largely on the nature of the ring substitution as well as on the half-wave reduction potential of the ring, its substituents, or the ether oxygen.

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Alfred J. Richard ˣ
Lemont B. Kier
Department of Pharmaceutical Chemistry
Medical College of Virginia
Virginia Commonwealth University
Richmond, VA 23298

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Calculation of Cutaneous Metabolic Rate Constant from Diffusion Model

Keyphrases □ Rotating disk—analysis, cutaneous metabolism of vidarabine, guinea pig, *in vitro* study □ Skin—metabolism of vidarabine, rotating-disk method, guinea pig, *in vitro* study □ Antiviral agents—vidarabine, cutaneous metabolism, rotating-disk method, guinea pig, *in vitro* study □ Vidarabine—cutaneous metabolism, analysis by rotating-disk method, guinea pig, *in vitro* study

To the Editor:

A method for the determination of the cutaneous metabolic rate constant for vidarabine (I) from a rotating-disk diffusion model was reported recently by Leung and Ando (1). They calculated the enzyme rate constant from the observed flux using:

$$k_m = 1 \Big/ \left[\frac{C_A(h)}{-F_A} - \frac{h}{D_{aqA}}\right](m) \qquad \text{(Eq. 1)}$$

where k_m is the first-order enzyme rate constant, $C_A(h)$ is the initial substrate concentration, F_A is the flux of the substrate in the system, h is the aqueous diffusion layer thickness, D_{aqA} is the aqueous diffusion coefficient, and m is the membrane thickness. Equation 1 was derived from Eq. 25 of a previous publication by Ando *et al.* (2) by taking the limit of $\theta \to 0$ such that $\tanh \theta \to \theta$:

$$\frac{1}{\kappa} = C_1 \tanh \kappa m \qquad \text{(Eq. 2)}$$

where:

$$\kappa = \sqrt{k_m/D_A}$$

$$C_1 = D_A\left[\frac{C_A(h)}{-F_A} - \frac{h}{D_{aqA}}\right]$$

$$\theta = \kappa m$$

and D_A is the diffusion coefficient of the substrate for the cutaneous tissue.

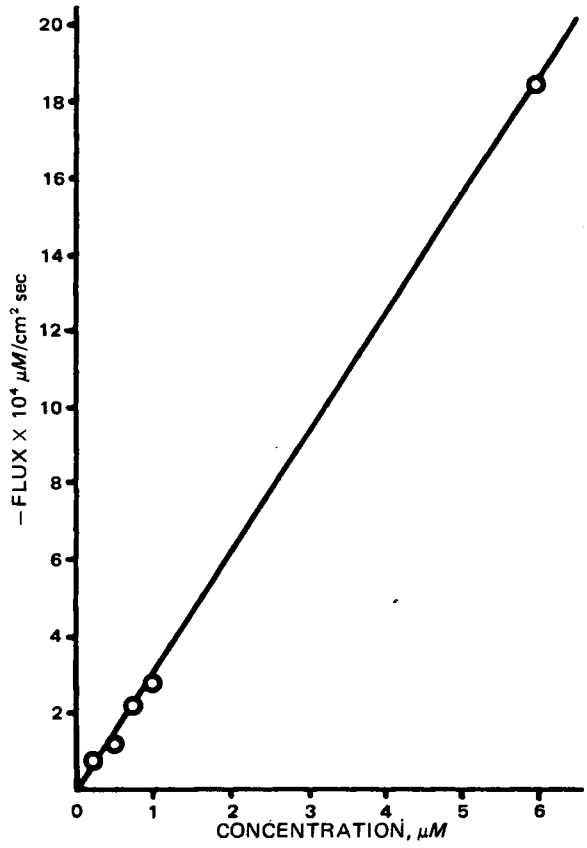

Figure 1—*Plot of flux* versus *substrate concentration. The slope of the linear regression line is 3.11 × 10⁻⁴ cm⁻² sec⁻¹ (r = 0.9997).*

