(20) T. Kametani and K. Fukumoto, *ibid.*, **1968,** 112.
(21) R. Mirza, *ibid.*, **1957,** 4400.
(22) A. R. Battersby, D. M. Foulkes, and R. Binks, *ibid.*, **1965,** 3323.
(23) M. Tomita and J. Kunitomo, *J. Pharm. Soc. Japan*, **80,** 1238 (1960).
(24) A. R. Battersby, P. Böhler, M. H. G. Munro, and R. Ramage, *Chem. Commun.*, **1969,** 1066.
(25) A. R. Battersby, R. Binks, and B. J. T. Harper, *J. Chem. Soc.*, **1962,** 3534.
(26) Huang-Minlon, *Chem. Ber.*, **69,** 1737(1963).
(27) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry, Volume I: Alkaloids," Holden-Day, San Francisco, Calif., 1964, p. 181.

ACKNOWLEDGMENTS AND ADDRESSES

Received January 4, 1971, from the *Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, CA 94122*

Accepted for publication February 19, 1971.

This work was supported by a research grant (MH-03487) from the National Institute of Mental Health, Bethesda, Md.

The authors are indebted to Dr. R. H. F. Manske for a sample of authentic coreximine.

Determination of Time Course of *In Vivo* Pharmacological Effects from *In Vitro* Drug-Release Testing

VICTOR F. SMOLEN

Abstract □ A mathematical approach is described which permits the computation of the temporal variation of pharmacological response intensity from the results of *in vitro* drug-release testing that linearly correlate or can otherwise be functionally related to *in vivo* drug availability. The development of optimized drug-release tests is suggested. Through the application of the described approach, such tests would permit the predictive estimation from *in vitro* data of the dependency of the pharmacological behavior of appropriate drugs on formulation factors. The dependency of the time course of pharmacological effects of a mydriatic drug on the drug-release characteristics of several types of dosage formulations is graphically shown.

Keyphrases □ Pharmacological response intensity correlation—*in vivo* drug availability □ Bioavailability time course determination—pharmacological data □ Drug-release tests, *in vitro*—optimization □ Absorption analysis, drug—theoretical considerations

It is well known that formulation factors can markedly influence the therapeutic activity and toxicity of pharmaceutical products. The advent of ever increasingly more effective and potent drugs emphasizes the imperative of concomitantly developing pharmaceutical dosage forms for optimal effectiveness, safety, and reliability. It is seldom practical to perform the exhaustive *in vivo* human testing of drug formulations that may be necessary to obtain a formulation possessing optimal drug-release characteristics and, therefore, the pharmacological response behavior that may be precisely desired. It is presently proposed that human testing of drug formulations for this purpose could be minimized if, instead of directly testing the many formulations themselves, *in vivo* studies are performed to establish "optimized *in vitro* drug-release tests" capable of predicting the *in vivo* bioavailability behavior of the drug as a function of formulation factors. Presently employed *in vitro* drug-release tests are generally inadequate for this purpose.

Morrison and Campbell (1), in their 1965 review article, concluded that: "It is apparent that *in-vitro* disintegration tests, as presently used, have certain inherent faults, and eventually must be modified or replaced by more critical tests of physiological availability." Aside from discrediting disintegration tests and replacing them by dissolution tests, apparently only limited progress has been reported since this time with regard to the modification and further development of *in vitro* tests that can reflect *in vivo* drug behavior.

It is often stated that the problem is quite complicated because a correlation of *in vivo* to *in vitro* release that is found with a particular test for a particular drug in a particular dosage form may not exist if another drug is substituted or the formulation altered. Few attempts have been reported to determine the limits to which this may be the case. The *in vitro* to *in vivo* drug availability correlations that have been found have always been after the fact. Quantitative correlations for a spectrum of dosage forms were well exemplified by the work of Cressman *et al.* (2) for one drug. The correlations, however, were of a single-point nature, *e.g.*, time for 50% of the drug to be released from the dosage form was correlated with the 50% release time *in vivo*.

