In Vitro Estimates of Topical Bioavailability

Keyphrases □ Bioavailability, topical—quantitative skin model assuming inactive stratum corneum and viable, enzyme-containing epidermis □ Topical bioavailability—quantitative skin model assuming inactive stratum corneum and viable, enzyme-containing epidermis □ Metabolism, drug—topically applied, quantitative skin model assuming inactive stratum corneum and viable, enzyme-containing epidermis □ Absorption, percutaneous—quantitative skin model assuming inactive stratum corneum and viable, enzyme-containing epidermis □ Diffusion, drug—quantitative skin model assuming inactive stratum corneum and viable, enzyme-containing epidermis

To the Editor:

Many disease conditions of the skin are due to pathogens or aberrant metabolism of the epithelial layer. Cold sores, for example, are caused by the Herpes simplex type I virus (1) which forms intraepithelial vesicles as a result of successive rounds of infection and proliferation. Psoriasis, on the other hand, is due to the uncontrolled proliferation of the basal epithelial cells. This abnormal growth probably results from a metabolic defect of the basal cells.

Since the epidermis is the most metabolically active layer of the skin (2, 3), any topical dosage form that delivers active drug components to intraepithelial sites should be formulated realizing that the skin is an active metabolizing barrier. In the past, the passive permeability properties of the stratum corneum were shown to constitute the major diffusional resistance of the skin (4). Several studies (5–7) also examined the *in vivo* percutaneous absorption of topical dosage forms. However, since urine was usually assayed as the main excretory route of the drug, the degree of cutaneous metabolism could not be clearly established because the metabolism of other tissues was included in the data. In one human skin study on the *in vivo* catabolism of hydrocortisone (7), the conclusion drawn on the basis of urine data was that hydrocortisone was probably rapidly metabolized in the skin, thus becoming physiologically inactive before it reached its site of action.

Direct evidence of the cutaneous metabolism of adrenal steroids (8) and hydrocortisone (9) was obtained *in vitro* by incubating viable human skin in a medium containing the steroid and assaying the medium by chromatographic methods. In another study (10), the cutaneous conversion of fluorouracil (I) to 5-fluoro-2′-deoxyuridine 5′-monophosphate (II) was verified *in vitro*. The intraepithelial metabolism of I was important to verify, since II is considered to be the drug form with antitumor activity. Thus a "prodrug," I, penetrated the stratum corneum (II, being more polar, would be expected to penetrate less effectively) and was converted by the enzymes of the epithelial cells into II, the active drug form.

These considerations emphasize the need for a quantitative model of the skin that can assess the metabolic barrier of the skin concurrent with cutaneous transport. One model, which treats the skin as a bilayer (11), assumes that the stratum corneum is inactive metabolically and that the epidermis contains a homogeneous distribution of enzymes. Some preliminary experimental results based on the model are discussed here. It is hoped that these and future studies will provide the experimental framework for evaluating this and more complex models of the viable epidermis.

In this system, the viable epidermis contains an enzyme, adenosine deaminase, which can convert the antiviral drug vidarabine (9-β-D-arabinofuranosyladenine) (III) into 9-β-D-arabinofuranosylhypoxanthine (IV). Skin from a 3-month-old hairless mouse[1] containing both the dermis and epidermis was removed and placed between two 3-ml chambers of a dialysis cell[2] (Fig. 1). Both the stratum corneum and the dermis side of the diffusion chamber could be used as the donor compartment for III in Dulbecco's phosphate-buffered saline (12). Five-microliter samples were taken at different times using a disposable microliter pipet[3], spotted onto TLC plates[4], and developed in chloroform–methanol–3% acetic acid (3:2:1) (13). Both III and IV were assayed.

When this solution was placed on the stratum corneum side of the diffusion cell and saline was placed on the receptor side, no metabolism of III was observed on the epidermis side and no label was found on the dermis side for time intervals up to 24 hr. Similarly, when the dermis side was used as the donor compartment, no drug penetrated into the receptor compartment. However, in this latter situation, rapid metabolism was observed in the donor compartment; 50% of the original III was in the hypoxanthine form after 2 hr (Fig. 2).

