Physical Model Evaluation of Topical Prodrug Delivery—Simultaneous Transport and Bioconversion of Vidarabine-5′-valerate II: Parameter Determinations

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Abstract □ Results of initial studies on methods for determining various model parameters are reported. By employing excised hairless mouse skin in a diffusion cell system, numerous model parameter values were deduced. The stratum corneum permeability was estimated from steady-state fluxes with preparations of heat-separated epidermal membranes. Determinations of dermis diffusivities and enzyme rate constants *in situ* involved considering the simultaneous transport and the enzyme processes and factoring the diffusivities and enzyme rate constants from the overall kinetics. Dermal diffusivities were on the order of 10^{-6} cm²/sec for vidarabine and its 5′-valerate ester. The enzyme rate constants were 1.70×10^{-3} sec^{-1} for the esterase and 8.68×10^{-3} sec^{-1} for the deaminase.

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

A physical model for the topical delivery of an ester prodrug of vidarabine (9-β-D-arabinofuranosyladenine) (I) was presented previously (1). The model treats the skin as a two-ply membrane composed of a stratum corneum and a viable cutaneous tissue. When the I prodrug is applied on the skin surface, the prodrug is transported across the stratum corneum and reaches the viable cutaneous tissue where the esterase converts some of the prodrug to I and the deaminase metabolizes a portion of the latter to its metabolite, 9-β-D-arabinofuranosylhypoxanthine (II). Concurrently, all three species diffuse toward the blood sink.

The mathematical problem of the simultaneous diffusion and bioconversion was solved, and the solution provided expressions for the concentration–distance profiles in the cutaneous tissue as well as for the transport rates of the three species into the bloodstream. Simulation calculations (Figs. 3 and 4 in Ref. 1) showed the influences of the stratum corneum permeability and the esterase activity on prodrug bioavailability[1]. Since knowledge of the actual parameters would make the proposed physical model useful for quantitative predictions and mechanism evaluations in topical bioavailability, it was desirable to develop methods for parameter determinations. These parameters include the stratum corneum permeability coefficients and the dermis diffusivities for the prodrug, the drug, and the metabolite; the enzyme rate constants for the esterase and the deaminase reactions; and the skin thickness.

The present paper reports the results of an initial investigation on methods for determining these parameters. Vidarabine-5′-*n*-valerate (III) was selected as the prodrug because preliminary experiments showed that III is significantly deacylated by the hairless mouse skin.

By employing excised hairless mouse skin in a well-designed *in vitro* experimental system, all parameters could be determined. The skin thickness could be measured with a micrometer by sandwiching the specimen between two glass slides. The stratum corneum permeability coefficients could be determined in a diffusion cell with preparations of the heat-separated epidermal membrane[2]. The evaluations of dermis diffusivities and the enzyme reaction rate constants, however, presented certain difficulties because of the simultaneous transport and the enzyme processes and because of the need to factor out the diffusivities and the enzyme reaction rate constants from the overall kinetics. The proposed procedures combined experiments and theory in a novel way and provided the means for determining the transport and enzyme activity parameters in the tissues.

THEORY

Aqueous Permeability Coefficient (P_{aq}) Determinations—Although the transport resistances arising from the aqueous diffusion layers generally do not greatly influence the overall transport and metabolism, estimations of them are desirable. The following procedures provide the means for approximating P_{aq}.

The aqueous diffusion layer thickness, h, may be determined by conducting a dissolution rate experiment with a reference solute (such as benzoic acid) for which both the solubility and the diffusivity are known. The data are plotted as $\ln [C_s/(C_s - C_t)]$ *versus* t, which gives a slope equal to (D/h) (area/volume) according to the dissolution rate equation:

$$\ln\left(\frac{C_s}{C_s - C_t}\right) = \left(\frac{D}{h}\right)\left[\frac{\text{area}}{\text{volume}}(t - t_l)\right] \qquad \text{(Eq. 1)}$$

where C_s is the solubility of benzoic acid, C_t is the concentration of benzoic acid at time t, t_l is the extrapolated lag time at which the first benzoic acid molecule theoretically appeared in the bulk solution, area is the dissolutional area of the benzoic acid pellet, volume is the bulk solution volume, and D is the aqueous diffusivity of benzoic acid (2). Therefore, h may be calculated from the slope.

