Kinetic Analysis of Relationship between Partition Coefficient and Biological Response

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Abstract □ Both nonequilibrium and equilibrium models were proposed to explain the optimal biological response to a set of congeners with respect to the oil–water partition coefficient (P). A detailed analysis of the kinetic model proposed by Hansch demonstrates the bilinear form of the model, with the initial slope of the logarithm of the concentration for 50% receptor binding *versus* log P having a slope of greater than one. This result is in contrast to the equilibrium model for an initial slope of less than one. Thus, a criterion is established for deciding whether equilibrium or nonequilibrium processes apply.

Keyphrases □ Partition coefficients—relationship with biological response, kinetic analysis □ Models, mathematical—relationship between partition coefficient and biological response, kinetic analysis □ Kinetics—analysis of relationship between partition coefficient and biological response

Hansch and Clayton (1) documented numerous examples of an optimal biological response to a set of congeners with respect to the oil–water partition coefficient. That is, a plot of the logarithm of the concentration at which a standard biological response is elicited *versus* the logarithm of the partition coefficient exhibits a maximum. Hansch and coworkers (2) attributed this maximum to nonequilibrium conditions resulting from transport across a series of water–oil membranes, and a kinetic model was developed to account for the maximum in the partition coefficient (2). Rather than extract model parameters from the experimental data, they fitted the data empirically with parabolic equations. While the parabolic equations have no direct relationship to the model, they are a convenient mathematical form for regression analysis.

McFarland (3) and later Kubinyi (4) employed a probability approach to account for the optimum in the partition coefficient, and they derived bilinear equations to fit the data. Higuchi and Davis (5) constructed an equilibrium model as an alternative explanation for the optimal biological response, and their model equation also has a bilinear form. Kubinyi (6) showed that bilinear equations fit the data better than parabolic equations. This finding is comforting to the model builder since none of the proposed models predicts a parabolic equation.

This paper compares the merits of the various models and proposes ways of determining whether equilibrium or nonequilibrium processes are responsible for the maximum with respect to the partition coefficient. First, the equilibrium and probability models are examined. Then the kinetic model of Hansch and coworkers (2) is examined in detail, and its bilinear form is demonstrated. The kinetic model reduces to the equilibrium model at infinite time and is consistent with what is known about transport. Difficulties in the assessment of the probability model predictions also are discussed. Time is not explicitly mentioned in the development of this model, and it appears to be a random walk-type model with probabilities obtained from the equilibrium.

MODELS

Equilibrium and Probability Models—For the models considered, the arrangement of membranes can be represented as in Fig. 1. An aqueous reservoir of the active compound is in contact with a series of n pairs of lipid–water membranes or compartments. In the last aqueous compartment, binding to a receptor occurs. The amount bound to the receptor is proportional to $C_{2n+1}P^{\alpha}$, where P is the lipid–water partition coefficient, C_{2n+1} is the concentration in the nth aqueous compartment, and α is a constant near unity that expresses a possible difference in the lipophilicity between the receptor site and the lipid compartment. Kubinyi (4) showed that the equilibrium model of Higuchi and Davis (5) could be expressed as:

$$C_r = \frac{aP^{\alpha}}{1 + \beta P} \qquad \text{(Eq. 1)}$$

where C_r is the concentration at the receptor site, a is a proportionality constant, and β is the ratio of the lipid volume to the aqueous membrane volume. Kubinyi (4) modified McFarland's (3) probability model to give:

$$C_r = \frac{bP^{n}P^{\alpha}}{(1 + \beta P)^{2n}} \qquad \text{(Eq. 2)}$$

where b is the proportionality constant. The slopes of log C_r *versus* log P predicted by these two models differ. The initial slopes are α and $n + \alpha$ for the equilibrium and probability models, respectively; the final slopes are $\alpha - 1$ and $\alpha - n$, respectively. In the equilibrium model, there is no unique maximum in P if $\alpha = 1$.

Kinetic Model and Solutions—The kinetic model as represented in Fig. 1 is characterized by the water-to-lipid rate constant, k, by the lipid-to-water rate constant, l, where $P = k/l$, and by the following:

$$\frac{dC_1}{dt} = -\frac{k}{\gamma V_w} C_1 + \frac{l}{\gamma V_w} C_2 \qquad \text{(Eq. 3)}$$

$$\frac{dC_i}{dt} = \frac{k}{\beta V_w} C_{i-1} - \frac{2l}{\beta V_w} C_i + \frac{k}{\beta V_w} C_{i+1} \quad i = 2, 4, \ldots 2n \qquad \text{(Eq. 4)}$$

$$\frac{dC_i}{dt} = \frac{l}{V_w} C_{i-1} - \frac{2k}{V_w} C_i + \frac{l}{V_w} C_{i+1} \quad i = 3, 5, \ldots 2n - 1 \qquad \text{(Eq. 5)}$$

$$\frac{dC_{2n+1}}{dt} = \frac{l}{V_w} C_{2n} - \frac{k}{V_w} C_{2n+1} \qquad \text{(Eq. 6)}$$

