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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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 $\theta \to 0$. 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 $\theta \to 0$ 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

distribution of the enzyme from the stratum corneum to the basal layer.

Adenosine deaminase is a salvage pathway enzyme in the skin (9) and converts the purine nucleoside adenosine to inosine. This intermediate may be degraded or possibly converted by other salvage pathway enzymes in the skin into guanosine (10). These salvage pathways permit re-utilization of purines and may be necessary since the continuous cellular renewal occurring in the epidermis requires active synthesis and breakdown of nucleic acids. Indeed, the absence of effective salvage pathways for the reutilization of inosine forms the biochemical basis for some inherited disorders (11, 12). Similar salvage pathways exist for the synthesis of pyrimidines in the skin (13, 14).

With respect to Dr. Yu's comments, there is every reason to believe that there is not a homogeneous or even a gradual distribution of the enzyme adenosine deaminase in the skin; rather, there is every reason to believe that the enzyme resides exclusively in the basal layer of the epidermis. The basal layer of the epidermis can be separated from the upper differentiated spinous and granular layers (15). Studies with these cells have shown that only the basal cells have proliferative capabilities and that only the basal cells can be used to establish successful primary cultures. Irreversible changes in the spinous and granular cells of the upper epidermal layers probably are responsible for the absence of mitosis in these cells. A concomitant change with this loss of mitotic ability in the upper epidermal layer is the complete loss of salvage pathway pyrimidine enzymes (16). Only the basal cells have these enzymes. For this reason, only the basal cells probably contain the purine salvage enzyme, adenosine deaminase.

Studies have shown that the basal cells incorporate thymidine 205-fold faster than do the upper epidermal cells in the spinous and granular layers (16). This observation raises serious questions as to the utility of measuring the epidermal diffusion coefficient, D_A, for the entire epidermis, as Dr. Yu evidently has done. The fact that the diffusion coefficient for a drug would be much slower in the spinous and granular layers is reasonable from considerations of epidermal differentiation. Basal cells would be expected to be the most permeable because they are the only cells undergoing mitosis. Essential nutrients must permeate these cells from the dermal capillaries. For this reason, one would suppose that the diffusion coefficient of the basal cells would be on the order of the diffusion coefficient of the dermis. In this way, nutrient transport would be optimized. The diffusion coefficient of the differentiating spinous and granular layer cells of the upper epidermis should be *intermediate* between that of the basal cells and that of the very impermeable stratum corneum.

Further support for a faster diffusion coefficient for the basal cells is evident from a comparison of the unesterified cholesterol content (with respect to total lipid) of the epidermal cells (17): basal cells, 8.4 ± 0.3; granular cells, 18.0 ± 2.6; and stratum corneum, 23 ± 4.7. Since unesterified cholesterol is found primarily in the plasma membrane of these cells (18) and since levels of unesterified cholesterol and sphingomyelin render membranes more lipophilic, less permeable, and less easily degraded (19), one might justifiably conclude that the basal cells are much more per-

meable than the spinous and granular cells.

With these facts on hand, let us examine Dr. Yu's criticisms. Dr. Yu assumed D_A values of 1.0×10^{-5} and 1.0×10^{-9} cm^2/sec and, using the value for the membrane thickness that we used, 20.85×10^{-4} cm, and our value of k_m, he computed θ values of 0.25 and 24.9, respectively. He claims that this result contradicts our $\theta \to 0$ assumption. This is not true. Dr. Yu is making an inappropriate comparison. We viewed the epidermal layer as a whole and felt that it would be pedantic to try to define the model any further experimentally because of the inherent limitations of the model. The distribution of the enzyme, adenosine deaminase, is not homogeneous throughout the epidermal layer but rather resides exclusively in the basal cell layer.

Furthermore, a determination of D_A for the entire epidermis, exclusive of the stratum corneum, as Dr. Yu evidently has done experimentally, is equally pedantic and misleading. As we have shown, the diffusion coefficient of the basal cell layer where the enzyme almost exclusively resides is most likely on the order of the diffusion coefficient of the dermis and not the epidermis as a whole. Therefore, determination of D_A for the entire epidermis as Dr. Yu has done is in error, because D_A is defined as the diffusion coefficient in the region of a homogeneous enzyme distribution.