In a previous report (3), a multiple-point quantitative *in vitro* to *in vivo* correlation procedure was recommended for providing criteria on which to gauge the adequacy of an *in vitro* drug-release test in reflecting *in vivo* drug availability. Also discussed was a quantitative approach to the development of *in vitro* drug-release tests, the expected limitations of such tests, and the manner in which the approach to the limits of applicability of a given test may be discerned. Levy and Hollister (4) and, more recently, Gibaldi and Weintraub (5), demonstrated that the required multiple-point linear correlations of amounts of drug released *in vitro* to the amounts of drug systemically absorbed *in vivo* (A_t) at corresponding times can be well accomplished for different dosage forms using the same dissolution test. The correlations can be further improved through suitably adjusting such test conditions

as rate of agitation, geometry of the apparatus, composition of the release media, solubility volume of a sink, and permeability of membranous sources.

The author suggested (3) that the methods of operation research (3, 6) can be applied to optimize these conditions and, therefore, design an *in vitro* release test to function optimally in reflecting *in vivo* drug availability for a spectrum of drug formulations possessing varying drug-release characteristics. The experimentation could be designed so that the predicted *in vivo* behavior of the drug computed from *in vitro* data would be within predetermined statistical confidence intervals. Briefly, the author's suggested approach may be further understood from considering that if an *in vitro* drug-release test perfectly simulates *in vivo* drug availability from several different types of dosage forms, all points on a plot of quantities of drug absorbed *in vivo versus* quantities released at corresponding times *in vitro* would ideally fall upon a line having a slope of unity and an intercept equal to zero. The linear correlation coefficient would also be computed as unity. The deviations of the slope and intercept of least-squares regression lines and linear correlation coefficients from these ideal values may be utilized as criteria on which to gauge the adequacy of an *in vitro* drug-release test at any stage of its development. A systematic repetition of the test conducted under varying test conditions will provide the data necessary for the expression of these criteria as empirical functions of the test conditions. These functions may then be utilized to compute the set of test conditions that are optimal in minimizing the deviations and, therefore, in reflecting the *in vivo* drug-availability behavior of the dosage forms.

In anticipation of the implementation of this approach resulting in the development of *in vitro* drug-release tests possessing a predictive capability with regard to the influence of formulation factors on drug bioavailability, it is the purpose of this communication to describe a means by which such tests could also be utilized with appropriate drugs to predict quantitatively the time course of their pharmacological activity. When the method to be described is applicable, pharmacological response characteristics such as onset and duration of action, maximum intensity of effect, time of maximum effect, and rates of dissipation of the response intensity could be estimated from *in vitro* drug-release testing. Obviously, such results may be expected, within the limits of biological variation, to apply specifically only to the subject(s) donating the *in vivo* data which the *in vitro* release test has been designed to simulate optimally.

THEORETICAL

Pharmacological Method of Drug-Absorption Analysis—A mathematical approach was developed by the author which provides the necessary link between the quantity of drug cumulatively absorbed (A_t) *in vivo* from a site of administration at any time and the observed intensity of the pharmacological response at the corresponding time following dosing. The method permits the determination of the time course of drug bioavailability to be determined entirely from pharmacological data. The method is applicable to any route of administration and any type of dosage form. It has been successfully applied to drugs administered by parenteral, oral, and ophthalmic routes. The confirmation of the applicability of the method in its simplest form, as presented here, can be directly accomplished from pharmacological data alone. The details of this procedure are presented elsewhere (7, 8).

A reverse application of the pharmacological method of drug-absorption analysis permits the computation of the time course of variation of the pharmacological response intensity from the temporal variation of A_t. Therefore, if A_t values for a spectrum of dosage formulations are linearly correlated—in the manner reported by Gibaldi and Weintraub (5)—or functionally related—in the manner reported by Levy and Hollister (4)—to analogous values, R_t, obtained from the results of *in vitro* drug-release testing, the time course of pharmacological activity can be computed from *in vitro* data.

