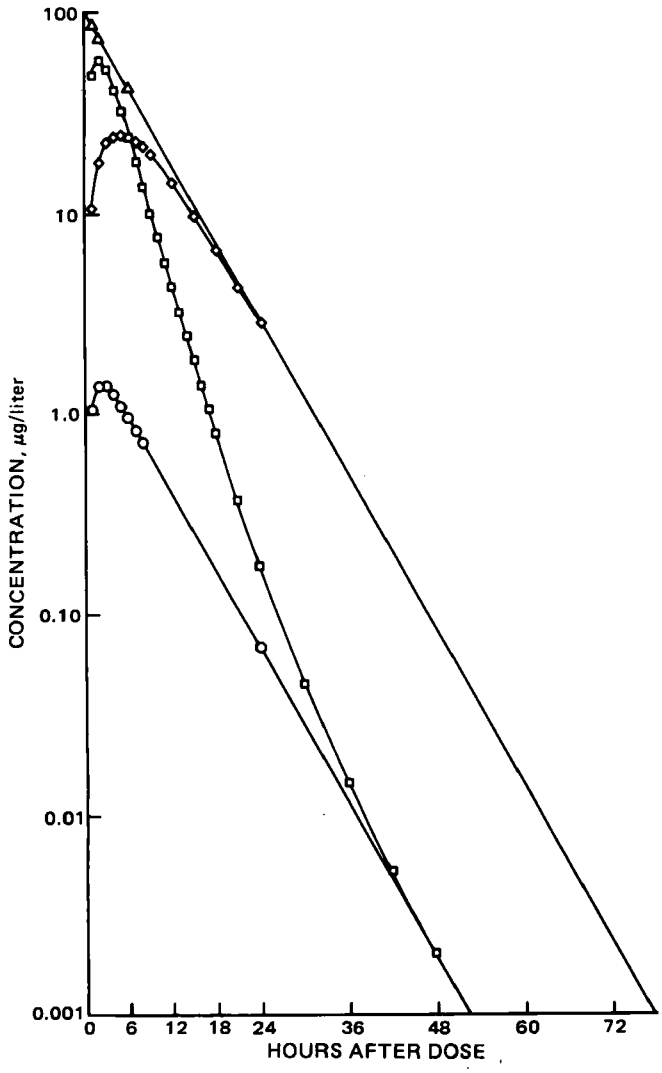


Figure 1—*Blood concentrations of drug and metabolite following oral (○ and □) and intravenous (△ and ◇) doses, respectively, simulated according to Case IVB.*

pass metabolism on various pharmacokinetic parameters has been delineated using simulation techniques.

APPENDIX

The following differential equations were used to simulate the plasma concentration–time data presented in this report.

$$\frac{dC_P^S}{dt} = Q_H \cdot (C_P^H - C_P^S)/V_P^S \qquad \text{(Eq. A-1)}$$

$$\frac{dC_M^S}{dt} = [Q_H \cdot C_M^H - (Q_H + CL_M^S) \cdot C_M^S]/V_M^S \qquad \text{(Eq. A-2)}$$

$$\frac{dC_P^H}{dt} = [k_a De^{-k_{at}} + Q_H \cdot C_P^S - (Q_H + CL_P^H) \cdot C_P^H]/V_P^H \qquad \text{(Eq. A-3)}$$

$$\frac{dC_M^H}{dt} = [Q_H \cdot C_M^S + CL_P^H C_P^H - Q_H C_M^H]/V_M^H \qquad \text{(Eq. A-4)}$$

Where D is the oral dose and the remaining terms have been identified in the text and legend to Scheme I. The $k_a De^{-k_{at}}$ term is the source of drug input for the oral dose. This source is not used for the IV dose, whereas the initial condition for C_P^S is set equal to D/V_P^S for the IV dose.

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

(2) W. A. Colburn, *J. Pharm. Sci.*, **70**, 969 (1981).

Wayne A. Colburn
Department of Pharmacokinetics and Biopharmaceutics
Hoffmann-La Roche Inc.
Nutley, NJ 07110

Received March 10, 1982.
Accepted for Publication January 5, 1983.

