Drug-Absorption Analysis from Pharmacological Data I: Method and Confirmation Exemplified for the Mydriatic Drug Tropicamide

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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 single, multiple, or continuous dosing of a drug by any route of administration. The results of such analyses permit an evaluation of the physiological availability characteristics of drugs from dosage forms and the elucidation of the kinetics and mechanisms operative in the passage of drugs across 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. The method is confirmed and illustrated for tropicamide, with results derived from the slow intravenous infusion of the drug to rabbits and the measurement of pupillary diameters. Agreement was observed between the quantities of drug known to be infused at any time and the amounts calculated wholly from pharmacological data on the basis of an assumed single-compartment model. The rapid appearance of the maximum mydriatic response following rapid intravenous dosing allows approximation of the biophase as a compartment kinetically indistinguishable from the central systemic compartment.

Keyphrases □ Drug-absorption analysis—pharmacological data □ Pharmacological response intensity—biophasic drug level relationship □ Tropicamide-produced mydriasis—drug-absorption analysis □ Dose–pharmacological effect relationship determination—pharmacological data □ Model, one compartment—tropicamide dose–effect relationship

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 an administration site 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 principal purposes of this report are to describe a basis for the use of temporal pharmacological response intensity data for drug-absorption analysis and to determine its applicability to the description of the biokinetic behavior of a mydriatic drug, tropicamide.

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 from a mathematical 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). Since these treatments rely on the periodic determination of the drug by direct chemical or radiological assay techniques, they may not be practical when the detection of very small quantities of very labile or potent drugs is required. In addition, these treatments have only been described 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, for example.

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 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 problems associated with the direct assay for drug levels in such locations could also be obviated. The development and confirmation of a basis for the utilization of pharmacological data for the absorption analysis of tropicamide are the subjects of the present report. Tropicamide was chosen as one of the model drugs in these studies because of the relative ease of following its mydriatic activity. Moreover, due to its nonionic character at physiological pH, it was anticipated to possess relatively uncomplicated biological transference properties.

Prior to the utilization of the presently described pharmacological method of drug-absorption analysis, several procedures should be performed to confirm its unmodified applicability to a particular drug system of interest. When the method is applicable, 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 areas under the curves of the results derived from the transformation of pharmacological measurements and plotted *versus* time have exactly the same interpretation with respect to physiological drug availability as similar curves obtained from blood level or urinary excretion rate *versus* time plots (2–3). In addition, when the sites of drug action are located in a compartment, termed the biophase, separate from the systemic circulation, the pharmacological method permits the determination of the "biophasic drug availability" as the fractional amount of the dose absorbed systemically that has entered and traversed the biophasic compartment. Analogous calculations of the

relative quantity of a topically administered drug that has penetrated directly to its vicinal biophase and the amount that is dissipated from the administration site by other routes, *e.g.*, systemic absorption, can also be performed. These and other aspects of the applicability of the pharmacological method to the performance of drug-absorption analysis for all routes of administration will be treated fully in subsequent reports.

THEORETICAL

Relationship of Pharmacological Response Intensity to Biophasic Drug Levels—The kinetic behavior of a drug in the body (*e.g.*, as regards its distribution, metabolism, and excretion) can most concisely be characterized in terms of compartment models. Data derived from the determination of the time course of variation of drug levels in a body compartment provide the necessary input for the construction of such biomathematical models. By treating the biophase as a compartment, its level of drug at any time following dosing is by definition uniform throughout its volume and is responsible for the observed intensity of the response to the drug. Since the biophase is a hypothetical concept, it is seldom possible to sample directly its drug content in the manner of blood, for example.

However, if a functional relationship between the level of drug in the biophase at any time and the observable intensity of drug response is known, the use of pharmacological data can, in appropriate cases, obviate the use of direct analytical methods for detecting the presence of the drug. Previous reports concerning the kinetics of pharmacological effects (4–10) invariably assumed that the intensity of a pharmacological effect is directly or otherwise simply related (*e.g.*, through hypothetical models for drug–receptor interaction) to the drug level in the biophase. In the present approach, the functional relationship between the pharmacological response intensity and biophasic drug levels is defined by the experimental intravenous dose–effect curve instead of through the postulation of hypothetical mechanisms for the dose–effect relationship. The intravenous dose–effect curve is utilized in the manner of a calibration curve to transform observed values of pharmacological response intensity (I) at any time into values of $f(I)$,[1] which are variables directly proportional to the quantity of drug in the biophase.

The present approach is developed on the consideration that the biokinetic behavior of many drugs is describable in terms of linear compartment models (11). The quantity of drug in the biophase following an intravenous dose, Q_{BS},[2] at any time, t, is given by the product of the dose, D, and a summation of exponential terms, as expressed by Eq. 1:

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

The A_i's and m_i's are equation parameters evaluated from the data; n denotes the number of compartments included in the model. An intravenous dose–effect curve may be constructed by plotting the intensity of the pharmacological response, which has been consistently recorded at a fixed time, t_r, following rapid intravenous dosing, as a function of the dose. Substituting the symbol $f(I)_{t_r}$ for D and the constant, β_{t_r}, for the summation, $\sum_{i=1}^{n} A_i e^{-m_i t_r}$, Eq. 1 can be rewritten at t_r as Eq. 2:

$$Q_{BSt_r} = f(I)_{t_r}\beta_{t_r} \qquad \text{(Eq. 2)}$$

Assuming Eq. 2 to hold at times other than t_r allows it to be rewritten as Eq. 3, which now provides a general relationship between the quantity of drug in the biophase and the intensity of response, where $Q_B = Q_{BS}$ and/or Q_{BB}:

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

[1] This symbol is read as "function of I"; I is not multiplied by f.

[2] The subscript, S, signifies that the quantity of drug, Q_{BS}, has reached the biophase *via* the systemic compartment. A subscript B, in place of S, indicates that the drug has entered the biophase directly from the site of administration without first being absorbed systemically.

