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Physical Model Evaluation of Topical Prodrug Delivery—Simultaneous Transport and Bioconversion of Vidarabine-5′-valerate I: Physical Model Development

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Abstract □ A physical model approach to the topical delivery of a vidarabine ester prodrug was investigated. It involved modeling, theoretical simulations, experimental method development for factoring and quantifying parameters, and, finally, employment of the deduced parameters to determine the steady-state species fluxes and concentration profiles in the target tissue. The present report describes the physical modeling and theoretical simulation aspects. The physical model for the simultaneous transport and bioconversion of a topically delivered prodrug was formulated assuming homogeneous enzyme distributions and constant diffusivities in the membrane. The mathematical problem was solved, and the solution yielded concentration profiles and fluxes of all species in the biomembrane. These results provided the prevailing levels of the prodrug, the drug, and the metabolite at the target site and the transport rates of all species into the bloodstream. Computations of concentration profiles and fluxes were carried out for a reasonable range of the parameters. The relative activities of the esterase and the deaminase enzymes, as well as the stratum corneum permeabilities, were important in influencing the concentration profiles and fluxes of all species.

Keyphrases □ Vidarabine valerate prodrug—topical dosage forms, physical models, pharmacokinetics □ Antiviral agents—vidarabine valerate prodrug, topical dosage forms, physical models, pharmacokinetics □ Prodrugs—vidarabine valerate, topical dosage forms, physical models, pharmacokinetics □ Models, physical—vidarabine valerate prodrug, topical dosage forms, mouse skin □ Pharmacokinetics—vidarabine valerate prodrug, topical dosage forms, physical models

A physical model that described simultaneous drug transport and bioconversion across the skin was reported recently (1). The model treated the skin as a two-ply laminate composed of the stratum corneum and the viable epidermis. Only the epidermis was assumed to contain enzymes responsible for metabolism; for mathematical simplicity, the enzyme distribution was assumed to be homogeneous. Two theoretical situations were investigated. In one, the stratum corneum was considered impermeable to the drug and the drug was allowed to penetrate from the dermis side of the epidermal membrane. In the other, the stratum corneum was assumed to be permeable and the drug penetrated the stratum corneum from the stratum corneum side of the tissue and then permeated the metabolizing epidermis. Preliminary experimental transport studies (2) on vidarabine (9-β-D-arabinofuranosyladenine, I) were conducted using full thickness hairless mouse skin. The metabolic rate constant was calculated for the first case from the rate of change in the drug concentration on the dermis side.

The present report describes the results of a more comprehensive investigation dealing with the physical model approach for the delivery of therapeutically active drugs in the skin, with emphasis on the simultaneous transport and bioconversion for the system: prodrug → drug → metabolite.

BACKGROUND

Compound I has broad-spectrum activity against DNA viruses (3–6) including the herpes simplex virus type I, which is the cause of recurrent herpes labialis ("cold sores"). When applied topically for the treatment of herpetic skin infection in the hairless mouse, the drug was ineffective, which was attributed to poor transepidermal penetration (7). When injected intradermally, I improved the course of the infection (8). Recent *in vitro* studies (2) of hairless mouse skin mounted in a diffusion cell found the stratum corneum to be relatively impermeable to I, in support of earlier observations by Fondak *et al.* (7).

Compound I has many suboptimal properties (9) including low aqueous solubility [0.5 mg/ml at 25° and 1.8 mg/ml at 37° (5)], low lipophilicity (log partition coefficient in *n*-pentanol–water of −0.48)[1], and rapid

[1] Dr. David C. Baker, Department of Chemistry, University of Alabama, University, AL 35486, personal communication.

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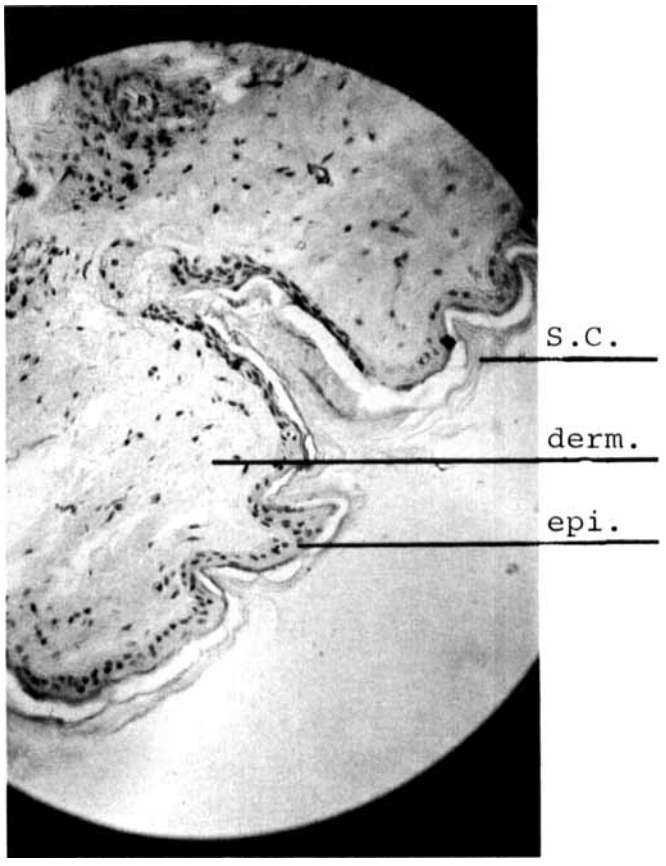


Figure 1—*Cross-section of hairless mouse skin (1040× magnification). Key: S.C., stratum corneum; derm., dermis; and epi., epidermis.*

conversion to 9-β-D-arabinofuranosylhypoxanthine (II) by adenosine deaminase (10–17). While the metabolite possesses antiviral activity, it is about one-thirtieth as potent as I (18–20). Because of these shortcomings, I appeared to be a good candidate for the prodrug approach.

