Quantitative Determination of Drug Bioavailability and Biokinetic Behavior from Pharmacological Data for Ophthalmic and Oral Administrations of a Mydriatic Drug

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Abstract □ A theoretical basis is developed for the performance of drug-absorption analysis from data obtained from the observation of the time course of pharmacological response intensity following a single dose of drug by any route of administration. The results of such analyses permit an evaluation of the physiological availability characteristics of drugs from pharmaceutical dosage forms and the elucidation of the kinetics and mechanisms operative in the passage of drugs across *in vivo* biological barriers. It is demonstrated that through the suitable use of intravenous dose–effect curves, the postulation of hypothetical models for pharmacon–receptor site interaction is obviated. The dose–effect curve graphically provides the relationship between the quantity of drug in body compartments (biophase) and the observed intensity of drug response. The use of pharmacological data, in contrast to the use of time course of drug level in body fluid data, has the advantage, in addition to not requiring a method of direct assay for the drug, of being applicable to cases where the drug first penetrates from a local site of administration to a vicinal site of action prior to reaching the systemic circulation. In the present study, kinetic pharmacological data for tridihexethyl chloride were transformed to obtain the temporal variation of biophasic drug levels, which in turn provided the input for least-squares fits to the linear two- and three-compartment models studied. Digital computer programs, employing an iterative systematized guessing method, were designed to aid in fitting the mathematical models. A triexponential fit was found to be the best with the constraints imposed on the model parameters. The method is further exemplified by its application to the determination of drug absorption following oral and ophthalmic administration.

Keyphrases □ Mydriatic drug—bioavailability, biokinetic behavior □ Tridihexethyl chloride—bioavailability, biokinetics □ Ophthalmic, oral administration—mydriatic drug bioavailability, biokinetics □ Pharmacological response data—biokinetics, mydriatic drug □ Biokinetics, mydriatic drug—pharmacological response data

A biokinetic method of analysis of *in vivo* data that does not involve *a priori* assumptions of kinetic models for the absorption of the drug from the site of administration has the distinct advantage of allowing the kinetics and mechanisms of the biological availability of the drug to be elucidated under *in vivo* conditions. The purpose of this report is to describe a basis for the use of temporal pharmacological response-intensity data for drug-absorption analysis. The method is exemplified by its application to the determination of the absorption of tridihexethyl chloride following oral and ophthalmic administration.

Generally the ability of the drug to reach its biological site(s) of action (contained in a body compartment termed the biophase) is either dosage form rate limited by its release from the dosage form or biological factor rate limited by biotransformation, permeation of biological barriers, and/or unavailability due to partitioning into body depots of relatively large solubility volume (1). Information concerning some of these processes *in vivo* can be gained by absorption analysis of temporal blood level and/or urinary excretion data through the application of the now familiar treatments first described by Wagner and Nelson (2) and further developed by others (3). These treatments, however, have been described only for cases where the drug is absorbed into a central, systemic compartment prior to or coincident with inducing its biological response. This is most often the intent of oral and parenteral administration. In contrast, the study of drug availability to a biophase vicinal to the site of administration following topical or parenteral use of the drug for local effects has not been described. Provided the appropriate tissue locations could even be unambiguously identified, their sampling for the periodic determination of drug levels could present intractable technical difficulties. If drug-absorption analysis could be performed using temporal pharmacological response-intensity measurements, the direct assay for drug levels in such locations could be obviated. The development of a basis for the utilization of pharmacological data for drug-absorption analysis and the determination of a suitable compartment model to describe the biokinetic behavior of tridihexethyl chloride are the subjects of the present report. Tridihexethyl chloride was chosen as one of the model drugs in these studies because of the relative ease of following its mydriatic activity. Furthermore, due to its quaternary ammonium structure, it was anticipated to possess interesting biological transference properties.

Previous investigations (4–10) concerned with the kinetics of pharmacological effects always adopted the sometimes tenuous assumption that the intensity of an observed pharmacological effect at any time is directly or otherwise simply related (*e.g.*, through a hypothetical model for drug–receptor interaction) to the level of drug in a peripheral tissue or other compartment (the biophase) containing the sites at which it acts to induce the observed response. The present approach, when applicable, does not require the postulation of hypothetical mechanisms for dose–effect relationships. It allows biophasic drug levels to be estimated at any time—following drug administration by any route—from the observed intensity of the induced pharmacological effects. The manner in which intravenous dose–effect curves may be applied to this purpose, as well as to obtain confidence in the applicability of the models to any given drug, is presented. Such procedures of indirect, or direct, verification should be performed prior to application of the method to any drug system. By applying the procedures described, it becomes entirely unnecessary to assay for drug concentration levels in body fluids or tissue compartments in order to perform analyses of drug absorption and availability into the systemic circulation or directly into the biophase. The principles developed are generally applicable; however, special

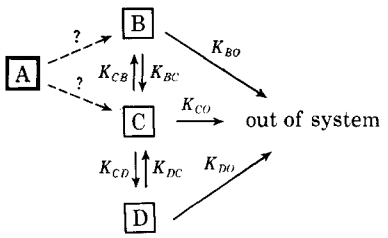
consideration is presently afforded to the treatment of ophthalmic drugs.

THEORETICAL

General—Physiological drug availability has been defined (2) as the ratio of the amount of a drug systemically absorbed from its site of administration when it is presented to the site in a readily available form as compared to other dosage forms. Methods of calculating the physiological drug availability from blood and urine data have become familiar (2, 3). The pharmacological response characteristics of a drug, however, are not necessarily predictable from its concentration in extracellular fluids *versus* time profile (3). A similar profile of drug concentration in its biophase (*i.e.*, the region(s) in the organism containing the primary sites of biological action[1]) could, in many instances, be of greater utility in this respect. The physiological drug availability could then be redefined as a similar ratio with respect to the quantities of drug absorbed into the biophase. Depending upon the specific drug of interest, the biophase may or may not constitute a compartment separate from the extracellular fluids of distribution. For example, Smolen and Schoenwald (11) found that the biophase and central compartment were practically indistinguishable for a nonionic mydriatic drug but constituted separate compartments for the presently studied cationic drug having a similar pharmacological activity. The difference in their behavior may be attributed to the relative rates at which the drugs can penetrate to their sites of action.

The general linear three-compartment model adopted in the present treatment represents the behavior of tridihexethyl chloride. The biophase is treated as a separate compartment; the concentration of drug in the biophase is by definition uniform throughout its volume. It is also precisely the concentration of drug responsible for the corresponding observed intensity of pharmacological response. Since the biophase is a hypothetical concept, it is seldom possible in practice to sample its drug content directly in a manner analogous to blood, aqueous humor, urine, *etc.* However, in appropriate cases (7), the quantity of drug in the biophase can be assumed as being reflected in the intensity of the pharmacological response to the drug at any time following its administration (Appendix I). If the functional relationship between the instantaneous pharmacological response intensity and the corresponding content of drug in the biophase was known, the temporal variation of the pharmacological response intensity could substitute for drug levels and provide the input necessary for a biokinetic analysis of the drug-transport system. Following certain assumptions, the requisite relationship between intensity of pharmacologic response and concentration can be obtained if the intravenous dose–response relationship is known and an appropriate compartment model of drug transport is constructed.

Compartment Model for Ophthalmic Drug Transference—As a first approximation at least, ophthalmic drug transference may be modeled by the linear compartment system shown in Scheme I where



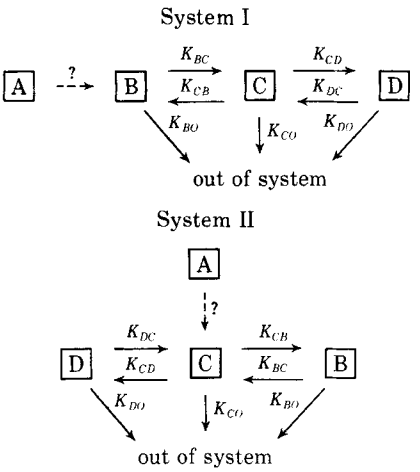
Scheme I—Compartment model for ophthalmic drug transference. Compartments A, B, C, and D correspond to the site of administration, biophase, systemic fluids of distribution, and tissue depot, respectively. The kinetics and mechanisms of drug transference from A to B and A to C are the phenomena to be elucidated from kinetic pharmacological data.

Compartments A, B, C, and D refer to the site of drug administration (*e.g.*, the surface of the eye), the biophase, the systemic fluids of distribution, and a peripheral tissue depot for the drug, respectively.

[1] See Appendix I.

The K_{ij}'s are first-order transfer constants. Only six of the seven constants can be computed from the combined pharmacological data obtained from intravenous dosing and the administration of the drug to a site from which it is directly absorbed into the biophase. The remaining constant must be assigned an arbitrary (*e.g.*, zero) value. This is unnecessary for single and two open-compartment models.

It can be assumed that any of the drug absorbed systemically, rather than directly into the biophase, will have equal access to both eyes of the animal. If the drug is administered only to the experimental eye, the other eye can serve as a control. The level of drug in the biophase which is responsible for the observed intensity of pharmacological response in the experimental eye at any time arises from the sum of the quantities of drug absorbed directly into the biophase and that which has arrived *via* the systemic route. Since this systemic contribution can be assumed to be the same for both the experimental and control eyes, the pharmacological response intensity in the experimental eye which is attributable to the level of drug in the biophase arising through direct absorption from the site of administration can be ascribed, at any time, to the difference in the intensities observed in the experimental and control eyes. However, this is strictly true only if the drug levels and intensities of effect are linearly related. As will be shown, the determination of whether a linear or some other form of functional relationship exists between the intensity of effect and quantity of drug in the biophase can be discovered from inspection of an intravenous dose–effect curve. In any event, the quantities of drug reaching the biophase directly or *via* the systemic circulation can be discerned. These considerations allow the compartment system shown in Scheme I to be resolved into the two systems depicted in Scheme II. By utiliz-



Scheme II—Resolution of the compartment model of ophthalmic drug transference into two systems which is possible through use of a control eye. Each system can be studied individually. System II is also generally appropriate for any other route of administration by which the drug enters the systemic circulation prior to penetrating into the biophase, e.g., *oral, parenteral, and scleral. System I is also applicable to other routes of administration besides ophthalmic when the pharmacological response resulting from drug entering B from C is negligible or can be independently measured.*

ing an experimental and a control eye, each system can be studied individually, although experimentally the data for each study are obtained simultaneously. Appendix II presents a further treatment of these considerations.