The aqueous diffusivities of I, II, or III may be estimated using:

$$D_i = \left(\frac{M}{M_i}\right)^{1/3} D \qquad \text{(Eq. 2)}$$

where i = I, II, or III; M is the molecular weight of benzoic acid; M_i is the molecular weight of species i; D is the aqueous diffusivity of benzoic acid; and D_i is the aqueous diffusivity of species i.

[1] Prodrug bioavailability is defined as the topically available concentration of the parent drug in the cutaneous tissue.

[2] Scotch-tape stripped skin was also employed. Data from these preparations and those obtained from full-thickness skin may be used to calculate the stratum corneum permeability coefficients.

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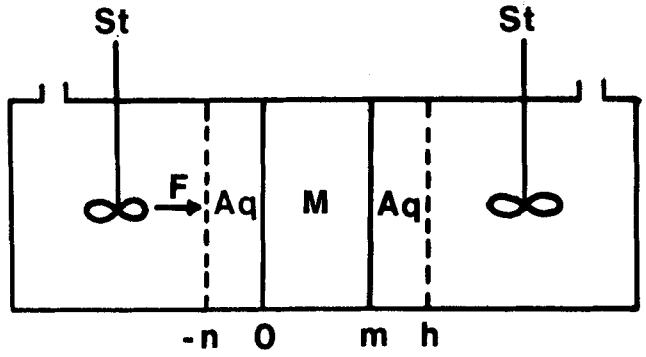

Figure 1—*Go-through experiment. Key: M, viable epidermis and dermis; Aq, aqueous diffusion layer; F, flux of diffusant; and St, stirrer.*

The P_{aq} of I, II, or III in the aqueous diffusion layer is simply:

$$(P_{aq})_i = \frac{D_i}{h} \qquad \text{(Eq. 3)}$$

Membrane–Water Partition Coefficient ($K_{m/w}$)—By the definition of partition coefficient, the following relationship exists:

$$(K_{m/w})_i = \frac{[C_m]_i}{[C_w]_i} = \frac{(M_m)_i/V_m}{(M_w)_i/V_w} \qquad \text{(Eq. 4)}$$

where, for species i, $[C_m]_i$ is the equilibrium concentration of i in the membrane, $[C_w]_i$ is the equilibrium concentration of i in the aqueous solution, $(M_m)_i$ is the mass of i in the membrane at equilibrium, $(M_w)_i$ is the mass of i in the aqueous solution at equilibrium, V_m is the membrane volume, and V_w is the aqueous solution volume.

The $(M_m)_i$ may be expressed in terms of $(M_w)_i$ and $(M_w^0)_i$, the total mass of i initially present in the aqueous solution:

$$(M_m)_i = (M_w^0)_i - (M_w)_i \qquad \text{(Eq. 5)}$$

Therefore, Eq. 4 may be rearranged to give:

$$(K_{m/w})_i = \left(\frac{[C_w^0]_i - [C_w]_i}{[C_w]_i}\right)\left(\frac{V_w}{V_m}\right) \qquad \text{(Eq. 6)}$$

where $[C_w^0]_i$ is the initial concentration of i in the aqueous phase. By assaying $[C_w^0]_i$ and $[C_w]_i$, $(K_{m/w})_i$ may be determined by Eq. 6.

Diffusivity and Permeability Coefficient Determinations—With a diffusion cell, the steady-state flux method may be used for the determinations of permeability coefficients of I, II, or III in different skin preparations (epidermal membrane or dermis) according to:

$$F_i = \frac{1}{\frac{1}{P_i} + \frac{2}{P_{aq}}} \Delta C_i \qquad \text{(Eq. 7)}$$

where F_i is the steady-state flux of diffusant i, P_i is the permeability coefficient of i, and ΔC_i is the concentration gradient of i across the membrane.

The diffusivity, D_i, may be related to P_i, $(K_{m/w})_i$, and m, the thickness of the membrane, by:

$$D_i = \frac{(P_i)(M)}{(K_{m/w})_i} \qquad \text{(Eq. 8)}$$

Simultaneous Determinations of Diffusivity and Enzyme Reaction Rate Constant in "Go-Through" Experiments—In the present studies, an inhibitor of the esterase activity was not employed. Therefore, a direct measurement of the prodrug diffusivity in the dermis was not possible. However, the diffusivity may be determined indirectly in the following manner. Figure 1 illustrates the go-through experiment that provides a situation for determining simultaneously the diffusivity of a prodrug and its esterase activity. The system consists of two identical aqueous diffusion layers and a membrane in between. The membrane is assumed to have a homogeneous enzyme distribution[3]. The diffusivity is assumed to be constant[3] through the membrane.