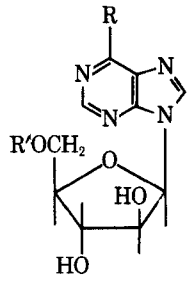
Vidarabine-5′-valerate (III) is an example of a model prodrug of I. Although preliminary experiments (2) indicated that the stratum corneum is almost impermeable to I, this barrier might be overcome by the 5′-ester prodrug. Because of its increased lipophilicity, this prodrug might penetrate the lipoidal stratum corneum more readily. After passing through the stratum corneum, the prodrug may be converted by esterase into the parent drug, which subsequently may be degraded by deaminases located in the viable cutaneous tissue. Since back-diffusion of I and II across the stratum may be negligible, a relatively high buildup of these species may result in the cutaneous tissue, depending on the ratio of the activities of the two enzymes.

In the case of topical I therapy for herpes simplex virus infection, it is believed that the higher the concentration of I in the target tissue, the more effective is the therapy. An appropriately designed prodrug may be able to achieve both a high stratum corneum permeability and good access to esterase activity, both of which lead to an enhanced bioavailability[2] compared to the parent drug.

For quantitative assessment of the efficacy and possible consequences of side effects, however, the mathematical problem of the simultaneous diffusion and enzyme reactions must be solved. The solution to this problem yields directly the concentration profiles of all species in the skin as well as the fluxes for all species. The former provides the prevailing levels of the prodrug, drug, and metabolite in the target sites; the latter yields a direct measure of the rate of prodrug, drug, and metabolite transport into the bloodstream.

THEORY

Considerations in General Model Development—A cross section of the hairless mouse skin used as the model is shown in Fig. 1. It consists



I: R = NH_2, R′ = H
II: R = OH, R′ = H
III: R = NH_2, R′ = $CH_3(CH_2)_3CO$

of the stratum corneum (~50 μm thick); the epidermis, which is approximately a layer or two of cells; and the dermis, which contributes most of the skin thickness. Accordingly, a model treating the skin as a two-ply laminate composed of a metabolically inactive stratum corneum and a metabolically active cutaneous tissue (epidermis and dermis) was formulated.

Homogeneous enzyme distribution will be assumed here for mathematical simplicity, while the more general case of nonhomogeneous enzyme distribution will be discussed in another report (21).

General Model Description—The physical model for the simultaneous diffusion transport and bioconversion of the prodrug, III, in the skin is shown in Fig. 2. It consists of a two-ply biomembrane composed of the stratum corneum of thickness s and the cutaneous tissue (epidermis and dermis) of thickness m followed by a blood sink. The following assumptions were made:

1. The relevant enzymes are homogeneously distributed in the cutaneous tissue and are not present in the dead stratum corneum tissue.
2. The stratum corneum is essentially permeable to the highly lipophilic prodrug and serves as a relatively natural barrier preventing the backflow of the more hydrophilic parent drug and its metabolite.
3. The enzyme reactions in the cutaneous tissue are simple, sequential first-order and irreversible reactions of prodrug → drug → metabolite (Scheme I):

$$\text{III} \xrightarrow{k_1} \text{I} \xrightarrow{k_2} \text{II}$$

Scheme I

4. Diffusivities of all species are independent of distance within the viable cutaneous tissue.
5. Steady-state conditions prevail.

Steady-State Flux and Concentration–Distance Expressions—The flux of the prodrug in the stratum corneum is:

$$F_V = P_{sc}[V(-s) - V(0)] \qquad \text{(Eq. 1)}$$

where F_V is the flux across the stratum corneum per unit area, P_{sc} is the permeability coefficient of the stratum corneum, and $V(-s)$ and $V(0)$ are the prodrug concentrations at $x = -s$ and 0, respectively.

The changes in concentrations of the prodrug, drug, and metabolite with distance x for $0 \le x \le m$ in the cutaneous tissue are described by:

$$D_V \frac{d^2V}{dx^2} - k_1 V = 0 \qquad \text{(Eq. 2)}$$

$$D_A \frac{d^2A}{dx^2} - k_2 A + k_1 V = 0 \qquad \text{(Eq. 3)}$$

$$D_H \frac{d^2H}{dx^2} + k_2 A = 0 \qquad \text{(Eq. 4)}$$

where V, A, and H are the concentrations of the prodrug, drug, and metabolite, respectively; D_V, D_A, and D_H are the diffusion coefficients in the cutaneous tissue; and k_1 and k_2 are the apparent first-order rate constants for the III → I and I → II reactions, respectively.