If the quantity of drug absorbed systemically relative to that absorbed directly into the biophase is appreciable, it may be an important factor contributing to any observed systemic side effects in addition to the intensity of the sought local pharmacological effect. Under such circumstances, the study of the kinetic processes involved in the systemic absorption route(s) may be as important as those operative in the direct absorption of the drug into the biophase.

A simplified compartmental model for drug absorption directly into the biophase is illustrated in Scheme III. Excluding the volume of the biophase, the remaining volume of distribution for the drug in the animal is assumed very large in comparison to the volume of the biophase. In this case a sink is provided for the drug into which

it is eliminated from the biophase. The backflow into the biophase can then be neglected to simplify the model, as shown in Scheme III. In the event the backflow was appreciable, it can be assumed that the quantity of drug influxed from this source at any time is equal for both the experimental and reference biophases. Therefore, the correction obtained for the control eye also takes account of the backflow of drug from the central compartment. The use of this correction, when necessary, allows the retention of the simplified model even in the event of an appreciable backflow of drug. However, when this is the case, the ability to distinguish between drug absorbed from the site of administration into the biophase and that reaching it by the systemic absorption from the site of administration is vitiated. The absorption analysis then could only be performed for the direct transference of drug into the biophase. Except for the instillation of inordinately high concentrations of drug into the eye, it has thus far been the author's experience with mydriatics that the correction for drug reaching the biophase by a systemic route is negligible.

Based upon these considerations, the fraction, F_{BT}, of the quantity of drug ultimately absorbed into the biophase, $Q_{BT\infty}$, which has been absorbed up to a time, t, can be expressed by Eq. 1, where Q_{BB} refers to the quantity of drug in the biophase at time t that has gained entrance by direct absorption. The elimination constant from the biophase in accordance with Scheme I is given by $K^*_{BO} = K_{BO} + K_{BC}$. It can be evaluated from kinetic data describing biophasic drug levels following the administration of an ophthalmic dose of the drug.

$$F_{BT} = \frac{Q_{BB} + K^*_{BO}\int_0^t Q_{BB}\,dt}{K^*_{BO}\int_0^\infty Q_{BB}\,dt} \qquad \text{(Eq. 1)}$$

In a manner analogous to physiological availability (2), the biophasic drug availability (BDA) and physiological drug availability (PDA) can be defined by Eqs. 2*a* and 2*b*.

$$\text{BDA} = \frac{\left[\int_0^\infty Q_{BB}\,dt\right]_{\text{exptl.}}}{\left[\int_0^\infty Q_{BB}\,dt\right]_{\text{standard}}} \qquad \text{(Eq. 2a)}$$

$$\text{PDA} = \frac{\left[\int_0^\infty Q_{BS}\,dt\right]_{\text{exptl.}}}{\left[\int_0^\infty Q_{BS}\,dt\right]_{\text{standard}}} \qquad \text{(Eq. 2b)}$$

The integrals in the numerator refer to some experimental (*e.g.*, ophthalmic or oral) formulation, while the denominators refer to a standard formulation to which the experimental formulation is being compared. The expression for PDA derives directly from a consideration of Eq. 3*a*.

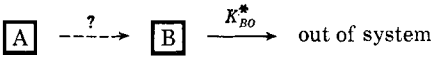
Equation 3*a* is appropriate for drug reaching the biophase by a systemic route (*e.g.*, by oral and parenteral dosing) or through scleral and/or mucosal absorption following instillation into the eye. Equations 3*a*–3*e* exemplify the type of expressions that are applicable to a three-compartment system for which the transfer constants have been determined or assumed. The derivation of an equation for a two-compartment model is presented in Appendix III. A discussion of the properties and application of Eq. 3*a* is presented in Appendix IV. Digital computer programs have been developed by the author for the routine numerical solution of such equations.

$$F_{ST} = \frac{R\frac{d^2Q_{BS}}{dt^2} + S\frac{dQ_{BS}}{dt} + TQ_{BS} + V\int_0^t Q_{BS}\,dt}{V\int_0^\infty Q_{BS}\,dt} \qquad \text{(Eq. 3a)}$$

$$RK_{BC}K_{DC} = 1 \qquad \text{(Eq. 3b)}$$

$$SK_{BC}K_{DC} = [K_{BC} + K_{BO} + K_{CB} + K_{CD} + K_{CO} + K_{DO}] \qquad \text{(Eq. 3c)}$$

$$TK_{BC}K_{DC} = [K_{DC}(K_{CB} + K_{BC} + K_{BO}) - K_{DC}K_{BC}(K_{BC} + K_{BO})(K_{CO} + K_{CO} + K_{CB}) + K_{DO}(K_{BC} + K_{BO} + K_{CB} + K_{CD} + K_{CO}) + K_{DC}K_{CO}] \qquad \text{(Eq. 3d)}$$



Scheme III—Simplified compartment system for the description of ophthalmic drug transference directly into, as well as its removal from, the biophase. In referring to Schemes I and II, the elimination constant from the biophase, K^*_{BO}, is seen to be related to other transfer constants by the equation, $K^*_{BO} = K_{BO} + K_{BC}$.

$$VK_{BC}K_{DC} = [K_{DC}K_{BC}K_{BO} - K_{BC}K_{CB}K_{DD} + (K_{CB} + K_{CD} + K_{CO})(K_{BC} + K_{BO})K_{DO} + K_{CO}K_{DC}(K_{BC} + K_{BO})] \qquad \text{(Eq. 3e)}$$

Through the use of pharmacological data, the cumulative amount of drug that has been absorbed into the biophase from the systemic circulation at any time following dosing by any systemic route can also be computed from Eq. 1. This amount, Q_{BST}, is composed of that quantity present in the biophase at any time and the quantity that has already been eliminated following its absorption from the systemic circulation. The fraction of the drug, F_{BST}, that has been absorbed systemically which has entered the biophase can readily be seen to be given at any time by the ratio of the numerator of Eq. 1 to the numerator of Eq. 3*a*. The limiting value of F_{BST}, *i.e.*, at $t = \infty$, may be termed the systemic biophasic availability, SBA, and be expressed simply by Eq. 4:

$$\text{SBA} = \frac{K^*_{BO}}{K_{BC}K_{DC}V} \qquad \text{(Eq. 4)}$$

Equations 1–4 would be of only academic interest if a means of estimating Q_{BB} and Q_{BS} at any time from the pharmacological response intensity did not exist.

Relationship of Pharmacological Response Intensity to Drug in the Biophase—Following intravenous administration, the quantity of drug in the biophase at any time will be given by Eq. 5 (9) in accordance with the three-compartment model shown in Scheme II. The symbol D refers to the dose of the drug. The relationships between the equation parameters, A_i's and m_i's, and the model parameters, K_{ij}'s, are shown in Appendix IV. Similar relations for biexponential models are given by Riggs (12) and Rescigno and Segre (13, 14).

$$Q_{BS} = D(A_1e^{-m_1t} + A_2e^{-m_2t} + A_3e^{-m_3t}) \qquad \text{(Eq. 5)}$$

A dose-effect relationship may be obtained by observing the intensity of response at a fixed time following rapid intravenous administration. A convenient and most propitious time to observe the response is the time ($t_{\max.}$) corresponding to the peak response intensity, $I_{\max.}$. The time, $t_{\max.}$, is independent of the dose for linear compartment models. This is easily verified by differentiating Eq. 5 and setting the derivative equal to zero.

The determination of the dose-effect relationship allows the dose to be expressed as a function of $I_{\max.}$; *i.e.*, $D = f(I_{\max.})$. Substituting this functional relationship for D into Eq. 5 allows it to be written as Eq. 6:

$$(Q_{BS})_{\max.} = f(I_{\max.})[A_1e^{-m_1t_{\max.}} + A_2e^{-m_2t_{\max.}} + A_3e^{-m_3t_{\max.}}] \qquad \text{(Eq. 6)}$$

The terms on the right-hand side of Eq. 6, other than $f(I_{\max.})$, are constant and will be included in an overall constant, β, as shown by Eq. 7:

$$(Q_{BS})_{\max.} = f(I_{\max.})\beta \qquad \text{(Eq. 7)}$$

Assuming that the relationship between the intensity of pharmacological response and the quantity of drug in the biophase, irrespective of its mode of entrance, given by Eq. 7 is a general relationship and holds at times other than $t_{\max.}$ allows Eq. 7 to be rewritten as Eq. 8 and Eq. 5 to be rewritten as Eq. 9:

$$Q_B = \beta f(I) \qquad \text{(Eq. 8)}$$

$$f(I) = \frac{f(I_{\max.})}{\beta}(A_1e^{-m_1t} + A_2e^{-m_2t} + A_3e^{-m_3t}) \qquad \text{(Eq. 9)}$$

Equation 9 predicts the response behavior of the drug as a function of time following an intravenous dose, while Eq. 8 applies irrespective of the route of administration.

Direct substitution of $f(I)$[2] from Eq. 8 for Q_{BS} or for Q_{BB} in Eqs. 1–4 will allow F_{BT}, BDA, F_{ST}, and PDA to be evaluated from pharmacological data alone. Most often the value of the constant, β, relating Q_B to $f(I)$ in Eq. 8, need not be known, since it cancels between the numerators and denominators and otherwise disappears from Eqs. 1–4 (Appendix IV).

The derivations of Eqs. 1 and 3*a*–3*e* are devoid of any assumptions concerning the transfer processes involved in the penetration of the drug into the biophase or the systemic compartment; they are, therefore, valid irrespective of the nature of these processes and could be utilized to elucidate the nature of their kinetics and mechanisms. Since $f(I)$ can be substituted for Q_{BB} and Q_{BS} in Eqs. 1 and 3*a*, respectively, the biokinetic analysis of drug absorption from the site of administration can be wholly accomplished from pharmacological response-intensity *versus* time data. Subtracting values of F_{BT} and F_{ST} from unity yields values of A_{RB} and A_{RS}, respectively, which refer to fractions of drug ultimately absorbed still remaining to be absorbed at the site of administration at any time. The temporal relationships of A_{RB} and A_{RS} can be utilized to provide insight into the kinetics and mechanisms of the absorption processes (2). This can best be accomplished through the construction of physical models to describe the involved transport processes while making comparisons of predicted behavior with that actually observed. In such physical models, the magnitude of the driving force for the passive transference of the drug across tissue barriers into the biophase or systemic compartment is most closely related to the quantity of drug remaining at the site of administration. This quantity is only equal to A_{RB}, or A_{RS} if there is no peripheral loss of the drug occurring from the absorption site; that is, *all* the drug leaves the administration site by the same route and is 100% available by this route. Generally this is not the case.

The integrals in Eqs. 1 and 3*a* are equal or directly proportional to the areas under curves of plots of $f(I)$ values *versus* time. The areas under curves obtained from pharmacological data can be given precisely the same interpretation and significance as the areas under curves resulting from similar plots of blood levels or urinary excretion rates (2).