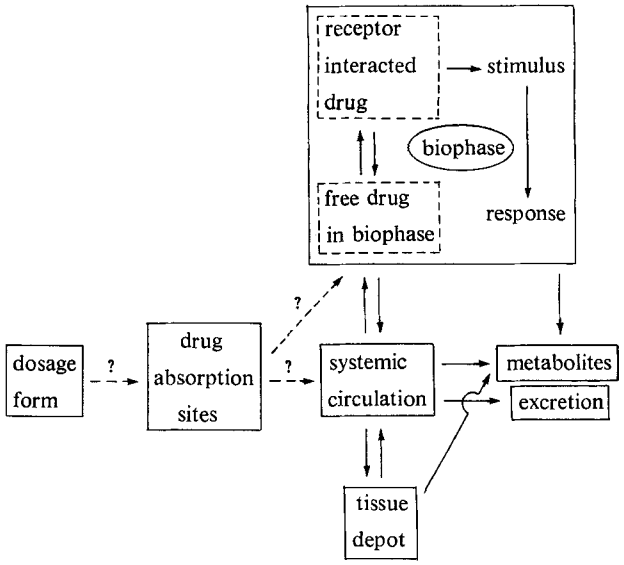

Figure 1—*Simplified compartmental illustration of the processes generally operative in affecting the pharmacological response characteristics of drugs. The purposes of drug absorption analysis are to elucidate the processes (designated by - - →) by which the drug is systemically absorbed, gains access to the biophase (which may or may not be a compartment distinct from the systemic circulation), and to determine the extent to which the absorption is efficient.*

The relationship between I and Q_B given by Eq. 3 applies at any time and irrespective of the route of administration. Substituting from Eqs. 2 and 3 into Eq. 1 allows the time course of change in the relative drug levels in the biophase following intravenous dosing to be described by Eq. 4. Equation 4 also applies to routes of administration other than the intravenous, provided the drug is absorbed from the site of its administration by an apparent first-order process, *i.e.*, passive diffusion. When this is known to be the case, data obtained from dosing by such routes can also be used in the construction of the dose–effect curve.

$$f(I) = \frac{f(I)_{t_r}}{\beta_{t_r}} \sum_{i=1}^{n} A_i e^{-m_i t} \qquad \text{(Eq. 4)}$$

Confirming the Applicability of the Approach—By referring to Fig. 1, it is implicit in the treatment that, first of all, 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 within the compartment designated as the biophase. The subsequent interaction of the drug with the receptor sites and the induction of the stimulus resulting in the observed response are sufficiently rapid that their extents are representative of instantaneous reversible equilibrium with the levels of drug in the biphase at any time. Second, all processes are entirely reversible, *i.e.*, nonhysteretic. And, third, the biokinetic behavior of the drug is describable in terms of a linear compartment model.

When these three conditions are not satisfied, the direct use of the dose–effect curve may not be appropriate and it may be necessary to derive other mathematical models to relate intensities of response to biophasic drug levels which are based on postulated mechanisms of drug–receptor site interaction. A difficulty in deriving such relations 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 maximum values of the response intensity ($I_{max.}$), 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 be approached would be such that the rate of change of the concentration of drug in the biophase is minimal (zero). This will occur when a steady-state drug concentration in the biophase

is achieved as, for example, through a slow intravenous infusion of a drug at a constant rate. Following a rapid intravenous dose, this time is obviously $t_{max.}$, *i.e.*, the time at which the maximal response, $I_{max.}$, is observed. A dose–effect curve constructed using $I_{max.}$ values corresponding to the plateaus of I *versus* time plots observed for differing rates of intravenous infusion or, if justified, to $t_{max.}$ following rapid intravenous dosing 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 to 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. As will be shown, the introduction of such complications into the model for tropicamide is not necessary.

When the previously stated conditions are satisfied, the functional relationship between the quantity of drug in the biophase and the observed response intensity is accurately given by Eq. 3. Obviously, in all cases a clearly defined graded intensity of pharmacological response must be measurable.

It is not generally sufficient to assume arbitrarily that the described conditions are met for any given drug and biological system. The applicability of the approach in any given case should be confirmed. The most obvious manner to accomplish this is through a comparison of results obtained by the actual measurement of drug levels (in, for example, plasma) with similar results calculated from pharmacological data. However, the greatest utility of the use of the pharmacological method is its applicability to systems where direct assay methods for the drug are not practical. However, other equally valid means may be applied to confirm the method. Two of these means are suggested by the form of Eqs. 3 and 4 and involve the comparison of the behavior of the data as actually observed with that predicted by the equations.

Congruency Test—The first test derives directly from inspection of Eq. 4, which predicts that plots of dose normalized values of $f(I)$ as a function of time should be independent of the dose. The values of $f(I)$ are normalized by division by the dose, which by definition equals $f(I)_{t_r}$. The curves so obtained for various doses should be congruent. This provides a test of the assumption of a linear compartment model.

$f(I)$ and β Ratio Tests—The second test becomes obvious from the consideration that the value of Q_B must be the same for any given value of I, irrespective of the dose or the time at which the value of I is observed. Since the choice of t_r for the selection of I values for use in the construction of intravenous dose–effect curves is arbitrary, any number of such curves can be constructed merely by selecting values of I corresponding to any number of values of t_r and plotting them as a function of dose. For two such curves using values of t_r and t_r', Eq. 3 will be $Q_B = f(I)_{t_r}\beta_{t_r}$ and $Q_B = f(I)_{t_r'}\beta_{t_r'}$ respectively. The values of Q_B for any given value of I are the same; therefore, the ratio of the two equations will equal unity and the result given by Eq. 5 is obtained:

$$\frac{f(I)_{t_r}}{f(I)_{t_r'}} = \frac{\beta_{t_r'}}{\beta_{t_r}} \qquad \text{(Eq. 5)}$$

It is, therefore, predicted that the ratio of $f(I)$ values, for any given value of I, obtained from the abscissas of any two intravenous dose–effect curves—differing only in the selection of the time of recording of the I values used in their construction—should be constant and equal to the inverse ratio of their β values. The observation of this predicted behavior of the data may be considered as an affirmation of the assumptions that the response to the drug is nonhysteretic and the instantaneous result of a continuous equilibrium between the free and receptor bound drug in the biophase.