These expressions can be rewritten as follows:

$$\frac{d^2V}{dx^2} = K_1^2 V \qquad \text{(Eq. 5)}$$

$$\frac{d^2A}{dx^2} - K_2^2 A = \left(-\frac{k_1}{D_A}\right) V \qquad \text{(Eq. 6)}$$

$$\frac{d^2H}{dx^2} = \left(-\frac{k_2}{D_H}\right) A \qquad \text{(Eq. 7)}$$

[2] In this study, prodrug bioavailability was defined as the topically available concentration of the parent drug in the cutaneous tissue.

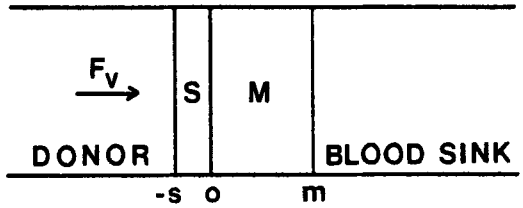


Figure 2—*Physical model for topical prodrug delivery in skin. Key:* M, *viable epidermis and dermis;* S, *stratum corneum;* F_V, *prodrug flux; and* s *and* m, *thickness of stratum corneum and epidermis–dermis layers.*

where:

$$K_1 = \sqrt{k_1/D_V} \qquad \text{(Eq. 8)}$$

$$K_2 = \sqrt{k_2/D_A} \qquad \text{(Eq. 9)}$$

The boundary conditions are:

$$P_{sc}[V(-s) - V(0)] = -D_V \left.\frac{dV}{dx}\right|_{x=0} \qquad \text{(Eq. 10)}$$

$$D_A \left.\frac{dA}{dx}\right|_{x=0} = 0 \qquad \text{(Eq. 11)}$$

$$D_H \left.\frac{dH}{dx}\right|_{x=0} = 0 \qquad \text{(Eq. 12)}$$

The last two conditions account for the absence of back-diffusion of species A and H across the stratum corneum. Because sink conditions are assumed, it follows that:

$$V = A = H = 0 \qquad \text{(Eq. 13)}$$

at $x = m$.

The details of the solutions to Eqs. 5–7 are given in the *Appendix*. However, the results are:

$$V(x) = -\beta \sinh K_1(m - x) \qquad \text{(Eq. 14)}$$

$$A(x) = \omega \sinh K_2(m - x) + \left(\frac{\beta}{(K_1^2 - K_2^2)}\right)\left(\frac{k_1}{D_A}\right)[\sinh K_1(m - x)] \qquad \text{(Eq. 15)}$$

$$H(x) = \left(\frac{k_2}{D_H}\right)\left[\frac{\omega}{K_2}(\cosh K_2 m) + \left(\frac{\beta}{(K_1^2 - K_2^2)K_1}\right)\left(\frac{k_1}{D_A}\right)(\cosh K_1 m)\right](m - x) - \frac{k_2}{D_H}\left[\frac{\omega}{K_2^2}[\sinh K_2(m - x)] + \left(\frac{\beta}{(K_1^2 - K_2^2)K_1^2}\right)\left(\frac{k_1}{D_A}\right)[\sinh K_1(m - x)]\right] \qquad \text{(Eq. 16)}$$

where $(0 \leq x \leq m)$, $(K_1 \neq K_2)$, and:

$$\beta = \frac{[-V(-s)](P_{sc})}{D_V K_1 \cosh K_1 m + P_{sc} \sinh K_1 m} \qquad \text{(Eq. 17)}$$

$$\omega = \left(\frac{-\beta K_1}{(K_1^2 - K_2^2)K_2}\right)\left(\frac{k_1}{D_A}\right)\left(\frac{\cosh K_1 m}{\cosh K_2 m}\right) \qquad \text{(Eq. 18)}$$

Since $A(x)$ and $H(x)$ in Eqs. 15 and 16 must be bounded for physical reasons, it is required that $K_1 \neq K_2$; otherwise, $A(x)$ and $H(x)$ will go to infinity.

In the case of $K_1 = K_2 = K$:

$$A(x) = \left(-\frac{\beta}{2}\right)\left(\frac{D_V}{D_A}\right)\left[\begin{matrix}\sinh K(m - x) + (Kx)[\cosh K(m - x)] \\ -(Km)(\operatorname{sech} Km)(\cosh Kx)\end{matrix}\right] \qquad \text{(Eq. 19)}$$

$$H(x) = \left(\frac{-\beta}{2}\right)\left(\frac{D_V}{D_A}\right)\left[\begin{matrix}-3 \sinh K(m - x) + (Km)(\operatorname{sech} Km)(\cosh Kx) \\ -(Kx)[\cosh K(m - x)] + 2K(m - x)(\cosh Km)\end{matrix}\right] \qquad \text{(Eq. 20)}$$

The fluxes of all species per unit area across the membrane system (Fig. 2) are readily found by evaluating the derivatives of Eqs. 14–16, 19, and 20 at $x = m$. Thus (see *Appendix*):

$$F_V = -\beta D_V K_1 \qquad \text{(Eq. 21)}$$

$$F_A = \omega D_A K_2 + \left(\frac{\beta D_A K_1}{(K_1^2 - K_2^2)}\right)\left(\frac{k_1}{D_A}\right) \qquad (K_1 \neq K_2) \qquad \text{(Eq. 22)}$$