MATERIALS AND METHODS

Materials—Crystalline tridihexethyl chloride[3] was dissolved in isotonic, pyrogen-free, sodium chloride solution prior to intravenous administration *via* the rabbit's marginal ear vein.

The rabbits used in the study were New Zealand white males, approximately 3–4 months of age. The rabbits were allowed a minimum of 2 days of recovery time between experiments. A preliminary screening of the rabbits was observed to reduce appreciably intersubject variation in the data. Twelve rabbits out of a total of 40 were chosen for use in the study on the basis of agreement between the maximum intensities of mydriatic response observed when the rabbits were administered the same ophthalmic dose and an approximately ED_{50} intravenous dose of drug. The pupillary response of the rabbits to controlled light intensities was also a criterion in their selection.

Measurement of Pupillary Diameters—The experimental arrangement employed in the present study is illustrated in Fig. 1. When measurements were made on both eyes, an additional light source and mirror were arranged symmetrically to those shown in Fig. 1. The light source(s) consisted of a 100-w. bulb inserted in a microscope lamp. The intensity of the light reflecting from the mirror(s) was measured with a light meter located near the experimental eye of the rabbit. A constant intensity of illumination was maintained by adjusting the light source with a rheostat. The mirror served to minimize the quantity of heat generated by the source that reached the cornea. The constancy of the lighting conditions was found to be an important factor affecting the reproducibility of experimental results. Prolonged illumination of the eye caused the pupillary diameter to dilate, following an initial constriction, and was therefore avoided. Consequently, the eye was illuminated only prior to a measurement. The minimum observed values of the pupillary diameters following illumination were assumed to correspond to full accommodation and were recorded along with the time of their observation.

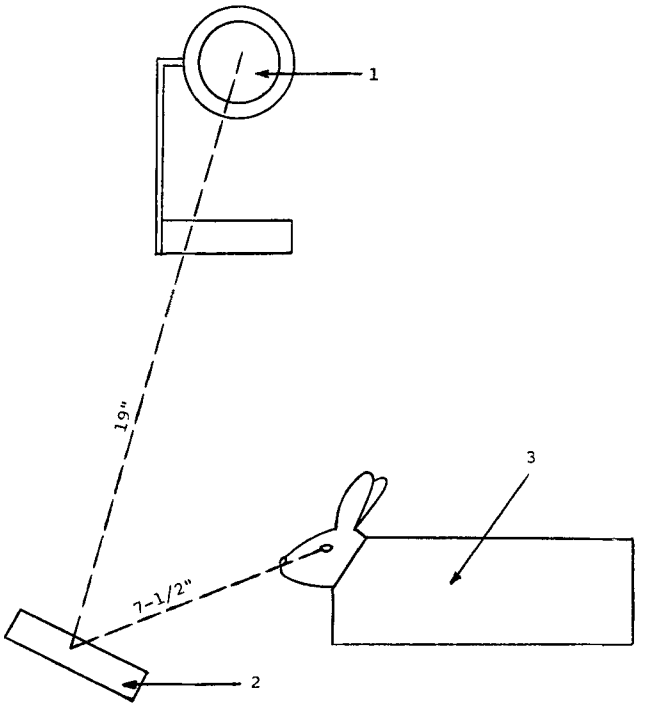


Figure 1—*Experimental arrangement for measurement of pupillary diameters. Key: 1, light source; 2, flat-faced mirror; and 3, rabbit in rabbit box.*

The diameters of the pupils were measured to within 0.1 mm., using a vernier caliper. The precision of the measurements increased with their magnitude, varying from approximately 5 to 55% mean error. The average mean error for the experiment was approximately 20%. The intersubject variation in replicated results was observed to be of approximately the same magnitude as intrasubject variation, therefore permitting the pooling of results obtained with different animals under the same experimental conditions otherwise.

RESULTS AND DISCUSSION

Relationship of Mydriatic Response Intensities to Biophasic Drug Levels—The values of $f(I)$ which are, by Eq. 8, directly proportional

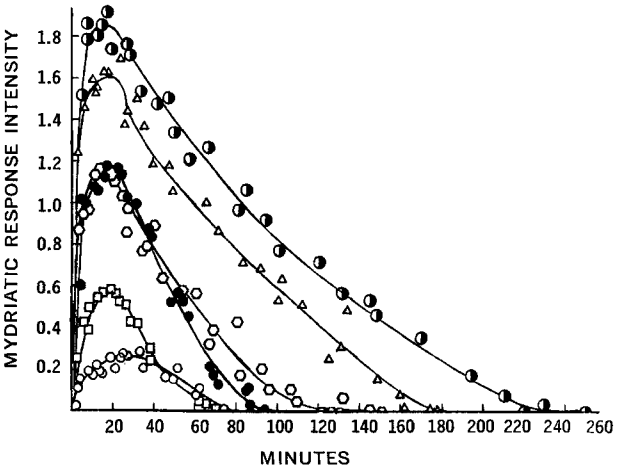


Figure 2—*Time course of mydriatic response intensity for several intravenously administered doses of tridihexethyl chloride to rabbits. Each curve is the result of measurements on a minimum of three separate rabbits. The intravenous doses employed, in mg./kg., were 0.17 (—○—), 0.22 (—□—), 0.30 (—●—), 0.45 (—○—), 0.95 (—△—), and 1.60 (—◑—).*

[2] Read as function of *I*, *not I* multiplied by *f*; the value of $f(I)$ is at all times directly related to the quantity of drug in the biophase; *i.e.*, $f(I) = Q_B/\beta$.

[3] Lot CL 19,813, supplied by Lederle Laboratories.

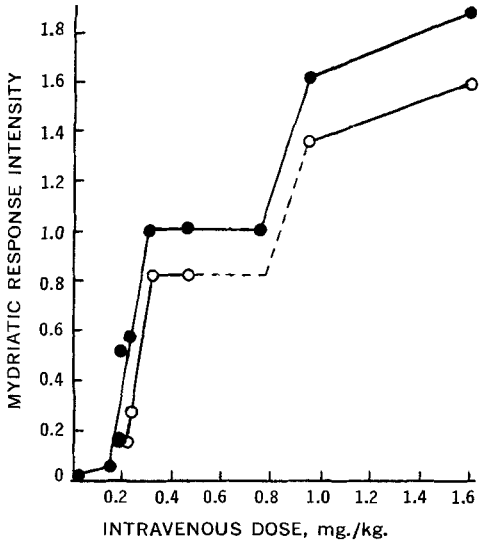


Figure 3—*Relationship of maximum mydriatic response intensity,* —●—●—, *and mydriatic response intensity observed at twice the observed time corresponding to the maximum intensity,* —○—○—, *to the intravenous doses of tridihexethyl chloride employed. The six points on each curve, which correspond to the same value on the abscissa, are the result of a minimum of three determinations on separate rabbits. The remaining points result from single determinations.*

to the quantities of drug in the biophase at any time can be obtained directly from intravenous dose *versus* effect curves. Such curves for tridihexethyl chloride are illustrated in Fig. 3. The experimental curves from which the values of $I_{max.}$ were obtained are shown in Fig. 2. The upper curve in Fig. 3 is a plot of $I_{max.}$ values *versus* dose, while the lower curve was constructed by plotting I values observed at times corresponding to double the experimental $t_{max.}$ values ($2t_{max.}$) as a function of dose. Values of $f(I)$ corresponding to observed values of I at any time may be read directly as the corresponding abscissae using either curve, provided it is used consistently. The choice of the time for the observed I values to be used in the construction of the intravenous dose–effect curve is arbitrary when the drug in the biophase at any time is continually in equilibrium with the processes responsible for the manifestation of the pharmacological response. $I_{max.}$ values corresponding to $t_{max.}$ are best chosen for use because, in addition to theoretical considerations, they often appear to be better defined by the experimental I *versus* time curves.

Indirect Verification for Postulated Use of Pharmacological Data to Reflect Biophasic Drug Levels—A direct means of verifying the use of pharmacological data for the absorption analysis of a particular drug can obviously be obtained through a comparison of the results obtained by its use with similar results based on the detection of the drug in body fluids. As in the present case, due to difficulties encountered in the assay of tridihexethyl chloride in body fluids, such a direct means of confirmation is not always convenient or practical. In fact, the use of pharmacological data possesses the greatest utility when this is indeed the case. However, confidence in the applicability of the techniques may still be obtained by comparing the behavior of the observed pharmacological results with that predicted by the postulated model. A method of direct confirmation using only pharmacological data can be performed through a comparison of the quantities of drug absorbed at any time calculated from the pharmacological data with known quantities administered by slow intravenous infusion. This was not done for tridihexethyl chloride; this test was, however, performed on tropicamide, another mydriatic drug, where exceptionally close agreement was observed between known and calculated quantities. The details of these results are reported (11).

Confidence in the assumption that biophasic drug levels can be monitored by observing the intensity of response, *i.e.*, all processes involved in the manifestation of the response are entirely reversible and nonhysteretic, can be gained by constructing more than one intravenous dose–effect curve. Each curve is constructed by plotting I values observed at a constant arbitrarily chosen elapsed period of

Table I—Mydriatic Response Results Observed for Intravenous Doses of Tridihexethyl Chloride

Dose, mg./kg.	Range of $t_{max.}$[a]	$I_{max.}$[b]	$I_{2max.}$[c]	$[f(I)_{max.}]/[f(I)_{2max.}]$[d]
0.140	—	0.052	—	
0.170	20–25	0.27 ± 0.006	0.18	0.8
0.180	—	0.52	—	—
0.220	15–22	0.58 ± 0.006	0.28	0.8
0.300	15–20	1.10 ± 0.05	0.82	0.8
0.450	14–20	1.14 ± 0.01	0.85	0.6
0.75	—	1.13	—	—
0.95	15–19	1.62 ± 0.01	1.38	0.9
1.60	17–19	1.90 ± 0.03	1.60	0.6
Averages				0.76

[a] The ranges of $t_{max.}$ were visually estimated from plots of I *versus* time for each replication on a different animal. [b] The mean error is indicated for values that are averages of a minimum of three determinations on different rabbits. The remaining values are single determinations. [c] These values were estimated from the curves in Fig. 2 at times corresponding to twice the average $t_{max.}$ [d] The doses in Column 1 were used as values of $f(I)_{2max.}$ for the calculation of the values of the ratios; $(I)_{max.}$ values were read as the abscissae corresponding to the $I_{2max.}$ values in Column 4 using the $I_{max.}$ *versus* dose curve shown in Fig. 3.

time, following the intravenous administration, as a function of the dose; *e.g.*, fractional or multiple values of $t_{max.}$ may be chosen. It can be predicted from Eq. 8 that for any given observed value of I, irrespective of the dose or time of its observation, the ratio of any two $f(I)$ values read from any two of the intravenous dose–effect curves should be constant. This is obvious when it is considered that the quantity of drug in the biophase must be the same for any given value of I. The value of the constant is determined by the ratio of β values corresponding to the times chosen for the construction of the intravenous dose–effect curves. The realization of this predicted behavior is evidenced by the relative constancy of the values listed in the fifth column of Table I. Considering the magnitude of the experimental error and its exaggeration in the computation of the ratios, the agreement of these values is interpreted as very reasonable.

