Table II—Relationship of Saliva, Plasma, and Plasma Water Concentrations of Acetazolamide in Five Subjects after Oral Administration of a 250-mg Dose

Subject	Saliva Concentration / Plasma Concentration × 100			Plasma Water Concentration / Plasma Concentration × 100	
	Mean	(± *SEM*)	Linear Regression Slope	Mean	(± *SEM*)
PJ	1.11	(±0.07)	1.12	3.02	(±0.32)
CA	0.70	(±0.10)	0.51	4.22	(±0.41)
SG	0.85	(±0.09)	0.77	10.74	(±0.73)
AS	0.94	(±0.14)	0.93	5.17	(±0.52)
TS	0.84	(±0.06)	0.92	5.35	(±0.40)
Pooled data	0.90	(±0.04)	0.88	5.66	(±0.58)

24 to 31 hr after administration of a single oral dose, the ratio of red blood cells to plasma levels was greater than 4:1. Therefore, to ensure accurate determination of plasma levels, plasma should be separated from blood immediately, before hemolysis occurs. An appreciable degree of hemolysis may lead to overestimation of plasma concentrations.

The significance of the slowly declining red blood cell levels during chronic therapy with the drug remains to be determined.

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Pharmacokinetic Analysis of Drug Concentration Data Obtained during Repetitive Drug Administration

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Abstract □ A digital computer curve-fitting method, designed to estimate pharmacokinetic model constants by utilizing all drug concentration–time data collected during repetitive dosing studies, was applied to data manifesting systematic dose-to-dose variability in one or another of the pharmacokinetic parameters. The method accurately determined dose-to-dose changes in absorption or elimination rate constants or in the apparent volume of distribution, and it would be useful for detecting phenomena such as self-induction and self-inhibition that may occur during multiple-dose administration. The method can also be used to analyze multiple-dose data of drugs exhibiting capacity-limited elimination and to obtain estimates of the Michaelis–Menten parameters.

Keyphrases □ Pharmacokinetics—model constants estimated by computer curve-fitting method, drug concentration data obtained during repetitive drug administration □ Computer curve-fitting method—pharmacokinetic model constants estimated, drug concentration data obtained during repetitive drug administration □ Repetitive drug administration—drug concentration data obtained, model constants estimated by computer curve-fitting method

A previous report (1) described the development of a digital computer method which utilized all plasma or blood concentration data collected during repetitive dosing studies to estimate pharmacokinetic model constants and, simultaneously, to fit the entire time course of drug concentration in plasma or blood. The method readily accommodates changes in dose or dosing interval during a multiple-dose regimen.

Several investigations found that pharmacokinetic parameters of certain drugs change in a systematic way

Table I—Average Parameter Estimates for 10 Sets of Errant Multiple-Dose Data that Can Be Described by First-Order Absorption and Simultaneous Michaelis–Menten and First-Order Elimination

	K_m[a], mg/liter	V_m[b], mg/hr	Vc[c], liters	Cl[d], liters/hr	k_a[e], hr^{-1}
Parameter value	0.961	0.388	101	0.405×10^{-3}	1.02
Computer estimate	1.08	0.407	103	0.413×10^{-3}	1.06
SD	0.281	0.053	6.0	0.009×10^{-3}	0.109
CV, %	26.0	13.0	5.8	2.1	10.3
Bias, %	12.4	4.9	2.0	2.0	3.9

[a]Michaelis constant. [b]Maximum elimination rate. [c]Apparent volume of distribution. [d]First-order clearance. [e]First-order absorption rate constant.

during repetitive drug administration (2–8). Self-induction, self-inhibition, and product inhibition of metabolism are possible causes of variation in elimination rate constants. Variability in absorption rate constants and/or the amount absorbed as well as changes in the apparent volume of distribution from dose to dose may also occur during a repetitive dose regimen.

Changes in elimination rate constants are usually detected by comparing the half-life of a drug after a single dose with that determined during the washout period following the last dose of a multiple-dose regimen. The interpretation of such results may be obscure if an inappropriate pharmacokinetic model is assigned to the data (9). In situations where self-induced changes in metabolism appear to be operative, little is known about the time course of these changes. No studies have been directed to detecting systematic variations in bioavailability or in the apparent volume of distribution from dose to dose during repetitive administration.