Assuming the biokinetics of the drug can be represented by a linear compartment model, the necessary mathematical relationships can be formulated as shown here. The quantity of drug in the biophase, Q_B, following a rapid intravenous dose, D, is given at any time by Eq. 1, where A_i and m_i are equation parameters and n denotes the number of body compartments. The biophase is defined as a hypothetical body compartment containing the sites of drug action:

$$Q_B = D \sum_{i=1}^{m} A_i e^{-m_i t} \qquad \text{(Eq. 1)}$$

An intravenous dose–effect curve may be constructed by plotting the intensity of pharmacological response, I, observed at an arbitrarily chosen time, t_r, as a function of dose. The functional relationship, $D = f(I_{t_r})$, between the dose and the intensity of response is graphically provided by the resulting curve. At a time t_r, Eq. 1 may be rewritten as Eq. 2, where β_{t_r} is constant:

$$Q_{Bt_r} = f(I_{t_r}) \sum_{i=1}^{m} A_i e^{-m_i t_r} = f(I_{t_r})\beta_{t_r} \qquad \text{(Eq. 2)}$$

Assuming Eq. 2 to be valid at times other than t_r, it can be rewritten as Eq. 3 to provide a general relationship between the observed intensity of response and the quantity of drug in the biophase:

$$Q_B = f(I)\beta_{t_r} \qquad \text{(Eq. 3)}$$

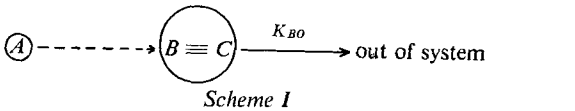
Equation 3 permits values of $f(I)$, which are directly proportional or equal to the quantity of drug in the biophase, to be obtained for any observed value of I directly from the corresponding value of the abscissa of the dose–effect curve irrespective of the time of observation, dose, and mode of administration. This is the case, provided, as generally assumed (9–11), that the pharmacological effect is an instantaneous, equilibrium, nonhysteretic result of the quantity of drug in the biophase at any time. Tests for the validity of this assumption as well as for the adequacy of a linear compartment model were described previously (7, 8).

Complete details of the pharmacological method of drug absorption analysis are presented in other reports. Briefly, however, the application of the pharmacological method routinely involves:

1. Experimental determination of the time course of pharmacological response intensity for several rapidly administered intravenous doses.
2. Construction of an intravenous dose–effect curve.
3. Transformation of observed I values, *via* the dose–effect curve, into $f(I)$ values normalized for dose.
4. Selection of a linear compartment model to simulate the biokinetic behavior of the system. The choice of the model, in addition to other criteria, is based upon the results of least-squares fits of sums of exponentials to the $f(I)/D$ *versus* time data.
5. Confirmation of the applicability of the method and the adequacy of the compartment model.

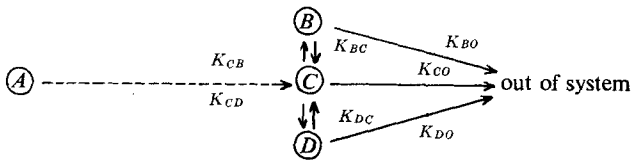
Upon the determination of a compartment model, suitable expressions for A_t as a function of $Q_B = \beta_{t_r} f(I)$ can be derived, which allow the cumulative amounts of drug systemically absorbed to be computed from the temporal variation of I observed following dosing by any route of administration. Similar results are also calculable for cases of, for example, percutaneous or transcorneal drug absorption directly into the biophase following topical application of the drug to the skin or eye, respectively, where it produces a local effect.

Equations 4 and 5 exemplify equations appropriate for the calculation of A_t for drugs whose biokinetic behavior can be described by single- and three-compartment models. The models are diagrammed in Schemes I and II, where C represents the central, plasma pool, compartment; B represents the biophase; D represents a pharmacologically inert depot compartment; the transfer constants are symbolized by the K_{ij}'s; and the site of administration is denoted by A. The calculation of A_t is independent of any assumptions concerning the nature of the kinetics and mechanisms of absorption. The single-compartment model treats compartments B and C as being kinetically indistinguishable and behaving as a single compartment, *i.e.*, $B \equiv C$.