Simulated Curve Fitting without Constraints on Model Parameters—It is obvious from inspection of Eq. 9 that a congruity in curves constructed by plotting normalized values of $f(I)$, *i.e.*, $f(I)/f(I_{max.})$ as a function of time is expected. The independence of such curves of the dose is a necessary condition of the verity of using pharmacological data to describe the biokinetic behavior of a drug. This condition arises from the assumption of linear compartment models. Prior to initiating an extended study of the biophasic availability of tridihexethyl chloride by the presently described methods, a premonitory indication of the applicability of such an approach for use with this first chosen drug was obtained with single determinations of the time course of mydriasis following the administration of intravenous doses of 0.3 and 0.45 mg./kg. to the same animal. The ratio of areas under the two plots of dose normalized values of $f(I)$ *versus* time was found to be 0.97. The average ratio of ordinates compared at 13 separate times was noted as 0.96. The deviation of these values from unity is, therefore, well within experimental error. Further confidence in the applicability of the method to tridihexethyl chloride was obtained from subsequent data obtained with a larger range of doses.

These results are summarily presented by the computer[4]-constructed composite plot of $f(I)/f(I_{max.})$ values *versus* time shown in Fig. 4. The scatter of points can largely be attributed to biological variation and the somewhat unrefined technique of pupillary diameter measurement employed in obtaining this particular set of early results. The use of animals further screened on the basis of their similitude of response to more than one preliminary test dose of the drug, as well as refinements in the method of pupillary diameter measurement, yielded considerably improved data in subsequent studies with tridihexethyl chloride and other mydriatic drugs. Despite the inprecision of the results plotted in Fig. 4, the points appear to scatter around the simulated least-squares biexponential and triexponential curves of best fit.

[4] The curves in Fig. 4 were constructed by a model 563 Calcomp Digital Incremental Plotter, California Computer Products Inc., Anaheim, Calif.

FORTRAN IV programs for use on a CDC 6500 digital computer were developed to obtain the unconstrained biexponential and triexponential curves of best fit to the experimental data. A method of systematized iterative guessing (15) was used in the programs. This method is advantageous because it converges rapidly and is devoid of the danger of merely reaching a local minimum in the sums of squares as can occur with other computational techniques (16). First approximations to the equation parameters—which are necessary to initiate the iterative computer programs—were obtained by the "peeling off" technique (17).

The simulated unconstrained curves in Fig. 4 serve only as references to which other constrained fits are to be compared. The values of the equation parameters do not necessarily correspond to physically allowable (*i.e.*, real and nonnegative) values for the transfer constants. The values of $t_{max.}$, the least sums of squares, and the average values of $\beta_2 t_{max.}/\beta t_{max.}$ for the biexponential fit are 11.6 min., 1.2217, and 0.7724, respectively. In the same order, the values for the triexponential fit are 11.7 min., 1.2227, and 0.7701. On the basis of these criteria alone, it would be difficult to choose between the biexponential and triexponential fits. The expected agreement of the $\beta_2 t_{max.}/\beta t_{max.}$ values with the average value of $f(I_{max.})/f(I_{2max.})$, equal to 0.76, is exceptionally good considering the magnitude of the involved errors.

The biexponential and triexponential equations, respectively, are:

$$f(I)/f(I_{max.}) = 1.1294\,[\exp(-0.014635t) - \exp(-0.26530t)] \quad \text{(Eq. 10)}$$

$$(I)/f(I_{max.}) = 0.39190\exp(-0.01293t) + 0.91438\exp(-0.015713t) - 1.3063\exp(-0.25870t) \quad \text{(Eq. 11)}$$

Biophasic Availability Characteristics of Tridihexethyl Chloride following Ophthalmic Dosing—Figure 5 contains semilogarithmic plots of I, $f(I)$, and A_{RB} as a function of time following ophthalmic dosing with 0.015% tridihexethyl chloride in a pH 7.4 isotonic phosphate buffered solution. The half-life of drug elimination from the biophase ($t_{1/2} = 0.693/K^*_{BO}$) is obtained from the latter linear segment of the log $f(I)$ *versus* time curve as equal to 132 min. This value can be compared to the apparent mydriatic half-life of 165 min., which is similarly obtained from the log I *versus* time curve. The mydriatic half-life corresponds to the rate of decay of the mydriatic response intensity. The mydriatic and elimination from the biophase half-lives, although related, obviously need not necessarily be the same value. For any given drug, the precise nature of the relationship between the decay of I and K^*_{BO} depends upon the form of the dose-effect relationship.

The values of F_{BT} used in the log A_{RB} *versus* time plot were calculated using Eq. 1 and the value of $K^*_{BO} = 0.00524$ corresponding to

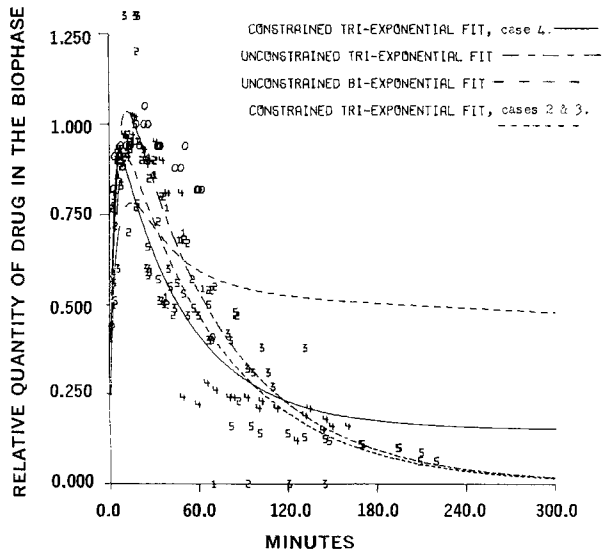


Figure 4—*Computerized least sums of squares fits to a composite plot of* f(I), *normalized for dose, representing the time course of variation of relative levels of tridihexethyl chloride in the biophase. The intravenous doses represented are 0.170 (—0—), 0.220 (—1—), 0.300 (—2—), 0.45 (—3—), 0.95 (—4—), and 1.60 (—5—) mg./kg.*

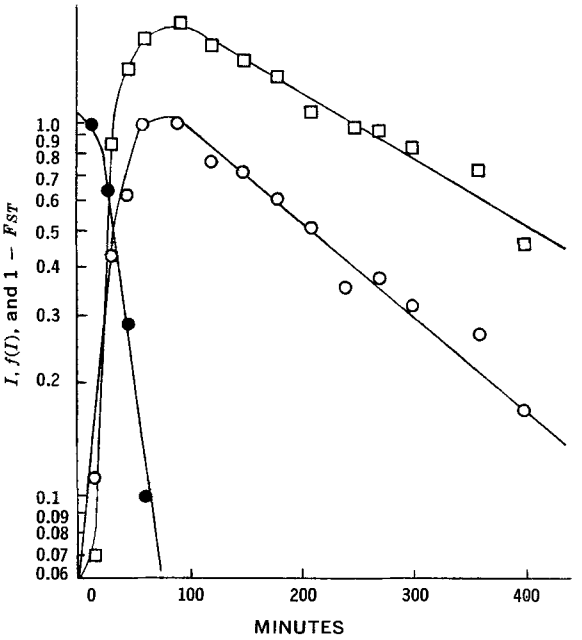
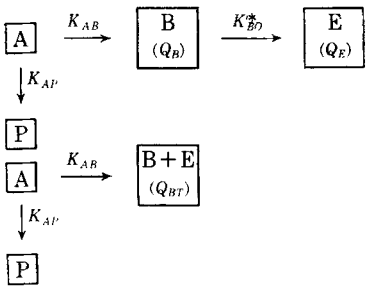


Figure 5—*Semilogarithmic plots of* I, *the mydriatic response intensity* (—□—); f(I), *the relative quantity of drug in the biophase* (—○—); *and* 1 − FST, *the fraction of drug remaining to be transcorneally absorbed into the biophase* (—●—), *as a function of time in minutes, following a 0.02-ml. ophthalmic dose of 0.015% tridihexethyl chloride (in pH 7.4 isotonic buffer) into the cul-de-sac of rabbit eyes. Each point is the average of 12 determinations on different rabbits. The absorption of the drug into the biophase is completed between 60 and 90 min.*

the biophase elimination half-life. The linearity of the log A_{RB} *versus* time plot is indicative of the operation of apparent first-order processes, such as passive diffusion, being responsible for the dissipation of drug from its ophthalmic site of administration. This conclusion justifies the construction of a linear compartment model to describe these processes. Following administration into the cul-de-sac of the eye, the dissipation of the drug from the site of administration occurs through transcorneal absorption into the biophase and through, what will be termed, peripheral loss, which can include scleral absorption and such loss of drug as can occur down the lacrimal nasal canal. By following this consideration, a compartment model such as is shown in Scheme IV can be applied to describe



Scheme IV—Equivalent compartment models pertaining to the dissipation of drug from its site of administration in the eye. The symbol P designates the compartment into which peripheral loss of the drug occurs; B represents the biophase; E is a compartment constituted by the combination of all body compartments other than those shown; A is the site of administration; Q_{BT} *represents the quantity of drug which has been transcorneally absorbed from A into the biophase;* $Q_{BT} = Q_B + Q_E$, *where* $Q_E + Q_B$ *is the sum of the quantities of drug present and having been eliminated from the biophase at any time.*

these processes. On the basis of these models, Eq. 12 can readily be derived:

$$\log A_{RB} = -\left(\frac{K_{AP} + K_{AB}}{2.303}\right)t \quad \text{(Eq. 12)}$$

Table II—Results of Constrained Computerized Fits to Two-Compartment Models

Dose, mg./kg.	Equation Parameters	Model Parameters	$\beta_{2t_{max.}}/\beta_{t_{max.}}$	$t_{max.}$	Sums of Squares
0.450	$A'_1 = 6.205$ $A'_2 = -6.205$ $m_1 = 0.03757$ $m_2 = 0.05010$	$K'_{BO} = 0$ (assigned) $K'_{CB} = 0.0833$ $K'_{CO} = 5.30 \times 10^{-5}$ $K'_{BC} = K^*_{BO}$ $= 0.00524$	0.894	22.7	1.709
0.950	$A'_1 = 5.268$	$K'_{BO} = 0$ (assigned) $K'_{CB} = 0.0939$ $K'_{CO} = 4.91 \times 10^{-6}$ $K'_{BC} = K^*_{BO}$ $= 0.00524$	0.816	20.4	2.033

The sum of the constants, $K_{AP} + K_{AB}$, represents the total proportional rate of loss of the drug from the site of administration. This value is obtained from the slope of the log A_{RB} *versus* t plot. In this manner, a half-life of 17 min. can be calculated to represent the total rate of loss of tridihexethyl chloride from the surface of the eye.