The enzymatic reactions involved are taken as simple first-order reactions for mathematical simplicity. Sink conditions on the receiver side and steady-state conditions are assumed to prevail throughout the experimental period. Based on these assumptions, the mathematical problem for the simultaneous diffusion and bioconversion may be set up and the differential equations describing the steady-state simultaneous diffusion and bioconversion of a prodrug may be written as:

$$D_V \frac{d^2V}{dx^2} - k_1 V = 0 \qquad \text{(Eq. 9)}$$

$$D_A \frac{d^2A}{dx^2} + k_1 V - k_2 A = 0 \qquad \text{(Eq. 10)}$$

$$D_H \frac{d^2H}{dx^2} + k_2 A = 0 \qquad \text{(Eq. 11)}$$

where V, A, and H are concentrations of the prodrug, drug, and metabolite, respectively; D_V, D_A, and D_H are diffusivities of the prodrug, drug, and metabolite in the membrane, respectively; and k_1 and k_2 are first-order rate constants for the bioconversion shown in Scheme I:

$$\text{prodrug} \xrightarrow[\text{esterase}]{k_1} \text{drug} \xrightarrow[\text{deaminase}]{k_2} \text{metabolite}$$

Scheme I

When an inhibitor[4] is used to block the deaminase activity, the situation is simplified and involves only the first step of the bioconversion. Then the differential equations become:

$$D_V \frac{d^2V}{dx^2} - k_1 V = 0 \qquad \text{(Eq. 12)}$$

$$D_A \frac{d^2A}{dx^2} + k_1 V = 0 \qquad \text{(Eq. 13)}$$

For the steady-state situation and sink conditions, the boundary conditions at $x = 0$ are:

$$-D_V \left.\frac{dV}{dx}\right|_{x=0} = P_{aq}[V(-n) - V(0)] \qquad \text{(Eq. 14)}$$

$$-D_A \left.\frac{dA}{dx}\right|_{x=0} = P_{aq}[0 - A(0)] \qquad \text{(Eq. 15)}$$

At $x = m$, they are:

$$-D_V \left.\frac{dV}{dx}\right|_{x=m} = P_{aq}[V(m) - 0] \qquad \text{(Eq. 16)}$$

$$-D_A \left.\frac{dA}{dx}\right|_{x=m} = P_{aq}[A(m) - 0] \qquad \text{(Eq. 17)}$$

With these boundary conditions, analytical solutions (*Appendix I*) for Eqs. 12 and 13 may be obtained. These solutions give concentration–distance profiles of the prodrug and drug in the domain of $0 \le x \le m$. The species flow into the receiver chamber is, by definition, the product of the diffusivity and the derivative of the concentration with respect to x at $x = m$. Therefore, the fluxes, F_{hV} and F_{hA}, are related to D_V and k_1 by:

$$F_{hV} = \frac{-\gamma P_{aq}}{(K_{m/w})_V} \qquad \text{(Eq. 18)}$$

$$F_{hA} = \frac{\gamma P_{aq}}{(K_{m/w})_V} + \psi \qquad \text{(Eq. 19)}$$

where:

$$K_1 = \sqrt{k_1/D_V}$$

$$\gamma = \frac{-\frac{P_{aq}V(-n)}{D_V K_1}}{\left(1 + \frac{P_{aq}^2}{D_V^2 K_1^2 (K_{m/w})_V^2}\right)\sinh K_1 m + \frac{2P_{aq}}{D_V K_1 (K_{m/w})_V}\cosh K_1 m}$$

$$\psi = \frac{-D_A}{\frac{P_{aq} m}{(K_{m/w})_A} + 2D_A}\left[\frac{\gamma k_1}{K_1}\left(1 + \frac{P_{aq}^2}{K_1^2 D_A D_V (K_{m/w})_A (K_{m/w})_V}\right)\sinh K_1 m + \frac{\gamma k_1 P_{aq}}{K_1^2}\left(\frac{1}{D_V (K_{m/w})_V} + \frac{1}{D_A (K_{m/w})_A}\right)\cosh K_1 m - \frac{\gamma k_1 P_{aq}}{K_1^2}\left(\frac{1}{D_V (K_{m/w})_V} - \frac{1}{D_A (K_{m/w})_A}\right)\right]$$

[3] A more general case with variations in diffusivity and enzyme reaction rate constants with respect to position in the membrane will be considered later.

