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# Relationship between Dose, Effect, Time, and Biophasic Drug Levels

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**Abstract** □ A detailed basis for the use of experimentally determined dose–effect curves, in the manner of calibration curves, to transform observed intensities of pharmacological response into biophasic drug levels at all times following dosing by any route is presented. The principles are graphically exemplified through the construction of dose–response–time surfaces. The conditions of applicability of the described approach are also discussed.

**Keyphrases** □ Drug levels (biophasic), dose, effect, and time—relationship, equations □ Pharmacological response, intensity—basis for transforming into biophasic drug levels at any time by any route, equations □ Dose effect curves—used to transform pharmacological response intensity into biophasic drug levels, equations

The use of biophasic drug level–time profiles, obtained from the transformation of observed pharmacological data, for the purposes of biokinetic systems analysis (1, 2), drug absorption analysis (1–5), and determination of the time course of pharmacological effects from the results of optimized *in vitro* drug release testing (3), was described in previous reports. The validity of implementing the pharmacological method to a particular drug system can be confirmed from observed pharmacological data alone; therefore, this approach can be implemented with drugs for which assays for their detection in biological media are difficult or nonexistent. In contrast to the use of data derived from direct assays for the drug, another advantage of the pharmacological approach is that its applicability to the performance of drug absorption analysis is not limited to systemic routes of administration alone. Provided biophasic drug levels sufficient to induce detectable magnitudes of pharmacological effects are achieved, both the systemic and biophasic (1, 2) drug availability can be computed for any route by which the drug is administered.

The use of pharmacological data is based upon the implementation of an experimentally observed dose–effect curve to provide a relationship between experimentally observed intensities of pharmacological response, $I$ (at any time following dosing by any route of administration), and the corresponding values of the quantities of drug in the biophase, $Q_B$. The justification for this procedure involves mathematical steps which were not made explicitly apparent in earlier reports. The purpose of the present article is to outline a more rigorous and detailed explication of this approach.

## THEORETICAL

**Experimental Data Required to Construct $I$–$Q_B$ Relationship**—The necessary data are constituted by observed $I$ time, $t$, profiles for varying doses, $D$, of drug administered either intravenously or by any route by which the drug is known, *a priori*, to be absorbed either directly to the site(s) of action in the biophase or to the systemic circulation by apparent first-order processes[1].

**Assumptions Implicit in Treatment and Confirmation**—For the pharmacological method of biokinetic analysis to be applicable in its simplest, unmodified form, as described earlier (1–3), the pharmacokinetic processes of drug absorption[1], distribution, and elimination should be nonsaturable (*i.e.*, dose independent) and, therefore, describable by a linear compartment model. Therefore, following dosing, $Q_B$ can be expressed in terms of a sum of exponential terms multiplied by $D$ (as given by Eq. 1, where $n$ is the number of terms, and $A_i$ and $m_i$ are equation parameters):

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

The confirmation of this condition is embodied in the congruency test and $\beta$-ratio test as previously described (1, 2).

The second condition which must be met for the accurate implementation of the unmodified approach requires that the intensity of pharmacological effect is instantaneously responsive to the quantity of drug in the biophase and is a nonhysteretic (single-valued) monotonic function of $Q_B$. The verity of the occurrence of this condition is indicated by a satisfactory conclusion to the $f(I)$ ratio test (1, 2).

**Construction of Dose–Effect Curve**—The dose–effect curve consists of a plot of the intensity of effect, $I_r$, (recorded consistently at any arbitrarily chosen time, $t_r$, following dosing), as a function of the dose. For both practical and theoretical reasons (1, 2), $t_r$ is best chosen as the time, $t_{max.}$, corresponding to the maximum observed response. If the discussed conditions are realized, the value of $t_{max.}$ is constant and dose independent.

**Relationship between $I$ and $Q_B$ at Any Time**—The basis for the relationship of $Q_B$ to $I$ and its inverse becomes apparent from a consideration of the following equations. Consider:

$$I = g(Q_B) \qquad \text{(Eq. 2)}$$

[1] The necessity of this condition applies only to the route of administration employed for the construction of the dose effect curve. The pharmacological method of drug absorption analysis (1, 2) is otherwise independent of the kinetics and mechanisms of drug absorption and can be applied to their elucidation.