Testing and Selection of Compartment Models—In contrast to the case of drug absorption occurring directly into the biophase, the computation of the cumulative amount of drug absorbed systemically requires the adoption of a biokinetic model, which may not necessarily be constituted of only a single compartment. It is seldom possible in a biokinetic study to determine uniquely a compartment model, since any model that is compatible with the data can always be interpreted as a degenerate case of a higher order model. Generally, for linear models, a model with the smallest number of compartments compatible with the data and physical reality (*i.e.*, the transfer constants, K_{ij}'s, must be real and nonnegative) giving the best fit, as judged by the sums of squares, is chosen (18, 19). Simulated curves should, as far as possible, be devoid of systematic deviations from those experimentally observed (19).

For the purpose of its application to drug-absorption analysis, the questions of whether the model ultimately chosen is unique and whether the compartments have a counterpart in biological reality are irrelevant. For the present application, the utility of a model is limited to its use for the calculation of the relative quantities of drug remaining at the site of administration to be absorbed at any time. For this purpose, any model consistent with the data to which physically real values of the model parameters can be assigned is suitable. The biological and physical reality of models to be used solely for the purpose of drug absorption analysis is further discussed in Appendix IV.

It is apparent from the maximum in the data points, shown in Fig. 4, that the biophase is constituted by a compartment distinguishable from the central systemic compartment; therefore, a minimum of two compartments is necessary to characterize the biokinetic behavior or tridihexethyl chloride. As previously discussed, the sum of K_{BJ}'s should equal the overall elimination constant from the biophase, K^*_{BO}, whose value has been obtained from the results of ophthalmic dosing. With this relationship and the results of intravenous dosing, four transfer constants can be calculated to char-

Table III—Averaged[a] Results for Triexponential Constrained Computerized Fits to Different Cases of Three-Compartment Models

Model Parameters	Equation Parameters	$\beta_{2t_{max.}}/\beta_{t_{max.}}$	$t_{max.}$, min.	Sums of Squares
	Case 1			
$K'_{BO} = 0$ $K'_{BC} = 0.00524$ $K'_{CO} = 0$ $K'_{CB} = 0.3785$ $K'_{CD} = 0.0464$ $K'_{DO} = 0.0200$ $K'_{DC} = 2.79 \times 10^{-4}$	$A'_1 = 0.5391$ $A'_2 = 0.706$ $A'_3 = -1.33$ $m_1 = 3.34 \times 10^{-4}$ $m_2 = 0.032$ $m_3 = 0.417$	0.867	12.2	0.9672 3.288[c]
	Case 2			
$K'_{BO} = 0$ $K'_{BC} = 0.00524$ $K'_{CO} = 0.0462$ $K'_{CB} = 0.379$ $K'_{CD} = 2.069 \times 10^{-4}$ $K'_{DO} = 0$ $K'_{DC} = 0.0203$	$A'_1 = 0.539$ $A'_2 = 0.706$ $A'_3 = -1.33$ $m_1 = 3.33 \times 10^{-4}$ $m_2 = 0.0320$ $m_3 = 0.412$	0.867	12.2	0.9672 3.288[c]
	Case 3[b]			
$K'_{BO} = 0.00519$ $K'_{BC} = 4.55 \times 10^{-5}$ $K'_{CO} = 0.0438$ $K'_{CB} = 0.651$ $K'_{CD} = 2.74$ $K'_{DO} = 0$ $K'_{DC} = 0.0052$	$A'_1 = 6.39 \times 10^{-4}$ $A'_2 = 0.943$ $A'_3 = -0.944$ $m_1 = 0.00520$ $m_2 = 0.00520$ $m_3 = 0.696$	0.9088	8.0	1.070
	Case 4			
$K'_{BO} = 0.00169$ $K'_{BC} = 0.0232$ $K'_{CO} = 0$ $K'_{CB} = 0.475$ $K'_{CD} = 0.0305$ $K'_{DO} = 0$ $K'_{DC} = 0.00741$	$A'_1 = 0.162$ $A'_2 = 0.909$ $A'_3 = -1.07$ $m_1 = 2.28 \times 10^{-4}$ $m_2 = 0.021$ $m_3 = 0.483$	0.863	9.8	0.7274 2.400[c]

[a] The results are averages of the values obtained from separate fits to data corresponding to six different doses. [b] Results for Case 3 were obtained only for data corresponding to the 0.300-mg./kg. dose. [c] Value of the sum of squares corrected to enable direct comparison to the value of 1.2227 obtained for the unconstrained fit to the pooled and averaged data.

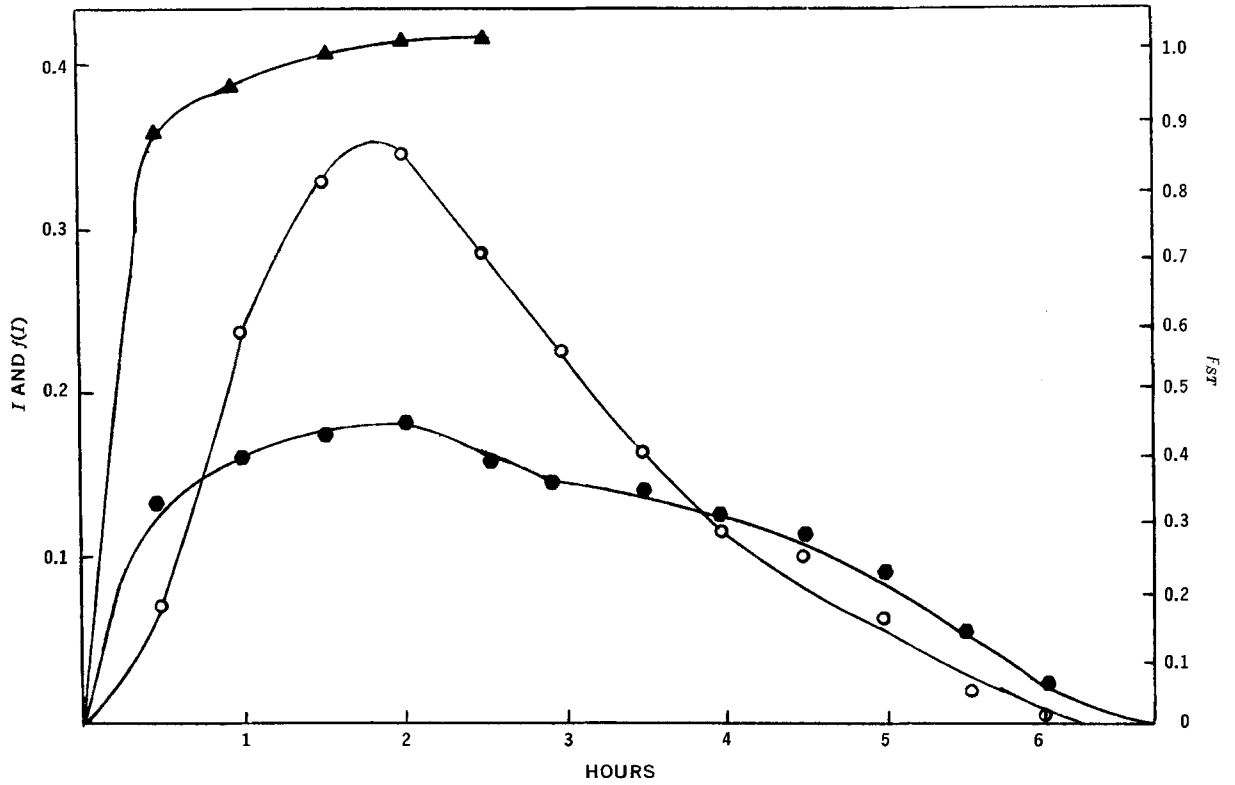


Figure 6—*The temporal variation of* I, *mydriatic response intensity* (—○—); *relative quantity of drug in the biophase,* f(I) (—●—); *and relative quantities of drug systemically absorbed,* F_{ST} (—▲—), *following oral dosing of rabbits with 35 mg./kg. tridihexethyl chloride. Each point is the average of a minimum of three determinations.*

acterize a biexponential model and six can be derived for a triexponential model with their corresponding three and five degrees of freedom, respectively (20).

Two-Compartment Model Fit—Eliminating Compartment D, shown in Scheme I, from consideration is tantamount to assuming that the biophase is indistinguishable from other tissue compartments. By setting $K_{BC} + K_{BO} = K^*_{Bo}$, all four transfer constants can be evaluated. This was accomplished by adding additional constraints on the values of the equation parameters such that the iterative systematized guessing computer program would compute the least-squares values of the transfer constants which are also nonnegative and nonimaginary; these values are included in the computer printouts. The values of the equation parameters obtained from the unconstrained least-squares fit to the data may be employed as the initial estimates for the constrained fit to the data. In the search for least-squares values of the equation parameters consistent with the constraints imposed on the transfer constants, each equation parameter is allowed to assume values in the limits between zero and nearly twice the value of its initial estimates. Exceeding these limits would serve no purpose, since the resulting theoretical curve would be grossly inconsistent with experimental results. When a fit to a model providing positive and real values for the transfer constants was not obtained within these limits, the model was discarded as a grossly inaccurate representation of the system's biokinetic behavior. This was found to be the case with 16 out of the 18 attempts in which data for six doses were separately fitted to each of three cases of two-compartment models. These included, in addition to the case where the evaluation of all four transfer constants was sought, two models in which K_{BO} and K_{CO} were set equal to zero, respectively. These results, shown in Table II, clearly indicate that a two-compartment model is inadequate in simulating the behavior of this system. Consequently, test fits to models with an additional two degrees of freedom were performed in an effort to obtain an improved fit to the data.