[4] An inhibitor of deaminase activity, covidarabine, was provided by Dr. T. H. Haskell, Warner-Lambert/Parke-Davis, Ann Arbor, MI 48106.

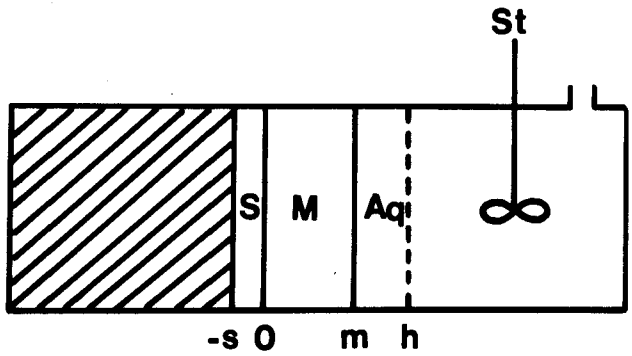

Figure 2—*In-and-out experiment. Key: M, viable epidermis and dermis; S, stratum corneum; Aq, aqueous diffusion layer; and St, stirrer.*

Similarly, species flow at $x = -n$ may be expressed by:

$$F_{-nV} = -\gamma\left[D_V K_1 \sinh K_1 m + \frac{P_{aq}}{(K_{m/w})_V}\cosh K_1 m\right] \quad \text{(Eq. 20)}$$

$$F_{-nA} = \gamma\left[D_V K_1 \sinh K_1 m + \frac{P_{aq}}{(K_{m/w})_V}\cosh K_1 m\right] + \psi \quad \text{(Eq. 21)}$$

where F_{-nV} is the prodrug transport rate from the donor chamber into the membrane and F_{-nA} is the back-diffusion rate of the drug out of the membrane and into the donor side.

Examination of Eqs. 18–21 demonstrates that:

$$F_{-nV} + F_{-nA} = F_{hV} + F_{hA} \quad \text{(Eq. 22)}$$

Equation 22 is a mathematical statement of the prevailing steady-state condition at which the net flux into the membrane at $x = -n$ is equal to the total flux out of the membrane at $x = h$.

Equations 18–21 may be used to determine simultaneously k_1 and D_V from the measured fluxes and the predetermined D_A. These equations are more than adequate since there are four equations and only two unknowns. In the conversion of the parent drug to its metabolite, the unknown reduces to k_2 only since D_A and D_H are predetermined. The equations involved in the k_2 determination are:

$$F_{hA} = -\frac{\delta P_{aq}}{(K_{m/w})_A} \quad \text{(Eq. 23)}$$

$$F_{hH} = \frac{\delta k_2 P_{aq}}{K_2^2 D_A (K_{m/w})_A} + \phi \quad \text{(Eq. 24)}$$

$$F_{-nA} = -\delta\left[D_A K_2 \sinh K_2 m + \frac{P_{aq}}{(K_{m/w})_A}\cosh K_2 m\right] \quad \text{(Eq. 25)}$$

$$F_{-nH} = \delta\left[D_A K_2 \sinh K_2 m + \frac{P_{aq}}{(K_{m/w})_A}\cosh K_2 m\right] + \phi \quad \text{(Eq. 26)}$$

where:

$$K_2 = \sqrt{k_2/D_A}$$

$$\delta = \frac{-\dfrac{P_{aq} A(-n)}{D_A K_2}}{\left(\dfrac{P_{aq}^2}{D_A^2 K_2^2 (K_{m/w})_A^2} + 1\right)(\sinh K_2 M) + \dfrac{2P_{aq}}{D_A K_2 (K_{m/w})_A}(\cosh K_2 m)}$$

$$\phi = \frac{-D_H}{\dfrac{P_{aq} m}{(K_{m/w})_H} + 2D_H}\left[\frac{\delta k_2}{K_2}\left(1 + \frac{P_{aq}^2}{K_2^2 D_H D_A (K_{m/w})_H (K_{m/w})_A}\right)\sinh K_2 m + \frac{\delta k_2 P_{aq}}{K_2^2}\left(\frac{1}{D_A (K_{m/w})_A} + \frac{1}{D_H (K_{m/w})_H}\right)\cosh K_2 m - \frac{\delta k_2 P_{aq}}{K_2^2}\left(\frac{1}{D_A (K_{m/w})_A} - \frac{1}{D_H (K_{m/w})_H}\right)\right]$$

Enzyme Reaction Rate Constants in "In-and-Out" Experiments—A modification of the go-through design (Fig. 2) involves substituting one side of the diffusion cell with an impermeable glass plate. In this case, the prodrug (or drug) in the bulk solution is allowed to penetrate from the dermis side and is metabolized by the cutaneous enzymes. The products then back-diffuse into the bulk phase.

