Limitations in Interpretation of Digoxin Absorption Using Averaged Pharmacological Response Intensities

J. H. GUSTAFSON [x], R. D. SCHOENWALD [*], and L. Z. BENET

Abstract □ In this paper, the interpretation of averaged pharmacological effect data for the cardiac glycoside digoxin is reconsidered through a comparison with theoretically simulated data. Four parameters, with their standard deviations calculated from the best-fit biexponential equation for the experimentally derived transformed pharmacological response intensity, were the basis for the random development of a series of 17 individual sets of data with average parameters and standard deviations identical to the values found experimentally. Parameters from these 17 data sets were averaged and randomly altered as a function of the standard deviations for each average. Loo–Riegelman analyses, modified for application to the peripheral compartment, and fraction remaining to be absorbed plots were made. Results of this comparison of experimental pharmacological simulated data indicate that the presence or absence of enterohepatic recycling may not be unambiguously determined here.

Keyphrases □ Digoxin—absorption, different methods of data interpretation, averaged pharmacological response data compared to simulated data with averaged parameters □ Analysis—*in vivo* experimental data, different methods of interpretation compared

When several identical studies are run on a series of individuals, each of whom may serve as his or her own control, the analysis may include: (*a*) determining the appropriate pharmacokinetic parameters for each subject from the data obtained for that subject, averaging parameters for all subjects, and treating the average of those parameters as descriptive of the group of subjects as a whole; or (*b*) averaging the experimental data for all individuals and treating this average to produce the parameters descriptive of the group as a whole.

Recently, both methods were utilized in a methaqualone study, and no significant differences were found between the values resulting from both methods of analysis (1). Still, in the analysis of parameters determined from averaged grouped data, the standard deviations around the individual averaged data points need to be considered when defining how specific an interpretation can be placed on the data (*i.e.*, averaged data with greater standard deviations may lead to a more uncertain interpretation than averaged data with smaller standard deviations due solely to the variation among the data points treated collectively). One recent study (2) utilized the method of treatment of averaged data in interpretating averaged pharmacological effect data for the cardiac glycoside digoxin.

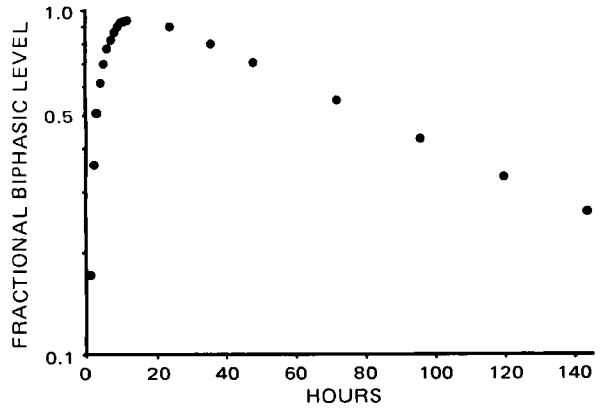

Figure 1—*Logarithm of the average of 17 individual ideal fractional biphasic levels as a function of time.*

Digoxin, a drug with a very low therapeutic ratio, recently was critically studied with respect to its possible varied bioavailability (3–6). Schoenwald (2) analyzed digoxin solely from averaged pharmacological effect data and suggested that his findings indicated enterohepatic recycling. Since the suggestion was totally based on averaged data, the intent of the present study was to evaluate the validity of the interpretation as to the prevalence of biliary recycling.

EXPERIMENTAL

Schoenwald's (2) method of treating the published pharmacological response data of Gold *et al.* (7) was followed. Gold *et al.* (7) studied the decrease in ventricular heart rate for 17 selected patients after administration of 1.2 mg of digoxin by either the oral or the intravenous route. The 17 subjects for the pharmacological effect study were selected from approximately 1000 adult patients. The patients selected had various common heart diseases, but "with few exceptions only those with a rapid ventricular rate during the period without digitalis" (7) were included. This restriction was intended to minimize the variation in heart rate sensitivity to digoxin.