Three-Compartment Model Fit—A total of seven transfer constants are shown to characterize the three-compartment model (Schemes I and II). Again, employing the constraint that $K_{BC} + K_{BO} = K^*_{Bo}$ allows six of these constants to be calculated from the data. The remaining constant(s) can be assigned an arbitrary value which can appropriately be taken as zero.

Attempts were made to fit the data for each dose to five cases of the three-compartment model shown in Schemes I and II. These cases included setting: (1) $K_{BO} = K_{CO} = 0$; (2) $K_{BO} = K_{DO} = 0$; (3) $K_{DO} = 0$; (4) $K_{DO} = K_{CO} = 0$; (5) $K_{BO} = 0$; and (6) $K_{CO} = 0$. Each case assumes negligible or zero contributions for different paths of drug elimination from the body. All the cases assume the operation of a peripheral, pharmacologically inert drug depot compartment, D, communicating with the central, systemic compartment.

Cases 1 and 2 provided fits to the data derived from all six doses studied. Case 3 was found compatible with only one of the six doses. The averaged results are summarized in Table III. The relations between the transfer constants and equation parameters for the three cases, to which it was found that the data could be fitted, are given in Appendix IV along with appropriate expressions for the calculation of Q_{BS} and F_{ST}.

Generally, the selection of a compartment model with regard to its adequacy in simulating the experimentally observed biokinetic behavior of a system has been based only upon the value for the sums of squares. However, in the present study the agreement between (*a*) calculated and observed values of $t_{max.}$ and (*b*) calculated values of $\beta_2 t_{max.}/\beta t_{max.}$ with the observed value of $f(I_{max.})/f(I_{2max.})$ serves as additional criteria for the selection of a model. Inspection of Table III, however, reveals that except for the transfer constants, the results for Cases 1 and 2 are identical and clearly demonstrate the lack of uniqueness of fitted compartment models. Figure 4 also contains a plot of the equation for Cases 1 and 2, where it can be compared to the scattered experimental values and the curves corresponding to the unconstrained least-squares fit to the data. The systematic inconsistency of the fit to Cases 1 and 2 clearly indicates their inadequacy in simulating the behavior of the system. To obtain an improved fit, the requirement that $K_{BC} + K_{BO} = K^*_{Bo}$ was removed and the data again fitted to Cases 1, 2, and 4. Cases 1 and 2 could not be fitted to any of the data, while Case 4 provided fits for each of the doses. The averaged results are reported in Table III. The corresponding curve shown in Fig. 4 demonstrates a considerable improvement over the fits obtained to Cases 1 and 2. However, none of the constrained fits to the two- and three-compartment models which were obtained is exceptionally good. Presumably, further improvement in the simulation of the data could be obtained by

complicating the model with additional compartments. However, no further attempts of this nature were pursued in the present study.

If Models 1 and 2 had provided an acceptable fit to the data, it would not have been possible to choose between them. A need for additional criteria on which to base the selection of a model is clearly indicated. Since the purpose of the model is its application in the computation of Q_{BS} and F_{ST} for systemic routes of administration, a model could best be chosen on the basis of the accuracy with which it allows known, experimental values of these quantities to be computed. The experimental results necessary for the implementation of this criterion, however, were not obtained in the present study.

Oral Administration of Tridihexethyl Chloride—Figure 6 illustrates results obtained by orally dosing rabbits with 35 mg./kg. of tridihexethyl chloride contained in gelatin capsules. The method employed in its administration has been reported (21). The values of F_{ST} plotted in Fig. 10 were calculated using relations appropriate for Case 4. These expressions are contained in Appendix IV.

SUMMARY AND CONCLUSIONS

All behavior of the data predicted as necessary to the verity of using pharmacological response-intensity data to describe the biokinetics of tridihexethyl chloride was observed. A three-compartment model provided the best simulation of the temporal variation of the biophasic drug levels. In general, when a reversible, gradual response to a drug is measurable and the applicability of the pharmacological technique is suspected, further confidence in its use may be readily gained from a minimum of pharmacological data obtainable from intravenous dosing with the drug of interest.

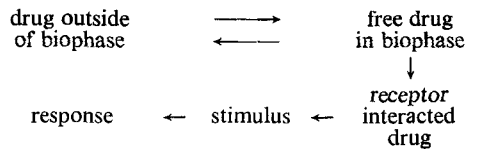
Unmodified, the presently described method may be expected to fail for cases where: (*a*) the biological response to the drug is not a continuous, single-valued function of the equilibrium drug level in the biophase, *e.g.*, it may exhibit hysteresis; (*b*) a continuously graded intensity of response is not detectable; (*c*) the effects of the drug are not immediately reversed by the removal of the drug from the biophase; and (*d*) the biokinetic behavior of the drug at some stage following its absorption into the central compartment or the biophase from its site of administration occurs by processes not describable by linear compartment modeling. Some of these conditions can be corrected for by suitable and sometimes simple modifications of the mathematical relations derived to describe the system behavior. In other cases, the innate behavior of the systems will preclude the use of pharmacological data for drug-absorption analysis. In every case, indirect or direct verification of the pharmacological method should be obtained prior to its application to new drugs or biological systems.

The sought therapeutic effect of some important classes of drugs, (*e.g.*, psychotherapeutic agents) cannot readily be quantitated or expressed as a continuous graded intensity of effect. However, many such drugs produce side effects, such as the hypothermic response to chlorpromazine, which can be continuously monitored and which may be appropriate for the implementation of the presently described pharmacological method of drug-absorption analysis. In this manner, the use of pharmacological data can provide an analytical tool which may allow the elucidation of the biokinetics and drug-absorption behavior of labile or highly potent drugs whose detection in corporeal fluids or tissues cannot be accomplished by direct chemical or radiological assay techniques. When the direct use of the observed pharmacological response intensity itself is inappropriate, it may occur that a rate of change—as expressed by numerically evaluated first or higher order derivatives of the observed response intensity with respect to time—or some other functional transformation of the pharmacological data may possess the behavior required for the application of the presently described method in an unmodified form. The use of transformed pharmacological response-intensity data in pharmacokinetic studies has been reported for anticoagulants (22, 23).

APPENDIX I

The rate processes responsible for the manifestation of a reversible pharmacological response may be diagrammed as in Scheme IA. The drug receptors are contained within the compartment termed the biophase.

The biokinetic treatment which is presently developed may be assumed to be strictly applicable when the rate-limiting process in the manifestation of the pharmacological response at any time is the drug's ability to gain access to its biological sites of action, *i.e.*, to penetrate into the biophase. The subsequent interaction with the receptor sites and the induction of the stimulus resulting in the observed response are sufficiently rapid that their extents are representative of reversible equilibrium with the levels of drug in the biophase at any time. As indicated by the results gained in the present study, this appears to be the case with tridihexethyl chloride. This is particularly indicated by the constancy of the $f(I)$ ratios read from the two different dose–effect curves shown in Fig. 3. With other drugs, if $f(I)$ ratios are clearly found not to be constant it may be suspected that the rate of flux of drug into and from the biophase from the systemic compartment is fast relative to the subsequent occurrence of the other processes necessary to the manifestation of the drug response. It might also be observed that due to the rapid passage of drug, the systemic and biophasic compartments are mathematically the same.



Scheme IA

When the described complications are met, it may become necessary to derive other mathematical models to relate intensities of response to biophasic drug levels which are based on a postulated mechanism of drug–receptor site interaction. As occurred with tridihexethyl chloride, this is not necessary if the rate-limiting step is merely the entrance and efflux of drug from the biophase. When it is necessary, however, a difficulty in deriving this relation becomes evident when it is considered that an equilibrium between the density of drug-affected receptor sites and the quantity of drug in the biophase cannot be expected. In fact, this precisely is the cause of the complication. However, some insight into the nature of the kinetic processes may again be gained from consideration of the intravenous dose–effect curve.

If the curve is constructed using $I_{max.}$ values, then considering I to be a monotonic increasing function of the equilibrium concentration (*i.e.*, concentration of drug in equilibrium with the receptor sites), the most propitious time to assume this equilibrium to obtain would be such that the rate of change of the concentration of drug in the biophase is minimal and therefore allows a maximum opportunity for the receptor sites to come into equilibrium with it. This time is obviously $t_{max.}$. The dose–effect curve using $I_{max.}$ values may then be assumed to characterize the equilibrium between drug-affected and free-receptor sites with free drug. This information may, in turn, be used to provide the insight necessary for the construction of a kinetic model to apply at other times besides $t_{max.}$ and provide the needed relationship between the observed intensity of response and quantity of drug in the biophase. Such a final relation is then valid irrespective of the path by which the drug gains entry to the biophase and can be used for drug-absorption analysis in lieu of the dose–effect curve which would, in this case, not be applicable for this purpose.

APPENDIX II

The resolution of the compartment system shown in Scheme I into that in Scheme II in practice can be accomplished by using a control eye. The verity of this approach can perhaps be further understood by considering for the moment that the quantity of the total drug absorbed systemically, Q_{TS}, is hypothetically in some way distinguishable from the quantity of the drug absorbed directly into the biophase[5], Q_{TB}. At any time the total quantity of drug absorbed from the site of administration, Q_T, is the sum of these quantities as given by Eq. 1A:

$$Q_T = Q_{TB} + Q_{TS} \qquad \text{(Eq. 1A)}$$

Mass balance dictates that Q_T at any time is the sum of the quantities of drug in the biophase, Q_B, occurring in the central compartment, Q_C, present in the depot compartment, Q_D, and which has been eliminated from the system, Q_E:

$$Q_T = Q_B + Q_C + Q_D + Q_E \qquad \text{(Eq. 2A)}$$

[5] This could be exemplified by the simultaneous administration of two hypothetical drugs differing only in the routes by which they are capable of gaining access to the biophase.