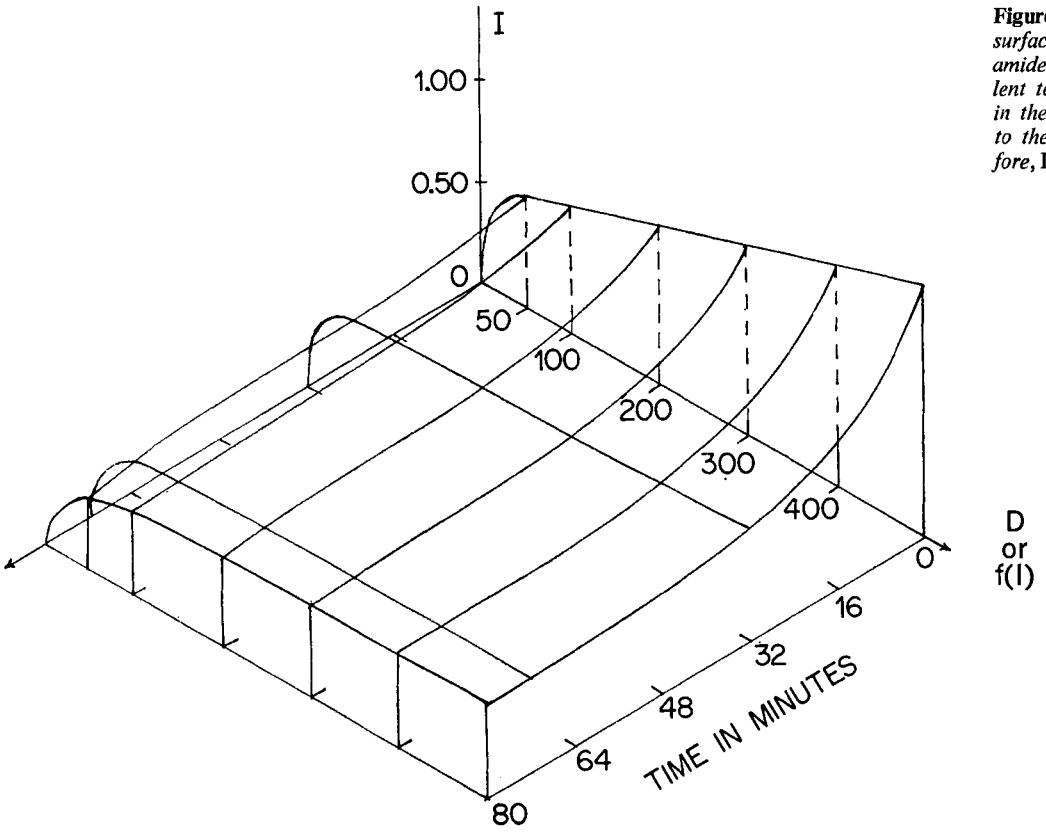


**Figure 1**—*Dose–response* (I)*–time surface for the mydriatic drug tropicamide. The dose,* D, *axis is equivalent to the relative quantity of drug in the biophase,* f(I), *axis. Referring to the* $t_r = 0$ *trace,* $\beta_{t_r} = 1$*; therefore,* $D = f(I) = Q_B$.

and inversely:

$$Q_B = G(I) \qquad \text{(Eq. 3)}$$

At $t = t_r$:

$$Q_{B,t_r} = G(I_{t_r}) \qquad \text{(Eq. 4)}$$

Recalling Eq. 1:

$$Q_{B,t_r} = D \sum_{i=1}^{n} A_i e^{-m_i t_r} \qquad \text{(Eq. 5)}$$

define:

$$\beta_{t_r} = \sum_{i=1}^{n} A_i e^{-m_i t_r} = \text{constant} \qquad \text{(Eq. 6)}$$

