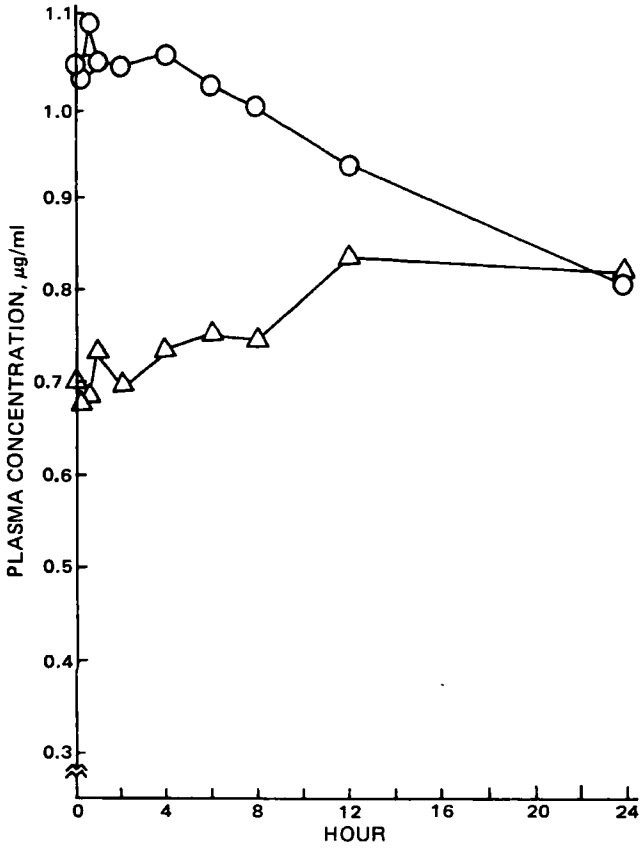

Figure 4—*Procainamide (○) and N-acetylprocainamide (△) plasma concentration–time profiles in a blood sample kept at ambient temperature.*

A second metabolite with shorter retention than *N*-acetylprocainamide (Fig. 2) was found in all chromatograms except those from samples obtained at earlier times. This unidentified metabolite was also found in many cases after the administration of procainamide to rabbits (5) and humans (6). Recent studies (7) have shown that desethyl procainamide and desethyl *N*-acetylprocainamide might be two additional metabolites of procainamide. However, identification of the unknown product was not made due to the lack of authentic samples. According to the relative peak heights, the appearance rate of this unidentified metabolite in plasma also followed a zero-order process in the first 12 hr of study.

To ascertain whether metabolism took place in plasma or blood cells, pooled plasma was spiked with procainamide to yield an initial concentration of 20 μg/ml and kept at ambient temperature for various periods of time. No metabolite formation could be found up to 48 hr, indicating that blood cells are the sole site of metabolism in whole blood. Since the plasma concentrations of procainamide were essentially identical during the study, no degradation of plasma samples could be assumed.

The results of the above *in vitro* studies suggest that the time between collection and centrifugation of a blood sample may have a considerable influence on the measured plasma levels of procainamide and its metabolites. This was supported by another experiment in which ~10 ml of blood was collected shortly before the next scheduled dose from an adult male patient on chronic oral procainamide therapy (Fig. 4). During the 24 hr of storage, the difference between the minimum and maximum plasma concentrations measured for procainamide was ~ 35% and that for *N*-acetylprocainamide was 24%. Similar effects also were observed from two rabbits, whose blood was collected in syringes[8] after intravenous dosing of procainamide.

In light of the results of this study, it appears that a prudent approach is to separate plasma as soon as the blood sample is collected; this might minimize the difference between the true *in vivo* plasma concentration and the measured *in vitro* concentration. More work is required in order to fully assess the potential significance of the present findings in the pharmacokinetic studies.

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Nonlinear Regression Approach for Determining Whether Absorption and Elimination Rate Constants are Equal in the One-Compartment Open Model with First-Order Processes

Keyphrases □ Pharmacokinetics—one-compartment open model, nonlinear regression analysis, absorption rate constants, elimination rate constants

To the Editor:

Recently Bialer reported a simple method for determining whether absorption and elimination rate constants are equal in the one-compartment open model with first-order processes (1). The basis for this method is that whenever the product of time of peak drug concentration (t_{max}) is equal to total area under curve (AUC) divided by the base of natural logarithm (e), the absorption rate constant (k_a) must be equal to the elimination rate con-

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stant (k_e). This method is mathematically sound, but when applied to clinical study data, t_{max} and C_{max} are difficult to determine with sufficient accuracy, and the true t_{max} and C_{max} may be missed with routine sampling protocol. Additionally, the calculation of the AUC requires an accurate estimate of the half-life of the terminal portion of the concentration–time curve to estimate the residual area remaining after the last measured concentration. The terminal half-life estimation requires fitting the terminal portion concentration–time of the curve or, preferentially, the entire profile by some standard fitting procedure to an *a priori* model. Thus, satisfaction of the criterion stated above may not be obvious. It is, therefore, of interest to see if there is an alternate method which can achieve the same result.

