Pharmaceutical Research, Vol. 5, No. 6, 1988

Research Article

Metabolite Formation Pharmacokinetics: Rate and Extent of Metabolite Formation Determined by Deconvolution

Michael D. Karol[1,2] and Scott Goodrich[1]

Received September 9, 1987; accepted January 19, 1988

A two-step analytic procedure to determine the rate and extent of metabolite production following administration of the parent compound is described. The procedure provides the rate and extent of metabolite production as a function of time by application of the general model independent approach of deconvolution. The metabolite unit impulse response function is obtained by implicit deconvolution of the metabolite data with a truncated constant-rate metabolite input function. Then the obtained unit impulse response function is used in an analytic deconvolution with metabolite data following administration of the parent compound to obtain the rate and extent of metabolite production. The input function is also deconvolved with metabolite data to obtain the unit impulse response function appropriate for prediction of metabolite levels given a selected input of parent compound. The expected profile following administration of the consecutive infusions of parent drug is shown for both parent and metabolite. The rationale for selection of deconvolution methods is discussed. The approach is applied to data for procainamide and *N*-acetylprocainamide from three human subjects. The results indicate that from 27 to 39% of the procainamide was converted to *N*-acetylprocainamide in these subjects.

KEY WORDS: procainamide; *N*-acetylprocainamide; NAPA; metabolite; deconvolution; model independent.

INTRODUCTION

The pharmacokinetics of metabolite production influence therapy in primarily two ways. First, since metabolism serves as a route of elimination, the concentration profile of the parent compound is affected by the metabolic process (1). Second, the metabolite may have activity. Sutfin and Jusko published an extensive review discussing 41 compounds which have active metabolites (2). In cases such as the conversion of acetylmethadol to noracetylmethadol the metabolite has greater activity.

Metabolite formation may not necessarily be describable by a simple first-order rate constant. More complex mechanisms may be involved. For example, in the production of oxazepan from diazepam, one of two intermediary compounds is produced, either *N*-desmethyldiazepam or oxydiazepam (2).

We present a method of examining the process of metabolite formation based solely on the qualities of linearity and time invariance. In contrast to traditional techniques, the rate and extent of metabolite production are determined as a function of time (1). Whereas previous methods give a final value, such as 28% metabolized, this approach provides an equation from which a plot of both the rate and the extent of metabolite production can be made. For example, such an analysis might indicate that at 2 hr 8% of the parent drug had been converted to metabolite, at 3 hr, 13%, and so on. One could also determine that point at which metabolite formation was at its peak rate by examining the rate plot or, more directly, by setting the first derivative of the rate function to zero and solving for time. This approach does not require complex compartmental models. The approach makes no assumptions with regard to the nature of the metabolite formation kinetics, only that the kinetics of the metabolite itself be linear and time invariant. It does not require that the site of elimination of the metabolite be the site of sampling. This is to say that it does not require that clearance be constant. It is assumed that the rate of metabolite delivery to the site of sampling is, for all practical purposes, the rate of metabolite production. In other words, the rate of transfer of the metabolite from the site where it is produced to the site of sampling is a relatively fast, non-rate-limiting step (3).

The second goal of this communication is to show that the same basic qualities of linearity and time invariance can be used to obtain a function useful in the prediction of metabolite concentrations which would result from some selected administration profile of the parent compound. For the previous objective no assumptions were needed regarding the nature of metabolic conversion. This goal, however, requires that the conversion be such that the relationship between the administered parent compound and the resulting metabolite be linear and time invariant.

[1] Department of Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, Arizona 85721.

[2] To whom correspondences should be addressed.

0724-8741/88/0600-0347$06.00/0

THEORETICAL BACKGROUND

In the most general sense, a system may be defined as an input–response pair. Typically, in pharmacokinetic applications, the input into the system is the rate of drug absorption or infusion, and the response is the resulting drug plasma or serum concentrations.