Since drug absorbed directly and systemically is being assumed to be distinguishable, similar relations exist for Q_{TB} and Q_{TS}; *i.e.*, at any time,

$$Q_{TB} = Q_{BB} + Q_{CB} + Q_{DB} + Q_{EB} \quad \text{(Eq. 3A)}$$

$$Q_{TS} = Q_{BS} + Q_{CS} + Q_{DS} + Q_{ES} \quad \text{(Eq. 4A)}$$

It is obvious that the sum of Eqs. 3A and 4A is equal to both Eqs. 1A and 2A and the quantity of drug in the biophase at any time is the sum of the quantities originating from systemic and direct absorption. Subtracting Eq. 4A from Eq. 2A produces a result equivalent to Eq. 3A, from which Eq. 5A can be obtained:

$$Q_{BB} = Q_B - Q_{BS} \quad \text{(Eq. 5A)}$$

Since the pharmacological response intensity, I, can be assumed to be a continuous function of the quantity of drug in the biophase over the range considered, the difference in $f(I)$ values observed for the experimental eye and that simultaneously observed for the control eye reflects the quantity of drug, Q_{BB}, in the biophase that arises by direct absorption. Therefore, Eq. 5A can be rewritten as Eq. 6A:

$$Q_{BB} = \beta[f(I)_{\text{exptl.}} - f(I)_{\text{control}}] \quad \text{(Eq. 6A)}$$

APPENDIX III

Equations 3*a* and 4 can readily be obtained by referring to Eq. 4A and to the compartment system in Scheme II. Equation 4A can be rewritten in differential form as Eq. 7A for the model reduced to two compartments by eliminating D; *i.e.*, K_{CD}, K_{DC}, and K_{DO} are set equal to zero.

$$dQ_{TS} = dQ_{BS} + dQ_{CS} + dQ_{ES} \quad \text{(Eq. 7A)}$$

Referring to System II in Scheme II, the following relations can be generated:

$$\frac{dQ_{BS}}{dt} = K_{CB}Q_{CS} - (K_{BC} + K_{BO})Q_{BS} \quad \text{(Eq. 8A)}$$

$$\frac{dQ_{ES}}{dt} = K_{CO}Q_{CS} + K_{BO}Q_{BS} \quad \text{(Eq. 9A)}$$

Equations 10A and 11A are obtained by solving for and expressing dQ_{CS} and dQ_{BS} in terms of Q_{BS}:

$$dQ_{CS} = \left[\frac{K_{BC} + K_{BO}}{K_{CB}}\left(\frac{dQ_{BS}}{dt}\right) + \frac{1}{K_{CB}}\left(\frac{d^2Q_{BS}}{dt^2}\right)\right]dt \quad \text{(Eq. 10A)}$$

$$dQ_{ES} = \left\{\frac{[K_{CO}(K_{BC} + K_{BO}) + K_{CB}K_{CO}]}{K_{CB}}Q_{BS} + \frac{K_{CO}}{K_{CB}}\frac{dQ_{BS}}{dt}\right\}dt \quad \text{(Eq. 11A)}$$

Substituting Eqs. 10A and 11A into Eq. 1 and integrating yield Eq. 12A:

$$Q_{TS} = \frac{K_{CO}(K_{BC} + K_{BO}) + K_{CB}K_{BO}}{K_{CB}}\int_0^t Q_{BS}\,dt + \left(\frac{K_{CB} + K_{BC} + K_{BO} + K_{CO}}{K_{CB}}\right)Q_{BS} + \frac{1}{K_{CB}}\frac{dQ_{BS}}{dt} \quad \text{(Eq. 12A)}$$

In the limit when $t = \infty$, Eq. 12A reduces to Eq. 13A:

$$Q_{TS\infty} = \frac{K_{CO}(K_{BC} + K_{BO}) + K_{CB}K_{BO}}{K_{CB}}\int_0^\infty Q_{BS}\,dt \quad \text{(Eq. 13A)}$$

In a similar manner, Eqs. 3*a*–3*e* can be derived for a three-compartment system.

APPENDIX IV

When the biophase is constituted, as in the present case with tridihexethyl chloride, by a compartment kinetically distinguishable from the central systemic compartment, the K_{iJ} values to be used in expressions for the cumulative amount of drug absorbed, *e.g.*, in equations such as 3*a*–3*e* and 12A, are not necessarily the values obtained from a computerized fit of a compartment model to the observed relationship of $f(I)/D$ to time. The computed values of (K'_{iJ})'s must be corrected for the fact that they are calculated from values of A'_i, where $A'_i = A_i/\beta$, rather than A_i values; see Eq. 9. No correction is necessary for single-compartment models or multiple-compartment models in which the biophase and central systemic compartments are identical; *i.e.*, $t_{\max.} = 0$ and, therefore, $\beta = 1$. Each K_{iJ} can be functionally related to the determined values of the (A'_i)'s, m_i's, and (K'_{iJ})'s and an unknown value of β. The value of β can be computed by considering that, for a rapid intravenous dose, Q_{TS} = dose at all times from $t = 0$ to $t = \infty$ and $f(I)$ at any time is given by the sum of exponentials providing the best fit for the rapid intravenous dosing data; *i.e.*, $f(I) = D\sum_{i=1}^{n} A'_i e^{-m_i t}$. The integral and n derivatives of $f(I)$ can obviously also be expressed in terms of sums of exponentials involving (A'_i)'s and m_i's. Substituting these theoretical expressions into an equation for Q_{TS} written in terms of (K'_{iJ})'s and β and equating Q_{TS} to the intravenous dose at all times leave only β unknown. Substituting an arbitrarily chosen value for the time ($t = 0$ or $t = \infty$ may be chosen to simplify the expression) allows a solution for β in terms of known values of (K'_{iJ})'s, (A'_i)'s, and m_i's. It may often be found that β drops out of equations for Q_{TS}, and its evaluation is then not necessary for the calculation of Q_{TS} and F_{ST}. In fact, in such a case the absolute value of β apparently cannot be computed.

Equations derived for the model parameters expressed in terms of the equation parameters—which were used in providing the constraints in the systematized iterative guessing computer programs—appropriate for the four cases of three-compartment model fits to the intravenous dosing data are given.

For all cases:

$$g = K'_{DC} + K'_{DO} = \frac{K'_{CB}m_1 + A_1(m_2 - m_1)(m_3 - m_1)}{K'_{CB}} \quad \text{(Eq. 14A)}$$

$$K'_{CB} = -m_1A'_1 - m_2A'_2 - m_3A'_3 \quad \text{(Eq. 15A)}$$

$$K^*_{BO} = K'_{BO} + K'_{BC} \quad \text{(Eq. 16A)}$$

The numerical value of K^*_{BO} is obtained experimentally from pharmacological data resulting from ophthalmic dosing.

Case 1:

$$K'_{BO} = 0 \quad \text{(Eq. 17A}a\text{)}$$

$$K'_{CO} = 0 \quad \text{(Eq. 17A}b\text{)}$$

$$K'_{BC} = K^*_{BO} \quad \text{(Eq. 17A}c\text{)}$$

$$K'_{CD} = m_1 + m_2 + m_3 - K'_{CB} - K'_{BC} - Q \quad \text{(Eq. 17A}d\text{)}$$

$$K'_{DO} = \frac{m_1m_2m_3}{K'_{BC}K'_{CD}} \quad \text{(Eq. 17A}e\text{)}$$

$$K'_{DC} = g - K'_{DO} \quad \text{(Eq. 17A}f\text{)}$$

$$K_{CB} = \beta K'_{CB} \quad \text{(Eq. 18A}a\text{)}$$

$$K_{BC} = K'_{BC} \quad \text{(Eq. 18A}b\text{)}$$

$$K_{BO} = K'_{BO} \quad \text{(Eq. 18A}c\text{)}$$

$$K_{CO} = K'_{CO} \quad \text{(Eq. 18A}d\text{)}$$

$$K_{CD} = K'_{CD} + (1 - \beta)K'_{CB} \quad \text{(Eq. 18A}e\text{)}$$

$$K_{DO} = K'_{CD}K'_{DO}\left[\frac{m_1m_2m_3}{-K'_{CD} + (1 - \beta)K'_{CB}}\right] \quad \text{(Eq. 18A}f\text{)}$$

$$K_{DC} = K'_{DC} + K'_{BO}\left[1 - \frac{K'_{CD}(m_1m_2m_3)}{K'_{CD} + (1 - \beta)K'_{CB}}\right] \quad \text{(Eq. 18A}g\text{)}$$

Case 2:

$$K'_{BO} = 0 \quad \text{(Eq. 19A}a\text{)}$$

$$K'_{DO} = 0 \quad \text{(Eq. 19A}b\text{)}$$

$$K'_{BC} = K^*_{BO} \quad \text{(Eq. 19A}c\text{)}$$

$$K_{DC} = g \quad \text{(Eq. 19A}d\text{)}$$

$$K'_{CO} = \frac{m_1 m_2 m_3}{K'_{DC} K'_{BC}} \quad \text{(Eq. 19A}e\text{)}$$

$$K'_{CD} = m_1 + m_2 + m_3 - K'_{CO} - K'_{CB} - K'_{DC} - K'_{BC} \quad \text{(Eq. 19A}f\text{)}$$

$$K_{BO} = K'_{BO} \quad \text{(Eq. 20A}a\text{)}$$

$$K_{DO} = K'_{DO} \quad \text{(Eq. 20A}b\text{)}$$

$$K_{BC} = K'_{BC} \quad \text{(Eq. 20A}c\text{)}$$

$$K_{CB} = \beta K'_{CB} \quad \text{(Eq. 20A}d\text{)}$$

$$K_{DC} = K'_{DC} \quad \text{(Eq. 20A}e\text{)}$$

$$K_{CO} = K'_{CO} \quad \text{(Eq. 20A}f\text{)}$$

$$K_{CD} = K'_{CD} + (1 - \beta) K'_{CB} \quad \text{(Eq. 20A}g\text{)}$$

Case 3:

$$K'_{DO} = 0 \quad \text{(Eq. 21A}a\text{)}$$

$$K'_{DC} = g \quad \text{(Eq. 21A}b\text{)}$$

$$\lambda = m_1 m_2 m_3 - K'_{CB} - K'_{DL} \quad \text{(Eq. 21A}c\text{)}$$

$$\mathrm{X} = m_1 m_2 + m_2 m_3 + m_1 m_3 - K'_{DC}(K^*_{BO} + K'_{CB}) - K^*_{BO}(\lambda - K^*_{BO}) \quad \text{(Eq. 21A}d\text{)}$$

$$\phi = \frac{m_1 m_2 m_3}{K'_{DC}} \quad \text{(Eq. 21A}e\text{)}$$

$$K'_{CO} = \frac{\mathrm{X} - \phi}{K'_{DC} - K^*_{BO}} \quad \text{(Eq. 21A}f\text{)}$$

$$K'_{CD} = \lambda - K^*_{BO} - K'_{CO} \quad \text{(Eq. 21A}g\text{)}$$

$$K'_{BO} = (\phi - K^*_{BO} K'_{CO})/K'_{CB} \quad \text{(Eq. 21A}h\text{)}$$

$$K'_{BC} = K^*_{BO} - K'_{BO} \quad \text{(Eq. 21A}i\text{)}$$

$$K_{DO} = K'_{DO} \quad \text{(Eq. 22A}a\text{)}$$

$$K_{DC} = K'_{DC} \quad \text{(Eq. 22A}b\text{)}$$

$$K_{CB} = \beta K'_{CB} \quad \text{(Eq. 22A}c\text{)}$$

$$K_{CO} = K'_{CO} + \frac{(K'_{DC} - K^*_{BO})(1 - \beta) K'_{CB}}{K'_{DC} - K^*_{BO}} \quad \text{(Eq. 22A}d\text{)}$$