Scheme I

$$A_t = \beta_{t_r}\left[f(I) + K_{BO}\int_0^t f(I)\,dt \right] \qquad \text{(Eq. 4)}$$



Scheme II

$$A_t = \beta_{t_r}\left[R\frac{d^2f(I)}{dt^2} + S\frac{df(I)}{dt} + Tf(I) + U\int_0^t f(I)\,dt \right] \qquad \text{(Eq. 5)}$$

where:

$$K_{CB}K_{DC}R = 1$$

$$\begin{aligned} K_{CB}K_{DC}S &= K_{BO} + K_{BC} + K_{DO} + K_{DC} + K_{CO} + K_{CB} + K_{CD} \\ &= m_1 + m_2 + m_3 \end{aligned}$$

$$\begin{aligned} K_{CB}K_{DC}T &= K_{DC}K_{CB} + K_{DC}K_{BC} + K_{DC}K_{BO} + K_{BC}K_{CO} \\ &\quad + K_{BC}K_{CD} + K_{BO}K_{CO} + K_{BO}K_{CB} + K_{BO}K_{CD} \\ &\quad + K_{DO}K_{BC} + K_{DO}K_{BO} + K_{DO}K_{CO} + K_{DO}K_{CB} \\ &\quad + K_{DO}K_{CD} + K_{CO}K_{DC} \\ &= m_1m_2 + m_2m_3 + m_1m_3 \end{aligned}$$

$$\begin{aligned} K_{CB}K_{DC}U &= K_{DC}K_{BC}K_{CO} + K_{DC}K_{BO}K_{CO} + K_{BC}K_{DO}K_{CO} \\ &\quad + K_{BO}K_{DO}K_{CO} + K_{BC}K_{DO}K_{DC} + K_{BO}K_{DO}K_{CO} \\ &\quad + K_DK_{BO}K_{CB} + K_{BO}K_{DO}K_{CB} \\ &= m_1m_2m_3 \end{aligned}$$

It is apparent that Eq. 4 is very similar to the expression made familiar by Wagner and Nelson (12). However, its applicability is extended to the utilization of pharmacological data in the calculation of A_t.

Values of A_t are obtained from $f(I)$ values through the numerical solution of Eqs. 3 and 4. For the purpose of relating *in vivo* pharmacological behavior to results of *in vitro* drug-release tests, it is necessary to solve such equations to yield $f(I)$, or Q_B, in terms of A_t. The solution of Eqs. 4 and 5 for Q_B can be accomplished readily through the use of Laplace transforms to yield Eqs. 6 and 7. Values of Q_B are directly proportional (Eq. 3) or equal (when t_r can be chosen as zero) to values of $f(I)$:

$$Q_B = A_t - K_{BO}e^{-K_{BO}t}\int_0^t A_te^{K_{BO}t}\,dt \qquad \text{(Eq. 6)}$$

$$\begin{aligned} Q_B = \frac{K_{CB}K_{DC}}{(m_1 - m_2)(m_2 - m_3)(m_3 - m_1)}\Bigg[& m_1(m_2 - m_3)e^{-m_1t} \times \int_0^t A_te^{m_1t}\,dt + m_2(m_3 - m_1)e^{-m_2t}\int_0^t A_te^{m_2t}\,dt + \\ & m_3(m_1 - m_2)e^{-m_3t}\int_0^t A_te^{m_3t}\,dt \Bigg] \end{aligned} \qquad \text{(Eq. 7)}$$