One problem arose when the dermis side of the skin was used as the donor compartment. If samples from the donor compartment were taken at different times, stored in holding vials, and assayed 12 hr later, the amount of metabolism observed was greater than that found if the samples were assayed immediately. Evidently, adenosine deaminase, the enzyme that metabolized III, was leaching out of the skin into the donor compartment. Thus, the amount of metabolized drug in the donor compartment

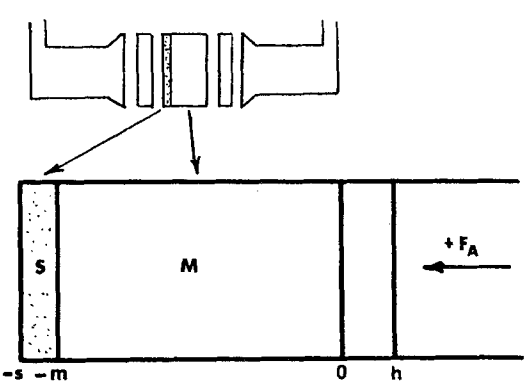

Figure 1—*Impermeable stratum corneum. Drug A moves in the negative* x *direction, first penetrating the aqueous diffusion* ($0 \leq x \leq h$) *and then the metabolizing epidermis* (M) *located at* $-m \leq x \leq 0$. *The stratum corneum* (S), located at $-s \leq x \leq -m$, *does not permit passage of A through it.*

[1] Jackson Laboratory, Bar Harbor, ME 04609.
[2] Karush-type dialysis chamber, Belco Glass Co., Vineland, NJ 08360.
[3] Drummon Scientific Co.; from A. H. Thomas, Philadelphia, PA 19105.
[4] Analtech, Newark, DE 19711.

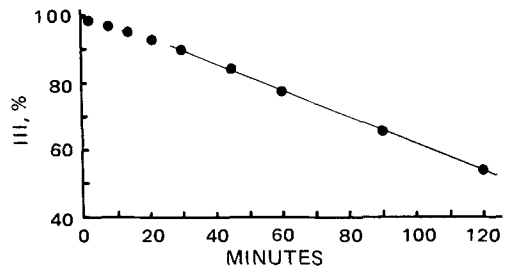

Figure 2—*Determination of* F_A. *Percent of III in the bulk solution was assayed with respect to time; the steady-state slope,* dC/dt, *was computed from the last five points. This value, −0.227, was corrected for bulk metabolism from Fig. 4 and was computed from* $F_A = (V/A)(dC/dt)$, *where* $V = 2.0\ cm^2$ *and* $A = 1.54\ cm^2$.

reflected not only the epidermal component but also a bulk phase metabolism due to leached enzyme. To correct for this bulk metabolism, a separate study was conducted.

Hairless mouse skin was prepared as already described, but only buffered saline was placed in both chambers of the diffusion cell. At 30-min intervals up to 2 hr, samples were taken from the dermis compartment. Compound III was immediately added to each sample, which was then assayed as a function of time for III metabolism. Figure 3 shows that the amount of enzyme in the bulk phase continuously increased with time. The slope of each curve was plotted in Fig. 4 as a function of sampling time to determine whether a linear relationship existed. Figure 4 shows that the slopes are highly linear with time so that:

$$\frac{dC_X(t)}{dt} = kt \qquad \text{(Eq. 1)}$$

or:

$$C_X(t) = C_X(0) + \frac{k}{2}t^2 \qquad \text{(Eq. 2)}$$

where C_X represents an upper bound to the amount of product, X, formed in the bulk aqueous phase, and k is the slope of the dC_X/dt *versus* time plot. The reason that $C_X(t)$ is an upper bound and not the actual amount of

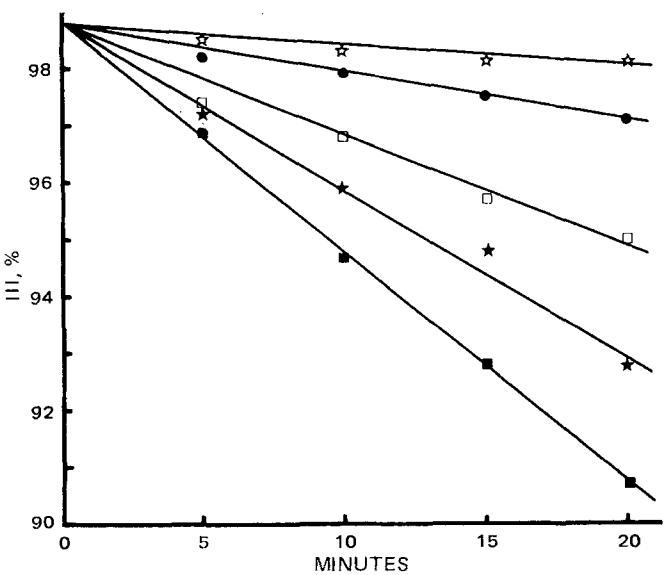

Figure 3—*Bulk phase metabolism. Metabolism of III in the bulk aqueous phase by enzymes leached out of the epidermal side of the diffusion cell at 5 (☆), 30 (●), 60 (□), 90 (★), and 120 (■) min. Solid lines were determined from linear regression fit forced through 98.8% purity for III.*