From the individual data of their studies, Gold *et al.* (7) presented a graph of the average decrease in ventricular rate as a function of time. These averaged data are treated by Eqs. 1 and 2 to calculate the transformed pharmacological response intensity, f':

$$F_I = \frac{I}{I_{max}} \qquad \text{(Eq. 1)}$$

$$f' = \frac{F_I}{1 - F_I} \qquad \text{(Eq. 2)}$$

where I = intensity of effect, I_{max} = maximum possible intensity of effect, and F_I is the fraction of maximum intensity attainable. The maximum intensity attainable for the dose given is 59.97 beats/min[1].

Following Schoenwald's procedure, treatment of the data was carried out on the normalized pharmacological response intensity, f, as given in:

$$f = f'/f'_{max} \qquad \text{(Eq. 3)}$$

where f'_{max} is the maximum value of the f' observed within a collected study. The normalized averaged pharmacological response intensity following a single intravenous injection to the 17 patients described by Gold *et al.* (7) was fit to equations containing both bi- and triexponential terms by Schoenwald (2). Reciprocal weighting was used to provide a biexponential and a triexponential least-squares fit of the data as a function of time, and the following

[1] Due to a misprint in Ref. 2, a value of 51.3 beats/min was designated as I_{max}. However, 59.97 was, in fact, the value used in the calculation.

expressions with accompanying mean sum of squares (SS) were obtained:

$$f = 2.842e^{-0.0144t} - 3.134e^{-1.200t} \qquad SS = 1.25 \times 10^{-2} \quad \text{(Eq. 4)}$$

$$f = 1.708e^{-0.0086t} + 2.138e^{-0.073t} - 3.478e^{-0.500t} \qquad SS = 1.03 \times 10^{-2} \quad \text{(Eq. 5)}$$

Schoenwald (2) pointed out that the triexponential fit (Eq. 5) only slightly improves the fit compared to the biexponential fit (Eq. 4), as judged by the similar sum of squares values. In fact, if the error mean squares (EMS) of the two fits were calculated, where the sum of squares is corrected for the number of degrees of freedom in each fit as given in Eq. 6, the fits are almost equivalent ($EMS = 9.62 \times 10^{-4}$ and 9.36×10^{-4} for the bi- and triexponential fits, respectively):

$$EMS = \frac{\sum_{i=1}^{n} w_i(f_i - \hat{f}_i)^2}{(n - p)} \quad \text{(Eq. 6)}$$

where:

f_i = experimentally determined transformed pharmacological effect at time t_i
$\hat{f}_i$ = computer estimated value for f_i
w_i = weighting factor for each value of f_i
n = number of data points to be fit, in this case 17
p = number of parameters to be estimated by the equation (*i.e.*, four and six in the bi- and triexponential equations, respectively)

The minimal model consistent with the intravenous results was chosen, and the values calculated by Schoenwald were used in Eq. 4. An attempt was made to computer fit the normalized pharmacological response intensity data observed following oral administration of digoxin to the 17 patients in the study of Gold *et al.* (7). The normalized oral data were fit to both bi- and triexponential equations, using the value of 59.97 for I_{max} as already discussed. The corresponding value of f_{max} calculated for the oral data from Eqs. 1 and 2 is 1.232, since the largest decrease in ventricular heart rate noted by Gold *et al.* (7) following oral dosing was 33.1 beats/min.

The best fit for the oral data is given in Eq. 7 for the biexponential fit and in Eq. 8 for a triexponential fit:

$$f_{oral} = -1.26e^{-0.227t} + 1.18e^{-0.0107t} \qquad EMS = 1.15 \times 10^{-2} \quad \text{(Eq. 7)}$$

$$f_{oral} = -1.41e^{-0.188t} + 1.16e^{-0.0189t} + 0.207e^{-(2.32 \times 10^{-10})t} \qquad EMS = 1.20 \times 10^{-2} \quad \text{(Eq. 8)}$$

Note here that the comparison of EMS values again indicates that the bi- and triexponential fits are almost equivalent, although here the biexponential fit has a slightly lower value for the EMS than the triexponential fit. In addition, as would be expected, the biexponential parameters have smaller coefficients of variation than the triexponential parameters.