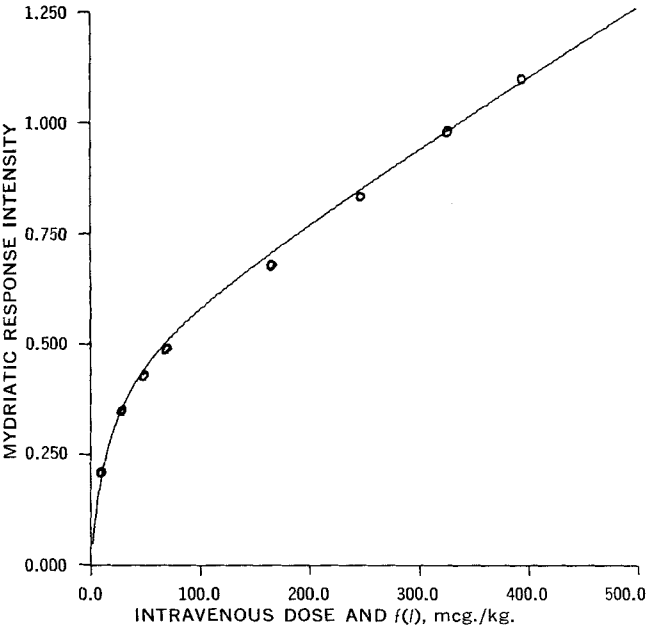


Figure 1—*Intravenous dose–effect curve for tropicamide illustrating the relationship of the mydriatic response intensity,* I, *to the dose and the quantity of drug,* $Q_B = f(I)$, *in the biophase.*

Assuming A_t can be directly or otherwise related to values of R_t, Eqs. 6 and 7 permit the calculation of $f(I)$ from the results of *in vitro* drug-release testing. The $f(I)$ values can be transformed graphically, *via* the dose–effect curve, or analytically to corresponding values of I. In this manner, the time course of pharmacological response intensity *in vivo* can be constructed from a succession of R_t values. The temporal variation of drug blood levels can be computed in a similar manner. For a single-compartment model, blood levels may be computed using Eq. 6 in its present form since the biophase and central compartments are identical and are equal to Q_B. For the three-compartment example, the level of drug in compartment C can be obtained from Eq. 8. The values of $f(I)$ for use in Eq. 8 are computed from A_t values using Eq. 7:

$$Q_C = \frac{\beta_{t_r}}{K_{CB}}\left[\frac{df(I)}{dt} + (K_{BO} + K_{BC})f(I) \right] \qquad \text{(Eq. 8)}$$

METHODS AND MATERIALS

The intravenous dose–effect curve, shown in Fig. 1, was determined using rabbits. Each point represents the average of a minimum of four replications on different animals. The method em-

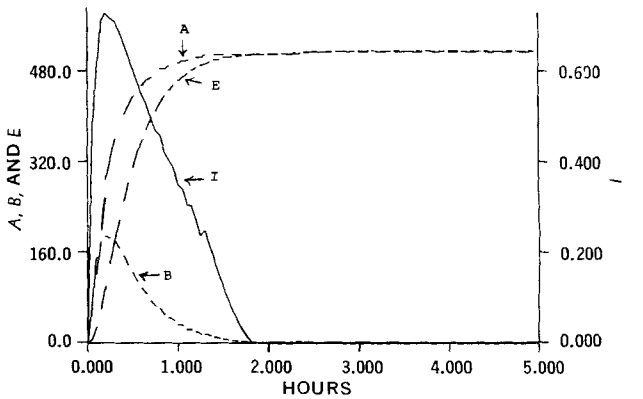


Figure 2—*Computerized plot of the time course of variation of the cumulative amount of drug excreted* (E), *the amount in the biophase* (B), *and the intensity of pharmacological response* (I), *computed from the cumulative amounts of drug absorbed* (A) *following dosing with a hypothetical fast-release dosage form.*

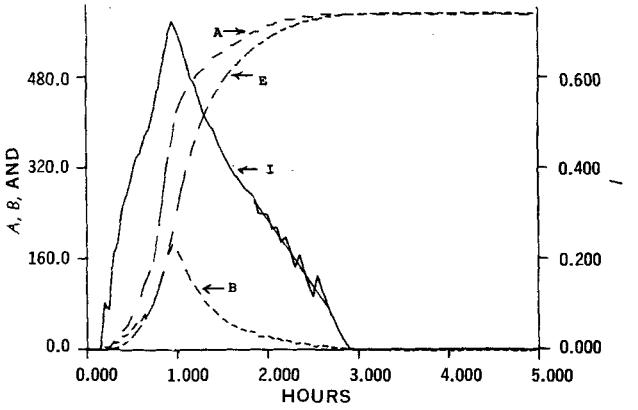


Figure 3—*Computerized plot of the time course of variation of the cumulative amount of drug excreted* (E), *the amount in the biophase* (B), *and the intensity of pharmacological response* (I), *computed from the cumulative amounts of drug absorbed* (A) *following dosing with a hypothetical slow-release dosage form.*

ployed for the measurement of pupillary diameters is presented elsewhere (8).