Here γ is the ratio of the volume of the aqueous reservoir to the volume of the aqueous compartment, V_w, and β is the ratio of the volume of the lipid compartment to V_w. Equations 3–6 can be written in matrix notation as:

$$\dot{\mathbf{C}} = \mathcal{J} \cdot \mathbf{C} \qquad \text{(Eq. 7)}$$

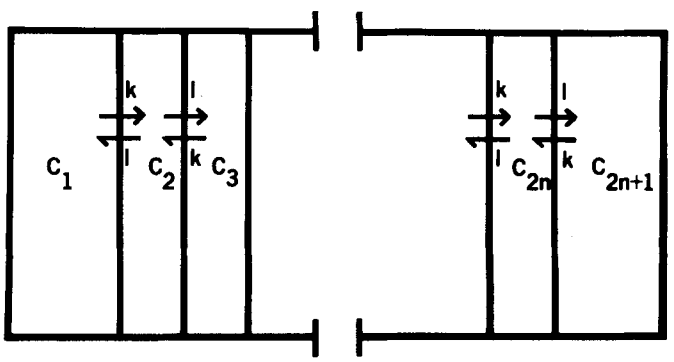

Figure 1—*Sequence of lipid–water barriers for transport from reservoir to receptor.*

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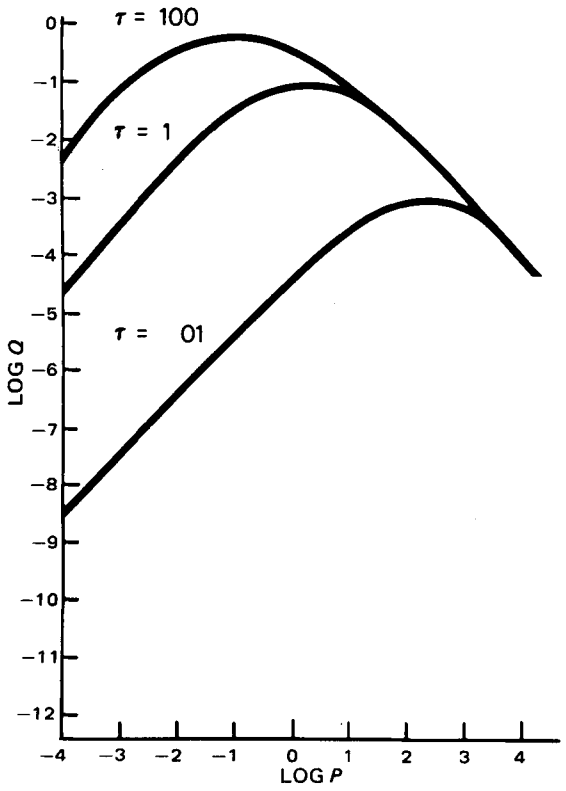

Figure 2—*Effect of time on Q(τ) for* n = *1, β = 1, and γ = 1.*

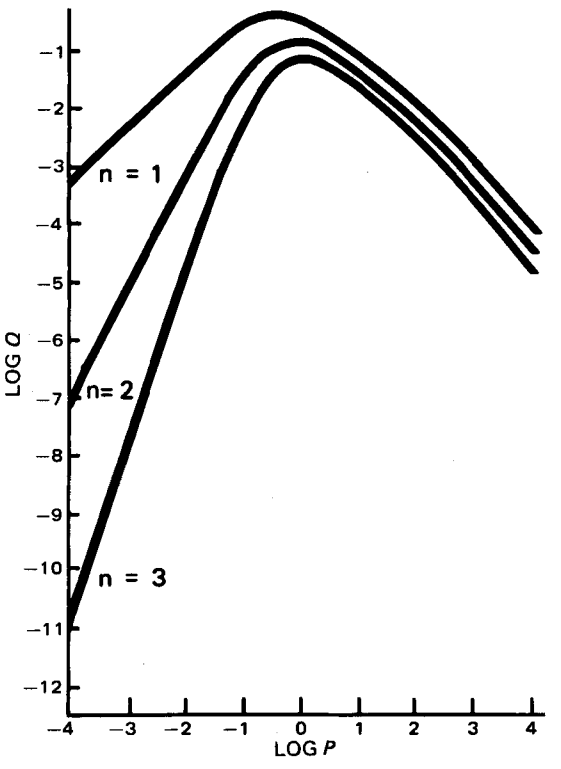

Figure 3—*Effect of* n *on Q(τ) for τ = 10, β = 1, and γ = 1.*

where $\dot{\mathbf{C}}$ is the time derivative of the $2n + 1$ concentrations, **C**, and $\mathcal{J}$ is the $2n + 1 \times 2n + 1$ matrix relating $\dot{\mathbf{C}}$ to **C**. If the eigenvalues of $\mathcal{J}$ are all distinct (7), then $\mathbf{C} = \mathbf{A}e^{\lambda t}$ is a solution where $\mathbf{A}_i$ is the eigenvector corresponding to the eigenvalue λ_i. The general solution for **C** is given by:

$$\mathbf{C} = \sum_{i=1}^{2n+1} f_i \mathbf{A}_i e^{\lambda_i t} \qquad \text{(Eq. 8)}$$

where the f_i values are determined by the initial conditions:

$$C_1(0) = C^{(0)} \qquad \text{(Eq. 9)}$$

and:

$$C_i(0) = 0 \quad i = 2, 3, \ldots 2n + 1 \qquad \text{(Eq. 10)}$$

The quantity of interest is $C_{2n+1}(t)$, which is proportional to the amount bound at the receptor. If:

$$A^{(50)} = aP^{\alpha}C^{(50)}_{2n+1}(t) \qquad \text{(Eq. 11)}$$

where $C^{(50)}_{2n+1}(t)$ is the concentration in the receptor compartment for which half of the sites, $A^{(50)}$, are occupied, then $C_1^{(50)}(0)$, the initial concentration necessary to give half-binding at time t and for the partition coefficient, P, can be estimated. That is:

$$C_1^{(50)}(0) = \frac{A^{(50)}}{Q_n(t)(aP^{\alpha})} \qquad \text{(Eq. 12)}$$

since $C_{2n+1}(t) = C_1(0)Q_n(t)$, where $Q_n(t)$ is independent of the initial concentration and represents the concentration fraction of initial drug in the receptor compartment. Thus, a plot of log Q_n *versus* log P reflects the dependence of $C_1^{50}(0)$ on P except for the additional slope α.

The solution (Eq. 8) of Eq. 7 is obtained easily for $n = 1$:

$$C_3(\tau) = C_1(0)Q_1(\tau) \qquad \text{(Eq. 13)}$$

where:

$$Q_1(\tau) = [2 + Pe^{-(P+2)\tau} - (P + 2)e^{-P\tau}]/(2P + 4) \qquad \text{(Eq. 14)}$$

For this solution, $\gamma = \beta = 1$, $V_w = 1$, and $\tau = lt$. For a short time, $Q_1(\tau)$ becomes:

$$\lim_{\tau \to 0} Q_1(\tau) = \frac{1}{2} P\tau^2 \qquad \text{(Eq. 15)}$$

which is linear in P and quadratic in τ. For values of $n > 1$, Eq. 8 was solved numerically (8); the results are shown in Figs. 2 and 3, where the bilinear nature of the function is clearly seen. The initial slopes ($P \ll 1$) are proportional to n, and the final slopes ($P \gg 1$) are all -1. As τ increases, the initial slopes all approach zero, the equilibrium value, and the position of the maximum with respect to P decreases. The fact that all of the final slopes are -1 is just a consequence of equilibrium. The