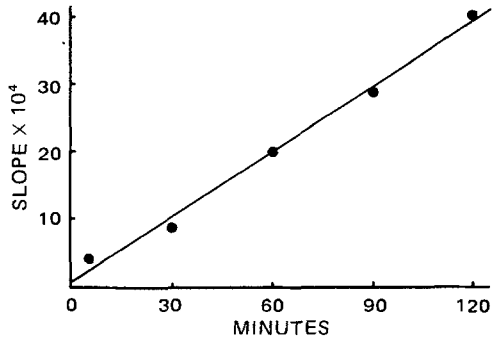

Figure 4—*Linearity of bulk phase metabolism slopes. Slopes determined from Fig. 3 are plotted against time to determine linearity. Linear regression gives an intercept of* $9.06 \times 10^{-5}\ min^{-1}$, *a slope of* $3.20 \times 10^{-5}\ min^{-2}$, *and a correlation coefficient of 0.996.*

product formed in the experiment of Fig. 2 is that the initial rate of enzyme reaction is usually faster than the reaction rate at later times. If the plots of Fig. 3 remained linear for times up to 2 hr, then $C_X(t)$ would represent the actual concentration of product formed, which would be reflected in Fig. 2. From Fig. 4, $k = 3.20 \times 10^{-5}\ min^{-2}$; therefore, at 20 min, $(k/2)t^2 = 0.0064$. The plots of Figs. 2 and 3 are linear up to 20 min, so that the flux determination from Fig. 2 can be corrected for bulk metabolism.

The model equation (11) that determines the loss of III from the bulk solution is:

$$\frac{1}{\kappa} = C_1 \tanh \kappa m \qquad \text{(Eq. 3)}$$

where:

$$C_1 = D_A\left[\frac{C_A(h)}{-F_A} - \frac{1}{P_{aqA}}\right] \qquad \text{(Eq. 4)}$$

and:

$$\kappa = \sqrt{\frac{k_m}{D_A}} \qquad \text{(Eq. 5)}$$

where m is the thickness of the skin sample, D_A is the diffusion coefficient of III in the skin, $C_A(h)$ is the concentration of III in the bulk solution, P_{aqA} is the permeability of III in the aqueous diffusion layer, and F_A is the flux of III into the skin. Since the flux of III from the bulk solution into the skin is considered negative, $-F_A$ is positive:

$$F_A = \frac{V}{A}\frac{dC_A}{dt} \qquad \text{(Eq. 6)}$$

where V is the volume of the bulk phase, and A is the area of the skin.

The initial flux of III can be estimated from Fig. 2 by using the steady-state slope of the last five points and correcting for the bulk metabolism using $(k/2)t^2$. The corrected slope is −0.227, so that F_A is 4.92×10^{-3}

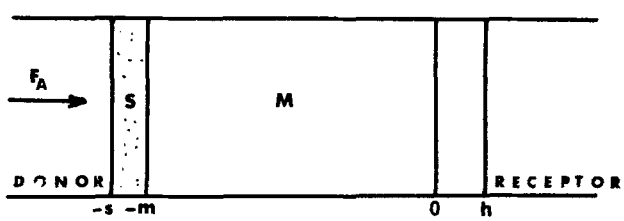

Figure 5—*Permeable stratum corneum. Drug A moves from left to right in the positive flux direction, first penetrating the stratum corneum* (S) *located at* $-s \le x \le -m$, *then the metabolizing epidermis* (M) *located at* $-m \le x \le 0$, *and then the aqueous diffusion layer at* $0 \le x \le h$.

amount/cm^2 sec, where $V = 2.0$ cm^3 and $A = 1.54$ cm^2. Because the full thick skin used also contained some subcutaneous tissue and dermis, there appeared to be a lag time of about 5 min (Fig. 1).