FORTRAN IV computer programs were prepared, and all calculations were performed with the aid of a CDC 6500 digital computer.

Figures 1–6 were constructed by a model 563 Calcomp Digital Incremental Plotter[1]. The jagged appearance of some curves is characteristic of the plotter.

RESULTS AND DISCUSSION

The dose–effect relationship for the mydriatic drug tropicamide was chosen to exemplify the theoretical considerations presented here. The confirmation of the applicability of the pharmacological method of drug absorption analysis to tropicamide was reported previously (8). The biokinetic behavior of this drug also has been determined to be well approximated by a single-compartment model in which the biophase and central systemic compartments are kinetically indistinguishable.

Figure 1 presents a plot of experimentally observed maximum mydriatic response intensities following the intravenous administration of the corresponding dose plotted on the abscissa. As discussed, the quantity of drug in the biophase, which in this case is identical to the central systemic compartment, can be obtained from observed mydriatic response intensities through the use of the dose–effect curve in a manner of a calibration curve. Alternative to the graphical estimation of the quantity of drug in the biophase, the curve in Fig. 1 may be described analytically by Eq. 9. The parameters, α = 1.0, K = 17.19 mcg./kg., R = 0.470 mcg./kg., and $P = 1.57 \times 10^{-3}$ kg./mcg., have biophysical significance as an intrinsic activity, drug–receptor association affinity constant, and density of receptor sites, respectively. The constant P has some connotation both as a distribution coefficient and an intrinsic activity. The manner in which these constants were evaluated was reported previously (8). The constant, β_{t_r}, has a value of unity, which is consistent in this case with the kinetic indistinguishability of the biophase and the central systemic compartment:

$$Q_B = \frac{\beta_{t_r}(I - \alpha R - KP) + [\beta(\alpha R + KP - I) + 4\beta^2 KPI]^{1/2}}{2\beta_{t_r}{}^2 p} \qquad \text{(Eq. 9)}$$

Transformation of observed values of I into corresponding values of Q_B can be accomplished directly through the use of the dose–effect curve or the application of an empirical equation fitted to the curve. No assumptions are necessary regarding the mechanism of the drug–receptor interaction. It is also not required to assign any physical significance to the parameters that would appear in an equation resulting from an empirical fit to the dose–effect curve.

The curves in Figs. 2–6 graphically exemplify the relationships between A_t, Q_B, I, and the quantity of drug, E, eliminated from the one-compartment system appropriate to describing the behavior of tropicamide. The curves were constructed using hypothetical values of A_t such as would be expected to be obtained from the results of optimized *in vitro* drug-release testing of dosage forms possessing the various drug-release properties indicated in the figures. The plotted A_t values were used for the computation of the remaining curves shown in each figure. Equations 6 and 9 were applied to

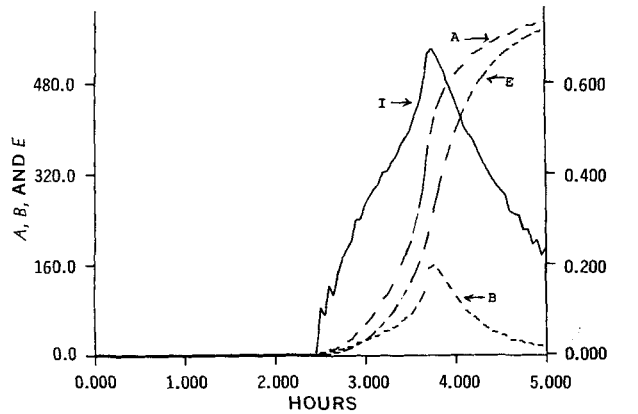


Figure 4—*Computerized plot of the time course of variation of the cumulative amount of drug excreted* (E), *the amount in the biophase* (B), *and the intensity of pharmacological response* (I), *computed from the cumulative amounts of drug absorbed* (A) *following dosing with a hypothetical enteric-coated oral dosage form.*

[1] California Computer Products, Inc., Anaheim, Calif.