In a one-compartment open model with first-order absorption and elimination the plasma–drug concentration (C_b) is defined generally by:

$$C_b = \frac{FDk_a}{V_d(k_a - k_e)}(e^{-k_e t} - e^{-k_a t}) \qquad \text{(Eq. 1)}$$

The relevance of this equation and the relationship between k_a and k_e have been discussed extensively in standard texts (2, 3). Generally speaking, when k_a is larger than k_e, the terminal phase reflects the process of elimination. The other case is the flip-flop model where k_a is smaller than k_e, and the terminal phase reflects the process of absorption. In the special case where k_a is equal to k_e Eq. 1 becomes mathematically irrelevant, since the derivation of Eq. 1 requires that $k_a \neq k_e$. In this case, the general equation describing the plasma–drug concentration has to be derived from the basic model setting $k = k_a = k_e$. By employing a standard technique such as Laplace transform, Eq. 2 can be obtained (1, 2):

$$C_b = \frac{FD}{V_d} kte^{-kt} \qquad \text{(Eq. 2)}$$

There are a number of techniques commonly used to obtain values for the rate constants of absorption and elimination. These include the manual method of residuals (feathering) and computer-based nonlinear regression techniques using least-squares criteria. The Wagner–Nelson method makes no specific compartmental model assumption and can also be used to estimate k_a. However, when $k_a = k_e$, absorption occurs throughout the whole process. Therefore, any technique that involves assuming the terminal phase reflects solely an absorption or elimination process will fail to reveal the true rate constants. This is not the case for nonlinear least-squares regression analysis. Provided that there is some minute difference between k_a and k_e, nonlinear regression may be applied to the data. To test this alternative, data with only rounding error were generated using Eq. 2 with FD/V_d = 10, $k = k_a = k_e = 0.5$ (Table I). Concentrations with 5% random noise are also listed in Table I.

Table I—Simulated Concentration Data Using Eq. 2 with $FD/V_d = 10$ and $k = k_a = k_e = 0.5$

Time	Concentration	Concentration with 5% Noise
0.25	1.10	1.10
0.5	1.95	1.95
1.0	3.03	3.18
2.0	3.68	3.68
3.0	3.35	3.52
4.0	2.71	2.85
5.0	2.05	2.05
6.0	1.49	1.42
8.0	0.73	0.69
10.0	0.34	0.36
12.0	0.15	0.14

With no *a priori* knowledge of the values of k_a and k_e, the two rate constants were estimated by using the method of residuals, the Wagner–Nelson method (4), the decision-making program AUTOAN (5), and the nonlinear regression program NONLIN (6). The results are listed in Table II.

Application of the graphical method of residuals revealed no problems except that the residual line seemed to show some curvature. The values for k_a and k_e obtained by this method were 0.87 and 0.39, respectively. The Wagner–Nelson method also revealed no problems and the values for k_a and k_e obtained by this method were 0.67 and 0.40, respectively. The same data were fitted by the decision-making program AUTOAN. Curve stripping, the first part of the AUTOAN output, indicated that the best number of exponential is 3 and the data are best described by a one-compartment open model with two first-order input steps, k_1 and k_2. The second part of the AUTOAN output using the 1969 version of NONLIN to fit the data according to this model yielded values for k_1, k_2, k_e, and R^2 of 0.707, 2.634, 0.450, and 0.855, respectively. Finally, using rate constants obtained from the method of residuals as initial estimates, and assuming the model described by Eq. 1, the 1974 version of NONLIN successfully converged to the real values. The goodness of fit was exemplified by the R^2 value and the small standard deviation of the parameters estimates (Table II).