A_t to CAD Comparison Test—The performance of this test of the applicability of the mathematical treatment to any given system involves the direct comparison of values for the amount, A_t, of drug absorbed calculated wholly from pharmacological data with actual known amounts, CAD, of drug cumulatively administered by slow intravenous infusion. Satisfactory agreement between the known and calculated quantities may be considered as a confirmation of all the assumptions implicit in the treatment as well as of the compartment model on which the calculation of A_t is based.

Table I—Steps in the Procedure, and Their Purposes, for the Application of Pharmacological Data to Drug Absorption Analysis

Step 1	
Procedure:	Experimental determination of the relationship between I and time for several intravenous doses
Purpose:	Collection of data needed: a. For the construction of intravenous dose-effect curves b. For the congruency test c. To fit a compartment model to simulate the biokinetics of the drug
Step 2	
Procedure:	Construct two intravenous dose–effect curves
Purpose:	a. Transformation of observed I values into $f(I)$ b. Performance of $f(I)$ ratio test
Step 3	
Procedure:	Derive a linear compartment model from a computerized, constrained, sums of exponentials fit to the $f(I)$/dose to time relationship
Purpose:	a. To obtain values of transfer constants (K_{iJ}'s) needed for the computation of the amount (A_t) of drug absorbed at any time following dosing by systemic routes; not needed for direct biophasic absorption[a] b. Calculation of β ratios for comparison to $f(I)$ ratios c. Performance of A_t to CAD comparison test (CAD = known cumulative amount of drug administered by slow intravenous infusion)
Step 4	
Procedure:	Apply the method to any route of administration
Purpose:	Elucidation of the kinetics and mechanisms operative in drug bioavailability

[a] The procedure in this case will be treated in a subsequent report in the present series.

Fitting a Compartment Model to the Data—Although desirable, a biomathematical, compartment model of the biokinetic behavior of a drug does not necessarily need to have a counterpart in biophysical reality in order to possess utility. Such models are merely mathematical abstractions of the actual biological processes. For the purposes of the present application to drug-absorption analysis, the questions of whether the model chosen to describe the data is unique and whether the compartments possess physiological reality are irrelevant. In fact, it is seldom possible 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, *i.e.*, one possessing an additional number of compartments. Therefore, the simplest model consistent with the data to which physically realizable values of the model parameters can be assigned (*i.e.*, transfer constants between compartments, K_{iJ}'s, must be real and nonnegative) is chosen.

For the present purposes, the general procedure for choosing a compartment model to describe the biokinetic behavior of the system is initiated by fitting a sum of exponential terms to a composite plot of $f(I)$/dose values as a function of time. The familiar graphical techniques (12, 13) of "peeling off" the exponentials may be used for this purpose to obtain values for the A_i's and m_i's constituting the equation parameters. Each exponential term in the summation corresponds to a compartment in the model. These hand-calculated values of the A_i's and m_i's are used as initial estimates in computer programs which are used to calculate least-square values of the A_i's and m_i's consistent with physically realizable values of the model parameters, *i.e.*, the K_{iJ}'s. The authors developed digital computer programs, using a method of systematized iterative guessing (14) for the fitting of data to a wide variety of multicompartment models. This method of curve fitting is advantageous because it converges rapidly and is devoid of the danger of merely reaching a local minimum in the sums of squares (11). The derivation of equations for relating the A_i's and m_i's to the K_{iJ}'s necessary for the computer programs is well described by Rescigno and Segre (15) as well as other authors (16, 17). In the present communication, the biokinetic behavior of tropicamide is simply described in terms of a single-compartment model in which the biophase is assumed to be kinetically indistinguishable from the central systemic compartment. A subsequent report will present results obtained from testing several

multicompartment models. Various criteria on which to base the selection of a model and judge its adequacy with regard to describing the drug biotransference behavior of pharmacologically responding systems will also be presented at that time.

Calculation of A_t for Systemic Absorption—The form of the equations for the calculation of the amount, A_t, of drug absorbed systemically from the site of administration at any time following dosing depends on the compartment model adopted to describe the distribution and elimination of the drug from the system (18, 19). When the ability of the drug to permeate tissue barriers is rapid, it may occur that the body can be treated as a single homogeneous compartment in which the biophase (as shown in Fig. 1) and other peripheral tissue depot compartments are kinetically indistinguishable from the central systemic compartment. In this case the value of A_t is simply given by Eq. 6 which resembles that of Wagner and Nelson (2). If t_r is chosen as zero, the value of β_{t_r} will be unity:

$$A_t = \beta_{t_r}\left[f(I) + K_e\int_0^t f(I)dt\right] \qquad \text{(Eq. 6)}$$

The transfer constant, K_e, represents the overall elimination constant from the system and is obtained from the slope of a linear semilogarithmic plot of $f(I)$ values as a function of time.

The procedural steps and their purposes in the application of the pharmacological method of drug-absorption analysis are summarily presented in Table I.

MATERIALS AND METHODS

Materials—Isotonic, phosphate-buffered, pH 7.4, tropicamide[3] solutions were prepared from double-distilled water for intravenous administration to rabbits.

To minimize intersubject variation, 3–4 month old, male, New Zealand white rabbits were screened with regard to: (*a*) the sensitivity of their pupillary response to varying light intensities; (*b*) the magnitude of their maximum mydriatic response to opththalmic and intravenous doses of tropicamide; and (*c*) the clarity of definition of their pupils. Four rabbits were ultimately selected from this group on the basis of observed similarities in their pupillary response behavior. These four rabbits were used throughout the remainder of the study.

Methods—Intravenous dosing of the rabbits was performed into their marginal ear veins. The doses ranged from 13.7 to 416.0 mcg./kg. and were contained in fluid volumes of 0.3 to 0.5 ml. which were rapidly injected.

