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Drug-Absorption Analysis from Pharmacological Data II: Transcorneal Biophasic Availability of Tropicamide

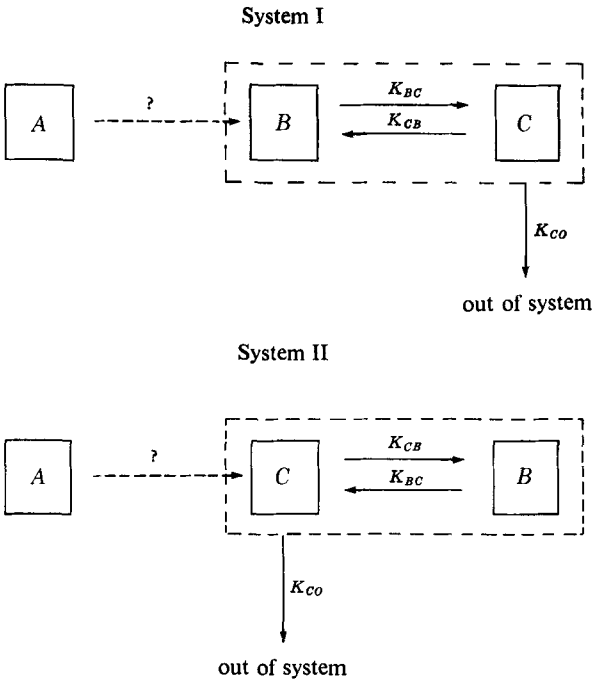
RONALD D. SCHOENWALD and VICTOR F. SMOLEN*

Abstract □ Following an ophthalmic dose, the mydriatic drug, tropicamide, may traverse the cornea to reach its vicinal sites of action (biophase) directly or indirectly gain access subsequent to its systemic absorption. The latter route of entry into the biophase may be attributed to fluid volume loss and scleral absorption. A means by which the relative quantities of the drug absorbed transcorneally directly into the biophase and indirectly following systemic absorption at any time can be discerned from concomitant measurements of pupillary diameters in drug-treated and untreated eyes is described. However, except at very high dosage, the observed magnitude of effects in the control eye were insufficient for this method to be practical for tropicamide. The determination of the total relative quantities of drug ultimately dissipated from the absorption site by routes other than transcorneal absorption into the biophase was, however, approximated from measurements performed on the treated eye alone. Semilogarithmic plots of the time course of transcorneal drug passage were linear, indicating the biophasic availability of tropicamide to occur through the operation of apparent first-order processes. A comparison of pharmacological and biokinetic parameters characterizing the mydriatic behavior of tropicamide administered in vehicles having a pH of 5.0 and 7.4 is presented.

Keyphrases □ Tropicamide mydriasis—transcorneal biophase parameters, vehicle pH influence, rabbits □ Pharmacokinetic parameters, biophase—transcorneal tropicamide mydriasis, vehicle pH influence □ Mydriatic response behavior, tropicamide—vehicle pH influence, rabbits

The influence of formulation factors on such pharmacological response characteristics as onset and duration of response, peak response intensity, time of peak response, and rate(s) of dissipation of effect is an important consideration in the development and evaluation of pharmaceutical products. A drug induces its biological effects when it enters its biophase compartment where it interacts with its receptor sites. The rates at which a drug enters and is subsequently dissipated from its biophase determine the time course of the induced response intensity and, therefore, the characteristics of its pharmacologic behavior. At the two extremes, the availability of a drug to its biological sites of action is either limited by its release from its dosage form or by such biological factors as the permeability of tissue barriers, metabolism, distribution of the drug into tissue depots, and excretion. When the drug is administered by other than parenteral routes, its biophasic availability, *i.e.*, the total quantity of drug that has penetrated to the biophase at any time, may also be severely affected by peripheral losses from the site(s) of absorption. The relative quantities of drug that are absorbed to contribute subsequently to the pharmacologic effect and the quantities that are peripherally eliminated from the site of administration are dependent upon the relative rates of the competing, simultaneously operative processes.

As is commonly the case with ophthalmic preparations, drugs are generally most rapidly available for absorption when administered in the form of their aqueous solutions. The composition of such solutions can influence the rates at which the drug becomes available to its biophase because it can determine the form of the drug and, therefore, its tissue permeability. Alternatively, the vehicle may exert an influence directly on the permeability properties of the tissue barriers which the



Scheme I—Resolution of a compartment model of ophthalmic drug transference into two systems which is possible through use of a control eye. System I depicts transcorneal passage of the drug from the site of administration, A, *directly into the biophase,* B. *System II is appropriate for the entry of the drug into the systemic circulation,* C, *prior to penetrating to the biophase. Although both absorptive processes are operating simultaneously, they can be studied individually. The enclosure of* B *and* C *within the broken lines is indicative of the kinetic indistinguishability of these compartments, as was found to be the case for tropicamide.*

drug must penetrate (1). The rates of peripheral drug loss can also be influenced by formulation factors, *e.g.*, by the inclusion of viscosity-imparting agents which affect the residence time of the drug vehicle at the absorption site(s) (2).

The application of conventional approaches utilizing direct assay methods in the study of *in vivo* transcorneal drug absorption can present intractable difficulties, while *in vitro* experimentation utilizing excised corneas may be poorly reflective of *in vivo* ophthalmic drug availability because of the difficulty of simulating the peripheral drug loss. Such peripheral dissipation of the drug occurs by fluid loss through the lacrimal drainage duct, scleral absorption into blood and lymphatic vessels, and aqueous flow following transcorneal passage. The purpose of the present study was to investigate the applicability of a previously described (3, 4) pharmacologic method of drug-absorption analysis to the elucidation of the biophasic availability and peripheral loss behavior of the mydriatic drug tropicamide following its administration into the eye.