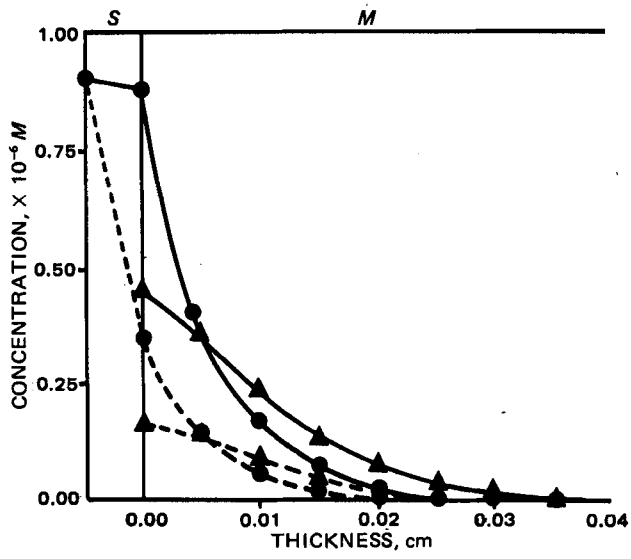


Figure 3—*Effect of III permeability across the stratum corneum on the concentration–distance profiles of III and I. Key:* ●, *prodrug; and* ▲, *drug* ($K_1 = K_2 = 163\ cm^{-1}$). *Solid lines denote the case of* $P_{sc} = 1 \times 10^{-2}$ *cm/sec; dotted lines denote the case of* $P_{sc} = 1 \times 10^{-4}$ *cm/sec.*

$$F_A = -\frac{\beta K^2 m D_V}{2} \tanh Km \qquad (K_1 = K_2 = K) \qquad \text{(Eq. 23)}$$

$$F_H = \frac{\omega k_2}{K_2}(\cosh K_2 m - 1) + \left(\frac{\beta k_2}{(K_1^2 - K_2^2)K_1}\right)\left(\frac{k_1}{D_A}\right)(\cosh K_1 m - 1) \qquad (K_1 \neq K_2) \qquad \text{(Eq. 24)}$$

$$F_H = \left(-\frac{\beta K D_H}{2}\right)\left(\frac{D_V}{D_A}\right)[2 \cosh Km - (Km) \tanh Km - 2] \qquad (K_1 = K_2 = K) \qquad \text{(Eq. 25)}$$

As can be seen in these equations, the concentration–distance expressions and fluxes are functions of membrane thickness, enzyme rate constants, prodrug donor concentration, species diffusivities in the cutaneous tissue, and the prodrug permeability coefficient in the stratum corneum.

THEORETICAL SIMULATIONS AND DISCUSSION

Computations were carried out for a reasonable range of parameter values (Table I). The stratum corneum permeabilities for III were arbitrarily chosen. The thickness of hairless mouse skin, used as the model skin, was determined previously to be 0.04 cm. The epidermis–dermis diffusivities of the prodrug, drug, and metabolite species were equated to 1.0×10^{-6} cm²/sec, which seems reasonable for drugs in tissues.

The apparent rate constant[3], k_2, for the I metabolism by deaminases was taken to be 2.66×10^{-2} sec^{-1}. The values of the k_1 rate constant for the cleavage of III by esterases were varied arbitrarily to demonstrate the effect of esterase activity on bioavailability. Equations 14–20 were used to obtain the concentration–distance profiles, and Eqs. 21–25 were used to estimate the fluxes. Computations centered about the stratum corneum permeability, P_{sc}, and the K_1 parameter, which is indicative of the esterase activity on the prodrug, since these phenomenological constants are considered to be the principal determinants in bringing about effective prodrug delivery in the dermis and concomitant high concentrations of the active species.

Figure 3 shows the effect of the P_{sc} of III on the concentration–distance curves of III and I. A higher P_{sc} leads to a higher I concentration in the dermis. Beyond the first 50-μm depth in the dermis, the I concentration buildup is higher than its precursor for both high and low stratum corneum permeability cases. Since the stratum corneum is relatively impermeable to topically applied I and since the I that enters the dermis is metabolized by deaminases, the prodrug approach allows for the advantageous buildup of I in the cutaneous tissue.

[3] This value of 2.66×10^{-2} sec^{-1} was obtained from preliminary *in vitro* kinetic studies of I in homogenates of hairless mouse skin.

Table I—Constants Used in Theoretical Computations Including the Calculated Fluxes of the Prodrug, Drug, and Metabolites [a]

K_1, cm^{-1}	K_2, cm^{-1}	P_{sc}, cm/sec	Flux, moles/sec III	Flux, moles/sec I	Flux, moles/sec II	All Species	Concentration–Distance Profile
163	163	1×10^{-2}	4.2×10^{-13}	1.4×10^{-12}	1.4×10^{-10}	1.42×10^{-10}	Fig. 3
163	163	1×10^{-4}	1.6×10^{-13}	0.53×10^{-12}	0.55×10^{-10}	0.56×10^{-10}	Fig. 3
54.5	163	1×10^{-4}	7.2×10^{-12}	0.89×10^{-12}	0.24×10^{-10}	0.32×10^{-10}	Fig. 4
490.5	163	1×10^{-4}	4.5×10^{-19}	0.25×10^{-12}	0.75×10^{-10}	0.75×20^{-10}	Fig. 4

[a] $V(-s) = 9 \times 10^{-7}$ M; $D_V = D_A = D_H = 1 \times 10^{-6}$ cm^2/sec; $m = 0.04$ cm; $k_1 = 2.66 \times 10^{-2}$ sec^{-1} when $K_1 = 163$ cm^{-1}; $k_1 = 0.3 \times 10^{-2}$ sec^{-1} when $K_1 = 54.5$; $k_1 = 24.1 \times 10^{-2}$ sec^{-1} when $K_1 = 490.5$; and $k_2 = 2.66 \times 10^{-2}$ sec^{-1} when $K_2 = 163$ cm^{-1}.