The relationship of an input to a response for a linear, time-invariant system is described by the convolution integral (4–6)

$$h(t) = \int_0^t f(T)\, g(t - T)dT \quad (1)$$

which may be stated in the following shorthand notation (6):

$$h(t) = f(t) * g(t) \quad (2)$$

where $h(t)$ denotes the response–time function, $f(t)$ denotes the input–time function, $g(t)$ denotes the unit impulse response (also known as the weighting function), and * denotes convolution.

In the context of pharmacokinetics, Eq. 2 may be stated as

$$c(t) = f(t) * c_\delta(t) \quad (3)$$

where $f(t)$ denotes the rate of drug input at any point in time, $c(t)$ denotes the resulting concentration vs time profile (the response), and $c_\delta(t)$ denotes the unit impulse response.

Solving for the function on the left side of the equation is referred to as convolution. Finding either element on the right is deconvolution. The typical procedure is as follows.

Drug is administered with a known rate of input, usually as a bolus or iv infusion. Thus, the input function $f(t)$ is known. The resulting drug concentration is measured, constituting the function $c(t)$. Given $f(t)$ and $c(t)$, the third function of the expression, $c_\delta(t)$ is determined by deconvolution. Once $c_\delta(t)$ is known, the system is completely characterized with regard to the input–response pair. For any known input the response may be determined. Conversely, for any measured response, the input needed to produce that response may be determined. An example of the latter is the determination of bioavailability profiles where the rate and extent of absorption are given as a function of time (8).

The input–response pair may be defined in other ways for other purposes. In this work, two different input–response pairs were used, defining two "systems." Each system was defined with different objectives in mind and the results require different assumptions for interpretation. The first system was defined as the input of metabolite (NAPA) paired with a resulting plasma metabolite (NAPA) concentration. With the system defined in this way, any production of metabolite is a system input. Thus, once the unit impulse response for the metabolite $[c_\delta(t)_N]$ is known, deconvolution may be applied to determine the rate and extent of metabolite production following administration of the parent compound. Rate and extent are given not only as final values but as a profile across time. The only requirement for the technique to be valid is that the pharmacokinetics of the metabolite be linear and time invariant. No assumptions need to be made with regard to the pharmacokinetics of the parent compound. Since the input into a system is external and not part of the system, no assumptions are needed with regard to the nature of the metabolic process. Thus, even if the metabolite was produced by Michaelis–Menton kinetics, the approach would still be valid. It is assumed that the produced metabolite is returned to the systemic circulation. If a sequential first-pass effect occurs, metabolite produced and eliminated before being returned to the systemic circulation would not be included as part of the metabolite produced (9).

The second system addressed in this work relates the administration of parent compound (procainamide) (input) to the resulting plasma concentration of metabolite (NAPA) (response). By defining a system in this way the expected metabolite concentration profile resulting from any given parent compound administration may be predicted without the necessity of complex modeling. The only assumption is that the relationship between parent compound administration and metabolite be linear and time invariant.

Deconvolution is accomplished by one of three basic means. They are analytic, numeric, and implicit. The analytical method provides an "algebraic" solution to the deconvolution problem. Examples of the analytic approach include the programs DECONV (8) and DCON (7). These programs contain within them a numeric root-finding algorithm, however. To use these programs, the functions to be deconvolved must be presented in the form of a sum of exponentials. The result is returned as a sum of exponentials.

Numeric methods include the point–point and the point–area algorithms (10,11). These techniques operate on actual data in a stepwise fashion starting with the first point. They require either equally spaced data or interpolation and perform best when data collection is dense.