The relationship given by Eq. 7 is defined by the form of the experimental dose–effect curve:

$$D = f(I_{t_r}) \qquad \text{(Eq. 7)}$$

Equation 8 is obtained by substituting Eqs. 6 and 7 into Eq. 5:

$$Q_{B,t_r} = \beta f(I_{t_r}) \qquad \text{(Eq. 8)}$$

Equation 8 holds for an unlimited number of values of $Q_{B,t_r}$ and $I_{t_r}$, which correspond to any dose, and can be compared to Eq. 4 to give Eq. 9:

$$Q_{B,t_r} = \beta_{t_r} f(I_{t_r}) = G(I_{t_r}) \qquad \text{(Eq. 9)}$$

Since Eq. 4 is a special case of Eq. 3 or generally:

$$Q_B = \beta_{t_r} f(I) = G(I) \qquad \text{(Eq. 10)}$$

It can be observed that $G(I)$ does not need to be a first-degree homogeneous function in $\beta_{t_r}$. The $f(I)$ can itself contain $\beta_{t_r}$ coefficients in an equation defining $f(I)$ analytically (*e.g.*, Eq. 14) and can depend upon the chosen value of $t_r$ which establishes $\beta_{t_r}$. It can also be observed that the value of $f(I)$ read from the abscissa of a dose–effect curve as corresponding to a given value of $i$ is not equal to the corresponding value of the quantity of drug in the biophase, $Q_B$, unless $\beta_{t_r}$ is unity. When $\beta_{t_r} < 1$, *i.e.*, at values of $t_r > 0$, the value of $f(I) > Q_B$, *i.e.*, since $Q_B = \beta_{t_r} f(I)$ and $\beta_{t_r} < 1$. When $t_r = 0$, then $G(I_{t_r}) = f(I_{t_r})$; otherwise, as shown, $G(I_{t_r}) = \beta_{t_r} f(I_{t_r})$. The explicit form of $f(I_{t_r})$ may contain terms that include $\beta_{t_r}$.

## RESULTS AND DISCUSSION

**Example of Relationships between $I$ and $Q_B$**—Equations 11 and 12 have been found applicable for describing the dose–effect relationship for the drug tropicamide (1, 3). In these equations, $I'_{max.}$ is the limiting value of $I' = I - PQ_B$ as $Q_B \to \infty$; $K$ can be hypothetically related to a drug–receptor association affinity constant; $P$ can be given a hypothetical connotation as the product of an intrinsic activity and distribution coefficient:

$$I = \frac{I'_{max.} K Q_B}{1 + K Q_B} + P Q_B \qquad \text{(Eq. 11)}$$

$$I = \frac{I'_{max.} K \beta_{t_r} f(I)}{1 + K \beta_{t_r} f(I)} + P \beta_{t_r} f(I) \qquad \text{(Eq. 12)}$$

Solving for $Q_B = \beta_{t_r} f(I)$, Eqs. 13 and 14 are obtained:

$$Q_B = \frac{\beta_{t_r}(I - I'_{max.} - KP) + [\beta_{t_r}(I'_{max.} + KP - I) + 4\beta_{t_r}{}^2 KPI]^{1/2}}{2\beta_{t_r}{}^2 P} \qquad \text{(Eq. 13)}$$

$$f(I) = \frac{(I - I'_{max.} - KP) + [\beta_{t_r}{}^{-1}(I'_{max.} + KP - I) + 4KPI]^{1/2}}{2\beta_{t_r}{}^2 P} \qquad \text{(Eq. 14)}$$

When $\beta_{t_r} = 1$, then both Eqs. 13 and 14 obviously reduce to Eq. 15:

$$Q_B = f(I) = \frac{(I - I'_{max.} - KP) + [(I'_{max.} + KP - I) + 4KPI]^{1/2}}{2P} \qquad \text{(Eq. 15)}$$