THEORETICAL

A basis for the transformation and subsequent utilization of pharmacological data, derived from monitoring the temporal dependency of the drug-response intensity following dosing, for the performance of compartmental analysis and the elucidation of drug bioavailability was described and confirmed for tropicamide in previous reports (3, 4). The applicability of the method to any particular drug can be determined from pharmacological data alone; therefore, the detection of the drug by direct assay is entirely obviated. With the exception of volumes of distribution, the method can be applied to determine all the types of pharmacokinetic results calculable from data obtained by directly sampling body fluids or tissue drug levels. Drug-absorption analysis using drug level in corporeal fluid data is limited to those routes of administration (*e.g.*, oral and parenteral) by which the drug is absorbed systemically, concomitantly, or prior to reaching its sites of action. The pharmacological method, when applicable, is not disadvantaged in this manner; it can be implemented for any route of administration to determine the time dependency of blood and/or biophasic drug levels as well as the quantities of drug absorbed from the site of administration into the blood and/or biophase for any route of administration. The method is particularly advantageous in its application to drugs that cannot readily be assayed and to the study of the bioavailability of drugs administered topically for localized effects.

When drugs are administered for local effects, the intended site of their action (biophase) is obviously located vicinally to the site of administration. The absorption of the drug and its subsequent disposition in the body may be envisaged ideally to be occurring as depicted in System I of Scheme I. Inevitably, however, some drug is absorbed systemically, as shown in System II of Scheme I; it directly enters the systemic circulation by the various previously mentioned routes prior to distributing into the biophase. This latter mode of entry is inefficient and generally undesirable. It may contribute to the occurrence of systemic toxicity and should be prevented as far as possible; this is important with local anesthetics, potent alkaloidal drugs used ophthalmically, *etc.*

The pharmacological effects and the relative quantities of the drug absorbed systemically and biophasically can be distinguished when the pharmacological effects can be simultaneously recorded from an untreated site of action symmetrical with the treated site to which the drug was administered; the untreated site serves as a control for comparison to the treated site. It can be assumed that the drug that is directly absorbed systemically, *i.e.*, without first passing through a biophase, gains equal access to both the symmetrical control biophase and the biophase located vicinally to the site of administration. This is well exemplified with the ophthalmic dosing of a mydriatic drug into one eye where the systemically absorbed drug may be expected to gain equal access to the iris in both eyes. The transcorneally absorbed drug may be assumed to enter the biophase (which anatomically may be constituted by a region of the iris) directly and prior to entering the systemic circulation.

The level of drug in the biophase that is responsible for the observed intensity of pharmacological response in the treated 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 treated and control eyes, the pharmacological response intensity in the treated eye that is attributable to the level of drug in its 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 treated and control eyes. The quantities of drug reaching the biophase directly or *via* the systemic circulation can, therefore, be discerned.

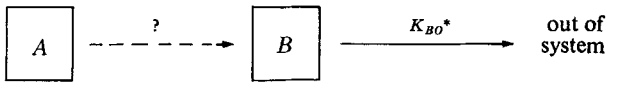
These considerations allow the resolution of the absorptive processes into the two systems depicted in Scheme I. By utilizing a treated and a control eye, each system can be studied individually, although experimentally the data for each study are obtained simultaneously. The relative quantity of drug in the biophase at any time resulting from direct biophasic absorption is simply obtained by converting the simultaneously observed values of I for both eyes to corresponding quantities of the drug in the biophase and taking their difference. As previously established (3, 4), the quantity of drug (Q_B) in the biophase compartment at any time following dosing by any route is provided by Eq. 1 where β is a constant. The details of the origin and evaluation of β were described in earlier reports (3, 4).

$$Q_B = \beta f(I) \qquad \text{(Eq. 1)}$$

The functional relationship between Q_B/β and I, *i.e.*, $f(I)$, is provided by an intravenous dose–effect curve, which is employed in the manner of a calibration curve. The curve for tropicamide was given previously (4), where it was also shown that the value of β for tropicamide is unity and Q_B may be read directly from the abscissa of the curve. As was discussed (4), this situation arises as a con-

sequence of the biophase and central systemic compartments for tropicamide being kinetically indistinguishable. This behavior can be attributed to the apparent rapidity with which tropicamide can permeate tissue barriers.

A simplified compartment model for drug absorption directly into the biophase is illustrated in Scheme II; its use can be justified



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

by the following considerations. By excluding the volume of the biophase, the remaining volume of distribution for the drug in the animal may be assumed very large in comparison to the volume of the biophase and, therefore, provides a sink for the drug into which it is effectively eliminated upon leaving the biophase. The backflow into the biophase can then be neglected to simplify the model (Scheme II). In the event the backflow is 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 obtaining from 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 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. However, since the biophase and systemic compartments have been found to behave identically in the case of tropicamide, such complications are of no further concern in this analysis.

