Calculating Rate Constants in Forward Drug Transfer Reactions

WILLIAM O. THOMPSON[▲] and SAMUEL T. CHRISTIAN*

Abstract □ A simple method for obtaining and processing drug transfer data derived from *in vitro* model cells such as the Schulman cell is presented. Simplified calculation procedures are developed for determining the rate constants that describe the transfer of the drug through the compartments. Transfer of the acidic drug salicylic acid from a pH 2.0 compartment through a lipid phase to a pH 7.4 compartment was utilized as the test system and example for the method. Theoretical *versus* experimental transfer curves are presented, along with statistical considerations for data of this type.

Keyphrases □ Rate constants—calculations and equations for forward drug transfer reactions, Schulman cell, salicylic acid □ Drug transfer (forward) reactions—calculations and equations for rate constants, Schulman cell, salicylic acid □ Schulman cell—calculations and equations for rate constants in forward drug transfer reactions, salicylic acid

In the area of pharmacokinetics and in the study of drug absorption, the use of *in vitro* or idealized model systems has become increasingly popular for simulation of *in vivo* transport, even with the deficiencies and cautions pointed out by Higuchi *et al.* (1) in extrapolating this type of data to *in vivo* situations. There are many situations arising in which an understanding of the factors influencing solute transport rates is of great importance in the design of new drugs with improved therapeutic properties. These studies appear to have particularly useful implications when one is dealing with a diffusion-limited, weakly acidic or weakly basic drug in which the transport can be regarded as two consecutive first-order reactions.

An investigator using these techniques would find useful a simplified and rapid method of calculation, particularly when measurement output is a continuous graph of concentration *versus* time such as is produced by continuous monitoring by means of flow cells in a recording spectrophotometer. In approaching this problem, we were surprised to find a general lack of statistical evaluation of the various kinetic parameters associated with the diffusion-limited transport investigated using the so-called Schulman cell (2, 3) or similar device (4). The general absence of these data very likely reflects the long periods of time required for a single experiment to be completed, the wide variability of constants derived from a given set of experiments, and the difficulty of obtaining the constants. The purposes of this study were: (*a*) to develop a simple method of calculation for the rate parameters from a plot of concentration *versus* time generated from a continuous monitoring system, and (*b*) to demonstrate the method with an example.

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

Scheme I

Table I—Statistical Evaluation of Rate Constants for the Salicylic Acid System

Run Number	Calculated k_1	Calculated k_2
1	0.9815	0.6617
2	0.8322	0.5958
3	0.9547	0.6497
4	1.0102	0.5856
5	0.8804	0.5242
6	0.9176	0.4707
Arithmetic mean	0.9294	0.5812
Standard deviation	0.0603	0.0669
95% Confidence interval	$0.8659 < k_1 < 0.9929$	$0.5177 < k_2 < 0.6447$

THEORETICAL

A drug transfer process that can be regarded as two consecutive first-order reactions may be described as shown in Scheme I, where A, B, and C are used for $A(t)$, $B(t)$, and $C(t)$, the concentrations of salicylic acid in compartments A, B, and C, respectively, at time t, as shown in Scheme II. Let V_A, V_B, and V_C equal the fluid volume in each of the three compartments. The three differential equations governing these first-order reactions are:

$$\frac{dA}{dt} = -k_1A \qquad \text{(Eq. 1)}$$

$$V_B\frac{dB}{dt} = k_1AV_A - k_2BV_B \qquad \text{(Eq. 2)}$$

$$V_C\frac{dC}{dt} = k_2BV_B \qquad \text{(Eq. 3)}$$

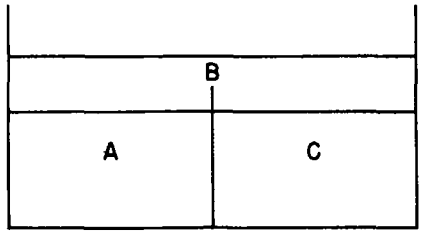
Initial conditions are set so that salicylic acid is only found in compartment A; thus, at time $t = 0$, $A(0) = A_0$ and $B(0) = C(0) = 0$. The solutions to Eqs. 1–3 as a function of time are as follows:

$$A(t) = A_0e^{-k_1t} \qquad \text{(Eq. 4)}$$

$$B(t) = \frac{V_AA_0k_1}{V_B(k_2 - k_1)}(e^{-k_1t} - e^{-k_2t}) \qquad \text{(Eq. 5)}$$

$$C(t) = \frac{V_AA_0}{V_C(k_2 - k_1)}(k_2 - k_1 - k_2e^{-k_1t} + k_1e^{-k_2t}) \qquad \text{(Eq. 6)}$$

Since the data output from the system consists of a continuous plot of the drug concentration in the A and C chambers as a function of time, as for example in Fig. 1, a convenient point for cal-



Scheme II—Sketch of Schulman-type in vitro *model cell*

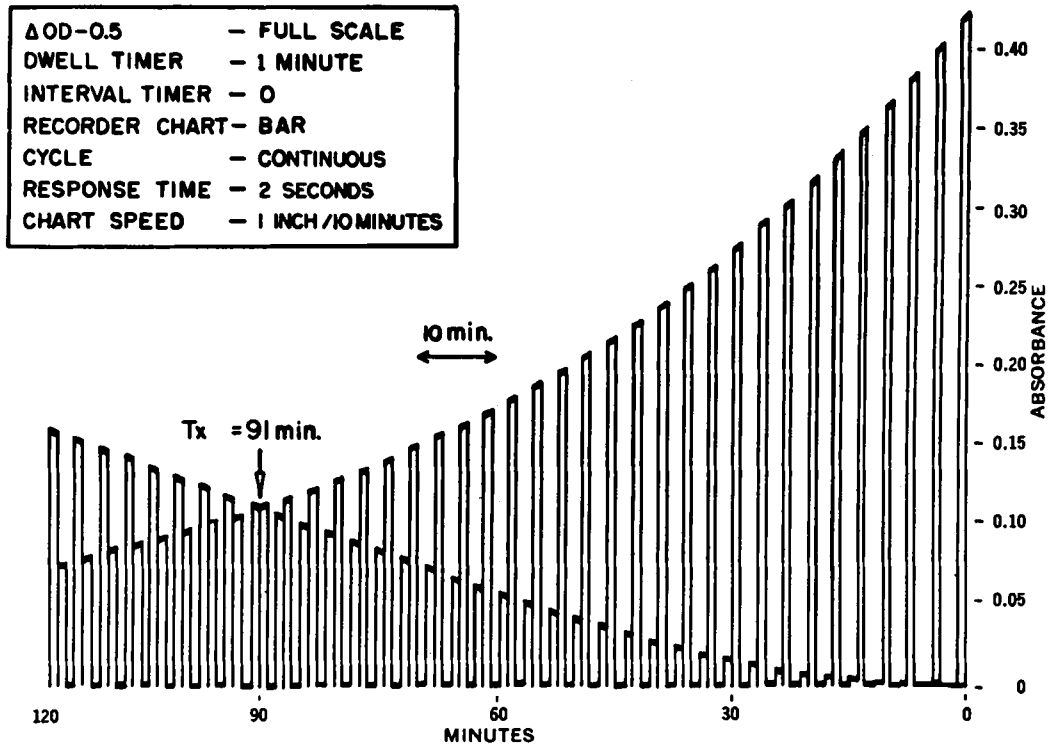


Figure 1—*Actual recorded data for a typical transfer run. Conditions are indicated in the figure insert.* T_x *indicates the time at which the salicylic acid concentrations in the* A *and* C *chambers are equal. The heavy line segment on the time axis represents the blank flow cell containing pH 2.0 buffer only. The starting salicylic acid concentration was 18.3 mcg./ml. in the* A *chamber.*

culation purposes is the point at which the concentration of drug in the A and C chambers is equal. If one designates the time at which this equilibrium occurs as the crossing time (t_x), then at t_x, $A(t_x) = C(t_x) = a$, where a is a constant. It then follows from Eq. 4 that:

$$k_1 = \frac{\ln A_0 - \ln a}{t_x} \qquad \text{(Eq. 7)}$$

where $\ln A_0$ is the natural (base e) logarithm function.