**Effect–Dose–Time Surfaces**—Equation 12 was used to construct the surface shown in Fig. 1. Equation 12 describes the trace of the surface in the $I$–$D$ plane at any value of $t$ corresponding to $t_r$. The values of the equation parameters, used in Eq. 12 to construct the surface in Fig. 1, were $K = 17.19$ mcg./kg., $P = 1.57 \times 10^{-3}$ kg./mcg., $I'_{max.} = 0.470$, and for $\beta_{t_r} = e^{-K_e t_r}$, $K_e = 0.071$ min.$^{-1}$. The values were observed to be appropriate for the mydriatic drug tro-

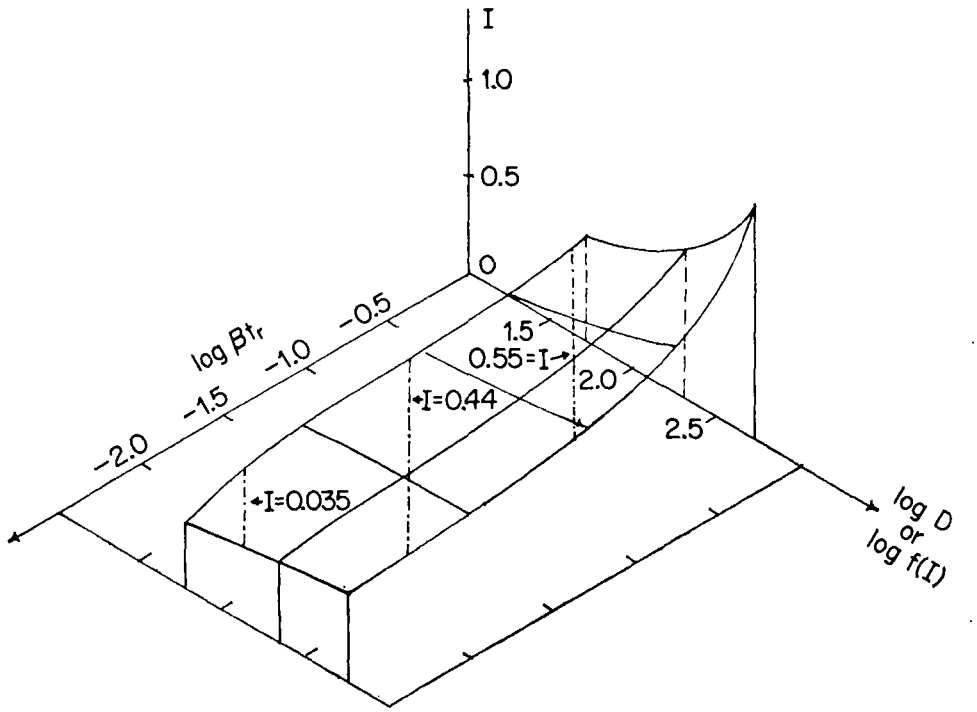


**Figure 2**—I–*log* f(I)–*log* $\beta_{t_r}$ *surface illustrating the effect of selecting different values of* $t_r$, *which determine the values of* $\beta_{t_r} = e^{-K_e t_r}$, *on the magnitude of* f(I) *values which correspond to given values of* I. *The sum of the coordinates for the lines of constant* I *drawn across the surface (- · -), corresponding to values of* I = *0.55, 0.44, and 0.035, in each case equals a constant value of* $Q_B$ *appropriate to each value of* I. *The equation for the projections of the surface lines of constant* I *onto the log* $\beta_{t_r}$–*log* D *plane is, for any value of* I, *given by: log* f(I) = *log* $Q_B$ − *log* $\beta_{t_r}$.

picamide (1). The pharmacokinetic behavior of tropicamide was found to be well described by a single-compartment model in which the biophase and central, systemic compartments behaved indistinguishably. This behavior is responsible for the trace in the *I–D* plane at $t = 0$ having the largest values of *I*. That the values of $f(I)$ which correspond to a constant value of *I* increase with the time, $t_r$, chosen to select values of *I*, *i.e.*, $I_{t_r}$, in the construction of the dose–effect curve can readily be seen upon inspection of the surface corresponding to any given value of *D*, which, since $\beta_{t_r} = 1$ at $t_r = 0$, is tantamount to $Q_B$ and $f(I)$.