Dr. Yu also questions the validity of our approximation since the $\theta \to 0$ assumption, strictly speaking according to him, implies that either $D_A \to \infty$, i.e., no diffusional barrier exists, or $M \to 0$, i.e., no membrane exists. This conclusion is incorrect; as shown in Table I, the error in our approximation is less than 0.0% for a value of $\theta = 0.11$. It is not necessary for the θ value to equal 0 for this accuracy. Therefore, one would never reach Dr. Yu's conclusion. Moreover, the condition $\theta \to 0$ does not necessarily mean that either $D_A \to \infty$ or $M \to 0$. In real skin, all of these variables change because real skin is not homogeneous. If we chose to make a Yu-type computation of θ, a more appropriate calculation would be based on the evidence I have presented. The thickness of guinea pig epidermis has been determined from the upper border of the stratum granulosum to the lower border of the basal cell layer (8). This measurement included one basal cell layer, one or two spinous cell layers, and one to three granular cell layers or a range of three to six cell layers (20, 21). If we take the number of cell layers to be four and divide the number of cell layers into the thickness of the epidermis, 20.85 μm (SE 0.40, n = 39), we obtain an approximate thickness for the basal cell layer of 5.21 μm. Since we have argued that this layer is the only one containing the enzyme adenosine deaminase and that the diffusion coefficient in this layer would be very close to the dermis, we can calculate θ using Dr. Yu's value for D_A for the dermis of 1.34×10^{-6} cm^2/sec from:

$$\theta = M \sqrt{k_m/D_A} \qquad \text{(Eq. 7)}$$

This calculation yields a θ value of 0.178. From Table I, we see that a θ of 1.8 will only produce an error of 0.9%. We can even improve on this result. In the previous calculation, we used the reported k_m value of 0.156 cm^{-1}, which was based on a membrane thickness of 20.85 μm. If k_m is recomputed based on a membrane thickness of 5.21 μm, then

$\theta = 0.039$. The approximation that we have made then gives an error of 0.05%.

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Elimination of Tablet Air Entrapment Using USP 1 Rotating-Basket Dissolution Apparatus

To the Editor:

Dissolution testing using the USP 1 rotating-basket apparatus (1) is subject to variability due to an artifact of the method. For certain dosage forms, this artifact results in entrapment of the test specimen in an air bubble at the top of the basket assembly. This air bubble is entrapped if the 2-mm vent hole in the top of the basket assembly is blocked by the tablet as the assembly is lowered into the dissolution fluid or if the bubble is positioned elsewhere on the top of the assembly and does not migrate toward the vent.

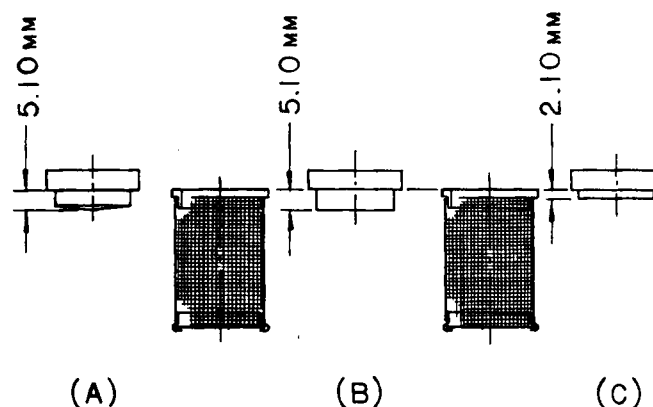Since the rim around the detachable part of the basket

Figure 1—*Conical head design (A), extended head design (B), and conventional head design (C).*

protrudes below the top of the assembly by about 3 mm, a dead space is available to prevent the bubble from migrating out of the basket at the edge (Fig. 1C). When a tablet is entrapped in this way, the apparent dissolution rate is very low and the dissolution run is invalid.

It was noted in our laboratories that this air entrapment occurred occasionally during dissolution tests on the nondisintegrating USP dissolution calibrator salicylic acid 300-mg tablet. A basket top was fabricated to eliminate the dead space around the basket rim (Fig. 1B), and this simple modification essentially eliminated the problem. However, the problem recurred during testing of an experimental film-coated tablet. For the film-coated tablets, the problem was so severe that several runs were needed to obtain valid six-tablet results. Use of the modified design (Fig. 1B) did not totally eliminate the problem.

To circumvent air entrapment with the film-coated tablets, the top of the basket assembly was modified further by fabricating a slight conical shape such that the center protrudes about 1 mm below the edge. This shape imparts enough of a slope to permit an entrapped bubble to escape and to allow the tablet to return to the bottom of the basket (Fig. 1A). The improvement is dramatic. For example, in one series of six-tablet runs, seven of 18 USP salicylic acid calibrators were air entrapped with the conventional head while the conical head design completely eliminated the problem. For the experimental film-coated tablets, 10 of 18 tablets were air entrapped with the conventional head but only one of 18 was air entrapped with the conical head design.

The conical head modification of the basket top design (Fig. 1A) may be of sufficient utility to warrant general use. It is not inconsistent with the present apparatus description (1); however, it may be advantageous to define explicitly the dimensions in the USP to permit its use.

(1) "Fifth Supplement to USP XIX and NF XIV," United States Pharmacopeial Convention, Rockville, Md., 1979, pp. 221, 222.

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