The method of residuals, the Wagner–Nelson method, curve stripping, and nonlinear regression are probably the

Table II—Comparison of Real and Estimated Values Obtained by the Method of Residuals, the Wagner–Nelson Method, AUTOAN, and NONLIN [a]

	FD/V_d	k_e	k_a	k_1	k_2	R^2
Real Values	10	0.500	0.500	—	—	—
Method of Residuals[b]	7.4	0.39	0.87	—	—	—
Wagner–Nelson Method[b]	7.7	0.40	0.67	—	—	—
AUTOAN[b]	5.79	0.450	—	0.707	2.634	0.855
	(23.01)	(1.937)	—	(3.937)	(8.343)	
AUTOAN[c]	6.64	0.695	—	0.771	1.030	0.718
	(67.17)	(6.86)	—	(13.05)	(11.85)	
NONLIN[b]	9.98	0.499	0.501	—	—	1.000
	(0.06)	(0.003)	(0.003)			
NONLIN[c]	10.31	0.506	0.501	—	—	0.997
	(0.89)	(0.042)	(0.049)			

[a] Standard deviation of the parameter estimates in parentheses. [b] Using data with only rounding error. [c] Using data with 5% noise.

most routine procedures for determining model rate constants. The first three methods are obviously bad choices when $k_a = k_e$. A nonlinear regression analysis program such as NONLIN, with the simplest model, successfully revealed the real values of k_a and k_e. Bialer's criteria (1) can serve as additional proof of the NONLIN output.

It should be emphasized that although nonlinear regression techniques successfully converged to the real rate constants used to generate the data in the example, this does not imply that Eq. 1 is the only model which can be fitted to the data. The problems associated with obtaining a reliable value for a pharmacokinetic parameter, such as absorption rate constants after oral administration, have been previously identified. For example, a multiple-compartment open model may also be collapsed to a one-compartment open model under certain conditions (7). In reality, the true model is rarely known, and in most cases one can not distinguish one model from another. However, this study demonstrated that if Eq. 1 represents a true model, nonlinear regression analysis separates the rate constants where other methods can not.

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Kinetic Interpretation of the Microparameters in Compartmental Modeling When Adjoining Compartments are Sampled

Keyphrases □ Pharmacokinetic analysis—compartmental modeling □ Compartmental modeling—kinetic interpretation of microparameters □ Diffusional transport hypothesis—compartmental modeling

To The Editor:

In linear compartmental modeling, the rate of mass transfer of drug from compartment i to compartment j is $k_{ij}x_i$, and for the reverse transfer $k_{ji}x_j$, where the k's are constant microparameters and the x's stand for the amounts in the compartments (1). It appears tempting to justify compartmental pharmacokinetic analysis by attaching special kinetic significance to the microparameters. However, it is well recognized that this type of modeling is merely an abstract mathematical way of accounting for the combined effect of many complex disposition processes, which are too difficult or impossible to consider individually, in order to explain the concentration profile in a sampled compartment; typically the blood. It is also recognized that in pharmacokinetic practice when dealing with prediction and adjustment of blood levels, in the calculation of dosage regimens and in the evaluation of drug input, there is no need for compartmental modeling. It would be irrational to do so, because the required calculations can (at least for dose-linear systems) be done simply on the basis of the principles of superposition, convolution, or deconvolution. However, there are cases in pharmacokinetics where more than one tissue compartment is sampled for the drug. A compartmental type of kinetic analysis is then definitely justified. The blood–brain barrier (BBB) transfer kinetics of theophylline in dogs has recently been investigated. In the analysis, the classical linear compartmental approach was avoided because it appears completely irrational to assume that the transfer across a membrane is proportional to amounts and not to a concentration differential. A model-independent approach combined with a more rational compartmental transport mechanism was applied instead. In analyzing the equations resulting from this approach an interesting relationship was discovered between the diffusion and binding parameters and the microparameters in a classical compartmental approach. It is of interest to communicate these findings which bring the classical compartmental modeling into a different perspective.

The Diffusion Approach: The diffusion rate of the drug across the BBB is proportional to the difference between the free drug concentrations on the two sides of the barrier:

$$\frac{d}{dt}[V_cC_c(t)] = K_1[F_sC_s(t) - F_cC_c(t)] \quad \text{(Eq. 1)}$$

Subscripts c and s denote cerebrospinal fluid (CSF) and serum, respectively; V, C, and F stand for volume, total drug concentration, and free (unbound) fraction, respectively; while K_1 is a positive diffusion constant. Equation 1 assumes that the drug is not metabolized in the CSF, which is consistent with our current knowledge about the metabolic systems present on the CNS side of the BBB (2). The equation can readily be solved by Laplace transforms to give the following expression relating the total concentration of the drug in the CSF to the total concentration in the serum:

$$C_c(t) = \left(\frac{F_cK_1}{V_c}\frac{F_s}{F_c}\right) C_s(t) * e^{(F_cK_1/V_c)t} \quad \text{(Eq. 2)}$$

where * denotes convolution.

The derivation of Eq. 2 assumes that F_c and F_s do not depend significantly on the drug concentration. The free fractions depend on the unbound protein concentration as well as on the affinity of the protein for the drug. Usually only a small fraction of the available binding sites is occupied at therapeutic drug concentrations; therefore, the

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