The third exponential term in Eq. 8 is so small that it may be considered negligible for all data points except those seen extremely early in the experiment. Thus, it seems appropriate to use the biexponential equations and the values given in Eq. 7 to simulate the oral data that would be randomly varied. This approach is consistent with the idea of using the minimal pharmacokinetic model as stated by Garrett (8): "The general operating rule in such pharmacokinetic analysis is to postulate the minimum number of compartments consistent with physiological reality."

One may question whether an appropriate oral fit of the data to a biexponential equation is valid when it is known that the intravenous data also are best described by a biexponential equation. The usually expected triexponential form for the oral data may degenerate to a biexponential equation when the absorption rate constant approaches a value equal to the sum of the exit rate constants from the peripheral compartment[2]. This may be the case with respect to the present data; however, irregularities in the apparent absorption profile preclude validation of this point without additional experiments.

The following discussion will be presented with respect to data manipulation using the parameters given in Eq. 7 for $I_{max} = 59.97$. The parameters are designated as Case I in Table I.

The constants in Case I were treated by random number multiplication of their standard deviations until a series of 17 individual constants were calculated having averages and standard deviations with values very close to the original constants and their standard deviations. Generally, this process was accomplished by multiplying a random number[3] by one- or twofold values of the given standard deviation values and adding that product to the given average value. Minor adjustments were made in selected randomly calculated constants to make the averages and standard deviations of the calculated constants equal to the values given in Table I, Case I.

Once 17 such sets of individual values for each parameter in Case I were developed, the parameters were randomly combined into 17 sets of A_1, A_2, r_1, and r_2, the constants in the biexponential equation, $f = A_1e^{-r_1t} + A_2e^{-r_2t}$. Each set of constants was then used to calculate f_i for this set of constants at 19 different time points utilized by Schoenwald (2), thereby providing ideal f values at 19 different times for 17 ideal individuals. The 17 ideal f values for each time point were then averaged, and individual standard deviations were calculated for each average.

To put the averaged ideal f values into a more "real" setting, the standard deviation for each averaged f multiplied by a random number was added to its averaged ideal f value. Three random number series were utilized: (*a*) $\mu = 0$, $\sigma = 0.5$; (*b*) $\mu = 0$, $\sigma = 1.0$; and (*c*) $\mu = 0$, $\sigma = 2.0$; μ is the mean and σ is the standard deviation. Thus, three new sets of averaged values were obtained; one series of values varied by $\sigma = 0.5$, one series of values varied by $\sigma = 1.0$, and one series of values varied by $\sigma = 2.0$. These series will be referred to as sigma 0.5, sigma 1, and sigma 2.

Calculations of intensity of effect for the series of averaged ideal f values and the three sigma series of f values were compared. For sigma 0.5, sigma 1, and the averaged ideal series, the differences between intensities at each time point from 1 to 48 hr inclusive ranged from 0.5 to 6.0 (average = 3) beats/min, with one "outlier"[4] variation of 11 beats/min.

A Loo–Riegelman (10) treatment, modified for application to the peripheral compartment (2), was then applied to the ideal data and the data from sigma 0.5, sigma 1, sigma 2, and Schoenwald's f values calculated from averaged data of Gold *et al.* (7). The values calculated by the Loo–Riegelman modification for the four series of f values were analyzed by plotting both the log fraction of the dose remaining to be absorbed *versus* time and the log absorption rate *versus* the midpoint of the time interval. A parallel treatment of parameters derived from the treatment of pharmacological response data (7) was also carried out utilizing $I_{max} = 51.3$ beats/min [the value reported by Schoenwald (2)] instead of the actual value, 59.97 beats/min, to test whether the results reported here may only have been due to the specific combination of random numbers used in the computer simulations.