The third technique is implicit deconvolution. For purposes of discussion, assume that the goal of this particular deconvolution is to find the unit impulse response function $c_\delta(t)$ in the expression

$$c(t) = c_\delta(t) * f(t) \quad (4)$$

The input function $f(t)$ is known and a functional form for $c_\delta(t)$ is assumed. The function $c_\delta(t)$ is analytically convolved with $f(t)$ to generate the expected form of the response function $c(t)$. This function will have parameters from only two sources, $c_\delta(t)$ and $f(t)$. The parameters from $f(t)$ are known. Therefore, all of the unknown parameters of $c(t)$ are the desired parameters of $c_\delta(t)$. By fitting $c(t)$ to the data, the parameters of $c_\delta(t)$ are determined implicitly.

PHARMACOKINETIC ANALYSIS

The primary objectives of this work are (i) to demonstrate a method of determining the rate and extent of production of a metabolite as a function of time given the administration of a parent compound and (ii) to present a technique of predicting expected metabolite levels resulting from an input of parent compound. No assumptions are made with regard to the process of metabolic conversion for the first objective. The only requirement for objective two is that there be a linear relationship between the input of parent compound and the metabolite concentration. For this objective, administration of the metabolite is not necessary.

For demonstration purposes, procainamide and *N*-acetylprocainamide data were chosen. For objective one, the first step is to define the appropriate system. In this case the input is the rate of appearance of metabolite (NAPA) and the

response is metabolite concentration. This system is defined mathematically by the convolution expression

$$c(t)_{NP} = c_\delta(t)_N * f(t)_{NP} \tag{5}$$

where $f(t)_{NP}$ is the rate of input of metabolite. In this case the metabolite is coming from the parent compound. $C(t)_{NP}$ is the concentration–time profile of metabolite resulting from that input. $c_\delta(t)_N$ is the unit impulse response which interrelates any input of the metabolite to a resulting concentration. To determine $f(t)_{NP}$ from $c(t)_{NP}$ data, $c_\delta(t)_N$ must be known. $c_\delta(t)_N$ may be determined from metabolite administration since the unit impulse response function of Eq. (5) is the same function as in Eq. (6) below.

$$c(t)_N = c_\delta(t)_N * f(t)_N \tag{6}$$

where $c(t)_N$ is the concentration profile of NAPA resulting from NAPA administration, and $f(t)_N$ is the NAPA input function. To apply Eq. (6) to the task of determining $c_\delta(t)_N$, NAPA must be administered at a known rate $f(t)_N$ and NAPA plasma levels are measured. By deconvolution, $c_\delta(t)_N$ is determined.

To select the appropriate deconvolution technique, the input function and the nature of the available data must be considered. For purposes of demonstration, the procainamide–NAPA data of Dutcher *et al.* (12) were selected. In their study, NAPA was administered by means of a short-term constant-rate infusion. Thus,

$$\begin{aligned} f(t)_N &= R_N \text{ for } 0 \leq t \leq T \\ &= 0 \quad \text{for} \quad t > T \end{aligned} \tag{7}$$

where R_N is the rate of NAPA input and T is the time where the input is terminated. This immediately rules out use of the analytic deconvolution program DECONV for two reasons. First, there is no way to present a discontinuous function to DECONV. Second, DECONV requires all functions to be represented as a sum of exponentials. A constant-rate input represented as a sum of exponentials is

$$f(t) = Ke^{-0t} \tag{8}$$

At one point of the DECONV program coefficients are divided by the time coefficients. Presentation of Eq. (8) to DECONV produces a "divide by zero" error.

Numerical procedures, such as the point–point or point–area algorithms present different problems. The NAPA profile was generated by a short-term infusion. Because all the input is completed before the first sample, the algorithms consider the input to be a bolus. This becomes apparent upon examination of the equation for the first point of the point–area deconvolution,

$$c_\delta(t) = \frac{c(T_1)}{\int_0^{T_1} f(t)dt} \tag{9}$$

$$= \frac{c(T_1)}{D} \tag{10}$$

The denominator is the amount administered from $t = 0$ to $t = T_1$, where T_1 is the time of the first data point. Since the measured concentrations after termination of the infusion are greater than those expected from a bolus of equal dose, the calculated values for $c_\delta(t)$ would contain within them a systematic error. In particular, they would be an underestimate of the true values. An analogous problem arises in the point–point algorithm. A second consideration is that it provides a numerical solution. This implies that the data relating to Eq. (6) must be collected at the same times as the data for Eq. (5); otherwise interpolation is necessary.