Factors Affecting the Accuracy of Estimated Mean Absorption Times and Mean Dissolution Times

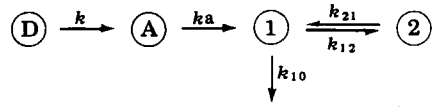
Keyphrases □ Statistical moment analysis—estimation of absorption and dissolution rates, effects of the sampling schedule, importance of the estimate of the terminal elimination rate constant (β)

To the Editor:

Recent discussions in the literature concerning the application of the concept of statistical moments to pharmacokinetic analysis has stimulated interest in the method and its potential utility in the evaluation of pharmacokinetic and bioavailability data. The most appealing aspect of statistical moment analysis is the potential for model-independent estimates of *in vivo* dissolution and absorption rates. A thorough discussion of this method and its potential applications was presented by Riegelman and Collier (1) and Yamaoka *et al* (2).

Using simulation techniques, the present study evaluates the ability of statistical moment analysis to provide accurate estimates of absorption and dissolution rates and the effects of sampling schedule, random error, and the estimate of the terminal elimination rate constant (β) on the accuracy of these estimates.

Simulations of drug concentration–time data corresponding to administration of an intravenous bolus, oral solution, and tablet dosage forms were generated by the CSSL-IV simulation program (3) based on the pharmacokinetic models presented in Scheme I. Unless otherwise specified, the parameter values presented in Table



Scheme I—Two-compartment pharmacokinetic model with sequential first-order dissolution and absorption, where k = *first-order dissolution rate constant and* k_a = *first-order absorption rate constant. To simulate intravenous data, the dose was entered into compartment 1; for oral solution data, the dose was entered into the absorption compartment (A); and for solid oral dosage form data, the dose was entered into the dissolution compartment (D).*

0022-3549/83/0600-0713$01.00/0

Table I—Parameter Values Used in the Simulations

Simulation	Dose, mg	V_1, liters	k, hr^{-1}	k_a, hr^{-1}	k_{10}, hr^{-1}	k_{21}, hr^{-1}	k_{12}, hr^{-1}
Intravenous	100	1.0	—	—	0.20	0.60	0.50
Liquid	100	1.0	—	3.0	0.20	0.60	0.50
Tablet	100	1.0	0.78	3.0	0.20	0.60	0.50

Table II—Sampling Schedules Used for Calculation of Parameters

Schedule	Sampling Times, hr
A	Every 0.05 hr for 36 hr
B	0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, 24, 36
C	0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24
D	0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, 96

I were used for all simulations. Four sampling schedules were evaluated (Table II) with schedule A providing parameter estimates corresponding to the theoretical values.

Areas under the concentration–time curves (AUC_0^{24}) and areas under the moment curve ($AUMC_0^{24}$) to 24 hr were calculated using the linear trapezoidal method. Extrapolations to infinity and calculations of mean residence time (MRT), mean absorption time (MAT), and mean dissolution time (MDT) were performed as described previously (1).

For those simulations in which random error was added to the concentration data, data corresponding to sampling schedule C were simulated and normally distributed random error with a coefficient of variation of ±10% was added to each concentration value. This was done for 18 data sets per group. Extrapolations of AUC_0^{24} and $AUMC_0^{24}$ to infinity were performed using the theoretical value of β (*i.e.*, 0.1 hr^{-1}) and the β value generated by linear regression of the transformed data from 8 to 24 hr. Two MDT values were then calculated, one using AUC_0^∞ and $AUMC_0^\infty$ extrapolated with the theoretical β (Footnote *b*, Table III) and one using AUC_0^∞ and $AUMC_0^\infty$ extrapolated with the β value corresponding to the transformed data (Footnote *c*, Table III).

The effect of sampling schedule on the accuracy of the calculated estimates of MAT and MDT is presented in Table IV. As expected, the values of MAT and MDT calculated from sampling schedule A correspond to the theoretical values; *i.e.*, the reciprocal values of MAT and MDT are equivalent to the values of k_a and k used to generate the theoretical data. Comparing the results from sampling schedules B and C with those from schedule A gives an indication of the accuracy of the estimates of MAT and MDT when these reduced sampling schedules are utilized.

In general, sampling schedule B provided reasonably good estimates of the theoretical values of MAT and MDT (Table IV). In many cases, however, the parameters calculated with schedule C (typical for many protocols) were relatively poor estimates of the theoretical values. The accuracy of MAT and MDT tended to decrease as the rate constants k_a and k increased and as the two-compartment characteristics of the concentration–time curve became more pronounced (as k_{21} varied from 0.6 to 0.1 hr^{-1}). Increased sampling during the absorptive phase (schedule B) improved the accuracy of MAT and MDT (compared with schedule C), and the improvement was most pronounced when a k_{21} value of 0.1 hr^{-1} was utilized and when k and k_a were large. It is obvious from these data that the sampling schedule is critical for obtaining accurate and meaningful estimates of mean absorption times and mean dissolution times.