RESULTS AND DISCUSSION

Figure 1 is a semilog plot of the average for each time point of the 17 individual ideal f values calculated from the randomly deviated biexponential parameters. Figure 2 is the same type of semilog plot for the following situations: (*a*) the f values for the Gold *et al.* (7) original averaged data, (*b*) the f values for sigma 0.5, (*c*) the f values for sigma 1, and (*d*) the f values for sigma 2. Corresponding plots of fraction remaining to be absorbed data are presented in Fig. 3.

All four calculations included negative values which could not be plotted on the logarithmic scale. The negative values are indicated by arrows on the abscissas of the plots in Fig. 3. A plot of the original data in this series as treated by Schoenwald (2) also demonstrated negative values, as did plots when $I_{max} = 51.3$ beats/min was used. Semilog plots of the absorption rate *versus* the midpoint time were too erratic to permit interpretation. Table I collates the

[2] R. A. Ronfeld and L. Z. Benet, to be published.

[3] All random numbers were from random number tables in the Appendix of Ref. 9.

[4] As defined by Table A-8e (Criteria for Testing for Extreme Mean) in Ref. 9, 11 beats/min is an outlier for hours 1–48 inclusive with P_{90}, and it is an outlier for hours 1–144 inclusive with P_{95}.

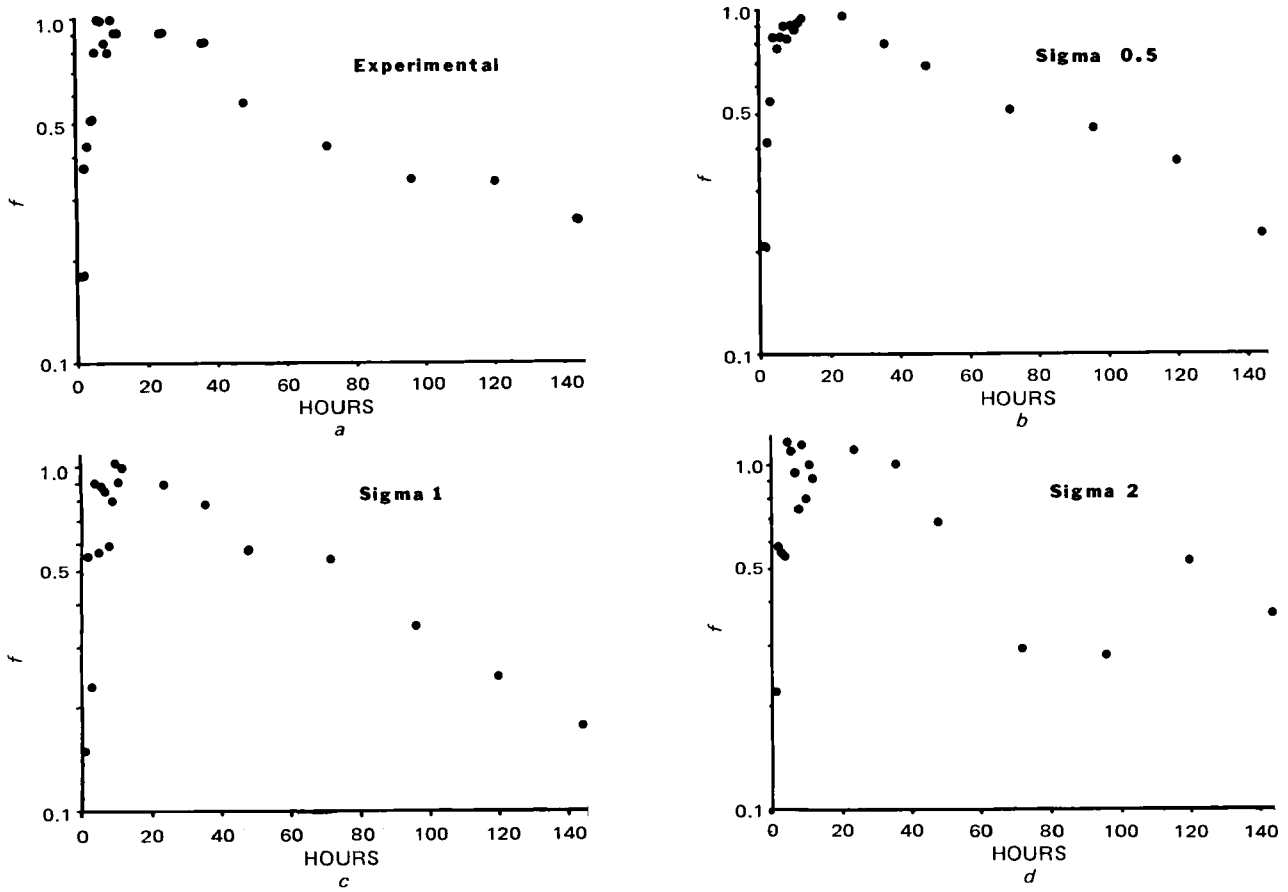

Figure 2—*Logarithm of fractional biphasic levels as a function of time for:* (a) *experimental data of Gold* et al. *(7),* (b) *sigma 0.5,* (c) *sigma 1, and* (d) *sigma 2.*

least-squares best-fit biexponential parameters with their corresponding standard deviations for the five semilog plots of pharmacological effect as a function of time presented in Figs. 1 and 2.

The graphs of the computer-generated data and the graph of the Gold *et al.* (7) data in Fig. 3 have similar general appearances. For example, all graphs have the increases and decreases of fraction remaining to be absorbed, which could be identified as enterohepatic recycling. The recycling aspect, however, was not included in the computer generation of data. For the computer-generated data, the increases and decreases in the fraction remaining to be absorbed are solely a function of the treatment of the ideal data. The treatment of ideal data here included only two factors: the averaging of individual ideal values and the random alteration of an average as a function of the standard deviation of that average. Thus, enterohepatic recycling is not a necessary interpretation of any of the data presented in Fig. 2.