Implicit deconvolution does not impose a systematic error and provides a solution as a function. It does require that the functional form of $C_\delta(t)_N$ be selected, however. In light of the fact that the analytic method could not solve this particular deconvolution and that the numerical approaches systematically bias the results, the implicit approach was taken. The unit impulse response function $C_\delta(t)_N$ was assumed to be adequately approximated by a sum of exponentials.

$$C_\delta(t)_N = \sum_{i=1}^{n} A_i e^{-\alpha_i t} \tag{11}$$

Given the input function $f(t)_N$ of Eq. (7) and $C_\delta(t)_N$ of Eq. (11), Eq. (6) was solved to provide an appropriate functional form for $c(t)_N$.

$$C(t)_N = \sum_{i=1}^{n} \frac{R_N A_i}{\alpha_i} \{e^{-\alpha_i(t-T)_+} - e^{-\alpha_i t}\} \tag{12}$$

where $(t - T)_+ = 0$ for $t - T < 0$, $t - T$ for $t - T \geq 0$. Equation (12) was fitted by means of nonlinear regression (FUNFIT) to the NAPA data resulting from NAPA administration (13). Since the parameters of Eq. (11), A_i and α_i, are contained within Eq. (12), the process of curve fitting implicitly performs deconvolution giving $c_\delta(t)_N$.

The second step is to do the deconvolution implied by Eq. (5) to obtain the function $f(t)_{NP}$. To do this, a sum of exponentials was fitted to the data to obtain a functional form $c(t)_{NP}$. Since both $c(t)_{NP}$ and $c_\delta(t)_N$ are represented by a sum of exponentials, deconvolution was done analytically by the routine DECONV.

The second objective was to provide a function which would predict the concentration vs time profile of the metabolite given a dosing scheme of the parent compound. The necessary assumptions for this objective differ from that for objective one. In this case the only assumption is that the relationship between the administration of parent compound and the metabolite be linear and time invariant. To achieve that objective the input function of parent compound $f(t)_p$ must be deconvolved with the concentration profile of NAPA resulting from procainamide administration $c(t)_{NP}$. That is to say that the expression

$$c(t)_{NP} = c_\delta(t)_{NP} * f(t)_p \tag{13}$$

must be solved for $c_\delta(t)_{NP}$.

For reasons discussed for objective one, analytic and numerical methods of deconvolution were excluded. $c_\delta(t)_{NP}$ was assumed to be adequately described by the sum of exponentials.

$$c_\delta(t)_{NP} = \sum_{i=1}^{m} B_i e^{-\beta_i(t-t_{LAG})} \tag{14}$$

for $t \geq t_{LAG}$ and $c_\delta(t)_{NP} = 0$ for $t < t_{LAG}$. As in the case of NAPA administration, procainamide was administered as a constant-rate short-term infusion. Convolution of Eq. (14) with this input function gives $c(t)_{NP}$:

$$c(t)_{NP} = \sum_{i=1}^{m} \frac{R_p B_i}{\beta_i} \{e^{-\beta_i(t-T-t_{LAG})_+} - e^{-\beta_i(t-t_{LAG})}\} \tag{15}$$

for $t \geq t_{LAG}$ where R_p is the rate of procainamide infusion $c(t)_{NP} = 0$ for $t < t_{LAG}$. By fitting Eq. (15) to the NAPA data resulting from procainamide administration, the desired function is implicitly obtained.