The importance of an accurate estimate of β when calculating various statistical moment parameters has been discussed previously (1, 2) and is demonstrated in our results. Using sampling schedule C and a k_{21} value of 0.2 hr^{-1}, 27% of AUC_0^∞ and 69% of $AUMC_0^\infty$ are due to extrapolation from the last data point to infinity using β. A ±10% error in β yields MDT estimates of −0.15 and 2.60 hr, respectively, compared with 1.07 hr when the correct β value is used. Utilizing sampling schedule D, only 1% of AUC_0^∞ and 6% of $AUMC_0^\infty$ are due to extrapolation, and the same ±10% error in β results in MDT estimates of 0.91 and 1.14 hr, respectively, compared with 1.01 hr when the

Table III—Mean Parameter Values Determined from Data Generated with Normally Distributed Random Error (CV ± 10%)

k, hr^{-1}	$1/k$,[a] hr	MDT[b], hr			β, hr^{-1}		MDT[c], hr		
		Mean	*SD*	*CV*	Mean	*SD*	Mean	*SD*	*CV*
0.60	1.67	1.56	0.39	25%	0.100	0.010	1.53	0.72	47%
0.72	1.39	1.27	0.42	32%	0.099	0.007	1.34	0.68	51%
0.78	1.28	1.26	0.33	26%	0.098	0.006	1.33	0.84	63%
0.90	1.11	1.05	0.24	23%	0.100	0.008	1.06	0.86	81%

[a] Theoretical value of MDT. [b] Calculated from AUC_0^∞ and $AUMC_0^\infty$ values in which extrapolation from 24 hr to infinity was performed using β = 0.1 hr^{-1}. [c] Calculated from AUC_0^∞ and $AUMC_0^\infty$ values in which extrapolation from 24 hr to infinity was performed using β determined by linear regression of the transformed data from 8 to 24 hr.

Table IV—Estimated Values of MAT and MDT from Data Generated for Sampling Schedules A, B, and C

Parameter Value, hr^{-1}	k_{21} = 0.1 hr^{-1}						k_{21} = 0.6 hr^{-1}					
	MAT, hr			MDT, hr			MAT, hr			MDT, hr		
	A	B	C	A	B	C	A	B	C	A	B	C
k = 0.4				2.50	2.45	2.11				2.50	2.46	2.26
0.7				1.43	1.36	1.05				1.43	1.39	1.25
1.0				1.00	0.91	0.62				1.00	0.96	0.84
k_a = 1	1.00	1.08	1.17	1.28	1.28	1.19	1.00	1.00	1.01	1.28	1.26	1.19
3	0.33	0.42	0.76	1.28	1.20	0.90	0.33	0.36	0.45	1.28	1.24	1.11
5	0.20	0.42	0.84	1.28	1.15	0.71	0.20	0.26	0.39	1.28	1.21	1.06
k_{10} = 0.1	0.33	0.40	0.69	1.28	1.23	1.00	0.33	0.29	0.42	1.28	1.30	1.24
0.3	0.33	0.41	0.77	1.28	1.18	0.90	0.33	0.36	0.49	1.28	1.25	1.14
0.5	0.33	0.44	0.81	1.28	1.15	0.88	0.33	0.37	0.54	1.28	1.19	1.14

correct value is used. Errors in the estimate of β are inherent due to biological and analytical variability, but their impact can be reduced drastically by expanding the sampling schedule to longer times. Since this is not always possible because of analytical and practical limitations, the use of statistical moments may not be appropriate if there is insufficient sampling to provide accurate and consistent estimates of β.

Routine analytical error in drug concentration data can affect estimates of MAT and MDT in two ways: first, by its effects on the calculation of $AUMC_0^t$ and AUC_0^t, which should be rather insignificant, and second, by its effects on the estimate of β, which in turn may affect the calculation of $AUMC_0^\infty$ and AUC_0^∞ significantly. The first case is illustrated in Table III by the values of MDT^b generated using the theoretical value of β (i.e. 0.1 hr^{-1}) for all extrapolations. The average ($n = 18$) estimated MDT values were good estimates of the theoretical values with coefficients of variation ranging from 23 to 32%. The effect of variations in β caused by random error and the subsequent impact on MDT^c is also shown in Table III. The average MDT^c values were again good estimates of the theoretical values but the coefficients of variation were quite large, ranging from 47 to 81%. With this sampling schedule ~8% of AUC_0^∞ and 31% of $AUMC_0^\infty$ were due to extrapolation, and this would not be uncommon for a typical bioavailability study. The large variability in these MDT values could affect the ability to statistically detect small differences in MDT values under such conditions.