The k_m of the deaminase enzyme in guinea pig epidermis was calculated to be 0.156 sec^{-1}, which contradicts the $\theta \to 0$ assumption by giving θ values of 0.25 and 24.9 for D_A values of 1.0×10^{-5} and 1.0×10^{-9} cm^2/sec, respectively. Obviously, the validity of Eq. 1 is questionable when D_A is smaller than 1.0×10^{-5} cm^2/sec. Strictly speaking, the $\theta \to 0$ assumption should never be valid because it leads to either $D_A \to \infty$, *i.e.*, no diffusional barrier exists, or $m \to 0$, *i.e.*, no membrane exists, since k_m is finite. In neither case will the simultaneous diffusion and metabolism theory

Table I—Fluxes [a] of I at Different Concentrations

Concentration, μM	Flux, %/cm^2 min	Mean Flux %/cm^2 min	Mean Flux $\mu M/cm^2$ sec
0.25	1.630 1.956 1.892	1.826	7.61×10^{-5}
0.50	1.888 0.797 1.164	1.283	1.07×10^{-4}
0.75	1.662 1.713 1.717	1.679	2.10×10^{-4}
1.0	1.691 1.921 1.230	1.616	2.69×10^{-4}
6.0	1.568 1.787 2.144	1.839	1.84×10^{-3}
600.0	0.388 0.399 0.346	0.378	3.78×10^{-2}

[a] Negative signs for all flux values, which indicate directions of the fluxes, are omitted in this table.

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apply. It would be a surprise if the calculation of k_m did not require a D_A value, as in the use of Eq. 1, since the model is based on the simultaneous diffusion and metabolism process.

The correct estimation of k_m can be drawn only from Eq. 2. From Eq. 2, one expects F_A to be a linear function of $C_A(h)$ for a given membrane. Figure 1 shows a plot of F_A *versus* $C_A(h)$. The data were taken from Table III of Leung and Ando's report (1) with a column added to list the mean fluxes in micromolar per square centimeter per second (Table I). With Eq. 2, the reciprocal of the slope of the line in Fig. 1 gives k_m values of 0.207, 1.148, 11.48, and 114.8 sec^{-1} for D_A values of 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9} cm^2/sec, respectively.

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Cheng-Der Yu
Institute of Pharmaceutical Science
Syntex Research
Palo Alto, CA 94304

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A Rebuttal and Some Insights into Cutaneous Metabolism and Diffusion

Keyphrases □ Rotating disk—analysis, cutaneous metabolism of vidarabine, guinea pig, *in vitro* study □ Skin—metabolism of vidarabine, rotating-disk method, guinea pig, *in vitro* study □ Antiviral agents—vidarabine, cutaneous metabolism, rotating-disk method, guinea pig, *in vitro* study □ Vidarabine—cutaneous metabolism, analysis by rotating-disk method, guinea pig, *in vitro* study

To the Editor:

Dr. Yu's communication (1) refers critically to the report of Leung and Ando (2); the basis for this criticism evidently stems from some of his recent research (3).

It appears from Dr. Yu's communication (1) that this research was based on a model derived by Ando *et al.* (4). One must assume that Dr. Yu used this model to measure k_m for the same enzyme and for the same drug as in the paper of Leung and Ando (2). The methodology is not stated; however, it is a good assumption that he used a diffusion cell similar to that used by Ando *et al.* (4) instead of the rotating disk in our system. It will be assumed that the D_A values for the epidermis that Dr. Yu cites for hairless mice skin were obtained after removal of the stratum corneum.

The basis of Dr. Yu's criticism is our interpretation of Eq. 25 of the model (4). His Eq. 2 is a rearrangement of this equation. In my comments, I shall show that Dr. Yu made two classical errors. First, he applied a model verbatim without due cognizance of the stated limitations of the model. Second, he made inappropriate comparisons.