No variations in the intravenous parameters used, *i.e.*, the parameters given in Eq. 4, were carried out since fraction remaining to be absorbed plots for oral ideal data did not show erratic changes in slope, even though these oral data were generated using values already different than the intravenous best-fit values. Further variance of the intravenous parameters should not markedly affect the plots seen in Fig. 3.

Perrier and Gibaldi (11) noted that fraction remaining to be absorbed plots can present a good estimate of the absorption rate when there is no lag time, there is some uniformity in absorption (*i.e.*, zero order, first order), and there is no parallel nonabsorptive loss of drug from the absorption site. The erratic plots of the fraction remaining to be absorbed for the Gold *et al.* (7) data, sigma 0.5, sigma 1, and sigma 2 demonstrate that the necessary underlying assumptions were not met by the data in any of these studies.

Negative fractions remaining to be absorbed were seen in three of the four sigma plots for I_{max} = 59.97 and also in the plot of the Gold *et al.* (7) data. A greater number of negative values were seen in three of the four sigma plots for the incorrect I_{max} = 51.3. Thus, negative values were considered to reflect the sensitivity of the fraction remaining to be absorbed function to nonideal variations in the data.

The protocol of this study included an *ab initio* correlation of the standard deviation of the least-squares-fitted grouped experimental data with the standard deviation of the arithmetic averages of groups of individual parameters. The data in Table I show this assumption to be realistic. Here, the range of values determined for the coefficients of the exponentials (*i.e.*, A_1 and A_2) from Case II to Case V is actually less than the range of values for the standard deviations of the parameters. With the exception of Case IV, the range of the values for the rate constant r_2 is less than the range of values for the respective standard deviations. With the exception of Case IV, the range of values for r_1 is within 14% of the range of values for the respective standard deviations. The comparison of the five cases presented in Table I is actually a comparison of their standard deviations.