Under certain conditions, a drug subject to capacity-limited metabolism will demonstrate pharmacokinetic characteristics similar to those of a drug subject to self-inhibition or product inhibition; *i.e.*, the half-life will appear to increase during multiple dosing. The characterization of Michaelis–Menten kinetics is usually attempted by analyzing data after administration of a large single dose, administration of different single doses, or administration of the last dose of a repetitive dose regimen. Although the most dramatic consequences of capacity-limited elimination are the unusual rate and extent of drug accumulation, little effort has been directed to the characterization of such kinetics by analyzing data obtained during repetitive drug administration.

Table II—Parameter Estimates for 10 Sets of Errant Data that Can Be Described by First-Order Absorption and Elimination (the Absorption Rate Constant Fluctuates during Repetitive Dosing)

Parameter	Parameter Value	Computer Estimate	*SD*	*CV*, %	Bias, %
$k_{a(1)}$[a]	1.98	1.95	0.15	7.7	−1.5
$k_{a(2)}$	2.92	2.84	0.65	22.9	−2.7
$k_{a(3)}$	1.98	1.96	0.31	15.8	−1.0
$k_{a(4)}$	2.92	2.99	0.79	26.4	+2.4
$k_{a(5)}$	1.98	1.93	0.26	13.5	−2.5
$k_{a(6)}$	2.92	2.78	0.50	18.0	−4.8
$k_{a(7)}$	1.98	2.07	0.21	10.1	+4.5
$k_{a(8)}$	2.92	3.01	0.78	25.9	+3.1
Kb[b]	0.346	0.351	0.005	1.4	+1.4
Vc[c]	99.7	98.7	1.78	1.8	−1.0

[a]The term $k_{a(n)}$ denotes the first-order absorption rate constant after the nth dose (hours^{-1}). [b]First-order elimination rate constant (hours^{-1}). [c]Apparent volume of distribution (liters).

Table III—Parameter Estimates for 10 Sets of Errant Data that Can Be Described by First-Order Absorption and Elimination (the Absorption Rate Constant Decreases during Repetitive Dosing)

Parameter	Parameter Value	Computer Estimate	*SD*	*CV*, %	Bias, %
$k_{a(1)}$[a]	3.14	3.29	0.658	20.0	+4.8
$k_{a(2)}$	2.71	2.91	0.783	26.9	+7.4
$k_{a(3)}$	2.10	2.13	0.372	17.5	+1.4
$k_{a(4)}$	1.53	1.52	0.136	8.9	−0.7
$k_{a(5)}$	1.02	1.00	0.069	6.9	−2.0
$k_{a(6)}$	0.509	0.500	0.020	4.0	−1.8
$k_{a(7)}$	0.507	0.519	0.029	5.6	+2.4
$k_{a(8)}$	0.505	0.495	0.029	5.9	−2.0
Kb	0.344	0.346	0.010	2.9	+0.6
Vc	100.7	100.2	3.1	3.1	−0.5

[a]The term $k_{a(n)}$ denotes the first-order absorption rate constant after the nth dose (hours^{-1}). [b]First-order elimination rate constant (hours^{-1}). [c]Apparent volume of distribution (liters).

The present report concerns the use of the previously described digital computer method (1) for detecting changes in pharmacokinetic model constants and for characterizing Michaelis–Menten kinetics during repetitive drug administration.

COMPUTER METHOD

The development of the computer method was detailed previously (1). The main element of the method is NONLIN (10), a nonlinear least-squares regression computer program specifically developed for pharmacokinetic applications, used in conjunction with DASCRU[1] (11), a subprogram that provides numerical solutions to differential equations.

To detect changes in pharmacokinetic constants during repetitive dosing, it is necessary to estimate independently model parameters for drug concentration data after each dose. While this approach is possible in principle, it requires considerably more data than are obtained in the usual metabolic or clinical study. Hence, it was decided that only one parameter would be estimated independently for each dose while all other parameters would be estimated by assuming that they were invariant from dose to dose. This procedure can be repeated for each parameter, and it becomes possible to detect systematic variability in absorption rate constants, amounts absorbed or volumes of distribution, and elimination rate constants.