These considerations justify the adoption of the single-compartment model shown in Scheme II. The quantity of drug that has been absorbed directly into the biophase from the site of administration up to a time, t, is given by Eq. 2. The elimination constant, K_{BO}^*, from the biophase, in accordance with Scheme I is given by $K_{BO}^* = K_{BO} + K_{BC}$. It can be evaluated from the postabsorptive time course of biophasic drug levels following the administration of an ophthalmic dose of the drug.

$$A_t = \beta_{t_0}\left[f(I) + K_{BO}^* \int_0^t f(I)dt\right] \qquad \text{(Eq. 2)}$$

Equation 2 is independent of any compartment models for the description of the biokinetic behavior of the drug in the system. The bioavailability characteristics of drugs directly into a vicinal biophase can, therefore, be determined with the use of Eq. 2 without any need for developing compartment models such as are necessary for the study of systemic drug absorption (3, 4). Only the intravenous dose-effect curve, used to transform I values into $f(I)$ values, and the data obtaining from the local administration of the drug are required. However, by considering that Eq. 1 is valid irrespective of the route of administration, it is conceivable that for drugs such as tropicamide, for example, the intravenous dosing necessary to construct the dose–effect curve could be obviated. Provided that the biophase and systemic compartments are known *a priori* to be identical (β, therefore, equals unity when maximum I values corresponding to zero time are used), the functional relationship given in Eq. 1 and between Q_B and I can be defined from data obtained by simultaneously monitoring the time course of the pharmacological response intensity and the corresponding blood levels of the drug following a single dose given by any route, irrespective of the kinetics and mechanisms involved in the absorption of the drug. The result of plotting values of I as a function of corresponding blood levels would be theoretically equivalent to an intravenous dose–effect curve and could be used in a similar manner for the conversion of I values into Q_B values, provided an appropriate volume of distribution could also be computed from the data. When the volume of distribution is not readily calculable, *e.g.*, when the actual dose of drug absorbed is not known, the dose–effect relationship would still provide relative values of Q_B. This method of determining the relationship between Q_B and I has the advantage of not requiring multiple intravenous dosing. On the other hand, it requires the detection of the drug in the blood. It will be further treated in a subsequent report.

If the quantity of drug absorbed systemically before entering into the biophase relative to that absorbed directly is appreciable, it may be an important factor contributing to any observed systemic side effects in addition to the intensity of the intended 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. As discussed previously, operation of the processes responsible for the systemic absorption of an ophthalmically administered drug, *i.e.*, its peripheral loss, is reflected in the response observed in the untreated control eye. Irrespective of the complexity of the involved processes, expressions for A_t, such as Eq. 2, are independent of the kinetics and mechanisms by which the drug becomes systemically absorbed. However, expressions for A_t are dependent upon the compartment models determined to describe best the biokinetic behavior of the drug in the body. These models are best determined from the results of intravenous dosing. In the simplest case, in which a single-compartment model is found adequate, Eq. 2 would be appropriate for the computation of the quantities of drug absorbed directly into the biophase as well as those amounts peripherally dissipated and subsequently absorbed systemically. Although the assumption of a single-compartment model was shown to be a reasonable approximation of the biokinetic behavior of tropicamide (4), this may not generally be the case (3).

MATERIALS AND METHODS

Materials—Tropicamide[1] solutions were prepared in concentrations of 0.010, 0.015, 0.020, and 0.030% w/v in an isotonic, pH 7.4 phosphate-buffered vehicle. A 0.015% w/v tropicamide solution, having a pH equal to 5.0, was also employed. It contained 1.313% w/v $NaNO_3$; the pH was adjusted by the dropwise addition of an appropriate volume of 2 N HNO_3. Four 3–4-month-old, white, New Zealand, male rabbits were chosen from among 20 rabbits screened on the basis of the similitude of their pupillary response to light intensity and to the mydriatic activity of tropicamide. These same rabbits were used throughout the study and were the same animals employed for the determination of the intravenous dose curve.

Methods—The technique employed for the measurement of pupillary diameters was described earlier (4). The mydriatic response intensity, I, is defined in terms of observed pupillary diameters by Eq. 3, where d_0 and d_t refer to values observed at time zero and t following dosing, respectively. The dose–effect curve, determined for the rabbits used in the present study, was reported (3). It was utilized for the conversion of I values into their corresponding $f(I)$ values.

$$I = \frac{d_t - d_0}{d_0} \qquad \text{(Eq. 3)}$$

The ophthalmic administration of tropicamide was performed by carefully pipeting 0.020-ml. volumes of the solutions into the lower conjunctival cul-de-sac of the right eyes of each of the four rabbits. The left eyes served as controls.

RESULTS

Mydriatic Response to Tropicamide—The control eyes of the rabbits were found to be entirely devoid of a detectable mydriatic response to the drug. This was generaly found to be the case, except at drug solution concentrations inordinately higher than those employed in the present experiment. The observed time and dose dependency of the mydriatic response in the tropicamide-treated eyes at pH 7.4 is graphically shown in the semilogarithmic plot in Fig. 1.

The time scale in Fig. 1, as well as that in the remaining figures, was corrected from real time by the subtraction of a consistently observed lag time of 6 min. This time likely corresponds to the ini-

[1] Supplied by Alcon Laboratories, Fort Worth, Tex.