***I*–log *f*(*I*)–log $\beta_{t_r}$ Surface**—Figure 2 explicitly depicts the dependency of $f(I)$ values on $\beta_{t_r}$. For any given value of *I*, there corresponds only one value $Q_B = \beta_{t_r} f(I)$; writing this relationship as $\log Q_B = \log \beta_{t_r} + \log f(I)$, it can be observed from Fig. 2 that the sum of the values of the $\log \beta_{t_r}$ and $\log f(I)$ coordinates corresponding to an $I$ = constant trace [as shown by the lines (- · -) corresponding to $I = 0.55$, 0.44, and 0.035, respectively] will for each value of *I* equal a constant value of $\log Q_B$. It should be recalled that when $0 < \beta_{t_r} < 1$, $\log \beta_{t_r} < 0$.

## SUMMARY AND CONCLUSIONS

The purpose of this report was to present a rigorous description of the relationship between the intensity of pharmacological response, the time following dosing, and the dose. This relationship has many practical consequences related to pharmacokinetic systems analysis.

Although dose–effect curves can be constructed using values of response consistently corresponding to any value of time following dosing, inspection of the surfaces reveals that the most propitious choice is the time, $t_{max.}$, corresponding to the maximum observed value of *I;* when the biophase and systemic compartments are kinetically identical, as in the present example, $t_{max.}$ is zero. The values of $t_{max.}$ corresponding to observed maximum intensities of response will be constant, provided that the biokinetic behavior of the drug can be sufficiently approximated by apparent first-order processes. Use of a $t_{max.}$ curve, in the manner of a calibration curve, allows all intensity values observed with doses equal to or below the largest used in the construction of an intravenous dose–effect curve to be converted into biophasic blood levels. It should be apparent that when the curve is constructed using, for example, intramuscular dosing where absorption is relatively slow, larger doses would generally be required to span the range of intensities obtained from smaller intravenous doses.

Based on the results of implementing multiple criteria for selection and screening 21 different cases of single-, two-, and three-compartment models (4), the pharmacokinetic behavior of tropicamide, used to exemplify the discussed principles, was found to be described by a compartment model in which the biophase and central, systemic compartments are kinetically identical (4). However, Eqs. 1–10 are entirely general and equally applicable to drugs exhibiting multiple-compartment pharmacokinetic behavior in which the biophase is constituted by a compartment kinetically distinguishable from the central plasma compartment. In fact, the generality of these relations permits the use of pharmacological data for the performance of drug absorption analysis with a considerable saving of time and effort by employing a systems analysis approach which obviates the construction of compartment models altogether (5).

As written, Eqs. 11 and 12 are specific for tropicamide. Their applicability may be extended to many other drugs by adding an exponent, *n*, to the equations and raising $Q_B$ to the *n*th power. Although convenient for use with a computer, such explicit analytical expressions for $f(I)$ are not necessary; observed *I* values can be graphically converted to $f(I)$ values using the dose–effect curve directly.

Relatively few continuously graded pharmacological responses are as well behaved and monitored as simply as the mydriatic response presently exemplified for tropicamide. The use of pharmacological data for pharmacokinetic systems analysis would be obviously limited if responses were restricted to only those effects that can be directly observed and utilized without further, or only minimal, treatment. However, when a readily and directly observable time course of drug effects is nonexistent or inappropriate for use, a more sophisticated method of recording and/or a mathematical transformation of the data may render it suitable. For example, spectral analysis (6) of drug-effected changes in biological signals (*e.g.*, as recorded electroencephalographically, electrocardiographically, *etc.*) appears to be a promising approach. When an assay for a drug in biological media does not exist or is difficult to perform, it can be considered that the development of a pharmacological method could instead provide the most expeditious means of accomplishing a pharmacokinetic analysis of the drug's behavior.