Also, since $A(t_x) = C(t_x)$, equating Eqs. 4 and 6 at time t_x yields:

$$e^{-k_1 t_x} = \frac{V_A}{V_C(k_2 - k_1)}(k_2 - k_1 - k_2 e^{-k_1 t_x} + k_1 e^{-k_2 t_x}) \qquad \text{(Eq. 8)}$$

Substituting for k_1 the solution from Eq. 7 into Eq. 8 and solving in terms of k_2, one has:

$$\alpha k_2 + \beta e^{-k_2 t_x} + \gamma = 0 \qquad \text{(Eq. 9)}$$

where:

$$\alpha = \frac{a(V_A + V_C)}{A_0} - V_A \qquad \text{(Eq. 10)}$$

$$\beta = \frac{V_A(\ln a - \ln A_0)}{t_x} = -k_1 V_A \qquad \text{(Eq. 11)}$$

and:

$$\gamma = V_A k_1 - [V_C k_1 a / A_0] \qquad \text{(Eq. 12)}$$

Equation 9 may be easily solved iteratively since the left-hand side, when considered as a function of k_2, *i.e.*, $f(k_2)$, is strictly monotonically increasing for all $k_2 \geq 0$. Furthermore, at $k_2 = 0$:

$$f(0) = \beta + \gamma = -V_C k_1 a / A_0 < 0 \qquad \text{(Eq. 13)}$$

Therefore, the function $f(k_2)$ is always negative at zero. Thus, if a particular trial value k_2^* produces $f(k_2^*) < 0$, k_2^* must be increased; if $f(k_2^*) > 0$, k_2^* must be decreased. For programming on a digital computer, the initial k_2 can be taken to be zero and then successively increased until $f(k_2)$ changes sign.

DISCUSSION

The solutions to Eqs. 7 and 13 for k_1 and k_2 were programmed on a laboratory-type digital computer[1]. Output from the computer was obtained both as a printout of k_1 and k_2 and as a plot of drug concentration in chambers A, B, and C *versus* time. This allows a direct comparison of the calculated (or theoretical) concentrations in A and C as a function of time and the concentration in A and C determined experimentally. This method has the added advantage of allowing the investigator to see quickly how close the experimental system approximates simple first-order kinetics.

As an example of the method, salicylic acid was transported from the A chamber (pH 2.0) through a layer of 20% decanol in cyclohexane (v/v) saturated with pH 2.0 buffer to the C chamber (pH 7.4). A continuous recording of the concentration in the A and C chambers was done with flow cells read by a recording spectrophotometer. A photograph of the data appears in Fig. 1. Points taken from the graph in Fig. 1 were plotted on Fig. 2, and the smooth lines were generated by a computer using the constants derived from the data of Fig. 1. As is evident from Fig. 2, transport seems to follow the model of two consecutive first-order reactions very well.

The concentration of salicylic acid in the B layer as a function of time is included in the figure, although this layer was not monitored.

After establishing the validity and convenience of the method, six independent runs were made on six different days to evaluate the statistical day by day variability of the rate constants. The results of these runs are shown in Table I. On each individual run, theoretical and empirical curves matched at least as well as those in Fig. 2, so the variabilty is between day to day determinations. Since errors are eliminated on a particular day once this system is in progress, it must be concluded that the between run variability is caused by random errors which are other than the more usual measurement variations. Variability in such systems becomes critical when one is comparing the transport properties of a closely related homologous series of compounds; for this reason, these data are presented. It is evident that comparisons from single determinations on different compounds with closely related rate constants must be made with extreme caution, especially if measurement variability is also introduced. The estimates of variability in these data can be used to provide future experimenters with a means of establishing appropriate sample sizes to detect specified differences with statistical confidence. The correlation coefficient $r = 0.6124$ between k_1 and k_2 is significant with 80% confidence on only six runs, which suggests a future area of research.