To demonstrate the utility of Eq. (14), the expected profile of metabolite following the administration of consecutive intermittent constant-rate infusions was determined and plotted (Fig. 2). This was based on the linear, time-invariant relationship described by

$$c(t)_{NPS} = C_\delta(t)_{NP} * f(t)_{PS} \tag{16}$$

where $f(t)_{PS}$ is the input rate of procainamide which was selected and $c(t)_{NPS}$ is the NAPA response function predicted to occur from the selected procainamide input. In the most general case, a series of consecutive intermediate constant-rate infusions $f(t)_{PS}$ is described by

$$f(t)_{PS} = \sum_{j=1}^{v} f_j(t) \tag{17}$$

where

$$f_j(t) \begin{cases} = 0 \text{ for } 0 \leq t < T_{j1} \\ = R_j \text{ for } T_{j1} \leq t \leq T_{j2} \\ = 0 \text{ for } T_{j2} < t < \infty \end{cases}$$

T_{ji} and T_{j2} are the starting and ending times of the jth constant-rate infusion, respectively, and v is the number of infusions. Convolution of Eq. (17) with Eq. (14) as indicated by Eq. (16) gives

$$C(t)_{NPS} = \sum_{i=1}^{m} \sum_{j=1}^{v} \frac{B_i R_j}{\beta_i} \{e^{-\beta_i(t-T_{j2}-t_{LAG})+} - e^{-\beta_i(t-T_{j1}-t_{LAG})+}\} \tag{18}$$

the equation used to generate the NAPA profile in Fig. 2. The procainamide profile in Fig. 2 was generated by first fitting an equation of the form of Eq. (12) to procainamide data to obtain $c_\delta(t)_p$. This was convolved with $f(t)_{PS}$ [Eq. (17)] to give a prediction equation of the form of Eq. (18). In the case of procainamide, t_{LAG} is zero, however.

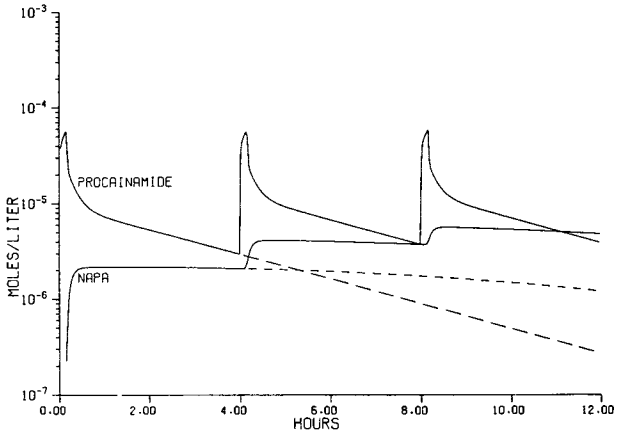

Fig. 2. Expected procainamide and NAPA levels resulting from three 10-min procainamide infusions. Dashed lines show expected profile if second and third infusions were not given.

RESULTS AND DISCUSSION

Equation (12) was fitted by means of the nonlinear regression program FUNFIT to the NAPA data resulting from NAPA administration (11). This provided the unit impulse response function $c_\delta(t)_N$. Correlation coefficients for the three subjects were 0.9994, 0.9998, and 0.9999, respectively. A simple sum of exponentials was fitted to the NAPA data resulting from procainamide administration (correlation coefficients 0.9043, 0.9635, 0.9558). Deconvolution of $c_\delta(t)_N$ with this sum of exponentials via the routine DECONV [solution of Eq. (5)] gave the rate of NAPA formation profiles shown in Fig. 1. The inset in Fig. 1 shows the rate of formation as units of millimoles per hour of NAPA for the three subjects up to 4 hr.