The results obtained from the rapid intravenous dosing were utilized in the construction of the dose–effect curves. Additional measurements of mydriatic response were performed on the four rabbits during the time course of a slow intravenous infusion of a 0.015% w/v pH 7.4 phosphate-buffered solution of tropicamide to the rabbit at a zero-order rate. The solution was infused using a Sigmamotor[4] model T8SH pump. The rate of infusion was found to fluctuate between 0.15 and 0.19 ml./min. The variable flow rate precluded the cumulative amount of drug infused at any given time to be accurately gauged from the duration of the experiment. To circumvent this difficulty, the infusion was monitored gravimetrically. A 10-ml. covered flask containing the tropicamide solution was placed on a Mettler (p20) balance. Polyethylene tubing[5] was inserted into the solution, led through the finger projections of the pump, and finally affixed to the rabbit's ear vein *via* a 27 gauge 1.27 cm. (0.5 in.) hypodermic needle. The balance was tared, and the time course of weight change of the solution was followed simultaneously with variations of pupillary diameters.

Figure 2 describes the experimental arrangement utilized for the measurement of the rabbit's pupillary diameters. The rabbits were restrained in a box. The measurements were performed using a vernier caliper accurate to 0.1 mm. The caliper was held against the cheekbone of the rabbit at a nearly constant distance from the eye. The experimental procedure was routinely initiated by adjusting the light intensity, provided by two Bausch and Lomb microscope illuminators used in conjunction with rheostats, to obtain constant predetermined, standard pupil diameters. This procedure was found to decrease significantly both intersubject and intrasubject variations in observed mydriatic responses. When measurements were performed on both eyes, an additional light source was arranged symmetrically to that shown in Fig. 2. The control of lighting conditions was found to be a very important factor, affecting the reproducibility of the experimental results. Prolonged illumination of the eye was found to cause the pupil to dilate, following an initial constriction, and was therefore avoided. Consequently, the eye was illuminated only prior to a measurement. The minimum values of the pupillary diameters observed within a 30–45-sec. period following illumination were found to correspond to full accommodation and were recorded along with the time of their observation.

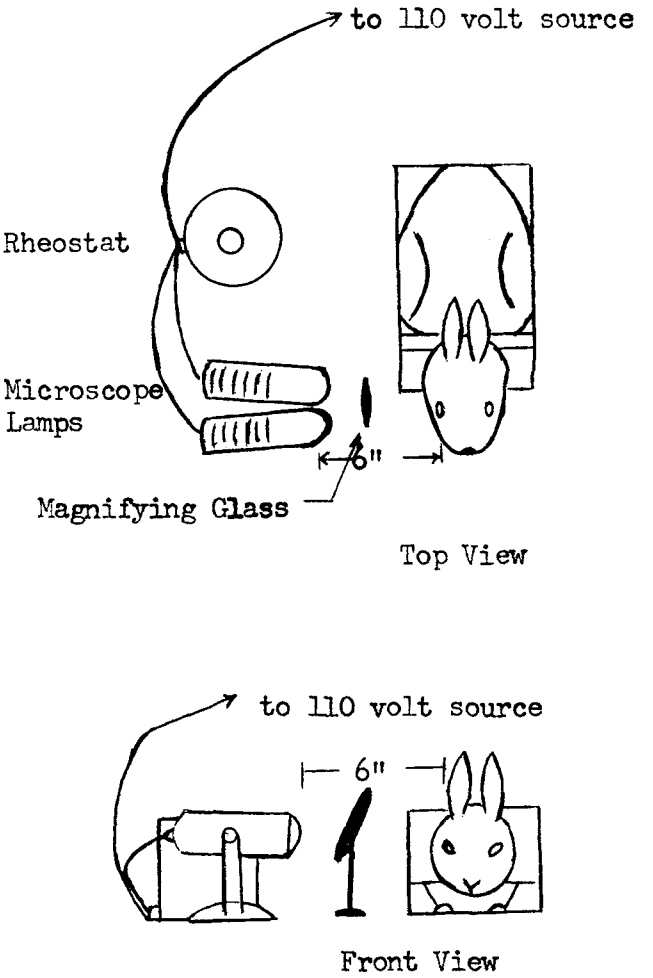

Figure 2—*Diagram of the experimental arrangement for the measurement of rabbit pupillary diameters.*

The mydriatic response intensity, I, was related to measured pupillary diameters at any time following the administration of the drug (d_t) and at time zero (d_0) by Eq 7:

$$I = \frac{d_t - d_0}{d_0} \qquad \text{(Eq. 7)}$$

An average error of precision of the $f(I)$ values, obtained through the conversion of observed I values *via* the $I_{max.}$ dose–effect curve, was calculated as less than 12%. Since all the $f(I)$ values were included in this estimate of error, it represents the overall average for the experiment. However, due to the constant error involved in the measurement of pupillary diameters, the percent average error of any value decreases with its magnitude. Mydriatic responses corresponding to drug-induced changes of less than 0.1 mm. in the diameter of the pupil could not be conveniently monitored. Therefore, responses of less than this magnitude were considered as subthreshold and recorded as zero.

[3] Tropicamide, Lot No. 2092, was supplied by Alcon Laboratories, Fort Worth, Tex.
[4] Middleport, N. Y.
[5] The tubing was animal tested PE 190: i.d. 0.119 cm. (0.047 in.), o. d. 0.170 cm. (0.067 in.); supplied by Clay-Adams, Inc., New York, N. Y.