$$K_{CD} = K'_{CD} + K'_{CB}(1 - \beta) + \frac{(K'_{DC} - K^*_{BO})(1 - \beta) K'_{CB}}{K'_{DC} - K^*_{BO}} \quad \text{(Eq. 22A}e\text{)}$$

$$K_{BO} = \frac{K'_{BO}}{\beta} - \left[\frac{K^*_{BO} K'_{CB}(K'_{DC} - K'_{BO})(1 - \beta)}{K'_{DC} - K^*_{BO}}{\beta K'_{CB}}\right] \quad \text{(Eq. 22A}f\text{)}$$

$$K_{BC} = K^*_{BO} - K_{BO} \quad \text{(Eq. 22A}g\text{)}$$

Case 4:

$$K_{BC} = + K_{BO} \neq K^*_{BO} \quad \text{(Eq. 23A}a\text{)}$$

$$K'_{CO} = 0 \quad \text{(Eq. 23A}b\text{)}$$

$$K'_{DO} = 0 \quad \text{(Eq. 23A}c\text{)}$$

$$K'_{DC} = g \quad \text{(Eq. 23A}d\text{)}$$

$$K'_{BO} = \frac{m_1 m_2 m_3}{K'_{DC} K'_{CB}} \quad \text{(Eq. 23A}e\text{)}$$

$$\mathrm{X} = m_1 m_2 + m_2 m_3 + m_1 m_3 - K'_{DC} K'_{BO} - K'_{BO} K'_{CB} - K'_{DC} K'_{CB} \quad \text{(Eq. 23A}f\text{)}$$

$$\Delta = (m_1 + m_2 + m_3 - K'_{DC} - K'_{BO}) K'_{DC} \quad \text{(Eq. 23A}g\text{)}$$

$$K'_{CD} = {}^1/_2[m_1 + m_2 + m_3 - K'_{CB} - 2K'_{DC} + \sqrt{(m_1 + m_2 + m_3 - K'_{CB} - 2K'_{DC})^2 - 4(\mathrm{X} - \Delta)}] \quad \text{(Eq. 23A}h\text{)}$$

$$K'_{BC} = m_1 + m_2 + m_3 - K'_{CD} - K'_{DC} - K'_{BO} - K'_{CB} \quad \text{(Eq. 23A}i\text{)}$$

$$K_{CO} = K'_{CO} \quad \text{(Eq. 24A}a\text{)}$$

$$K_{DO} = K'_{DO} \quad \text{(Eq. 24A}b\text{)}$$

$$K_{DC} = K'_{DC} \quad \text{(Eq. 24A}c\text{)}$$

$$K_{CB} = \beta K'_{CB} \quad \text{(Eq. 24A}d\text{)}$$

$$K_{BO} = \left(\frac{K'_{BO}}{\beta}\right) \quad \text{(Eq. 24A}e\text{)}$$

$$K_{CD} = {}^1/_2[m_1 + m_2 + m_3 - \beta K'_{CB} - 2K'_{DC} + \sqrt{(m_1 + m_2 + m_3 - \beta K'_{CB} - 2K'_{DC}) - 4\{[m_1 m_2 + m_2 m_3 + m_1 m_3 - K'_{DC} K'_{BO} - \beta K_{CB}(K_{BO} + K_{DC})] - \Delta\}}] \quad \text{(Eq. 24A}f\text{)}$$

$$K_{BC} = K'_{BC} + K'_{CD} - K_{CD} + K'_{BO}\left(1 - \frac{1}{\beta}\right) + K'_{CB}(1 - \beta) \quad \text{(Eq. 24A}g\text{)}$$

Calculating the cumulative amount of drug systemically absorbed, the general equation for Q_{TS} which includes all elimination paths, *i.e.*, K_{BO}, K_{CO}, and $K_{DO} \neq 0$, can be derived as:

$$Q_{TS} = \beta\left[R\frac{d^2 f(I)}{dt^2} + S\frac{df(I)}{dt} + Tf(I) + U\int_0^t f(I)dt\right] \quad \text{(Eq. 25A}a\text{)}$$

where:

$$K_{CB} K_{DC} R = 1 \quad \text{(Eq. 25A}b\text{)}$$

$$K_{CB} K_{DC} S = K_{BO} + K_{BC} + K_{DO} + K_{DC} + K_{CO} + K_{CB} + K_{CD} \quad \text{(Eq. 25A}c\text{)}$$

$$K_{CB} K_{DC} T = K_{DC} K_{CB} + K_{DC} K_{BC} + K_{DC} K_{BO} + K_{BC} K_{CO} + K_{BC} K_{CD} + K_{BO} K_{CO} + K_{BO} K_{CB} + K_{BO} K_{CD} + K_{DO} K_{BC} + K_{DO} K_{BO} + K_{DO} K_{CO} + K_{DO} K_{CB} + K_{DO} K_{CD} + K_{CO} K_{DC} \quad \text{(Eq. 25A}d\text{)}$$

$$K_{CB} K_{DC} U = K_{DC} K_{BC} K_{CO} + K_{DC} K_{BO} K_{CO} + K_{BC} K_{DO} K_{CO} + K_{BO} K_{DO} K_{CO} + K_{BC} K_{DO} K_{CD} + K_{BO} K_{DO} K_{CD} + K_D K_{BO} K_{CB} + K_{BO} K_{DO} K_{CB} \quad \text{(Eq. 25A}e\text{)}$$

These relations can also be written in terms of the equation parameters shown.

$$K_{CB} K_{DC} S = m_1 + m_2 + m_3 \quad \text{(Eq. 26A}a\text{)}$$

$$K_{CB} K_{DC} T = m_1 m_2 + m_2 m_3 + m_1 m_3 \quad \text{(Eq. 26A}b\text{)}$$

$$K_{CB} K_{DC} U = m_1 m_2 m_3 \quad \text{(Eq. 26A}c\text{)}$$

For a case in which any of the K_{iO}'s = 0, the equation for Q_{TS} is a degenerate case of the preceding. The relationships between the m_i's and the coefficients (R, S, T, and U) are not altered. This can be seen from inspection of the equation written in the following form:

$$\frac{Q_{TS}}{D} = \left[\frac{\beta}{K_{CB} K_{DC}}\right]\frac{d^2\left[\frac{f(I)}{D}\right]}{dt^2} + (m_1 + m_2 + m_3)\left[\frac{d\left[\frac{f(I)}{D}\right]}{dt}\right] + (m_1 m_2 + m_2 m_3 + m_1 m_3)\left[\frac{f(I)}{D}\right] + (m_1 m_2 m_3)\left\{\int_0^t\left[\frac{f(I)}{D}\right]dt\right\} \quad \text{(Eq. 27A)}$$

$$\frac{Q_{TS}}{D} = \left[\frac{\beta}{K_{CB} K_{DC}}\right]\vec{\theta} \quad \text{(Eq. 28A}a\text{)}$$

Three of the four cases for the three-compartment model have $K_{DO} = 0$. From these equations,

$$K_{DC} = m_1 + \left[\frac{A_1(m_2 - m_1)(m_3 - m_1)}{K_{CB}}\right] \quad \text{(Eq. 28A}b\text{)}$$

Since

$$K_{CB} = -m_1A_1 - m_2A_2 - m_3A_3 \quad \text{(Eq. 28Ac)}$$

$$= \beta K'_{CB} \quad \text{(Eq. 28Ad)}$$

for these cases,

$$K_{DC} = K'_{DC} \quad \text{(Eq. 28Ae)}$$

Inserting these equalities into Eq. 28A*a*,

$$\frac{Q_{TS}}{D} = \left[\frac{\beta}{\beta K'_{CB}K'_{DC}}\right]\theta \quad \text{(Eq. 29A}a\text{)}$$

$$= \left[\frac{1}{K'_{CB}K'_{DC}}\right]\theta \quad \text{(Eq. 29A}b\text{)}$$

Therefore, β does not enter as a factor into the equation for Q_{TS} when $K_{DO} = 0$. However, referring to Eqs. 18A*a*–18A*g* for Case 1, it is found that $K_{DC} \neq K'_{DC}$, as occurs for Cases 2, 3, and 4; a correction for β is, therefore, required. Appropriate substitutions using the equations for Case 1 into Eqs. 28A*a*–28A*e* and setting Eq. 28A*a* equal to unity at all times for a rapid intravenous dose allow solution for β. The solution given is appropriate for both the cases, $K_{BO} = 0$, and $K_{BO} = K_{CO} = 0$.

$$\beta = \left\{\frac{(K'_{CD} + K'_{CB})\,\theta\,(K^*_{BO} - Q) - K'_{CB}\,g(K^*_{BO} - g)\,(K'_{CD} + K'_{CB}) + K'_{CB}\,\zeta}{K'_{CB}\,\theta\,(K^*_{BO} - g)}\right\} \quad \text{(Eq. 30A}a\text{)}$$

$$\zeta = m_1m_2m_3 - g(m_1m_2 + m_2m_3 + m_1m_3 - gK^*_{BO} - K^*_{BO}K'_{CD} - K^*_{BO}K'_{CO}) \quad \text{(Eq. 30A}b\text{)}$$

The derivation of expressions for Q_{TS} is based on mass balance and requires the adoption of a compartment model. However, it is apparent from the mentioned considerations that the model parameters, K_{iJ}'s, can be replaced by equalities and Q_{TS} can be expressed entirely in terms of equation parameters, *i.e.*, the A_i's and m_i's. The values of the A_i's and m_i's corresponding to a model are generally distorted from their unconstrained least-squares values by the imposed constraint that requires the corresponding K_{iJ}'s to be positive and real in order to be consistent with physically realizable values. The unconstrained least-squares values of the A_i's and m_i's obviously provide the best representation of the experimental results.

Compartment models are not necessarily unique and realistic descriptions of the biokinetic behavior of a system. It is suggested that for the purposes of absorption analysis, it may be justifiable to abandon all pretenses of requiring compartment models to possess physical reality. On this basis an expression for Q_{TS} could be derived to correspond to a generalized compartment model and expressed entirely in terms of unconstrained least-squares values of A_i's and m_i's without concern to whether these values correspond to physically realizable values for the model parameters. Although the model may be completely fictitious for this reason, more realistic values for Q_{TS} might conceivably be obtained. The justification for this approach could only be confirmed experimentally through the comparison of known and computed values of Q_{TS}. The required experimental results were not obtained in the present study.

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