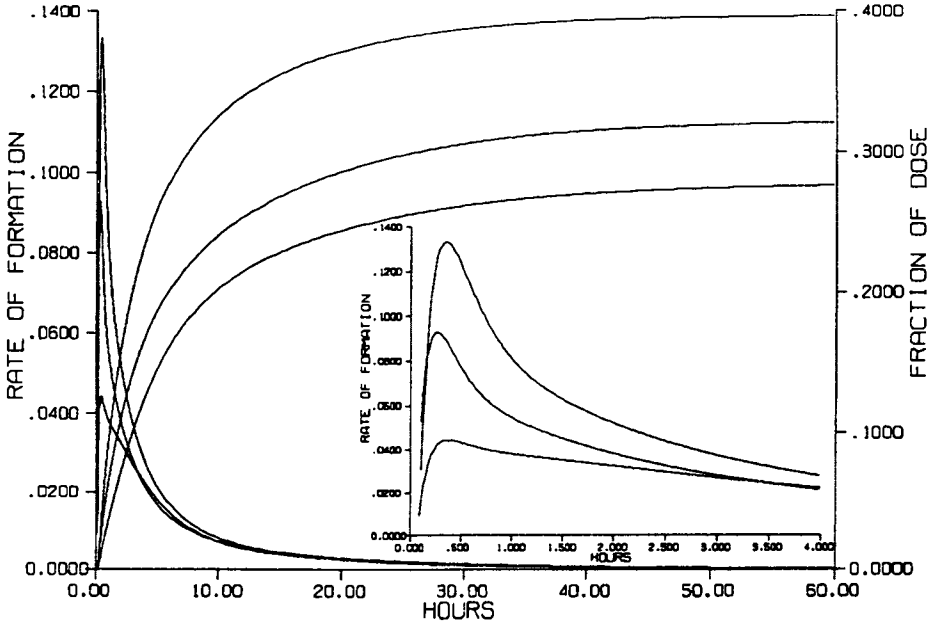

Fig. 1. Rate and extent of NAPA production. Inset: Rate of NAPA production up to 4 hr.

The larger plot in Fig. 1 shows the same information with the time start extended to 60 hr. Superimposed on this plot are three profiles showing the extent of conversion of procainamide to NAPA. This is stated in terms of the fraction of administered procainamide which is converted to NAPA. The functional forms of the rate and extent profiles are given in Veng-Pedersen's publication on DECONV (his equations 23 and 22, respectively) and are restated here for convenience of the readers (8).

$$F(t) = \frac{D}{100} \sum_{i=1}^{L} U_i(-V_i)e^{-V_i(t-t_{LAG})+} \quad (19)$$

$$PCT(t) = U_0 + \sum_{i=1}^{L} U_i e^{-V_i(t-t_{LAG})+} \quad (20)$$

$F(t)$ is the rate of conversion and $PCT(t)$ is the extent. The terms U_i and V_i are results from DECONV. D is the dose of procainamide administered and should be stated in terms of moles in this application. The values of U_0 represent the extent of metabolite production as time approaches infinity. In these examples the subjects had 39.7, 32.2, and 27.8% of the procainamide dose converted to NAPA, respectively.

Equation (15) was fitted to the NAPA data resulting from procainamide administration (correlation coefficients 0.9770, 0.9856, 0.9849) and the results were incorporated into Eq. (18). Equation (18) could then be used to determine the NAPA profile given the administration of procainamide. Figure 2 shows the NAPA concentration vs time profile given three 10-min constant-rate infusions of procainamide for subject 1. The initial infusion was set to be equal to that used to generate the original data. Subsequent infusions were set at a rate necessary to produce peak values of procainamide equal to that following the first infusion. Also shown in this figure is the procainamide profile which would result from the same input. Dashed lines indicate how the profiles would appear if only the initial infusion had been given.

Over the short duration of 4 hr, NAPA levels appear virtually constant. As subsequent doses are given NAPA levels rise, and at 11 hr the NAPA levels become greater than that of procainamide. If only one dose had been given, this would have occurred at 5 hr.

SUMMARY

Deconvolution techniques may be applied to provide insight to metabolite production. Figure 1 shows both the rate and the extent of metabolite production and Fig. 2 shows both the parent and the metabolite levels anticipated to result from a selected administration profile of parent compound. In these examples, procainamide and the metabolite NAPA were examined. Both compounds exhibit antiarrhythmic activity, although the metabolite is estimated to be only one-third to one-sixth as active as the parent compound (14–16).