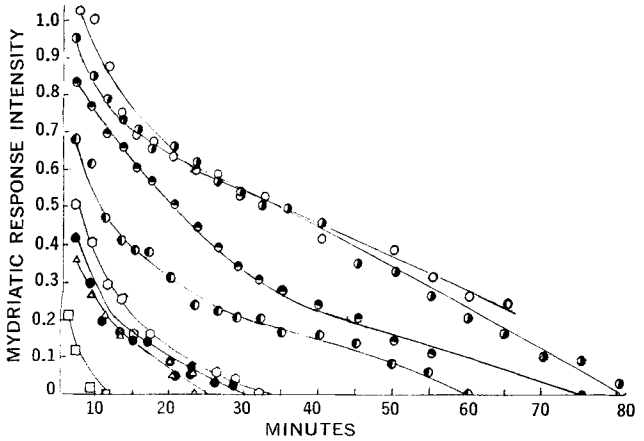

Figure 3—*Time course of mydriatic response intensity for rapidly administered intravenous doses of tropicamide. Each point is the average of a minimum of four determinations on four rabbits. The doses represented are 13.7 (□), 25.0 (△), 50.0 (●), 66.7 (○), 167 (◐), 250.5 (◓), 334 (◑), and 416.5 (○) mcg./kg. Continuous monitoring immediately following the dose revealed that, on the average, the maximum response occurred at* $t_{max.} = 1.88 \pm 0.72$ *min.*

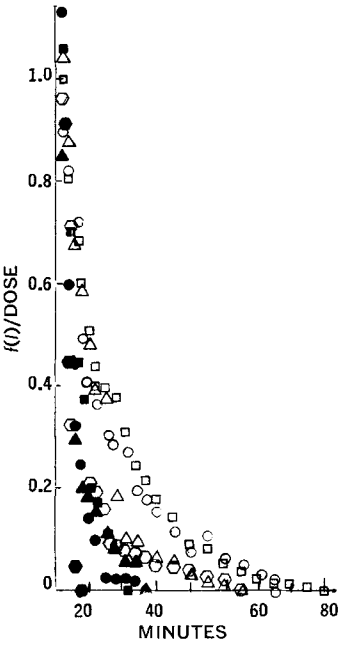

Figure 5—*The temporal variation of dose normalized relative quantities of drug in the biophase,* f(I). *Each value is the average of a minimum of four replications on four rabbits for doses of 13.7 (●), 25.0 (■), 50.0 (▲), 66.7 (●), 167.0 (○), 250.5 (△), 344.0 (□), and 416.0 (○) mcg/kg.*

RESULTS AND DISCUSSION

Mydriatic Response to Rapid Intravenous Dosing—Figure 3 graphically demonstrates the time and rapid intravenous dose dependency of the mydriatic response. The results are averages of four replications performed on the four rabbits screened for use in the study. The maximum values on the curves were recorded in each case following the continuous monitoring of the pupillary diameters immediately after dosing until the maximum response was observed to develop. The rapid appearance of the maxima, within 2 min. following dosing, precluded the accurate observation of submaximal values at prior times. The prompt development of the maximal responses is reflective of the rapidity with which the *de facto* unionized tropicamide ($pk_B \simeq 9.0$) can traverse tissue barriers to enter the biophase from the blood. Morphologically the biophase is likely constituted by some location in the iris.

Intravenous Dose–Effect Curves and the *f(I)* Ratio Test—The precision of observed values of the mydriatic response intensity increases with their magnitude. Therefore, dose–effect curves constructed by plotting the maximum observed values of I as a function of the dose produce the most precise relationship. The time, $t_{max.}$, corresponding to each $I_{max.}$ value is constant and independent of dose; this can readily be verified by differentiating Eq. 1 and setting the derivative equal to zero at $t = t_{max.}$. When $t_{max.}$ is experimentally observed to be constant, as in the present case with tropicamide, this observation may be taken as a further indication that a linear compartment model is adequate for simulating the biokinetic behavior of the drug. The choice of a constant time, t_r, at which to observe values of I for use in the construction of intravenous dose–effect curves is, therefore, consistent with the use of $I_{max.}$ values. The value of $t_{max.}$ observed for either I *versus* t or $f(I)$ *versus* t plots will of necessity be the same, provided the relationship between I and Q_B—as demonstrated by the intravenous dose–effect curve—is a single-valued, continuously increasing function. The $t_{max.}$ dose–effect curve was used for the routine conversion of I values into $f(I)$ values.

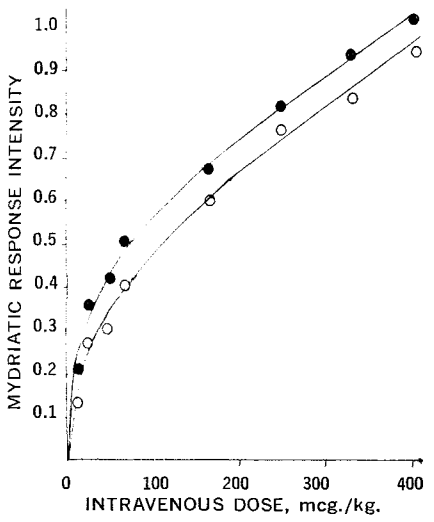

Figure 4—*Dose–effect relationships resulting from the intravenous dosing of rabbits with tropicamide. The upper curve (●) represents the maximum intensities. The lower curve (○) is a plot of the intensities observed at a time corresponding to twice the observed time of the maximum response. Each point is the average of a minimum of four replications using four animals.*

The choice of a time for the selection of I values for the construction of a second intravenous dose–effect curve may conveniently be taken as a multiple of $t_{max.}$. Figure 4 contains dose–effect curves constructed with I values corresponding to values of $t_{max.}$ and $2t_{max.}$ of 2 and 4 min., respectively. The values used in the preparation of these curves are listed in Table II.

The average ratio of $f(I)$ values, taken from the $t_{max.}$ and $2t_{max.}$ curves for I values arbitrarily chosen on the $2t_{max.}$ curve corresponding to the doses, is 1.52 ± 0.20 average error. Similar results are obtained when $f(I)$ ratios are computed from dose–effect curves constructed with I values observed at other times. Considering the exaggeration of experimental errors which occurs in the conversion of I values into $f(I)$ as well as in the calculation of the ratios, the agreement of the $f(I)_{2t_{max.}}/f(I)_{t_{max.}}$ ratios to constancy is considered acceptable. These results of the $f(I)$ ratio test are, therefore, considered as confirming the assumed nonhysteretic and reversible nature of the relationship between biophasic drug levels and the mydriatic response.