The solution to the mathematical problem for such an in-and-out system provides equations for the determination of the enzyme reaction rate constants from the observed fluxes. The mathematics are similar to those for the go-through case but with different boundary conditions. For the determination of k_1 in the presence of covidarabine, the relevant differential equation is:

$$D_V \frac{d^2V}{dx^2} - k_1 V = 0 \quad \text{(Eq. 27)}$$

and the boundary conditions at $x = 0$ are:

$$-D_V \left.\frac{dV}{dx}\right|_{x=0} = 0 \quad \text{(Eq. 28)}$$

At $x = m$, they are:

$$-D_V \left.\frac{dV}{dx}\right|_{x=0} = P_{aq}[V(m) - V(h)] \quad \text{(Eq. 29)}$$

The solution (*Appendix II*) to Eq. 27 gives the concentration–distance profiles of the prodrug in the domain $0 \le x \le m$. Taking the derivative at $x = m$ and relating the derivative to the flux by Fick's law give:

$$\frac{1}{(K_{m/w})_V K_1 \tanh K_1 m} = D_V\left[\frac{V(h)}{-F_{hV}} - \frac{1}{P_{aq}}\right] \quad \text{(Eq. 30)}$$

where F_{hV} is the flux of the prodrug transporting into the dermis from the bulk solution and $(K_{m/w})_V$ is the partition coefficient of the prodrug between the dermis and the aqueous phase. Therefore, k_1 may be determined from the observed flux using Eq. 30. A similar expression may be obtained for k_2:

$$\frac{1}{(K_{m/w})_A K_2 \tanh K_2 m} = D_A\left[\frac{A(h)}{-F_{hA}} - \frac{1}{P_{aq}}\right] \quad \text{(Eq. 31)}$$

where F_{hA} is the flux of the drug transporting into the dermis from the bulk solution and $(K_{m/w})_A$ is the drug partition coefficient between the dermis and the aqueous phase.

EXPERIMENTAL

Materials—^{3}H-2-I[5] and ^{3}H-2-III[6] were used as received after the purity was checked by TLC. ^{3}H-2-II was made from the enzyme conversion of ^{3}H-2-I by a commercial adenosine deaminase[7] followed by TLC examination of purity. Compounds I[8], II[8], and III[9] were used to make a 1-μg/μl aqueous solution for UV visualization of the respective TLC spots. Covidarabine was dissolved in distilled water to make a 5-μg/ml solution before use.

The liquid scintillation counter cocktail[10] was stored in a dark place, and 5 ml was transferred by a repipet into each vial for the scintillation counting. Male hairless mice[11], ~8–12 weeks old, were employed. Normal saline[12] was used for all experiments as a bulk phase solution. A dermatome[13] was employed for slicing the skin.

Diffusion Cells—A modified Karush-type diffusion cell[14] was further modified by a local glass shop; a 3-cm stirring shaft was attached for each half-cell. The stirrer, made of stainless steel and equipped with a small polytef propeller ~8 mm in length, was driven by a 150-rpm constant-speed motor[15]. The sampling ports, one for each half-cell, were 1 cm in length. The volume of each half-cell was ~3 ml. The effective diffusion area was 1.767 cm^2.

With the membrane in between, the two cell halves were assembled as shown in Fig. 3 using a No. 18 spring clamp. The assembled cell was then immersed in a 37° water bath so that the stirring and sampling ports were the only cell components above the water bath surface. A cell was then ready for a go-through experiment. A smaller cell, 1.5-ml half-cell volume and 0.7126 cm^2 diffusion area, also was used in permeability experiments.