Case II, the averaged ideal data, has very small standard deviations for all parameters; this should be so in the ideal situation. Case I, based on the Gold *et al.* (7) data, is true experimental pharmacological effect data. Cases III, IV, and V are based on random

Table I—Parameters with Standard Deviations for the Least-Squares Exponential Fit of: Case I, Data of Gold *et al.* (7); Case II, Averaged Values of 17 Ideal Data Sets; Case III, Sigma 0.5; Case IV, Sigma 1; and Case V, Sigma 2

Case	A_1 (*SD*)	r_1 (*SD*)	A_2 (*SD*)	r_2 (*SD*)
I	−1.26	0.227	1.18	0.0107
	(0.10)	(0.048)	(0.10)	(0.0013)
II	−1.22	0.212	1.18	0.0107
	(0.01)	(0.004)	(0.01)	(0.0014)
III	−1.18	0.286	1.12	0.0101
	(0.04)	(0.029)	(0.04)	(0.0007)
IV	−1.30	0.155	1.31	0.0139
	(0.26)	(0.051)	(0.27)	(0.0031)
V	−1.27	0.354	1.11	0.0108
	(0.13)	(0.128)	(0.15)	(0.0026)

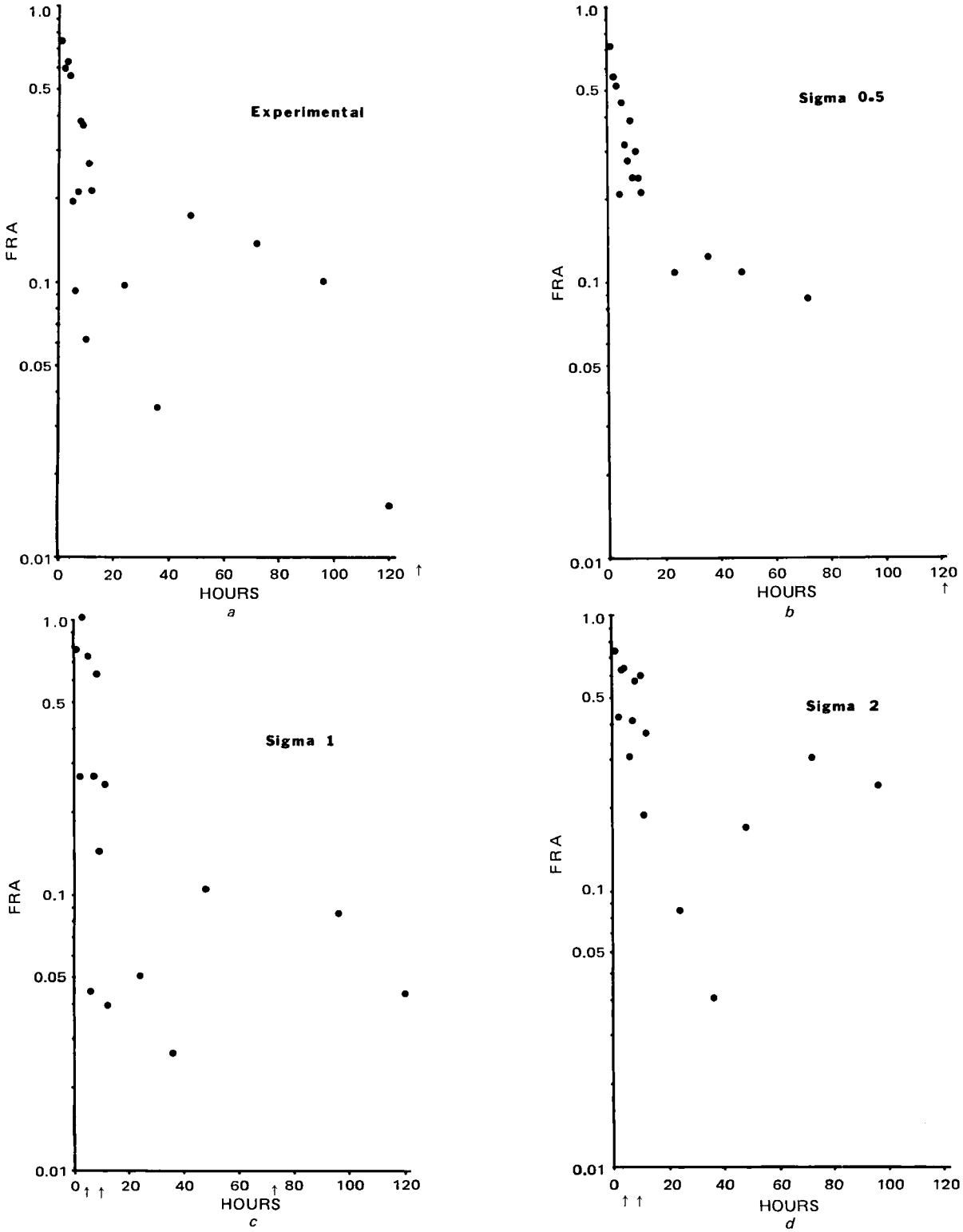

Figure 3—*Logarithm of fraction remaining to be absorbed (FRA) as a function of time for:* (a) *experimental data of Gold* et al. *(7),* (b) *sigma 0.5,* (c) *sigma 1, and* (d) *sigma 2. Arrows along the abscissa indicate negative values.*

alterations of Case II data as described under *Experimental.* The standard deviations in Case V are much greater than those in Case I, and the standard deviations in Case III are all smaller than the values for Case I. The standard deviations in Case IV are all greater than the standard deviations in Case I, although they are closer in value to the values for Case I than are the values for Case V. For the theoretical development cited here, the data from these five cases demonstrate that a sigma between 0.5 and 1.0 would give similar standard deviations as are given for the true experimental data of Case I. It is the similarity of standard deviations of similar parameters that permits this comparison of the theoretically determined data with the experimentally determined data.