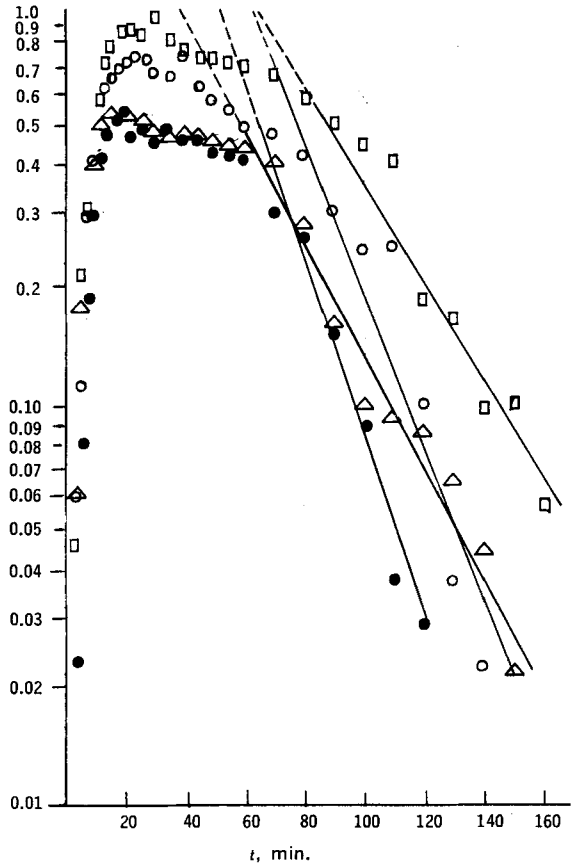


Figure 1—*Time course of mydriatic response following the ophthalmic administration of 0.02 ml. of 0.010* (●), *0.015* (△), *0.020* (○), *and 0.030* (□) % *w/v, pH 7.4, solutions of tropicamide. Each point represents the average of four determinations on different rabbits.*

tial loading of the drug into the biological barriers it must traverse, as well as the development of a level of drug in the biophase sufficient to elicit an observable threshold response. Each point in Fig. 1 represents the average of four determinations on different rabbits. The intrasubject variation of such results was previously discussed (3, 4) to be of the same magnitude as the intersubject variation, therefore justifying the averaging of the values replicated on different rabbits. The intersubject reproducibility of response may be attributed to the careful screening of the rabbits, which was performed prior to their selection for this study. The relative error of the measurements of pupillary diameter diminishes with the magnitude of the values. The overall average error was approximately 10%.

Time Course of Biophasic Drug Levels—Transformation of the I values, plotted in Fig. 1, into corresponding biophasic drug levels was accomplished through the implementation of the intravenous dose–effect curve as previously described (3). The results, replotted for each dose, are shown in Fig. 2. Relative to Fig. 1, the transformation of the data can be observed to have resulted in a somewhat enhanced linearity of the latter postabsorptive segments of the curves.

Biophasic Drug-Elimination Rates—Adopting the compartment model depicted in Scheme II permits the computation of apparent first-order biophasic elimination half-lives from the slopes of the latter linear segments of the semilogarithmic plots contained in Fig. 2. Linear least-squares regression values are listed in Table I. The expected independence of these values on dose and vehicle composition is indicated by their observed similarity, which lends credence to the assumption of the linear compartment model shown in Scheme II. The plot of dose normalized, *i.e.*, Q_B/D, values[2] shown in Fig. 3 provides a least-squares best value for K_{BO}* equal to 0.051 min.$^{-1}$.

[2] Since $\beta = 1$ and $f(I)_{max.}$ = dose, Q_B/D in reference to tropicamide is tantamount to $f(I)/f(I)_{max.}$. See *Reference 4* for further details.

Table I—Biophasic Elimination (t_B) and Apparent Mydriatic Response Dissipation (t_M) Half-Lives Observed for Ophthalmically Administered Tropicamide

pH of Vehicle	Tropicamide Concentration, % w/v	t_B[a]	t_M[a]
7.4	0.010	17.7	13.3
7.4	0.015	15.7	21.4
7.4	0.020	19.8	16.0
7.4	0.030	13.3	24.6
5.0	0.015	21.8	47.3

[a] Linear least-squares regression values.

Dissipation of Peak Mydriatic Effect—A semilogarithmic plot of averaged $I/I_{max.}$ values for each pH 7.4 solution is also shown in Fig. 3 for comparison to the corresponding plot of averaged Q_B/D values. The dissimilarity in the $I/I_{max.}$ and Q_B/D *versus* time curves emphasizes the necessity of transforming observed I values into Q_B or $f(I)$ values in order to draw conclusions concerning the biotransference behavior of a drug from pharmacological data. For example, assuming a linearity for the latter part of the $I/I_{max.}$ *versus* t plot allows a half-life of 50 min. to be ascribed to the dissipation of the peak mydriatic response intensity as compared to a value of 13.6 min. for the biophasic drug-elimination half-life. If the two quantities were directly related, the half-lives would be identical. The relationship, however, is again provided in this case by the intravenous dose–effect curve, as can be seen from the following considerations. The biophasic elimination constant, K_{BO}*, and the