To estimate a parameter for individual doses independently, the DFUNC section of NONLIN requires that each dose uses a separate in-

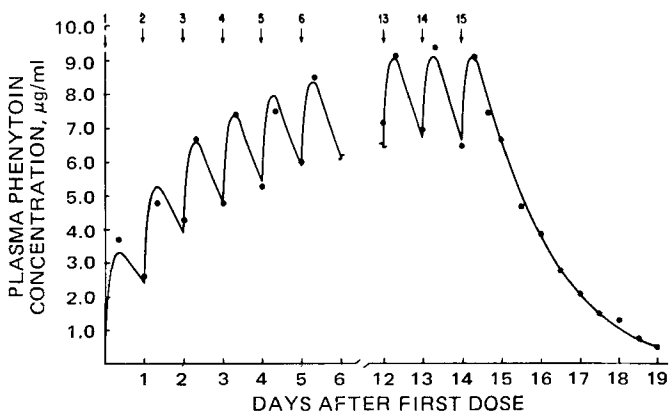

Figure 1—*Curve fit to plasma phenytoin concentrations during repetitive oral dosing, 300 mg/day for 14 consecutive days. Each point is the mean of determinations in 12 subjects. Data were fit using Program I.*

[1] Available from IMSL, 6200 Hillcroft, Houston, TX 77036.

Table IV—Parameter Estimates for 10 Sets of Errant Data that Can Be Described by First-Order Absorption and Elimination (the Elimination Rate Constant Increases during Repetitive Dosing)

Parameter	Parameter Value	Computer Estimate	SD	CV, %	Bias, %
k_a[a]	1.00	1.03	0.129	12.5	+3.0
V[b]	100.0	101.4	7.5	7.4	+1.4
$K_{(1)}$[c]	0.043	0.042	0.018	42.9	−2.3
$K_{(2)}$	0.087	0.086	0.009	10.5	−1.1
$K_{(3)}$	0.130	0.129	0.007	5.4	−0.8
$K_{(4)}$	0.173	0.172	0.013	7.6	−0.6
$K_{(5)}$	0.260	0.260	0.016	6.2	0.0
$K_{(6)}$	0.346	0.338	0.021	6.2	−2.3
$K_{(7)}$	0.346	0.348	0.026	7.5	+0.6
$K_{(8)}$	0.346	0.346	0.026	7.5	0.0

[a] First-order absorption rate constant (hours^{-1}). [b] Apparent volume of distribution (liters). [c] The term $K_{(n)}$ denotes the first-order elimination rate constant after the nth dose (hours^{-1}).

Table V—Parameter Estimates for 10 Sets of Errant Data that Can Be Described by First-Order Absorption and Elimination (the Elimination Rate Constant Decreases during Repetitive Dosing)

Parameter	Parameter Value	Computer Estimate	SD	CV, %	Bias, %
k_a[a]	1.00	1.02	0.149	14.6	+2.0
V[b]	100.0	100.9	8.8	8.7	+0.9
$K_{(1)}$[c]	0.779	0.777	0.063	8.1	−0.3
$K_{(2)}$	0.693	0.700	0.065	9.3	+1.0
$K_{(3)}$	0.606	0.610	0.038	6.2	+0.7
$K_{(4)}$	0.519	0.528	0.043	8.1	+1.7
$K_{(5)}$	0.433	0.446	0.039	8.7	+3.0
$K_{(6)}$	0.346	0.347	0.027	7.8	+0.3
$K_{(7)}$	0.346	0.350	0.029	8.3	+1.2
$K_{(8)}$	0.346	0.349	0.026	7.4	+0.9

[a] First-order absorption rate constant (hours^{-1}). [b] Apparent volume of distribution (liters). [c] The term $K_{(n)}$ denotes the first-order elimination rate constant after the nth dose (hours^{-1}).

tegration routine. Therefore, an F subroutine was included for each dose. The integration subprogram DASCRU was called using the F subroutine corresponding to the number of the dose.

Specific programs[2] were developed to fit data described by the following one-compartment pharmacokinetic models: I, constant first-order absorption and constant simultaneous Michaelis–Menten and first-order elimination; II, variable first-order absorption and constant first-order elimination; III, constant first-order absorption and variable first-order elimination; and IV, constant first-order absorption and elimination but variable apparent volume of distribution.

APPLICATIONS

Ideal drug concentration–time data after repetitive dosing were generated by means of appropriate equations satisfying the conditions of each program. Each data set consisted of five values during the first dosing interval, four values during the last interval, and two values for each of the six interceding intervals. A normally distributed random absolute error of 10% was introduced into each data set as previously described (1). Ten errant data sets were generated for each ideal data set. The errant data and the appropriate differential equations served as input for Programs I–IV.