Mathematical models, whether they be empirically or theoretically derived, may be misleading when obtained from averaged data since they represent averaged data and not necessarily any of the individual data. An example of this type was reported (12) in which two subjects were found to absorb drug according to a single first-order process; however, first-order rate constants differed considerably, *i.e.*, 0.0693 and 0.00866 min^{-1}. The averaged data for these two subjects could best be fit by a biexponential relationship.

This finding would artifactually lead one into concluding that absorption was occurring by two consecutive first-order processes. It should not be concluded that averaging of individual data is inappropriate, but its primary function should be to reduce the variance so that an accurate theoretical interpretation becomes more feasible.

CONCLUSION

Minor random deviations from averages of ideal data have been shown to be capable of producing results similar to those expected from enterohepatic recycling when the ideal data exclude that phenomenon. Thus, the definition of enterohepatic recycling from real pharmacological data probably requires an experimental study before confirmation can be made.

REFERENCES

(1) R. K. Nayak, R. D. Smyth, J. H. Chamberlain, A. Polk, A. F. DeLong, T. Herczog, P. B. Chemburkar, R. S. Joslin, and N. H. Reavey-Cantwell, *J. Pharmacokinet. Biopharm.*, **2**, 107(1974).

(2) R. D. Schoenwald, *J. Pharm. Sci.*, **63**, 411(1974).

(3) A. G. Hibble, P. Isaac, and D. G. Grahame-Smith, *Lancet*, **2**, 90(1972).

(4) J. Lindenbaum, M. H. Mellow, M. O. Blackstone, and V. P. Butler, Jr., *N. Engl. J. Med.*, **285**, 1344(1971).

(5) T. R. D. Shaw, M. R. Howard, and J. Hamer, *Lancet*, **2**, 303(1972).

(6) J. G. Wagner, M. Christensen, E. Sakmar, D. Blair, J. D. Yates, P. W. Willis, III, A. J. Sedman, and R. G. Stoll, *J. Amer. Med. Ass.*, **224**, 199(1973).

(7) H. Gold, M. Cattell, T. Greiner, L. W. Hanlon, N. T. Kwit, W. Modell, E. Cotlove, J. Benton, and H. L. Otto, *J. Pharmacol. Exp. Ther.*, **109**, 45(1953).

(8) E. R. Garrett, *J. Pharmacokinet. Biopharm.*, **1**, 341(1973).