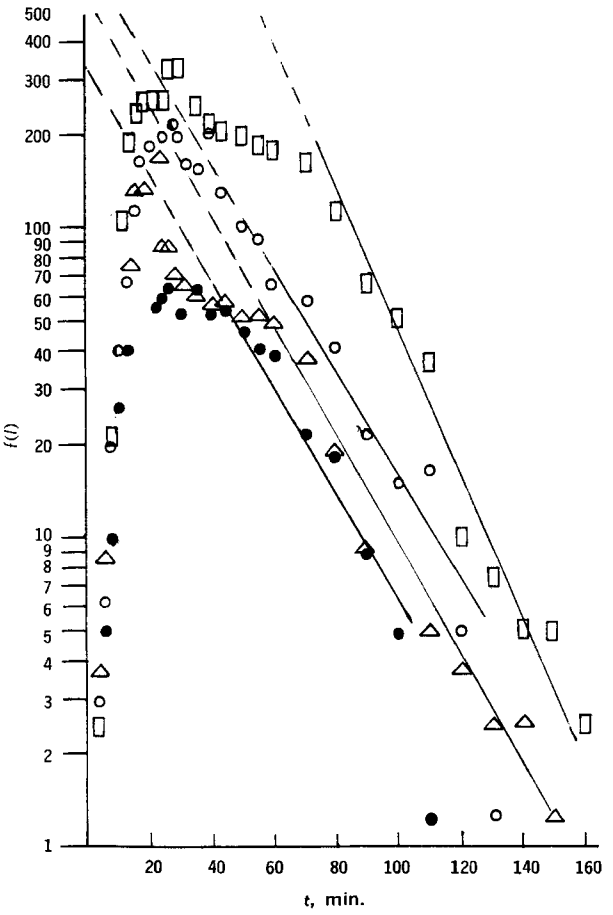


Figure 2—*Temporal variation of biophasic drug levels following ophthalmic dosing of 0.020 ml. of 0.010* (●), *0.015* (△), *0.020* (○), *and 0.030* (□) % *w/v, pH 7.4, tropicamide solutions. Each point is the average of four determinations performed on different rabbits. The time axis is corrected from real time by the subtraction of a 6-min. lag time.*

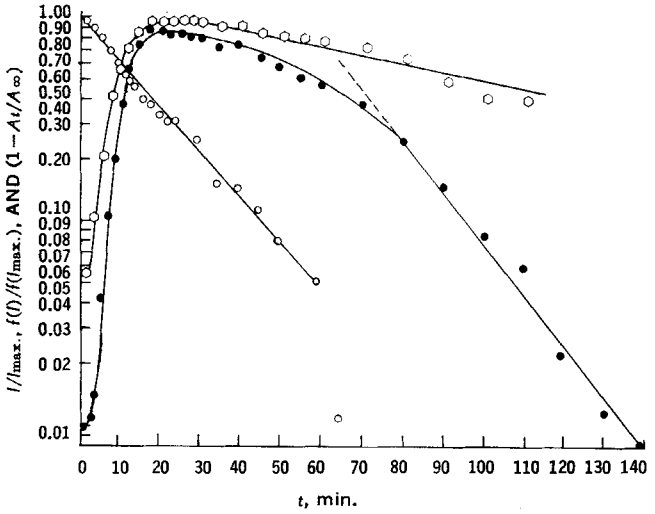


Figure 3—*Semilogarithmic plots of normalized values of* $I/I_{max.}$, *the relative mydriatic response intensity* (○); $f(I)/f(I_{max.})$, *the relative amount of drug in the biophase* (●); *and* $(1 - A_t/A_\infty)$, *the fraction of drug remaining to be transcorneally absorbed into the biophase* (○); *as a function of time following the ophthalmic administration of 0.020 ml. of 0.010, 0.015, 0.020, and 0.030% tropicamide solutions in pH 7.4 isotonic buffer to rabbits. Each point is the average of 16 determinations.*

apparent mydriatic response dissipation constant, K_M, may be defined by Eqs. 4 and 5, respectively, and related by Eq. 6:

$$\frac{dQ_B}{dt} = -K_{BO}^* Q_B \qquad \text{(Eq. 4)}$$

$$\frac{dI}{dt} = -K_M I \qquad \text{(Eq. 5)}$$

$$K_M/K_{BO}^* = \frac{d(\log I)}{d(\log Q_B)} \qquad \text{(Eq. 6)}$$

It is readily derived, as given by Eq. 6, that the ratio of the two constants at any value of I is directly equal to the instantaneous slope of a log–log plot of the dose–effect curve which, as discussed previously, is tantamount to a plot of log I *versus* log Q_B. The value of K_M may be expected to be constant and directly related to K_{BO}^* only for values of I corresponding to linear regions of such a plot. The log–log plot of the previously reported (4) dose–effect curve for tropicamide, shown in Fig. 4, approximates linearity for the majority of its extent. As expected from considering the deviations from linearity, the slope of the least-squares line drawn in Fig. 3, having a

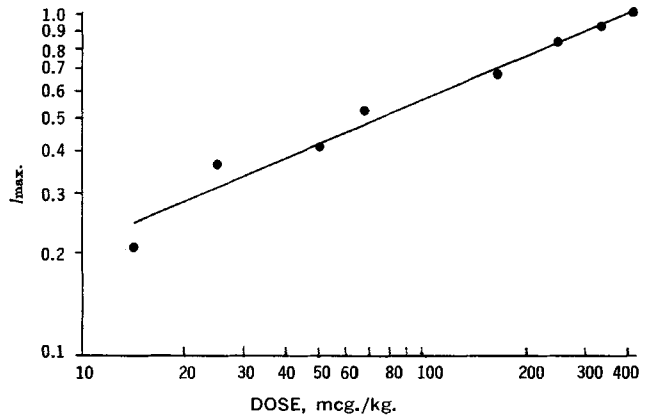


Figure 4—*Log* $I_{max.}$ versus *log dose plot for the rapid intravenous administration of tropicamide. The maximum response* ($I_{max.}$) *was observed at 2 min. following dosing. Each point is the average of four determinations on different rabbits.*