Overall, the results of these studies suggest that the statistical moment approach to the analysis of bioavailability data may offer an attractive alternative to C_{max} and t_{max} or to the model-dependent methods for assessing the rate of drug absorption. However, accurate results and meaningful conclusions using this method are very much dependent on the experimental design of the studies from which the data are generated. To obtain optimal information, frequent sampling during the absorption phase is necessary. In addition, sufficient sampling during the terminal elimination phase is needed to minimize the impact of extrapolation error and to provide an accurate estimate of β. MAT and MDT values calculated from data generated *via* a less than rigorous experimental design may yield poor estimates of the actual values and can result in misleading conclusions. Therefore, an understanding of the influence of the various factors affecting the accuracy of the calculated MAT and MDT values, along with a well-defined pharmacokinetic profile of the drug, are essential to achieve an experimental design which will allow for the determination of meaningful estimates of mean absorption and dissolution times.

(1) S. Riegelman and P. Collier, *J. Pharmacokinet. Biopharm.*, **8**, 509 (1980).

(2) K. Yamaoka, T. Nakagana, and T. Uno, *ibid.*, **6**, 547 (1978).

(3) "CSSL IV Users Guide and Reference Manual," Nielsen Associates, 1976.

Romulus K. Brazzell ˣ
Stanley A. Kaplan
Department of Pharmacokinetics and Biopharmaceutics
Hoffmann-La Roche Inc.
Nutley, N.J. 07110

Received July 28, 1982
Accepted for Publication January 27, 1983

Plasma Inorganic Sulfate Concentrations in Pregnant Women

Keyphrases □ Sulfate concentration—effect of pregnancy in humans □ Plasma sulfate concentrations—effect of pregnancy in humans

To the Editor:

The physiological process of sulfation has important biosynthetic and detoxification functions. It is necessary in the developing fetus for the formation of glycosaminoglycans, a structural component of cartilage and other tissues, and cerebroside sulfate, a constituent of the brain (1, 2). The sulfated glycosaminoglycans may also be involved in cell differentiation (2).

The biotransformation of certain phenolic drugs to sulfate conjugates is limited by the availability of inorganic sulfate (3, 4); the formation of such drug conjugates can cause depletion of endogenous inorganic (free) sulfate in animals and humans (4–6). Decreased sulfate availability in pregnant rats, due to administration of salicylamide, which produces sulfate depletion concomitant with salicylamide sulfate formation (3, 5), has been associated with decreased availability and decreased tissue incorporation of sulfate in the fetuses and may be the cause of teratogenic effects (7, 8). Depletion of endogenous sulfate can decrease the rate of sulfation of phenolic drugs, such as acetaminophen, and thereby decrease their clearance (4), while hypersulfatemia, such as occurs in renal dysfunction, can facilitate drug sulfate conjugate formation and thereby increase drug clearance (9). The maternal level of endogenous inorganic sulfate may, therefore, affect fetal development and fetal exposure to certain drugs and other xenobiotics.

The plasma or serum concentrations of most electrolytes tend to decrease slightly during pregnancy (10). We have found only one report concerning the effect of pregnancy on serum sulfate concentrations: Tallgren observed concentrations of 0.592 ± 0.275 mmole/liter (mean ± *SD*) in 118 Scandinavian women during their third trimester and 0.263 ± 0.091 mmole/liter in 42 age-matched nonpregnant controls (11). On the other hand, Lin and Levy recently determined serum sulfate concentrations in 20-day pregnant rats and nonpregnant controls and found no apparent difference between the two groups[1]. The effect of pregnancy on endogenous sulfate concentrations in women was, therefore, reexamined.

Seven Caucasian women in their third trimester of pregnancy and nine Caucasian nonpregnant women of similar age were the subjects in this study. They were medication-free for at least 1 week before the study and were in apparent good health. None of the controls were taking an oral contraceptive. Nine-milliliter blood samples were drawn from an antecubital vein into 10-ml capacity plastic disposable syringes containing 1 ml of trisodium citrate solution each. The citrated blood was centrifuged in plastic tubes at 12,000×*g* for 15 min at 25°, and the plasma was frozen pending assay. Inorganic sulfate concentration was determined by a modification (12) of the turbidimetric method of Berglund and Sörbo (13), using

[1] Results to be published.

0022-3549/83/0600-0715$01.00/0