Using the value for F_A, we can compute that $C_A(h)/-F_A = 2.03 \times 10^4$ sec/cm, where $V = 2.0$ cm^3 and $A = 1.54$ cm^2. A reasonable upper limit for P_{aqA} might be estimated as $P_{aqA} \doteq 5 \times 10^{-4}$ cm/sec, which corresponds to a 200-μm aqueous diffusion layer with a diffusion coefficient for III of 10^{-5} cm^2/sec. When assuming a III epidermal diffusivity of 10^{-6} cm^2/sec, $C_1 \doteq 1.8 \times 10^{-2}$ cm.

Finally, plotting each side of Eq. 3 *versus* κ for an epidermis of 300 μm, we find that $\kappa = 82$ or $k_m \doteq 6.7 \times 10^{-3}$ sec^{-1} from the intersection of the two curves. This calculation for the linear enzyme parameter, k_m, assumed some typical values for the diffusion coefficient of III in the epidermis and aqueous diffusion layer and the thicknesses of these layers. Experiments are planned to determine these parameters directly. In addition, the validity of assuming a first-order enzyme reaction must be investigated.

The determination of the metabolic parameter, κ, can be used to predict to what degree metabolism affects the bioavailability of the drug when it is administered topically. Figure 5 shows an *in vitro* situation relevant to the clinical application of a topical dosage form. The drug passes from the topical vehicle through the stratum corneum, S, into the metabolizing epidermis, M, and then through the aqueous diffusion layer into the receptor compartment. Suppose that there is a virus lesion halfway through the epidermal layer of 100 μm. How might drug bioavailability be affected by the fact that the epidermis metabolizes the drug?

For the application of a topical drug, the relevant equations (11) are:

$$C_{AM}(x) = -\frac{P_{SA}(P_{aqA}\sinh\kappa S - D_A\kappa\cosh\kappa X)C_A(-s)}{D_A\kappa(P_{SA}+P_{aqA})\cosh\kappa M + [P_{SA}P_{aqA}+(D_A\kappa)^2]\sinh\kappa M} \quad \text{(Eq. 7)}$$

and:

$$C_{A0}(x) = -\frac{\left(\frac{1}{D_A/X}-\frac{1}{P_{aqA}}\right)C(-s)}{\frac{1}{P_{aqA}}+\frac{1}{P_{SA}}+\frac{1}{D_A/m}} \quad \text{(Eq. 8)}$$

where $C_A(-s)$ is the concentration of III in the vehicle at the stratum corneum boundary; $C_{AM}(x)$ and $C_{A0}(x)$ are the concentrations of III at the distance X for a metabolizing and a nonmetabolizing epidermis, respectively; and P_{SA} is the III permeability of the stratum corneum. For P_{SA} values of 10^{-6} and 10^{-7} cm/sec and for the computed κ:

$$\frac{C_{AM}(-m/2)}{C_{A0}(-m/2)} = 0.181 \quad \text{(Eq. 9)}$$

$$\frac{C_{AM}(-m/2)}{C_{A0}(-m/2)} = 0.183 \quad \text{(Eq. 10)}$$

These results show that, for a metabolizing membrane, the bioavailability of III due to metabolism does not depend strongly on the stratum corneum permeability and that the concentration of the active drug, III, is only 18% of the level that would have existed at the site of the lesion (halfway through the epidermis) had there been no metabolism.

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Antineoplastic Agents L: Isolation and Characterization of Sphyrnastatins 1 and 2 from the Hammerhead Shark *Sphyrna lewini*

Keyphrases □ *Sphyrna lewini*—hammerhead shark, potential antineoplastic constituents isolated from body fluids, muscle tissue, and liver, identified □ Sphyrnastatins—isolated from hammerhead shark, preliminary evaluation of antineoplastic activity □ Antineoplastic activity—preliminary evaluation of sphyrnastatins isolated from hammerhead shark □ Marine antineoplastic agents, potential—sphyrnastatins isolated from hammerhead shark

To the Editor:

Hammerhead sharks of the Sphyrnidae family (cartilaginous fishes of the class Chondrichthyes) have inhabited the oceans for 181–350 million years (1) and represent a most successful evolutionary achievement. Sharks are rarely affected by diseases (2) and, to our knowledge, hammerhead sharks have not been found with cancer. In 1966, we began a long-term study (3) of marine animals for the purpose of developing cancer chemotherapeutic drugs (4). Early in this investigation, it was found that extracts of certain marine vertebrates exhibited a confirmed level of activity against the National Cancer Institute's PS (murine lymphocytic leukemia) screening system.