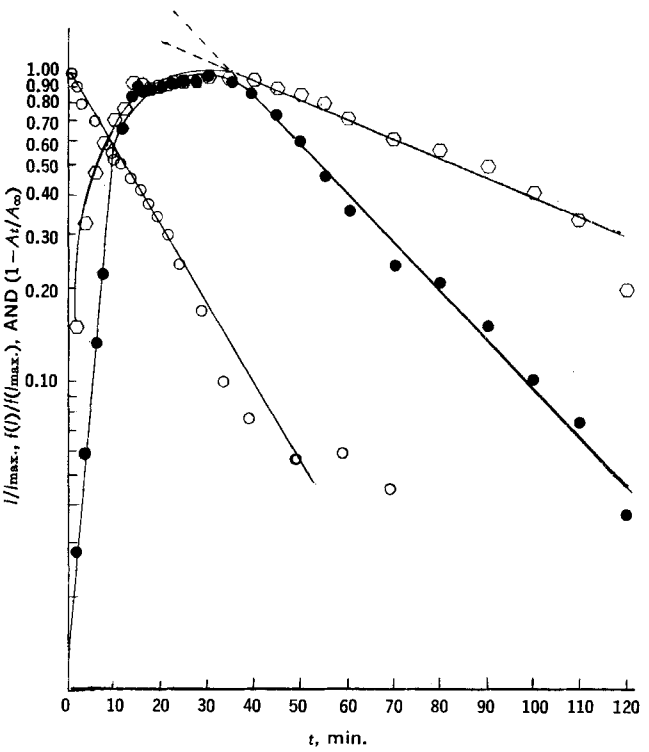


Figure 5—*Semilogarithmic plots of the time dependency of the relative mydriatic response intensity,* $I/I_{max.}$ (○); *the relative quantities of drug in the biophase,* $f(I)/f(I_{max.})$ (●); *and the fraction of drug remaining to be transcorneally unabsorbed,* $(1 - A_t/A_\infty)$ (○), *following ophthalmic dosing of 0.02 ml. of 0.015% w/v tropicamide in a pH 5.0 vehicle. Each point is the average of four determinations replicated on different rabbits.*

value of 0.43, only approximates the ratio of K_M/K_{BO}^*, equal to 0.72, calculated from the average half-lives listed in Table I.

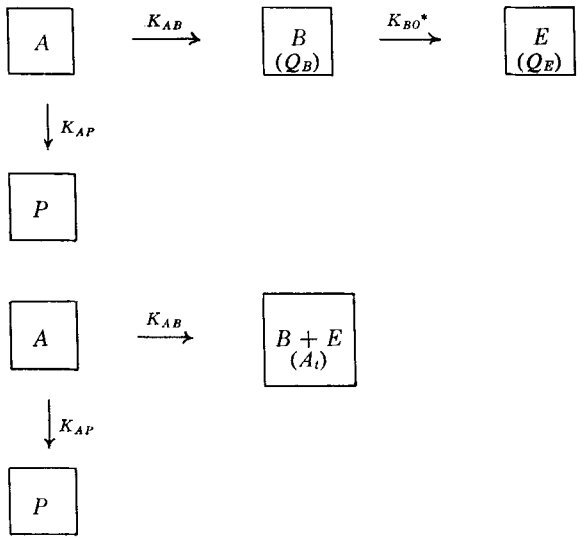
Biophasic Availability and Peripheral Loss of Ophthalmically Administered Tropicamide—Figures 3 and 5 present plots of log $(1 - A_t/A_\infty)$ *versus* t for the results obtained at pH 7.4 and pH 5.0, respectively. Values of A_t were computed numerically, using Eq. 2, with the aid of a CDC 6500 digital computer. The linearity of the plots is indicative that the clearance of the drug from the ophthalmic site of administration through both peripheral loss and absorption into the biophase can be described to occur by apparent first-order processes such as passive diffusion. Following this consideration, the compartment models, shown in Scheme III, can be applied to describe these processes. On the basis of these models, Eq. 7 can readily be derived:

$$\log (1 - A_t/A_\infty) = -\left(\frac{K_{AP} + K_{AB}}{2.303}\right)t \qquad \text{(Eq. 7)}$$

Table II—Pharmacokinetic Parameters Characterizing the Transcorneal Biophasic Availability (BA) of Tropicamide[a]

pH	Tropicamide Concentration, % w/v	$K_{AB} + K_{AP}$	r^b	K_{AB}	K_{AP}	Percent BA[c]
7.4	0.010	0.0687	0.9766	0.0348	0.0340	50.3
7.4	0.015	0.0528	0.7465	0.0230	0.0300	43.3
7.4	0.020	0.0613	0.9065	0.0454	0.0159	74.9
7.4	0.030	0.0387	0.9905	0.0294	0.0093	57.4
5.0	0.015	0.0667	0.9937	0.0461	0.0206	73.8

[a] The solutions were approximately isotonic; 0.02 ml. of each were instilled into the cul-de-sac of rabbits. Each parameter listed in the table is the average of four determinations on different rabbits. [b] Pearson r linear correlation coefficients for plots of log $(1 - A_t/A_\infty)$ *versus* time. [c] The values of percent BA represent the percent of the dose of drug transcorneally absorbed directly into the biophase.



Scheme III—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;* A_t *represents the quantity of drug transcorneally absorbed from* A *into the biophase; and* $A_t = Q_B + Q_E$, *where* $Q_E + Q_B$ *is the sum of the quantities of drug present and having been eliminated from the biophase at any time.*

The sum of the constants, $K_{AP} + K_{AB}$, represents the total proportional rate of loss of the drug from the site of administration. In accordance with Eq. 2, $K_{AP} + K_{AB}$ values can be obtained from the slopes of log $(1 - A_t/A_\infty)$ *versus* time plots; values obtained at pH 7.4 and 5.0 are compared in Table II.