The relative influence of esterase and deaminase activities on the tissue concentrations is shown in Fig. 4. When the esterase activity ($k_1 = 24.1 \times 10^{-2}$ sec^{-1}) is nine times higher than the deaminase activity ($k_2 = 2.66 \times 10^{-2}$ sec^{-1}), the overall tissue concentration of the active parent drug is markedly enhanced. Despite the unfavorable situation where the esterase activity is nine times smaller than the deaminase activity, the prodrug delivery system is still better than the parent drug itself since the stratum corneum is almost impermeable to I.

In all of the cases shown in Figs. 3 and 4, the stratum corneum permeability is the dominant factor affecting the prodrug and parent drug levels in the dermis. From the standpoint of overall prodrug design for I, ester prodrug lipophilicity should be optimized to improve stratum corneum permeability, but lability to cleavage by esterases and enzymatic deamination of the parent drug are also important. Thus, methods for assessing relevant enzymic activities in the skin become an important issue in evaluating prodrug bioavailability for topical delivery. Theoretical and experimental studies to assess quantitatively the *in situ* activities of esterases and deaminases in skin are in progress.

The fluxes of all three species that enter the bloodstream are direct measurements of potential toxicity. Table I shows that the total flux of all three species is a function of both stratum corneum permeability and enzyme activity. At constant enzyme activities, an increase in stratum corneum permeability results in an increase in the total flux. With the same stratum corneum permeability, an increase in esterase activity is accompanied by an increase in the total flux. Although a higher stratum corneum permeability and a greater esterase activity are favorable factors for a higher active species level in the skin, the accompanied elevation of total flux could mean higher systemic toxicity. From the therapeutic viewpoint, bioavailability and toxicity are both important. The compromise between the bioavailability factors (stratum corneum permeability and esterase activity) and toxicity factor (total flux) becomes one criterion for designing a successful prodrug.

Bioavailability of topically applied III can be assessed readily on a quantitative basis when the concentration–distance profiles (Fig. 3 or 4) are known. For example, the theoretical results in Fig. 4 focus attention on the concentration of the therapeutically active vidarabine species within the first 10 μm of the tissue (epidermis) where the herpes simplex virus is believed to proliferate in cold sores. The I concentration is ~40% of the topically applied prodrug concentration. In contrast, the available I level is only 6% of the applied prodrug level when the esterase activity is less than the deaminase activity. As indicated in Fig. 3, the bioavailability of I is 50% in the high stratum corneum permeability case and 20% in the lower permeability situation.

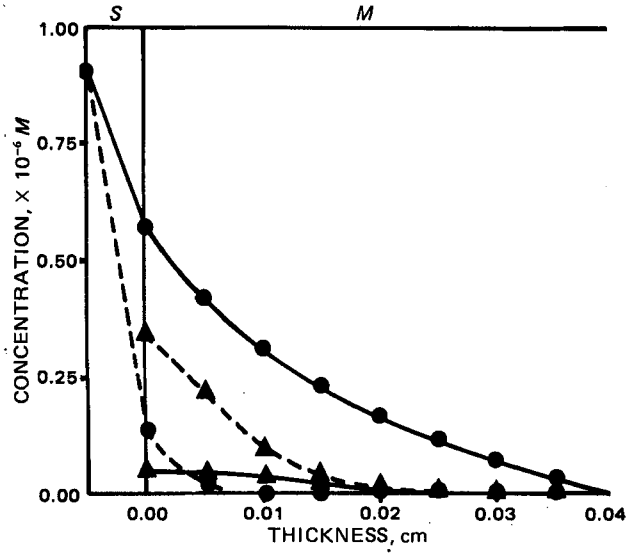


Figure 4—*Effect of esterase and deaminase activities on the concentration–distance profiles of III and I. Key:* ●, *prodrug; and* ▲, *drug* ($P_{sc} = 1 \times 10^{-4}$ *cm/sec*). *Solid lines denote the case when* $K_1 = 54.5$ cm^{-1} *and* $K_2 = 163$ cm^{-1}; *dotted lines denote the case when* $K_1 = 490.5$ cm^{-1} *and* $K_2 = 163$ cm^{-1}.

APPENDIX: MATHEMATICAL SOLUTIONS TO SKIN TRANSPORT MODEL (Fig. 2)