Table I shows the average computer-estimated pharmacokinetic parameters for data described by first-order absorption and simultaneous Michaelis–Menten and first-order elimination. Good agreement between the pharmacokinetic parameters used to generate the ideal data and the means of the parameter estimates that best describe the errant data was obtained.

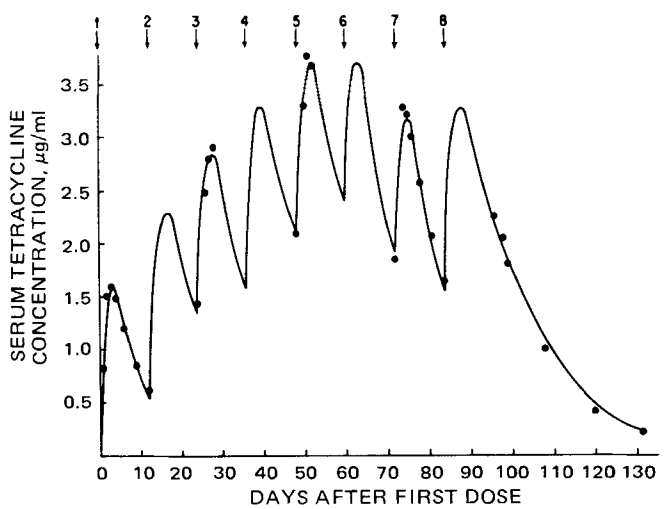

Figure 2—*Curve fit to mean serum tetracycline concentrations during repetitive oral dosing of 500 mg twice a day for eight consecutive doses to 12 healthy subjects. Data were fit using Program III.*

[2] Information concerning these programs may be obtained by writing the authors.

Programs II, III, and IV, which were intended to characterize dose-to-dose variation in the absorption rate constant (Tables II and III), in the elimination rate constant (Tables IV and V), and in the amount absorbed or the apparent volume of distribution (Table VI), respectively, also provided highly acceptable results.

The computer programs were evaluated further by determining their applicability to previously published experimental data. In a multiple-dose trial, subjects were given 300-mg oral doses of phenytoin once daily for 14 days (12). Blood samples were obtained during the first 6 days and the last 3 days of dosing as well as for 3 days following the last dose. These data were evaluated using Program I, which assumes first-order absorption and simultaneous Michaelis–Menten and first-order elimination. The computer fit to the average drug concentration data is shown in Fig. 1. The correlation coefficient was 0.996. The parameter estimates are listed in Table VII.

Several investigations indicated that the elimination of phenytoin in humans is capacity limited and can be described by Michaelis–Menten kinetics or simultaneous first-order and Michaelis–Menten kinetics (13–15). The values of the pharmacokinetic parameters determined in the present study differ considerably from previous estimates. For example, the K_m value of 20.7 mg/liter is three to five times larger than those values previously reported from single-dose data. The V_m value is about 100% larger than previously reported average values but comparable to values observed in certain individuals (14). The ratio of V_m/K_m is comparable to that reported by Gerber and Wagner (13) but much smaller than the ratios found by others (14, 15). In view of the narrow therapeutically effective range of plasma concentrations found with phenytoin, additional studies are needed to determine which set of parameter estimates is more realistic in predicting the relation between the dose and steady-state concentration of the drug.

Doluisio and Dittert (8) found that the average apparent half-life of

Table VI—Parameter Estimates for 10 Sets of Errant Data that Can Be Described by First-Order Absorption and Elimination (the Apparent Volume of Distribution Increases during Repetitive Dosing)

Parameter	Parameter Value	Computer Estimate	SD	CV, %	Bias, %
k_a[a]	0.782	0.778	0.019	2.4	−0.5
K[b]	0.360	0.362	0.004	1.1	+0.6
$V_{(1)}$[c]	68.0	68.0	1.29	1.9	0.0
$V_{(2)}$	73.4	73.1	2.27	3.1	−0.4
$V_{(3)}$	82.7	82.8	3.16	3.8	+0.1
$V_{(4)}$	94.9	94.2	3.88	4.1	−0.7
$V_{(5)}$	108	108	4.31	4.0	0.0
$V_{(6)}$	123	122	4.06	3.3	−0.8
$V_{(7)}$	143	141	3.16	2.2	−1.4
$V_{(8)}$	165	167	3.77	2.3	+1.2

[a] First-order absorption rate constant (hours^{-1}). [b] First-order elimination rate constant (hours^{-1}). [c] The term $V_{(n)}$ denotes the apparent volume of distribution after the nth dose (liters).