Congruency Test—A plot of average $f(I)$ values, normalized by dividing by their corresponding dose, is contained in Fig. 5. Ideally, curves drawn through each set of points corresponding to each dose should be congruent. However, the smaller values, which derive from the lowest doses, fail to coincide closely with the results corresponding to the higher doses. In addition to these particular values being derived from the smallest and least precise of the measurements, their deviant behavior may be attributed to the high sensitivity of the iris in response to low biophasic drug levels, as evidenced by the relatively large initial slopes of the dose–effect curves. This characteristic of the mydriatic response behavior becomes manifest at low dosage as an apparent premature disappearance of the effect relative to high doses. In reality the response has merely rapidly declined to below the limits of detection. Therefore, the apparent deviant behavior of the smallest doses is attributed to the experimental limitations that preclude the mensura-

Table II—Mydriatic Response Results for Rapid Intravenous Dosing of Rabbits with Tropicamide

Dose, mcg./kg.	$t_{max.}$[a]	$I_{max.}$[b]	I at $2t_{max.}$[b]	$\frac{f(I)_{2t_{max.}}}{f(I)_{t_{max.}}}$
13.7	2,2,2,1	0.215 ± 0.015	0.119 ± 0.063	1.52
25.0	2,2,1,1	0.364 ± 0.016	0.274 ± 0.060	1.72
50.0	2,2,2,2	0.420 ± 0.031	0.306 ± 0.060	1.92
66.7	2,2,4,2	0.510 ± 0.050	0.409 ± 0.044	1.67
167.0	2,1,1,2	0.671 ± 0.065	0.609 ± 0.120	1.39
250.5	1,2,1,1	0.831 ± 0.038	0.765 ± 0.012	1.17
334.0	2,1,2,2	0.940 ± 0.064	0.837 ± 0.080	1.47
416.0	1,2,4,4	1.017 ± 0.131	0.955 ± 0.251	1.16
Averages	1.88 ± 0.72			1.52 ± 0.20[b]

[a] Values, corresponding to individual rabbits, were recorded to the nearest minute. [b] Recorded ± average error.

tion of the subthreshold intensities of response which correspond to these lowest biophasic drug levels. Excluding these points, curves drawn through the remaining values would appear decidedly similar. The random scatter of points around the single curve shown in Fig. 6 may be taken as a further indication that the conditions of the congruency test are satisfied.

Single-Compartment Model for Tropicamide—Figure 6 is a semilogarithmic plot of average $f(I)$ values, which have been normalized for dose, as a function of time. A linear correlation coefficient of 0.990 was calculated for the points plotted in Fig. 6. The least-squares regression line is plotted in the figure. This line represents a fit to a single-compartment model, *i.e.*, $B + C \rightarrow$, where B and C denote the biophase and systemic circulation, respectively. Even though the convexity of the curve toward the abscissa indicates that additional compartments communicating with the biophase are operative, the single-compartment model may in some cases be preferred for its simplicity, provided the results derived on its assumption are reasonably accurate. Adoption of the linear single-compartment model in effect assumes that all kinetic processes are apparent first order and nonsaturable as well as that mixing of the drug between the systemic circulation, the biophase, and all other regions of the body is sufficiently rapid that these regions are kinetically indistinguishable. Ideally, in accordance with this model the distribution of the drug throughout the body should occur instantaneously. In adopting the model, the observed lag time of approximately 2 min. between the time of rapid intravenous dosing and the appearance of the maxima in the I and $f(I)$ *versus* time curves is subtracted from real time. On the corrected time scale, $I_{max.}$ now occurs at time zero; the value of the constant β_{t_r}, appropriate for use with the $I_{max.}$ dose–effect curve, is unity.

A comparison of the ratio of $f(I)$ values, derived from dose–effect curves constructed for two different times with the inverse ratio of their corresponding β values in accordance with Eq. 5, provides a criterion for judging the accuracy of a compartment model in simulating the change in biophasic drug levels in the period between the times corresponding to the values of I used in the construction of the dose–effect curves. The relatively poor agreement of the β ratio value of 1.17, computed on the basis of the single-compartment model, to its $f(I)$ ratio, previously noted as 1.52, reflects the inaccuracy of the model in simulating the biokinetic behavior of the system in the period of 2–4 min. Inspection of Fig. 6 reveals that the values of $f(I)$ occurring prior to 6 min. are among the largest of the values that systematically deviate from the least-squares regression line representing the fit of the data to the model. The utility of performing a comparison for an expanded time period is vitiated by the magnification of errors in the $f(I)$ ratio which occurs from the necessary use of smaller, and less precise, values of $f(I)$. The β ratio test was found to be a more useful criterion in its application to a cationic mydriatic drug (18, 19) having a longer onset and duration of action.

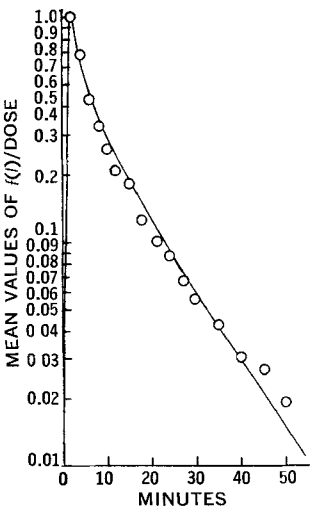

Figure 6—*Relationship of mean, normalized for dose, values of* f(I) *to time following intravenous dosing of rabbits with tropicamide. The points represent the average of four replications on different animals for each intravenous dose of 13.7, 25.0, 60.0, 66.7, 167.0, 250.5, 334.0, and 416.0 mcg./kg. The straight line is a least sums of squares regression line representing a fit of the data to a single-compartment model; the half-life for the overall elimination of the drug from the animal is 9.73 min.*

Resolution of the Dose–Effect Curve—Figure 7 demonstrates the resolution of the $I_{max.}$ intravenous dose–effect curve into apparent linear and hyperbolic components. The separation was performed by the extrapolation of the latter linear segment of the experimental curve to the ordinate and its subsequent transposition through the origin. The hyperbolic curve is obtained as the difference between the linear and the experimental curves.