If a mydriatic response attributable to peripheral drug loss had been detectable in the control eye, a similar plot of log $(1 - A_t/A_\infty)$ *versus* t performed for the control eye would be describable by Eq. 7 and identical to the results observed for the treated eye. Values of A_∞ would be calculable for both eyes, and their sum would be expected to equal the dose. Since Q_B values were obtained from the use of an intravenous dose-effect curve, the dose, D_e, computed from the sum of A_∞ values would correspond to an ophthalmically equivalent intravenous dose, which may not necessarily be the same as the actual ophthalmic dose administered. In any event, having values for D_e allows the resolution of individual values for K_{AP} and K_{AB} and a computation of the relative quantities of the drug absorbed directly into the biophase and dissipated peripherally from the application of Eq. 8 and 9:

$$\frac{K_{BO}^* \int_0^\infty Q_B\, d}{D_e} = \frac{A_\infty}{D_e} \qquad \text{(Eq. 8)}$$

$$\frac{K_{BO}^* \int_0^\infty Q_B\, dt}{D_e} = \frac{K_{AB}}{K_{AB} + K_{AP}} \qquad \text{(Eq. 9)}$$

Since no response was observed in the control eyes, D_e obviously cannot be computed from the sum of the A_∞ values computed separately for the two routes of drug loss. However, taking advantage of the kinetic indistinguishability of the biophase and central systemic compartments, as previously described for tropicamide (4), permits values of D_e to be obtained by extrapolating the latter linear segment of the log Q_B *versus* time curves forward to a value of the ordinate that corresponds to a real time, $t_{max.}$, equal to 1.88 min. The value of the ordinate at 1.88 min. on the extrapolated line provides the value of D_e. This can be understood by noting that 1.88 min. is the time corresponding to the maxima in the curves of I *versus* time obtained for rapid intravenous dosing, and that by definition: $Q_{B,\,max.} = f(I_{max.}) = \text{dose}$ (3).

Table III—Pharmacologic Response Parameters Characterizing Tropicamide-Induced Mydriasis following Ophthalmic Dosing[a]

pH of Vehicle	Tropicamide Concentration, % w/v	Onset[b]	$t_{max.}$[b]	Maximum Intensity	Duration[b]
7.4	0.010	10.5	17	0.603	58.5
7.4	0.015	8.8	20.5	0.722	61.0
7.4	0.020	9.2	22.5	0.7803	73.3
7.4	0.030	10.2	27.0	0.9363	86.0
5.0	0.015	6.1	14.5	0.695	79.9

[a] Each value represents the average of four replications on different rabbits. [b] Time in minutes.

In addition to the fact that some of the administered drug may not be absorbed either peripherally or transcorneally but simply washed out of the eye by tear fluids, the use of D_e instead of the actual ophthalmic dose is necessary because the mixing of the drug between tissues that actually comprise the biophase and the remainder of the body is less than instantaneous. When the drug is administered vicinally to the biophase, the drug is initially concentrated at the receptor sites before becoming mixed and diluted throughout the volume of the compartment. The actual ophthalmic dose absorbed, D_a, can be related to D_e by the equation: $D_e/V_s = D_o/V_B$, where V_B describes the volume of distribution of the biophase containing the drug receptor sites while V_s includes the entire distribution volume of the body that is being assumed to behave indistinguishably from the biophase. Since $V_s > V_B$, an intravenous dose hypothetically equivalent to an ophthalmic dose in producing the same sequence of mydriatic activity is correspondingly larger than an ophthalmic dose by a factor of V_s/V_B. Since the Q_B values measured for ophthalmic dosing are obtained from the intravenous dose–effect curve, they correspond to equivalent intravenous doses rather than the actual ophthalmic doses. In terms of ideal compartment theory, which assumes instantaneous mixing in all compartments, this treatment obviously presents a paradox. However, the justification for this approach is that it provides an explanation for the observed behavior and allows the calculation of quantities otherwise unattainable.

Pharmacologic Parameters Characteristic of Tropicamide Mydriasis—The onset of mydriasis (defined as the time following dosing required for the development of one-half the peak response intensity), the duration of mydriasis (defined as the time interval between one-half maximal responses), the peak response intensity, and the time of peak response are listed in Table III for each ophthalmic solution employed in the present study. The duration and maximum intensity exhibit a consistent increase with the concentration of tropicamide in the pH 7.4 solutions. Values for the onset and time of maximum intensity appear randomly variable.

Biokinetic Parameters Characterizing Tropicamide Mydriasis—The results of the biokinetic analysis of tropicamide-induced mydriasis are listed in Table II. In agreement with the assumed linear compartment models, no consistent pattern of dose dependency is apparent for the pH 7.4 buffers. The differences in the tabulated values of $K_{AP} + K_{AB}$, K_{AP}, K_{AB}, and the percent biophasic availability were found by t-tests to be statistically insignificant at well above the 5% level of confidence.