Flux of Prodrug across Stratum Corneum—The steady-state flux per unit area, F_V, of the prodrug within the stratum corneum is given by Fick's law:

$$F_V = \frac{D_{sc}}{h_{sc}}[V_{sc}(-s) - V_{sc}(0)] \qquad \text{(Eq. A1)}$$

where the subscript *sc* refers to the stratum corneum region. The other terms are self-explanatory. If it is assumed that the partition coefficients of the prodrug for stratum corneum–water and stratum corneum–cutaneous tissue are the same:

$$K_p = \frac{V_{sc}(-s)}{V(-s)} = \frac{V_{sc}(0)}{V(0)} \qquad \text{(Eq. A2)}$$

then the flux becomes:

$$F_V = P_{sc}[V(-s) - V(0)] \qquad \text{(Eq. A3)}$$

where the permeability coefficient is:

$$P_{sc} = \frac{D_{sc}K_p}{h_{sc}} \qquad \text{(Eq. A4)}$$

Cutaneous Concentration–Distance Expressions for Prodrug—To begin, Eq. 5 is employed:

$$\frac{d^2V}{dx^2} = K_1^2V \qquad \text{(Eq. A5)}$$

with $K_1 = \sqrt{k_1/D_V}$. The general solution is:

$$V(x) = C_1 \sinh K_1x + C_2 \cosh K_1x \qquad (0 \le x \le m) \quad \text{(Eq. A6)}$$

where C_1 and C_2 are the integration constants. By applying the stratum corneum–cutaneous interphase transfer and sink boundary conditions, *i.e.*:

$$P_{sc}[V(-s) - V(0)] = -D_V\frac{dV}{dx}\bigg|_{x=0} \qquad \text{(Eq. A7)}$$

and:

$$V = 0 \qquad \text{at } x = m \qquad \text{(Eq. A8)}$$

to Eq. A5, the integration constants are:

$$C_1 = \frac{-[V(-s)](P_{sc}\cosh K_1m)}{D_VK_1\cosh K_1m + P_{sc}\sinh K_1m} \qquad \text{(Eq. A9)}$$

and:

$$C_2 = \frac{[V(-s)](P_{sc}\sinh K_1m)}{D_VK_1\cosh K_1m + P_{sc}\sinh K_1m} \qquad \text{(Eq. A10)}$$

Thus, the particular solution is:

$$V(x) = -\beta \sinh K_1(m - x) \qquad \text{(Eq. A11)}$$

where:

$$\beta = \frac{[-V(-s)](P_{sc})}{D_VK_1\cosh K_1m + P_{sc}\sinh K_1m} \qquad \text{(Eq. A12)}$$

Cutaneous Concentration–Distance Expressions for Drug: Case

of $K_1 \neq K_2$—The solution to Eq. 6 is sought. With Eq. 14 (or A12), the equation becomes:

$$\frac{d^2A}{dx^2} - K_2^2A = (\beta)\left(\frac{k_1}{D_A}\right)[\sinh K_1(m-x)] \qquad \text{(Eq. A13)}$$

with $K_2 = \sqrt{k_2/D_A}$. This nonhomogeneous equation is conveniently solved by the method of undetermined coefficients. The homogeneous solution is:

$$A(x)_h = C_1 \sinh K_2x + C_2 \cosh K_2x \qquad (0 \leq x \leq m) \qquad \text{(Eq. A14)}$$

whereas the particular solution is:

$$A(x)_p = (C_3\beta)\left(\frac{k_1}{D_A}\right)[\sinh K_1(m-x)] \qquad \text{(Eq. A15)}$$

where C_1, C_2, and C_3 are arbitrary constants. In finding C_3, the first and second derivatives of Eq. A15 are taken and applied to Eq. A13, whereupon:

$$C_3 = \frac{1}{K_1^2 - K_2^2} \qquad \text{(Eq. A16)}$$

The general solution, which is the sum of $A(x)_h$ and $A(x)_p$, becomes:

$$A(x) = C_1 \sinh K_2x + C_2 \cosh K_2x + \left(\frac{\beta}{(K_1^2 - K_2^2)}\right)\left(\frac{k_1}{D_A}\right)[\sinh K_1(m-x)] \qquad \text{(Eq. A17)}$$

With the boundary conditions accounting for the impermeability of the stratum corneum with respect to the drug and the sink conditions:

$$D_A \left.\frac{dA}{dx}\right|_{x=0} = 0 \qquad \text{(Eq. A18)}$$

and:

$$A = 0 \qquad \text{at } x = m \qquad \text{(Eq. A19)}$$

the integration constants are:

$$C_1 = \frac{\beta K_1}{(K_1^2 - K_2^2)K_2}\left(\frac{k_1}{D_A}\right)(\cosh K_1m) \qquad \text{(Eq. A20)}$$

and:

$$C_2 = -\frac{C_1 \sinh K_2m}{\cosh K_2m} \qquad \text{(Eq. A21)}$$

After substitution of C_1 and C_2 into Eq. A17 and algebraic rearrangement, the concentration–distance expression for the drug is:

$$A(x) = \omega \sinh K_2(m-x) + \frac{\beta}{(K_1^2 - K_2^2)}\left(\frac{k_1}{D_A}\right)[\sinh K_1(m-x)] \qquad \text{(Eq. A22)}$$

where:

$$\omega = \frac{-\beta K_1}{(K_1^2 - K_2^2)K_2}\left(\frac{k_1}{D_A}\right)\left(\frac{\cosh K_1m}{\cosh K_2m}\right) \qquad \text{(Eq. A23)}$$

and $K_1 \neq K_2$.

Cutaneous Concentration–Distance Expression for Drug: Case of $K_1 = K_2$—As shown in the previous section, a necessary criterion for the existence of Eq. A22 is that $K_1 \neq K_2$. To obtain an expression for $A(x)$ for the case of $K_1 = K_2$, a different approach is used, *e.g.*, the variation of parameters method.