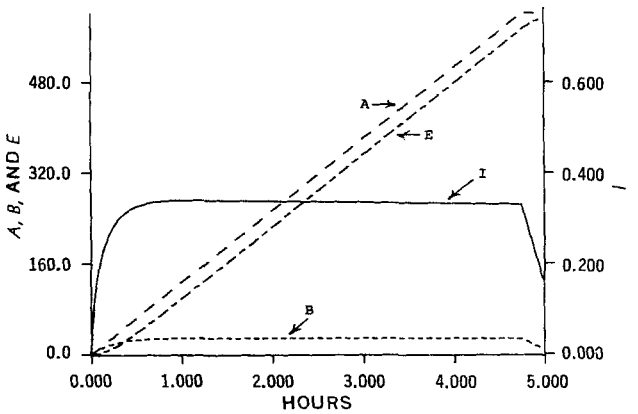


Figure 5—*Computerized plot of the time course of variation of the cumulative amount of drug excreted* (E), *the amount in the biophase* (B), *and the intensity of pharmacological response* (I), *computed from the cumulative amounts of drug absorbed* (A) *following dosing with a hypothetical zero-order sustained-release dosage form.*

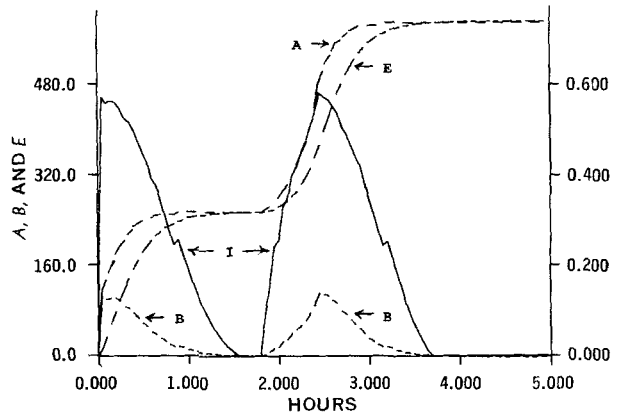


Figure 6—*Computerized plot of the time course of variation of the cumulative amount of drug excreted* (E), *the amount in the biophase* (B), *and the intensity of pharmacological response* (I), *computed from the cumulative amounts of drug absorbed* (A) *following dosing with a hypothetical repeat-action oral dosage form.*

compute Q_B and I, respectively. Values of E were simply obtained as the differences between computed values of Q_B and their corresponding values of A_t.

SUMMARY AND CONCLUSIONS

An approach is suggested for the development of "optimized *in vitro* drug-release tests" having a maximum capability of predicting *in vivo* drug bioavailability from dosage forms as a function of formulation factors. Such tests, appropriately applied with caution, could minimize the extent of human testing required to develop drug products with optimal *in vivo* drug-release characteristics. It is further demonstrated that with appropriate drugs for which a continuously graded pharmacological response intensity is observable, the results of optimized drug-release testing can be utilized to compute the time course of pharmacological activity.

The development of such a capability represents the ultimate in *in vitro* drug-release testing that can be sought. However, because of practical considerations such as the large magnitudes of intersubject and intrasubject variation commonly observed with *in vivo* data and the large amount of experimentation that may be necessary to develop optimized tests providing *in vivo* correlations within acceptable statistical limits, *in vitro* drug-release tests may remain constrained in their applicability. An intrinsic limitation is their applicability only to those cases where the systemic availability of the drug is rate limited by its release from the dosage form (3). Obviously, the development and subsequent application of *in vitro* drug-release tests should be undertaken with an awareness of such limitations. However, the inclusion of an appropriate membrane (13) as a permselective barrier in the *in vitro* drug-release testing apparatus could ultimately allow this latter serious limitation to be surmounted.