(9) W. J. Dixon and F. J. Massey, Jr., "Introduction to Statistical Analysis," 2nd ed., McGraw-Hill, New York, N.Y., 1957, pp. 366–380, 457–461.

(10) J. C. K. Loo and S. Riegelman, *J. Pharm. Sci.*, **57**, 918(1968).

(11) D. Perrier and M. Gibaldi, *ibid.*, **62**, 225(1973).

(12) G. Levy and L. E. Hollister, *ibid.*, **53**, 1446(1964).

ACKNOWLEDGMENTS AND ADDRESSES

Received December 16, 1974, from the *Department of Pharmacy, School of Pharmacy, University of California, San Francisco, CA 94143*

Accepted for publication May 7, 1975.

Presented in part at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, New Orleans meeting, November 1974.

Supported in part by Project Grant GM 16496 from the Institute of General Medical Sciences, National Institutes of Health. Ms. Gustafson received support from Training Grant in Pharmaceutical Chemistry GM 00728 from the National Institutes of Health. Ms. Gustafson also received support as a Fellow of the American Foundation for Pharmaceutical Education during 1974–1975.

* Present address: Alcon Laboratories, Fort Worth, TX 76101

ˣ To whom inquiries should be directed.

Anaerobic Photodecomposition of an Acridan Drug through Energy Transfer

GEORGE A. DIGENIS ˣ, SHUKRI SHAKHSHIR *, M. A. MIYAMOTO ‡, and H. B. KOSTENBAUDER

Abstract □ Anaerobic irradiation of 2-chloro-9-(3-dimethylaminopropyl)acridan phosphate (1:1) (III*a*) with filtered visible light (300–400 nm) resulted in its quantitative conversion to its acridine derivative IV. Photoproduct IV exerted significant product catalysis on the reaction rate at concentrations of 5×10^{-6} *M*. The anaerobic photodecomposition of III*a* to IV was catalyzed by the monosodium salt of riboflavin 5-phosphate (I*a*). Loss of I*a* was insignificant relative to that of III*a*, and reagents known to serve as quenchers of the triplet state of I*a* retarded the reaction. Fluorescence spectra of I*a* in the presence of III*a* and 1×10^{-4} *M* KI indicated no quenching of the I*a* singlet excited state. No deuterium isotope effect was noticed when III*a* and its deuterated derivative III*b* were subjected to anaerobic photodecomposition in the presence of I*a*. It is suggested that the anaerobic photodecomposition of III*a* by visible light in the presence of I*a* proceeds *via* a triplet–triplet energy transfer from I*a* to III*a*.

Keyphrases □ Photodecomposition, anaerobic—acridan derivative converted to acridine derivative in visible light through energy transfer, riboflavin 5-phosphate as catalyst □ Acridans—2-chloro-9-(3-dimethylaminopropyl)acridan phosphate converted to acridine derivative in visible light through energy transfer □ 2-Chloro-9-(3-dimethylaminopropyl)acridan phosphate—anaerobic photodecomposition to acridine derivative

Flavin coenzymes participate in various enzymatic dehydrogenation reactions. The overall dehydrogenation can be represented by Scheme I, where I*a* and A are the oxidized forms of riboflavin and substrate, respectively, and I*b* and AH_2 represent the reduced forms of the coenzyme and substrate, respectively (R = ribityl group).

Several classes of such reactions are known including: (*a*) alcohol dehydrogenation (glucose oxidase and lactate dehydrogenase); (*b*) amine dehydrogenation, commonly referred to as oxidative deamination (amino acid oxidases); (*c*) dehydrogenation alpha and beta to a carbonyl (succinate dehydrogenase); and (*d*) dihydronicotinamide dehydrogenation (NADH dehydrogenase).

BACKGROUND

Based on model studies, a mechanism was proposed for amine and alcohol dehydrogenations. This mechanism involves the formation of a carbanion-like intermediate (II, Scheme II) formed after the addition of an alcohol or amine at the C_{4a}-position of the flavin (1).