In pursuing the case of $K_1 = K_2 = K$, it follows that the enzyme rate constants are $k_1 = D_VK^2$ and $k_2 = D_AK^2$ such that Eq. A13 can be rewritten as:

$$\frac{d^2A}{dx^2} - K^2A = \left(\frac{\beta K^2 D_V}{D_A}\right)[\sinh K(m-x)] \qquad \text{(Eq. A24)}$$

By the method of the variation of parameters, the homogeneous solution is:

$$A(x)_h = C_1 \sinh Kx + C_2 \cosh Kx \qquad \text{(Eq. A25)}$$

and the particular solution is:

$$A(x)_p = u_1 \sinh Kx + u_2 \cosh Kx \qquad \text{(Eq. A26)}$$

In determining the parameters u_1 and u_2, which are variables in x, the following set of auxiliary equations is identified:

$$u_1' \sinh Kx + u_2' \cosh Kx = 0 \qquad \text{(Eq. A27)}$$

and:

$$u_1'K \cosh Kx + u_2'K \sinh Kx = \left(\frac{\beta K^2 D_V}{D_A}\right)[\sinh K(m-x)] \qquad \text{(Eq. A28)}$$

where u_1' and u_2' are derivatives of u_1 and u_2. After solving for u_1' and u_2' by determinants and then integrating, one gets:

$$u_1 = \frac{\beta D_V}{D_A}\left[\sinh Km\left(\frac{\sinh 2Kx}{4} - \frac{Kx}{2}\right) - \frac{(\cosh Km)(\cosh^2 Kx)}{2}\right] \qquad \text{(Eq. A29)}$$

and:

$$u_2 = \frac{\beta D_V}{D_A}\left[\cosh Km\left(\frac{\sinh 2Kx}{4} - \frac{Kx}{2}\right) - \frac{(\sinh Km)(\sinh^2 Kx)}{2}\right] \qquad \text{(Eq. A30)}$$

The substitution of Eqs. A29 and A30 into A26 and subsequent algebraic manipulation give rise to a simple expression for $A(x)_p$:

$$A(x)_p = -\frac{\beta D_V}{2D_A}(Kx)\cosh K(m-x) \qquad \text{(Eq. A31)}$$

The general solution is:

$$A(x) = A(x)_h + A(x)_p \qquad \text{(Eq. A32)}$$

or:

$$A(x) = C_1 \sinh Kx + C_2 \cosh Kx - \frac{\beta D_V}{2D_A}(Kx)\cosh K(m-x) \qquad \text{(Eq. A33)}$$

After applying the boundary conditions found in Eqs. A18 and A19, the integration constants are:

$$C_1 = \left(\frac{\beta D_V}{2D_A}\right)(\cosh Km) \qquad \text{(Eq. A34)}$$

and:

$$C_2 = \frac{\beta D_V}{2D_A}[(Km)\,\text{sech}\,Km - \sinh Km] \qquad \text{(Eq. A35)}$$

Finally, the drug concentration as a function of distance in the cutaneous tissue for the case of $K_1 = K_2 = K$ is expressed by:

$$A(x) = -\frac{\beta D_V}{2D_A}[\sinh K(m-x) + (Kx)\cosh K(m-x) - (Km)\,\text{sech}\,Km \cosh Kx] \qquad \text{(Eq. A36)}$$

Cutaneous Concentration–Distance Expressions for Metabolite—For the case of $K_1 \neq K_2$, utilization of Eqs. 7 and 15 provides the start in the derivation of $H(x)$. Thus:

$$\frac{d^2H}{dx^2} = -\frac{k_2}{D_H}\left[\omega \sinh K_2(m-x) + \left(\frac{\beta}{K_1^2 - K_2^2}\right)\left(\frac{k_1}{D_A}\right)[\sinh K_1(m-x)]\right] \qquad \text{(Eq. A37)}$$

Upon integrating twice, one obtains:

$$H(x) = -\frac{k_2}{D_H}\left[\left(\frac{\omega}{K_2^2}\right)[\sinh K_2(m-x)] + \left(\frac{\beta}{(K_1^2 - K_2^2)K_1^2}\right)[\sinh K_1(m-x)]\right] + C_1x + C_2 \qquad \text{(Eq. A38)}$$

whereby the integration constants C_1 and C_2 are evaluated from the following boundary conditions:

$$D_H \left.\frac{dH}{dx}\right|_{x=0} = 0 \qquad \text{(Eq. A39)}$$

and:

$$H = 0 \qquad \text{at } x = m \qquad \text{(Eq. A40)}$$

Accordingly:

$$C_1 = -\frac{k_2}{D_H}\left[\frac{\omega}{K_2}(\cosh K_2m) + \left(\frac{\beta}{(K_1^2 - K_2^2)K_1}\right)\left(\frac{k_1}{D_A}\right)(\cosh K_1m)\right] \qquad \text{(Eq. A41)}$$

$$C_2 = -C_1m \qquad \text{(Eq. A42)}$$

The final solution becomes:

$$H(x) = \frac{k_2}{D_H}\left[\left(\frac{\omega}{K_2}\right)(\cosh K_2 m) + \left(\frac{\beta}{(K_1^2 - K_2^2)K_1}\right)\left(\frac{k_1}{D_A}\right)(\cosh K_1 m)\right](m - x) - \frac{k_2}{D_H}\left[\left(\frac{\omega}{K_2^2}\right)[\sinh K_2(m - x)] + \left(\frac{\beta}{(K_1^2 - K_2^2)K_1^2}\right)\left(\frac{k_1}{D_A}\right)[\sinh K_1(m - x)]\right] \quad \text{(Eq. A43)}$$

where $K_1 \neq K_2$.

