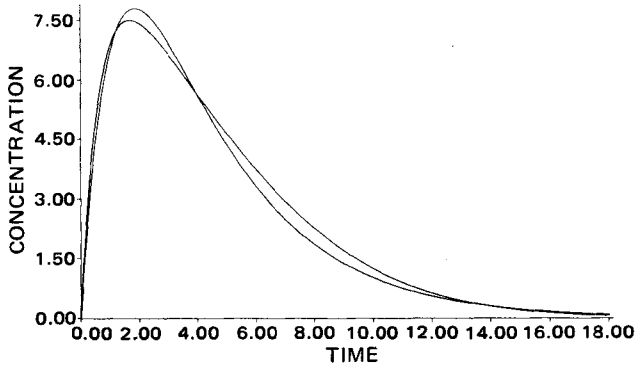

Figure 4—*Curves of the Michaelis–Menten elimination model for the* (V, K) *values (1561, 5165) and (2, 5).*

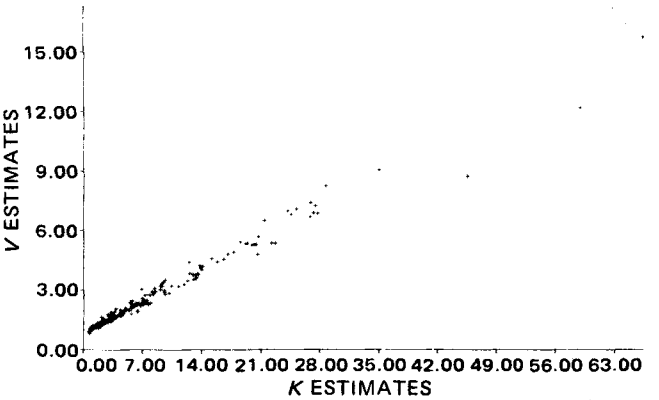

Figure 5—*Distribution of* K *and* V *estimates,* N = *180.*

Table III—Summary of Parameter Estimates for Simultaneous Fitting, Case 7

Parameter	Parameter or Sample Value	Sample Statistics					
		Minimum	P_{10}	Mean	Median	P_{90}	Maximum
V	2.00	1.68	1.81	2.00	2.00	2.18	2.39
K	5.00	3.47	4.01	4.99	4.96	5.90	6.62
$s(V)$	(0.138)	0.076	0.098	0.135	0.130	0.183	0.217
$s(K)$	(0.712)	0.627	0.822	1.141	1.120	1.540	1.966

K remained the same when D = 10, 20, and 40. Data were simulated for three experiments (D = 10, 20, and 40); these three observed curves, with a total of n = 33 observations, were fit simultaneously to one set of K_A, V, and K values and three D values. This procedure gave much better parameter estimation. The distributions of the V and K estimates were symmetric about the true values with no extreme estimates. The results for V and K are shown in Table III. The correlation of the V and K estimates was reduced to 0.767.

When comparing Table III with Table II, it must be noted that the data sets of Table III had 33 observations while those of Table II had only 11. The improvement in estimation of V and K was not due only to the larger sample sizes. To confirm this fact, Cases 8 and 9 (Table I) also had 33 observations per data set but, in these cases, the observations consisted of three replications at each sampling time used in Cases 3–6. Although the variability was reduced markedly and there were no extreme estimates, the distributions were still skewed to the right and the correlations were still very high (0.991 for Case 8 and 0.983 for Case 9).

DISCUSSION

The mathematical development and computer simulation indicate that it is not possible to estimate V and K of a Michaelis–Menten-type pharmacokinetic model with any precision from a single-dose experiment. Furthermore, the asymptotic theory standard deviations, as computed by a nonlinear regression program, are misleading in that they underestimate the uncertainty in the estimates. [Although the NONLIN program (9) was used in this study, this result would be true for any computer program that estimates the variance–covariance matrix of the estimates from the matrix of partial derivatives.] This observation may explain why in many studies the estimate of between-subject variability is so much larger than the estimate of within-subject variability of the estimates (10). It may be an artifact of estimation rather than a characteristic of the drug. These difficulties in estimating Michaelis–Menten parameters also make the comparison of algorithms for estimation less meaningful, as in Ref. 11.

In many situations, estimating V and K for the purpose of comparing values between studies (*i.e.*, in different disease states) is useless if the estimates are made from observations after only one dose of drug. However, the simulations indicate that if estimations can be made with observations obtained after two or more dose levels, then the estimations are much improved. To do this, it must be assumed that the biological system, like the computer system, can generate data with the same values of K_A, V, and K at different doses and different times. It is not certain that this assumption is valid.

Although the Michaelis–Menten-type models are not useful for estimation of V and K with observations from only one dose, they may have other value. They may be useful to show that a system is better described by a nonlinear pharmacokinetic model than by a linear model, for describing data, and for prediction.

The size of these simulations (N = 200 for most of them), while much larger than most computer simulations reported in the pharmaceutical sciences literature, is about minimum for validity. These simulations cover only a few points in the entire space of these models. Future research extending these simulations would be useful in evaluating these models. In particular, the errors assigned in these simulations were relatively small for pharmacokinetic data. Larger (and more realistic) errors could only make estimation more difficult. Development of other nonlinear models that avoid these computational problems also would be useful.

REFERENCES

(1) C. Marmasse, "Enzyme Kinetics," Gordon and Breach, New York, N.Y., 1977.

(2) K. J. Laidler and P. S. Bunting, "The Chemical Kinetics of Enzyme Action," 2nd ed., Clarendon, Oxford, England, 1973.

(3) J. G. Wagner, *J. Pharmacokinet. Biopharm.*, **1**, 103 (1973).

(4) D. D. M. Tong and C. M. Metzler, *Math. Biosci.*, **48**, 293 (1980).

(5) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975.

(6) G. E. P. Box and W. G. Hunter, *Technometrics*, **4**, 301 (1962).

(7) J. M. Chambers and J. E. Ertel, in "1975 Proceedings of the ASA Statistical Computing Section," American Statistical Association, Washington, D.C., 1976, p. 151.

(8) Y. Bard, "Nonlinear Parameter Estimation," Academic, New York, N.Y., 1974, chap. 7.

(9) C. M. Metzler, G. L. Elfring, and A. J. McEwen, *Biometrics*, **30**, 562 (1974).

(10) E. Martin, T. N. Tozer, L. B. Sheiner, and S. Riegelman, *J. Pharmacokinet. Biopharm.*, **5**, 579 (1977).

(11) K. C. Yeh and K. C. Kwan, *J. Pharm. Sci.*, **68**, 1120 (1979).

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