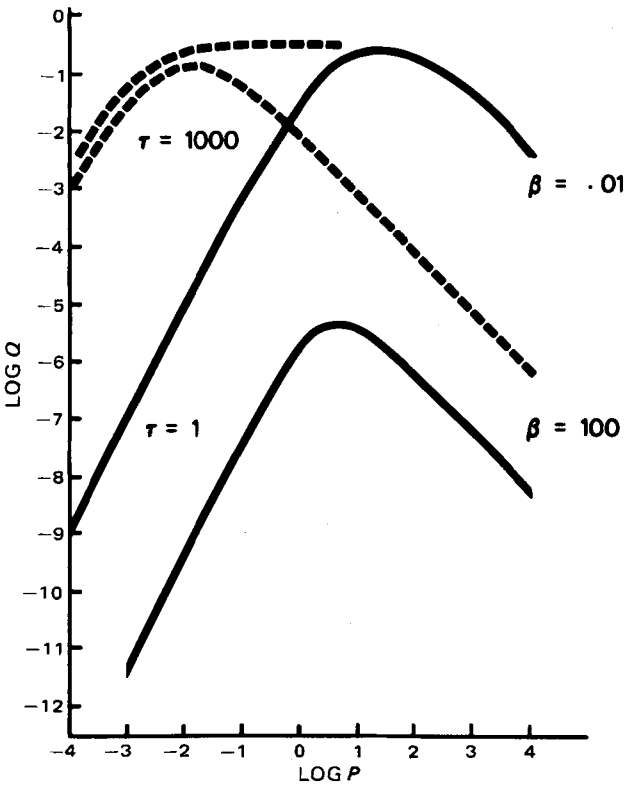

Figure 4—*Effect of β on Q(τ) for* n = *2 and γ = 1.*

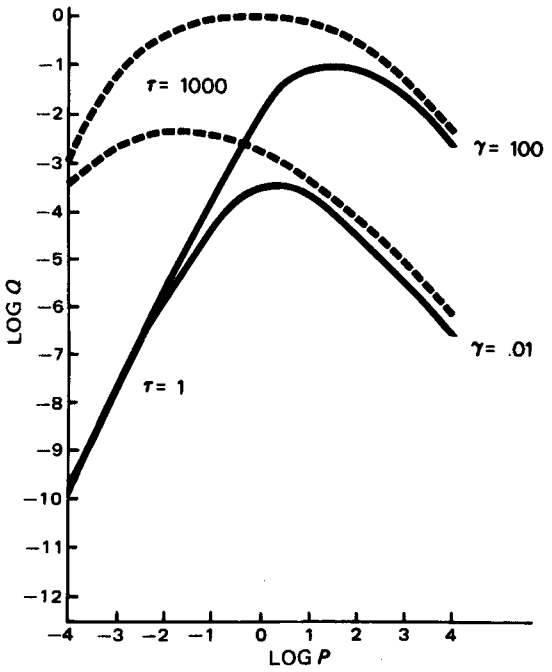

Figure 5—*Effect of* γ *on* $Q(\tau)$ *for* n = 2 *and* $\beta = 1$.

position of the maximum increases only slightly with n. In Figs. 4 and 5, the results are shown for different values of β and γ. The decrease in $Q(\tau)$ with an increase in the partition coefficient occurs at higher partition coefficient values for the smaller values of τ. This decrease occurs at lower partition coefficient values for larger β or smaller γ values.

DISCUSSION

The kinetic model accounts for transport in the steady-state approximation for diffusion across unstirred boundary layers at lipid–water interfaces, and it ignores differences in diffusion rates between the lipid phase and the aqueous phase. However, this model does approximate the effects of nonequilibrium on the concentration at the receptor site. The kinetic model predicts a time dependence for P_{max}, where P_{max} decreases with time. The probability model and the kinetic model both predict an initial slope of $n + \alpha$ for the dependence of log $1/C_1^{50}(0)$ on log P, where the equilibrium model predicts an initial slope of α. Thus, if the initial slope is greater than or equal to one, nonequilibrium processes obtain; if the slope is less than one, the equilibrium model can account for the observations. To understand the nonequilibrium models better, Kubinyi's (6) modification of Eq. 2 must be examined. He relaxed the parameter restrictions in Eq. 2 by writing:

$$C_r = \frac{bP^x}{(1 + \beta P)^y} \qquad \text{(Eq. 16)}$$

where x and y are freely adjustable instead of being related by $y = 2n$ ($n = 1, 2, \ldots$) and $x = n + \alpha$ where $0 \leq \alpha \leq 1$. Thus, Eq. 16 becomes a mathematical form to fit data rather than an equation to extract the model parameters n and α. Most of the data chosen by Kubinyi (6) to be fit by Eq. 16 had $x \leq 1$. This finding indicates that the equilibrium model is probably the better choice of operative process even though, with only one adjustable parameter, the data are not as well fit by Eq. 1.

Hansch and Clayton (1) summarized a variety of possible mechanisms to account for the portion of the curve that has the negative slope in log P. However, as they pointed out, biological data for molecules with large P values are difficult to interpret because of the low water solubility. Yalkowsky and Flynn (9) showed that the maximum steady-state flux drops off as $P^{-\delta}$, where δ is similar in magnitude to α and is an indicator of the decrease in water solubility with increased partition coefficient. Their model is essentially one of water layers (and, hence, water solubility) controlling the transport for molecules with high partition coefficients. Since binding to the receptor is proportional to P^{α}, one expects a final slope near zero and an initial slope of $1 + \alpha$ for cases where their model is applicable.

Since biological data are somewhat difficult to obtain and interpret for large partition coefficient values, the data for smaller partition coefficient values are interpreted most confidently. From the kinetic model analysis presented in this report, it is probable that nonequilibrium processes are involved when the initial slope is greater than one and that equilibrium processes obtain when this slope is less than unity. However, the kinetic model at longer times has a wide maximum, and the model could be stretched to fit data with initial slopes of less than one. Biological data covering a wide range of partition coefficients are needed to distinguish between nonequilibrium and equilibrium processes, especially in the small partition coefficient region.

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