In the case of $K_1 = K_2 = K$, Eqs. 7 and 19 are combined to give the following homogeneous differential equation:

$$\frac{d^2H}{dx^2} = \frac{\beta K^2 D_V}{2D_A}\left[\begin{matrix}\sinh K(m - x) + (Kx)\cosh K(m - x)\\ -(Km)\,\text{sech}\,Km\cosh Kx\end{matrix}\right] \quad \text{(Eq. A44)}$$

The solution to Eq. A44 is given by:

$$H(x) = \frac{\beta D_V}{2D_A}\left[\begin{matrix}2\sinh K(m - x) - (Km)\,\text{sech}\,Km\cosh Kx\\ -\sinh Km[(Kx)\sinh Kx - \cosh Kx]\\ +\cosh Km[(Kx)\cosh Kx - \sinh Kx]\end{matrix}\right] + C_3 x + C_4 \quad \text{(Eq. A45)}$$

where, with the boundary conditions given by Eqs. A39 and A40:

$$C_3 = \left(\frac{\beta K D_V}{D_A}\right)(\cosh Km) \quad \text{(Eq. A46)}$$

and:

$$C_4 = \left(-\frac{\beta D_V}{D_A}\right)[(Km)\cosh Km] \quad \text{(Eq. A47)}$$

After substitution of the evaluated constants C_3 and C_4 back into Eq. A45 and simplification of the algebra, the final expression is:

$$H(x) = -\frac{\beta D_V}{2D_A}\left[\begin{matrix}-3\sinh K(m - x) + (Km)\,\text{sech}\,Km\cosh Kx\\ -(Kx)\cosh K(m - x) + 2K(m - x)\cosh Km\end{matrix}\right] \quad \text{(Eq. A48)}$$

Total Flux of All Species across Membrane System—The total fluxes of the prodrug V, parent drug A, and metabolite H are readily found by applying the following relationships:

$$F_V = -D_V\left.\frac{dV(x)}{dx}\right|_{x=m} \quad \text{(Eq. A49)}$$

$$F_A = -D_A\left.\frac{dA(x)}{dx}\right|_{x=m} \quad \text{(Eq. A50)}$$

$$F_H = -D_H\left.\frac{dH(x)}{dx}\right|_{x=m} \quad \text{(Eq. A51)}$$

Without going into the details of the mathematics, the final flux expressions are given in Eqs. 21–25.

REFERENCES

(1) H. Y. Ando, N. F. H. Ho, and W. I. Higuchi, *J. Pharm. Sci.*, **66,** 1525 (1977).

(2) *Ibid.*, **66,** 755 (1977).

(3) F. M. Schabel, Jr., *Chemotherapy (Basel)*, **13,** 321 (1968).

(4) R. J. Suhadolnik, "Nucleoside Antibiotics" Wiley–Interscience, New York, N.Y., 1970, pp. 123–169.

(5) W. M. Shannon, in "Adenine Arabinoside: An Antiviral Agent," D. Pavan-Langston, R. A. Buchanan, and C. A. Alford, Jr., Eds., Raven, New York, N.Y., 1975, pp. 1–44.

(6) B. J. Sloan, in *ibid.*, pp. 45–94.

(7) A. A. Fondak, A. E. Friedman, and R. J. Klein, in "Antivirals with Clinical Potential," abstracts from a symposium at Stanford University, Stanford, Calif., Aug. 26–29, 1975.

(8) M. Lieberman, T. W. Schafer, and P. E. Came, *J. Invest. Dermatol.*, **60,** 203 (1973).

(9) D. C. Baker, T. H. Haskell, and S. R. Putt, presented at the American Chemical Society, Chicago meeting, Aug. 28–Sept. 2, 1977.

(10) J. J. Brink and G. A. LePage, *Cancer Res.*, **24,** 312 (1964).

(11) *Ibid.*, **24,** 1042 (1964).

(12) M. Hubert-Habart and S. S. Cohen, *Biochim. Biophys. Acta*, **59,** 468 (1962).

(13) G. A. LePage and I. G. Junga, *Cancer Res.*, **25,** 46 (1965).

(14) R. Koshiura and G. A. LePage, *ibid.*, **28,** 1014 (1968).

(15) J. G. Cory and R. J. Suhadolnik, *Biochemistry*, **4,** 1729 (1965).

(16) S. Frederiksen, *Arch. Biochem. Biophys.*, **113,** 383 (1966).

(17) A. Block, M. J. Robins, and J. R. McCarthy, *J. Med. Chem.*, **10,** 908 (1967).

(18) B. J. Sloan, J. K. Kielty, and F. A. Miller, *Ann. N.Y. Acad. Sci.*, **284,** 60 (1977).

(19) P. M. Schwartz, C. Shipman, Jr., and J. C. Drach, *Antimicrob. Agents Chemother.*, **10,** 64 (1976).

(20) J. D. Connor, L. Sweetman, S. Carey, M. A. Stuckey, and R. Buchanan, *ibid.*, **6,** 630 (1974).

(21) J. L. Fox, C. D. Yu, W. I. Higuchi, and N. F. H. Ho, *Int. J. Pharm.*, in press.

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