REFERENCES

(1) A. B. Morrison and J. A. Campbell, *J. Pharm. Sci.*, **54,** 1(1965).

(2) W. A. Cressman, C. A. Janicki, P. C. Johnson, J. T. Doluisio, and G. A. Braun, *ibid.*, **58,** 12(1969).

(3) V. F. Smolen, *Hosp. Pharm.*, **4,** 12(1969).

(4) G. Levy and L. E. Hollister, *J. Pharm. Sci.*, **53,** 1451(1964).

(5) M. Gibaldi and H. Weintraub, *ibid.*, **59,** 725(1970).

(6) D. J. Wilde and C. S. Beightler, "Foundations of Optimization," Prentice Hall, Englewood Cliffs, N. J., 1967, p. 1.

(7) V. F. Smolen, *J. Pharm. Sci.*, **60,** 354(1971).

(8) V. F. Smolen and R. D. Schoenwald, *ibid.*, **60,** 96(1971).

(9) G. Levy, *Clin. Pharmacol. Ther.*, **7,** 362(1966).

(10) J. G. Wagner, *J. Theoret. Biol.*, **20,** 173(1961).

(11) E. R. Garrett, A. J. Agren, and H. J. Lambert, *Int. J. Clin. Pharm.*, **1,** 1(1967).

(12) J. G. Wagner and E. Nelson, *J. Pharm. Sci.*, **53,** 1392(1964).

(13) V. F. Smolen and D. E. Hagman, to be published.

ACKNOWLEDGMENTS AND ADDRESSES

Received August 10, 1970, from the *Biophysical Pharmaceutics Area, Department of Industrial and Physical Pharmacy, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, IN 47907*

Accepted for publication February 12, 1971.

The assistance of Mr. Richard Hartley is acknowledged.

Problems Associated with Analysis of Pharmacokinetic Models

WILFRED J. WESTLAKE

Abstract □ When a pharmacokinetic model is fitted to blood levels of a drug, the estimates of the pharmacokinetic parameters are likely to be subject to considerable error. These errors are probably unimportant as long as the model is used only to predict blood levels. However, when the parameters are used to predict other features of the system (*e.g.*, tissue drug levels), considerable errors in prediction may result. An example based on simulated data is devised to illustrate this possibility. It is further suggested that if the pharmacokinetic parameters are derived for the purpose of predicting the blood levels for different regimens or formulations of the drug—such as multiple-dose regimens or sustained-release capsules—this end can be met more expeditiously by using purely empirical techniques.

Keyphrases □ Pharmacokinetic models—analysis problems □ Blood levels, drugs—parameter predictions □ Lithium carbonate pharmacokinetics—two-compartment model □ Tissue drug levels—prediction errors

Over the past few years, it has become fashionable to conduct studies in which blood levels of a drug are determined at various times after administration; the resulting data then are fitted to a pharmacokinetic model. An example is the two-compartment model with oral administration, in which the usual assumptions of first-order kinetics lead to the expression:

$$C(t) = \frac{k_a D}{V_c}\left[\frac{(\alpha - k_{21})}{(\alpha - \beta)(k_a - \alpha)}e^{-\alpha t} + \frac{(k_{21} - \beta)}{(\alpha - \beta)(k_a - \beta)}e^{-\beta t} - \frac{(k_a - k_{21})}{(k_a - \alpha)(k_a - \beta)}e^{-k_a t}\right] \quad \text{(Eq. 1)}$$

for the drug concentration in the central (plasma) compartment. The constants in this expression are the rate constants k_a (gut to plasma compartment), k_{12} and k_{21} (plasma compartment to tissue compartment and vice versa), k_e (elimination from plasma compartment), and D/V_c (amount of drug absorbed divided by the volume of distribution of the plasma compartment). The parameters α and β are defined by the relations $\alpha\beta = k_e k_{21}$ and $\alpha + \beta = k_e + k_{12} + k_{21}$. The usual problem is to find the values for these five constants which best fit Eq. 1 to a set of empirical data. This is an exercise in nonlinear least squares, usually carried out with a computer program which utilizes iterative schemes for finding the best fit.