The hyperbolic component of the curve is suggestive of Langmuirian adsorption and conforms to the fundamental tenets of the familiar drug–receptor occupation theory (20) for the operation of a single type of receptor (21). In accordance with these views, the hyperbolic contribution to the intensity of response (I_h) is directly related to the extent of receptor occupation and approaches a maximum, equal to αR, with the saturation of the receptor sites; this is expressed mathematically by Eq. 8, where Q_B is the quantity of drug in the constant volume of the biophase that is responsible for the total intensity of effect. The symbols α, R, and K_D refer to the intrinsic activity, total quantity of receptor sites, and the dissociation constant of the drug–receptor complex, respectively.

$$I_h = \frac{\alpha R Q_B}{K_D + Q_B} \qquad \text{(Eq. 8)}$$

The linear component of the dose–effect curve may be interpreted as a result of an apparently nonsaturable process such as could

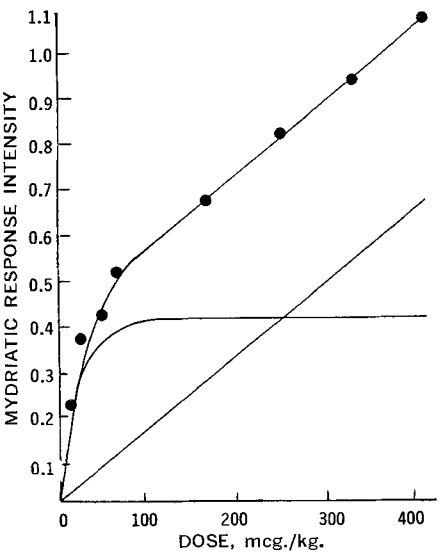

Figure 7—*Resolution of the* $I_{max.}$ *intravenous dose–effect curve for tropicamide into linear and hyperbolic components.*

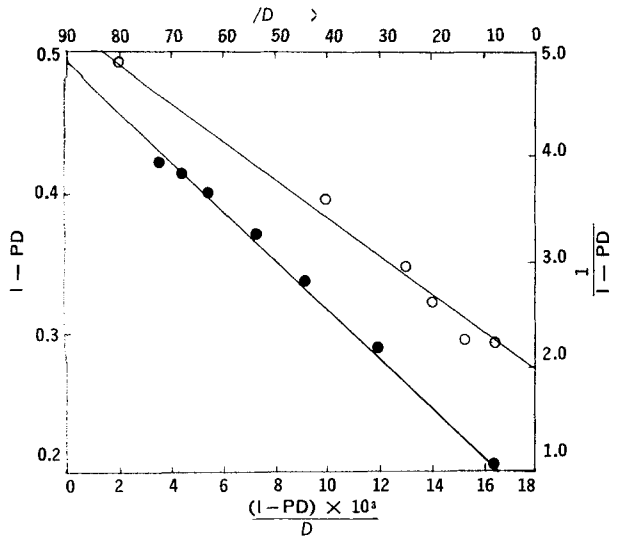

Figure 8—*Scatchard-* (●) *and reciprocal-* (○) *type linearized plots of the hyperbolic component resolved from the* I_{max}. *intravenous dose–effect curve of tropicamide for rabbits.*

conceivably involve the partitioning of the drug into some "intrabiophasic" location. The partitioning would be required to occur at rates sufficiently rapid for the continuous maintenance of distribution equilibrium throughout the volume of the biophase. Based on this surmise, the direct proportionality constant, P, relating the linear contribution to the response intensity (I_l) in Eq. 9 has some connotation both as a distribution coefficient and an intrinsic activity:

$$I_l = PQ_B \qquad \text{(Eq. 9)}$$

By combining Eqs. 8 and 9, the observed response intensity can be expressed by Eq. 10, in which Q_B is substituted by the equivalent product βD as defined by Eq. 2:

$$I = \frac{\alpha R \beta D}{K_D + \beta D} + P\beta D \qquad \text{(Eq. 10)}$$

Since β equals unity, P can be directly evaluated from the slope of the linear component curve in Fig. 7. Equations 11 and 12 are linearized forms of Eq. 10, which permit the evaluation of the equation parameters from the slopes and intercepts of the straight lines shown in Fig. 8. The curves in Fig. 8 result from plots of $I - P\beta D$ *versus* $(I - P\beta D)/D$ and $1/(I - P\beta D)$ *versus* $1/D$ and are analogous to Scatchard and reciprocal plots, respectively.

$$I - P\beta D = \alpha R - \frac{K_D}{\beta}\left(\frac{I - P\beta D}{D}\right) \qquad \text{(Eq. 11)}$$

$$\frac{1}{I - P\beta D} = \frac{1}{\alpha R} + \frac{K_D}{\alpha R \beta}\left(\frac{1}{D}\right) \qquad \text{(Eq. 12)}$$

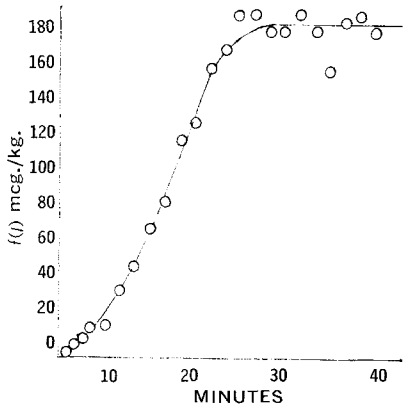

Figure 9—*The variation of* f(I), *the relative quantity of drug in the biophase, during the slow intravenous infusion of 0.015% tropicamide in an isotonic pH 7.4 solution at an average rate of 0.17 ml./min. The results are the average of four determinations on different rabbits.*