Influence of Vehicle pH on Mydriatic Response Behavior—Reference to Figs. 1 and 5 allows a comparison of the time dependency of biophasic drug levels following dosing with 0.015% w/v solutions of the drug in vehicles having a pH of 7.4 and 5.0, respectively. As indicated by the similarity in biophasic elimination half-lives listed in Table I, the postabsorptive phases of the curves are quite similar. The absorptive behavior of tropicamide at pH 7.4 and 5.0 is reflected in the log $(1 - A_t/A_\infty)$ plots shown in Figs. 4 and 5, respectively. A comparison of the pharmacological and biokinetic parameters for the 0.015% solutions of tropicamide of pH 5.0 and 7.4 showed them to be statistically insignificant at the 95% level of confidence. However, the onset, percent biophasic availability, and time of maximum effects exhibited statistical differences between confidence levels of 75 and 92%.

At pH 7.4, the drug exists in an essentially unionized form, whereas at pH 5.0 it is approximately 50% ionized. On this basis, a more rapid penetration of the drug into the cornea may be expected to occur at pH 7.4, relative to pH 5.0, due to the larger concentration of the more permeable unionized form of the drug (5, 6). If the pH of the vehicle can be assumed to have an influence at all, it can be

concluded from the values of K_{AB} and percent biophasic availability listed in Tables II and III that it is opposite to that expected on the basis of this consideration alone. The increased percent biophasic availability observed at pH 5.0 as equal to 73.8%, relative to 43.3% at pH 7.4, may be speculatively attributed to the interaction of tropicamide cations with anionic binding sites affixed to the colloids composing the corneal tissue; the tissue binding of the drug may function to retard peripheral drug loss relative to transcorneal absorption and provide a reservoir for the drug from which it may be subsequently more efficiently biophasically available. A similar mechanism was found responsible for the pH-enhanced effectiveness of procaine as a corneal anesthetic (1). The small magnitude of the effect of administering the drug at 5.0 relative to 7.4 may be a consequence of the dilution of the unbuffered pH 5.0 vehicle with lacrimal fluid, causing its rapid buffering to a physiological pH of 7.4.

SUMMARY AND CONCLUSIONS

A basis for the resolution of the rates and relative amounts of ophthalmically administered tropicamide which are transcorneally absorbed and peripherally dissipated by other routes has been described and applied using temporal pharmacological data. The previously established kinetic indistinguishability of the biophase and systemic compartments permitted the resolution to be accomplished despite the absence of a detectable mydriatic effect in the control eye. The biokinetic analysis of the pharmacological results permitted the mydriatic behavior of tropicamide to be described in terms of quantitative parameters. Although an insufficient number of replications (four replications on each of five ophthalmic solutions) was performed to draw firm, statistically based, conclusions regarding the effects of vehicle pH, the present study does exemplify a quantitative approach to the evaluation and design of ophthalmic drug vehicles.

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*To whom communications should be addressed.

Cardiovascular Effects of Bulbocapnine

T. FAKOUHI, T. D. DARBY*, and R. S. McCUTCHEON†

Abstract □ Upon intravenous injection of bulbocapnine into the dog, myocardial ventricular contractile force was altered and the mean arterial blood pressure was markedly lowered. Following a 50-mg./kg. dose of bulbocapnine, the blood pressure and contractile force effects of 5-hydroxytryptamine became negligible. Injections of norepinephrine, epinephrine, and isoproterenol in doses of 1 mcg./kg. and of ethylnorepinephrine in a dose of 50 mcg./kg. showed reduced effects on mean arterial blood pressure and contractile force. The effects of none of these were reversed by bulbocapnine. Animals treated with bulbocapnine, 25 mg./kg. i.p. for 5 days, became more sensitive to injections of 5-hydroxytryptamine, norepinephrine, epinephrine, and isoproterenol, as was seen in consistently altered diastolic blood pressure and contractile force effects.

Keyphrases □ Bulbocapnine—cardiovascular effects, dogs □ Catecholamine cardiovascular activity—bulbocapnine effect □ 5-Hydroxytryptamine cardiovascular activity—bulbocapnine effect

This investigation examines the changes produced in some of the cardiovascular responses of 5-hydroxytryptamine and representative catecholamines in the dog following treatment with bulbocapnine, an alkaloid, 3,4-methylenedioxy-6-apomorphine, found in the tubers of *Corydalis cava* (1). The use of the last-named agent was of interest following the reports of Walaszek and Chapman (2) that bulbocapnine depresses

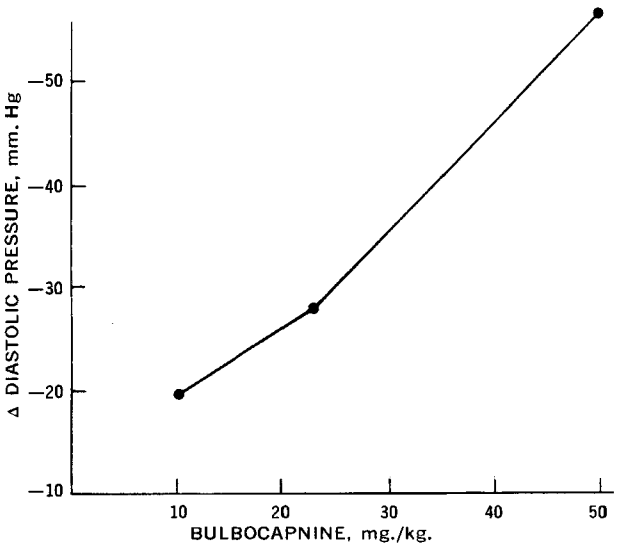

Figure 1—*Blood pressure responses to bulbocapnine. Key:* ●, *diastolic change.*

the hypertensive response to norepinephrine and reverses the response to epinephrine in cats and dogs. These authors classified the alkaloid as an α-blocking agent, using the terminology of Ahlquist (3). Since the