The basic equation Dr. Yu disagrees with is Eq. 1 in his communication. We arrived at this equation by taking the limit of tanh $\theta \rightarrow 0$. The basic validity of this approximation is evident from the nature of the power series expansion for tanh θ (5):

$$\tanh\theta = \theta - \frac{\theta^3}{3} + \frac{2}{15}\theta^5 - \frac{17}{315}\theta^7 + \ldots + (-1)^{n-1}\frac{2^{2n}(2^{2n}-1)}{(2n)!}B_{2n-1}\theta^{2n-1} + \ldots \quad \text{(Eq. 1)}$$

where $|\theta| < \pi/2$ and B_{2n-1} are Bernoulli's numbers. The approximation of:

$$\tanh\theta \simeq \theta \quad \text{(Eq. 2)}$$

as $\theta \rightarrow 0$ is one of the most common forms of linearization. The basis for this approximation is that as θ becomes small, θ^3 decreases much more rapidly. For this reason, in the simple pendulum (6, 7):

$$F = mg\sin\theta \quad \text{(Eq. 3)}$$

can be replaced by:

$$F = -mg\theta \quad \text{(Eq. 4)}$$

for small θ since (5):

$$\sin\theta = \theta - \frac{\theta^3}{3!} + \frac{\theta^5}{5!} - \frac{\theta^7}{7!} + \ldots \quad \text{(Eq. 5)}$$

Using this approximation, $\theta \times \tanh\theta$ of Dr. Yu's Eq. 2 becomes:

$$\theta \times \tanh\theta = \theta^2 - \frac{\theta^4}{3} + \frac{2}{15}\theta^6 - \frac{17}{315}\theta^8 + \ldots \quad \text{(Eq. 6)}$$

In Table I, we show the percent error in using θ^2 instead of $\theta \times \tanh\theta$ computed from the first four terms of the series. Although θ^2 always overestimates $\theta \times \tanh\theta$, values of $\theta > 0.75$ are reasonable considering that the biological variability in determining the skin thickness in unmatched guinea pigs has a standard deviation of 18% for eight or more animals (8). Note also that $\theta = 0.11$ gives a 0.0% error.

The major thrust of Dr. Yu's criticism is that he believes that D_A must be measured to determine the linear metabolic rate constant, k_m. Current methodology, including Dr. Yu's, does not justify this degree of refinement. He is, as I have stated before, applying the model verbatim without due cognizance of the stated limitations of the model. In the model that I derived (4), the skin was envisioned as a two-ply laminate composed of the relatively impermeable stratum corneum and the viable epidermis. The distribution of enzymes in the epidermis was assumed to be homogeneous. A caveat was given: "The epidermis contains most of the catabolic enzymes that render the drug inactive by metabolism, but it is not clear how the distribution of catabolic enzymes might vary with epidermal depth. Presumably, one would expect a gradual increase in metabolic activity as the basal epithelial layer is approached from the stratum corneum."As we shall see, the distribution of the enzyme adenosine deaminase, the most likely catabolic enzyme responsible for the metabolism of 9-β-D-arabinofuranosylhypoxanthine (I), is not homogeneous; apparently, there is not even a gradual

Table I—Approximation of $\theta \times \tanh\theta$ by θ^2 and Percent Error

θ	θ^2	$\theta^4/3$	$2(\theta^6/15)$	$17(\theta^8/315)$	$\theta \times \tanh\theta$	Percent Error
0.90	0.8100	0.2903	0.0708	0.0232	0.5673	42.8
0.75	0.5625	0.1054	0.0237	0.0054	0.4754	18.3
0.71	0.5041	0.0847	0.0171	0.0035	0.4330	16.4
0.50	0.2500	0.0208	0.0020	0.0002	0.2310	8.2
0.25	0.0625	0.0013	0	0	0.0612	2.1
0.18	0.0324	0.0003	0	0	0.0327	0.9
0.11	0.0121	0	0	0	0.0121	0.0

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