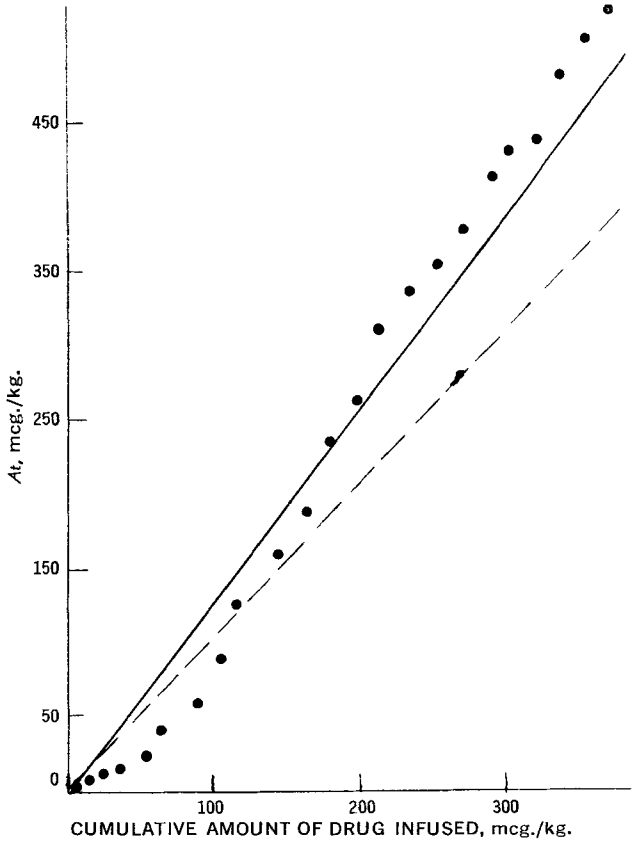

Figure 10—*A comparison of actual cumulative amounts of tropicamide administered to rabbits by slow continuous intravenous infusion to amounts,* A_t, *calculated from pharmacological data on the basis of a single-compartment model. The slope of the least-squares regression line (solid) forced through the origin is 1.29. The linear correlation coefficient is 0.994. The dotted line represents the theoretically perfect correlation with a slope of unity.*

The values of the model parameters, as computed from least-squares regression values of slopes and intercepts, are listed in Eqs. 13–15.

$$K_D = 17.33 \text{ mcg./kg.} \qquad \text{(Eq. 13)}$$

$$\alpha R = 0.496 \text{ mcg./kg.} \qquad \text{(Eq. 14)}$$

$$P = 1.57 \times 10^{-3} \text{ kg./mcg.} \qquad \text{(Eq. 15)}$$

Although the analysis of the intravenous dose–effect curve for tropicamide suggests some interesting aspects relative to the mechanisms of the pharmacological action of the drug, such information is not required and is in fact irrelevant to the use of the pharmacological method of drug-absorption analysis when the conditions of the $f(I)$ ratio and congruency tests are satisfied.

Comparison of Known and Calculated Amounts of Slow Intravenously Infused Drug—Figure 9 graphically demonstrates the variation of $f(I)$ values during the time course of the slow intravenous infusion of tropicamide at a zero-order rate of approximately 10 mcg. $kg.^{-1}$ $min.^{-1}$. A steady state was attained after approximately 20 min. The infusion was terminated at the end of 40 min.

Using the $f(I)$ values in Fig. 9 and the previously obtained value of 0.0713 for K_e, Eq. 8 was implemented for the calculation of A_t for each time corresponding to the known values of the cumulative amount of drug infused. Figure 10 compares normalized values of the known cumulative amounts of drug administered by slow intravenous infusion with the similar amounts of drug calculated from the pharmacological data. Ideally the plot in Fig. 10 should be linear with a zero intercept and a slope of unity as represented by the dashed line. The least-squares regression line plotted in the figure has been forced through the origin and has a slope of 1.29 The linear correlation coefficient for the relationship between the calculated and experimentally known values of A_t is 0.994. Although

there are obvious deviations, considering that the calculated values were derived entirely from the measurement of pupillary diameters, these results may be considered as providing additional confirmation of the applicability of the pharmacological method of analysis to this system. The majority of the results indicate that the single-compartment model represents a fair estimation of the kinetic behavior of tropicamide. However, the appearance in some instances of apparent systematic deviations of the data from values predicted on the basis of the single-compartment model suggests that the simulation of the biokinetic behavior of tropicamide might be improved with the adoption of a multicompartment model. The results obtained from the development and testing of 12 two-compartment and 9 three-compartment models will be the subject of a subsequent report.

SUMMARY AND CONCLUSIONS

The principal purpose of the present report has been the introduction of a method, and the means by which it may be confirmed, for the performance of drug-absorption analysis entirely from pharmacological data. A direct means of confirming the applicability of the described method to any particular pharmacologically responding system can most obviously be obtained through a comparison of results derived by its use with similar results based on the detection of the drug in corporeal fluids. However, the greatest utility of the pharmacological method obtains when such direct measurements are not convenient or practicable. As exemplified for tropicamide, confidence in the applicability of the techniques may alternatively be obtained by comparing the behavior of the observed pharmacological results with that predicted by a postulated model. In the present case, the conformancy of the results for tropicamide was concluded as sufficient for verity in the application of the method to the absorption analysis of this drug.

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 or 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.

Generally, results derived from the use of pharmacological data corresponding to the sought clinical response to the drug are of particular interest. However, when the sought therapeutic response cannot be quantitated or is otherwise not amenable to treatment by the methods described, data obtained from the monitoring of a side effect of the drug may be utilized instead. In this manner the same information that is commonly derived from blood level and urinary excretion data can still be obtained from pharmacological results. In this case the utilization of pharmacological data merely serves as an analytical tool which can be applied in lieu of directly assaying for the drug in body fluids or tissues. Since confidence in the applicability of the pharmacological data for this purpose is obtainable from the pharmacological data itself, there may be no necessity for performing direct assays, even in those instances with drugs whose direct detection in the body is practicable. The choice between the use of direct assay or pharmacological data may in each case depend upon the expected relative accuracy, reproducibility, and ease with which experimental results can be obtained.

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