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# Anticholinergic Agents Based on Ariëns' Dual Receptor Site Theory: Nonester Antagonists

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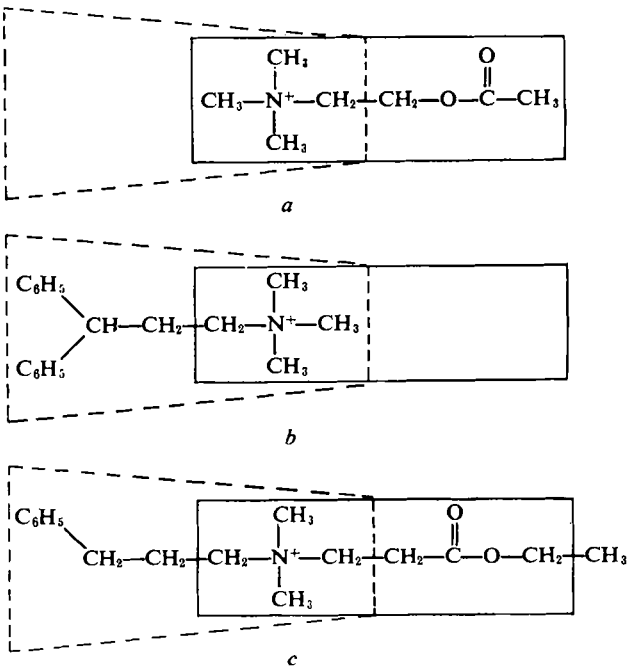
**Abstract** □ Additional compounds were prepared and evaluated for antimuscarinic activity as a test of Ariëns' dual receptor site theory. Consideration of the theory led to the conclusion that if an agonist moiety was added to the structure of classical muscarinic antagonists, then there might be an increased affinity for the receptor. Eleven such quaternary compounds, modeled after typical nonester antimuscarinic agents, were prepared. The $pA_2$ values against acetylcholine were determined on rat jejunum and compared to the activities of the classical quaternary antagonists. Only the compounds having an agonist moiety modeled after methylfurtrethonium consistently gave a significant increase in activity. The results do not allow a conclusion as to the validity of Ariëns' dual receptor site theory. Possible explanations for the results are considered.

**Keyphrases** □ Anticholinergic agents—synthesized, pharmacological screening, related to Ariëns' dual receptor site theory □ Receptor sites, Ariëns' dual theory—tested, nonester antagonists prepared, evaluated for antimuscarinic activity □ Antimuscarinic activity—nonester antagonists prepared and evaluated, Ariëns' dual receptor site theory tested

The long-term goal of the present research is to gain information concerning the nature of the subsites involved in binding various portions of agonist and antagonist molecules to the muscarinic receptor. Classical antimetabolite theory states that competitive antagonists should resemble agonists closely and bind with the same subsites. Many known pharmacodynamic antagonists do not closely resemble the agonist they antagonize, even though the inhibition is competitive and the antagonist is presumed to bind to the same receptor site. Furthermore, parallel modifications of the structure of agonists and antagonists often result in different effects on activity.

To account for these anomalies, Ariëns and Simonis (1–3) proposed that many known antagonists of muscarinic, histaminergic, and α-adrenergic agents are binding to sets of subsites on the receptor surface which overlap but do not include all of the subsites used by the agonist. If acetylcholine is bound to a portion of the muscarinic receptor surface as represented in Scheme I*a*, then Ariëns proposed that a quaternary antagonist might bind as shown in Scheme I*b*.

If the classical antimuscarinic agents are bound to the receptor as shown in Scheme I*b*, then the esteratic subsite normally involved with acetylcholine is left exposed. Compounds that mimic the structure of classical antagonists and have an additional agonist moiety connected through the quaternary nitrogen might have greater affinity if all portions could bind simultaneously to their respective subsites. Ariëns (3) reported that ethyl 3-[methyl(phenylpropyl)amino]-propionate methobromide was a weak antagonist, presumably binding to the receptor as shown in Scheme I*c*




*Scheme I—Proposed modes of binding of agonists and antagonists to the muscarinic receptor*