[1] Digital Equipment PDP-12.

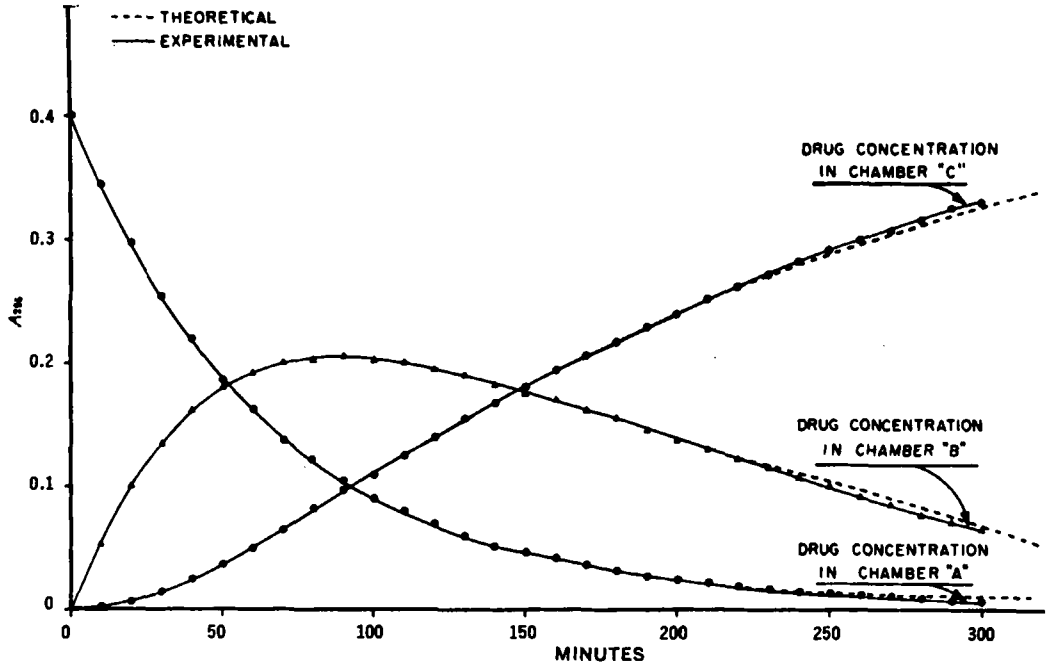


Figure 2—*Theoretical* (- - -) versus *experimental* (——) *salicylic acid transfer curves. Even though the organic layer was not monitored, the concentration–time curve was calculated from input data for comparison* (▲). *Theoretical curves were generated from Eqs. 1–3. The starting salicylic acid concentration for this experiment was 18 mcg./ml.*

The original intention was to utilize methods of calculation already present in the literature (5). However, by using the integrated rate equations which were reported as describing the forward transfer of an acidic drug, it was found that these equations did not describe the actual transfer process. It was then determined that the equations (5) do not satisfy the differential equations for the transfer process and hence are invalid.

REFERENCES

(1) W. I. Higuchi, G. Abdel-Halim, and A. B. Bikhazi, *Fed. Proc.*, **29**, 1327(1970).

(2) A. M. Agostini and J. H. Schulman, in "Surface Activity and the Microbial Cell," Gordon and Beach, New York, N. Y., 1965, p. 37.

(3) M. A. Augustine and J. Swarbrick, *J. Pharm. Sci.*, **59**, 314 (1970).

(4) S. A. Khalil and A. N. Martin, *ibid.*, **56**, 1225(1967).

(5) J. Perrin, *J. Pharm. Pharmacol.*, **19**, 25(1967).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 3, 1971, from the *National Institute of Mental Health, Addiction Research Center, Lexington, KY 40507*

Accepted for publication August 9, 1972.

• Present address: Department of Psychiatry, University of Alabama, Birmingham, AL 35233

▲ To whom inquiries should be directed.