As seen in Fig. 2, metabolite and parent compound levels do not parallel each other during the drug-loading phase. Thus, an understanding of the metabolite levels may be of value since therapeutic activity may not necessarily parallel parent drug levels in all cases.

ACKNOWLEDGMENTS

The authors wish to express their sincere appreciation to Arthur J. Atkinson, Jr., and his co-workers for supplying the procainamide and NAPA data.

NOMENCLATURE

General Terms

- $h(t)$ A response function
- $g(t)$ A unit impulse response (weighting) function
- $f(t)$ An input function
- $c(t)$ A response function in units of concentration
- $c_\delta(t)$ A unit impulse response (weighting) function as determined via $c(t)$

Specific Terms

- $f(t)_P$ Rate of procainamide input
- $f(t)_N$ Rate of NAPA input
- $f(t)_{NP}$ Rate of production of NAPA given procainamide administration
- $c(t)_P$ Concentration profile of procainamide resulting from procainamide administration
- $c(t)_N$ Concentration profile of NAPA, resulting from NAPA administration
- $c(t)_{NP}$ Concentration profile of NAPA resulting from procainamide administration
- $c(t)_{NPS}$ Concentration profile of NAPA predicted to result from a selected procainamide administration
- $c_\delta(t)_P$ Unit impulse response function for procainamide based on procainamide input
- $c_\delta(t)_N$ Unit impulse response function for NAPA based on NAPA input
- $c_\delta(t)_{NP}$ Unit impulse response function for NAPA based on procainamide input
- t_{LAG} Lag time between administration of the first molecule of parent compound and appearance of the first molecule of metabolite

REFERENCES

1. M. Gibaldi and D. Perrier. *Pharmacokinetics*, 2nd ed., Marcel Dekker, New York, 1982.
2. T. Sutfin and W. Jusko. In G. Wilkinson and M. Rawlins (eds.), *Drug Metabolism and Disposition in Clinical Pharmacology*, MTP Press, Boston, 1985, pp. 91–159
3. S. K. Pang and K. C. Kwan. *Drug Metab. Disp.* 11:79–84 (1983).
4. P. Veng-Pedersen. *J. Pharm. Sci.* 70:32–38 (1981).
5. M. D. Karol, P. Veng-Pedersen, and R. E. Brashear. *J. Pharmacokinet. Biopharm.* 11:373–387 (1983).
6. D. Cutler. *J. Pharmacokinet. Biopharm.* 6:227–241 (1978).
7. W. Gillespie and P. Veng-Pedersen. *Biopharm. Drug Dispos.* 6:351–355 (1985).
8. P. Veng-Pedersen. *J. Pharmacokinet. Biopharm.* 8:463–481 (1980).
9. S. K. Pang. *J. Pharmacokinet. Biopharm.* 13:633–663 (1985).
10. J. Wagner, *J. Pharmacokinet. Biopharm.* 3:457–471 (1975).
11. D. Vaughan and M. Dennis. *J. Pharm. Sci.* 67:663–665 (1978).
12. J. S. Dutcher, J. M. Strong, S. V. Lucas, W. Lee, and A. Atkinson, Jr. *Clin. Pharmacol. Ther.* 22:447–457 (1977).
13. P. Veng-Pedersen. *J. Pharmacokinet. Biopharm.* 5:513–531 (1977).
14. R. F. Minchin, K. F. Ilett, and J. W. Patterson. *Eur. J. Pharmacol.* 47:51–56 (1978).
15. E. E. Bagwell, T. Walle, D. E. Drayer, *et al. J. Pharmacol. Exp. Ther.* 197:38–48 (1976).
16. R. D. Reynolds, W. E. Burmeister, S. V. Calzadilla, *et al. Proc. Soc. Exp. Biol. Med.* 169:156–160 (1982).