Table VII—Pharmacokinetic Model Parameters Derived from Fitting Multiple-Dose Phenytoin Data Assuming First-Order Absorption and Simultaneous Michaelis–Menten and First-Order Elimination

	K_m[a], mg/liter	V_m[b], mg/hr	V[c], liters	Cl[d], liters/hr	k_a[e], hr^{-1}
Computer estimate	20.7	0.605	72.7	7×10^{-4}	0.320
SD of estimate	5.6	0.119	2.5	4×10^{-4}	0.072

[a] Michaelis constant. [b] Maximum elimination rate. [c] Apparent volume of distribution. [d] First-order clearance. [e] First-order absorption rate constant.

tetracycline in 10 subjects increased from 6.3 hr (after the first dose) to 10 hr (after the eighth dose) during 4 days of repetitive oral dosing (every 12 hr). Although at least one blood sample was taken during each dosing interval, pharmacokinetic analysis was limited to the data obtained from the first and last doses.

The average serum concentration–time data from this study were fit using Program III, which accommodates dose-to-dose variation in the first-order elimination rate constant. The computer fit of the data is shown in Fig. 2. The correlation coefficient was 0.997. The parameter estimates are listed in Table VIII.

A substantial change in the elimination rate constant is evident when the first dose is compared with each subsequent dose. The elimination rate constants for Doses 2–8 tend to fluctuate about a mean value of 0.078 hr^{-1} ($t_{1/2} \simeq 9$ hr), which is much smaller than the value estimated after the first dose.

Table VIII—Pharmacokinetic Model Parameters Derived from Fitting Multiple-Dose Tetracycline Data Assuming Constant First-Order Absorption and Variable First-Order Elimination

Parameter	Computer Estimate	*SD* of Estimate
k_a[a]	0.563	0.046
V/F[b]	191.1	4.6
$K_{(1)}$[c]	0.164	0.010
$K_{(2)}$	0.083	0.007
$K_{(3)}$	0.086	0.014
$K_{(4)}$	0.066	0.007
$K_{(5)}$	0.061	0.010
$K_{(6)}$	0.089	0.006
$K_{(7)}$	0.099	0.005
$K_{(8)}$	0.061	0.003

[a] First-order absorption rate constant ($hours^{-1}$). [b] Ratio of the apparent volume of distribution, V, to the fraction, F, of the dose absorbed (liters). [c] The term $K_{(n)}$ denotes the first-order elimination rate constant after the nth dose ($hours^{-1}$).

The reason for this apparent change in half-life remains obscure. Whether it is due to a real change in the pharmacokinetics of the drug upon repetitive dosing or to a mathematical artifact that arises from selecting the wrong pharmacokinetic model (9) is not known. The present analysis suggests that if an inhibitory mechanism is operative, it is fully induced after the first dose.

The described analyses of hypothetical and experimental multiple-dose data further support the utility and flexibility of the recently reported digital computer-fitting method (1).

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Metal-Ion Interaction with Penicillins: Kinetics of Complexation of Nickel(II)

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Abstract □ The kinetics of complexation of nickel(II) with some penicillins and related compounds show that the zwitterionic form of the ligand has very low reactivity compared to the anionic form. The resolved rate constants are interpreted in terms of binding to the ring nitrogen and carboxyl group and not to the side chain.

Keyphrases □ Penicillin G—and related compounds, complexation with nickel(II) ions, reaction kinetics □ Nickel(II)—complexation with penicillin G and related compounds, reaction kinetics □ Complex formation—penicillin G and related compounds with nickel(II) ions, reaction kinetics □ Kinetics, reaction—complexation of nickel(II) with penicillin G and related compounds □ Metal ions—nickel(II), complexation with penicillin G and related compounds, reaction kinetics □ Antibacterials—penicillin G and related compounds, complexation with nickel(II) ions, reaction kinetics

Penicillin antibiotics interact with metal ions in a complex manner. Copper(II) has a very pronounced effect on the hydrolysis rate of the β-lactam ring (1, 2), which is not shown by other first-row transition metal ions (3, 4).