A specially designed glass cell (Fig. 4) was employed for the in-and-out experiments. The cell consisted of a glass plate and a modified half-cell of a regular diffusion cell. The glass plate was for blocking the stratum

[5] New England Nuclear, Boston, MA 02118.
[6] Courtesy of Dr. A. J. Glazko, Warner-Lambert/Parke-Davis, Ann Arbor, MI 48106.
[7] Boehringer Mannheim GmbH, 15069 EAAT, Germany.
[8] Calbiochem, Los Angeles, CA 90054.
[9] Courtesy of Dr. D. C. Baker, Warner-Lambert/Parke-Davis, Ann Arbor, MI 48106.
[10] Aquasol, New England Nuclear, Boston, MA 02118.
[11] HRS/J mice, Jackson Laboratory, Bar Harbor, ME 04609.
[12] Sodium chloride irrigation (0.9%), Abbott Laboratories, North Chicago, IL 60064.
[13] Castroviejo, a small electrical dermatome, Storz Instrument Co., St. Louis, MO 63110.
[14] Bellco, Vineland, N.J.
[15] Hurst, Princeton, Ind.

Table I—Permeability Coefficients of I–III in Full-Thickness, Hairless Mouse Skin

Mouse	Permeability Coefficients[a], $\times 10^8$ cm/sec I	II	III
801034[b]	2.96	1.82	—
611031	2.33	—	5.43
611031 after 6 hr of soaking in saline	1.87	—	—

[a] Calculated from steady-state fluxes using Eq. 7, where P_{aq} was 1.05×10^{-3} cm/sec. [b] Raw data are shown in Fig. 12.

corneum side of the skin so that no drug could escape from that side. The half-cell opening was 0.635 cm in diameter, providing a 0.317-cm^2 effective contact area between the skin and the bulk solution, which was 1 ml in volume. The cell was equipped with two ports, one for the stirrer and the other for sampling. The two parts of the cell were held together by a No. 12 spring clamp. The assembled cell was immersed in a 37° constant-temperature water bath in the same way as was the go-through cell, and the cell contents were stirred continuously at 150 rpm.

Procedure for Skin Preparations—A male hairless mouse, ~6–12 weeks of age, was sacrificed by snapping the spinal cord at the neck. A square section of the abdominal skin, ~3 cm in each dimension, was excised from the animal with surgical scissors. After the incision was made, the skin was lifted and the adhering fat and other visceral debris were removed carefully from the under surface. Prior to removing the skin, a uniform circle was made on the abdomen with a marker pen, using the diffusion opening of a half-cell as the template. This circle marked the precise skin section to be positioned between the half-cells. After the skin was mounted between half-cells and clamped, the excess skin was trimmed.

For a dermis preparation, the top 50–150 μm of the abdominal skin was removed with a dermatome before any incision was made. Although the dermatome was preset at 100 μm, the thickness of the removed layer

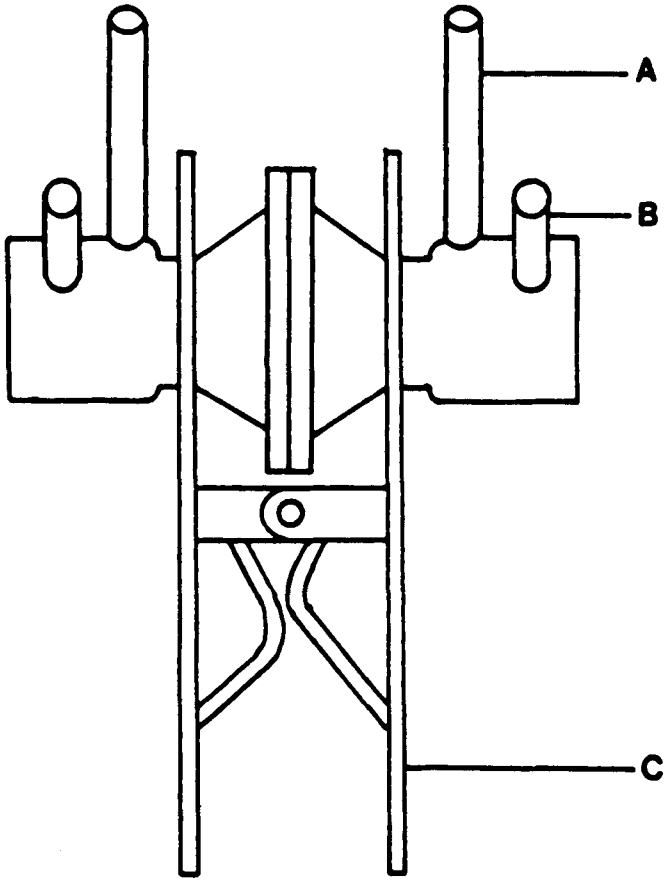

Figure 3—*Diffusion cell for the go-through experiment. Key: A, stirrer port; B, sampling port; and C, spring clamp.*

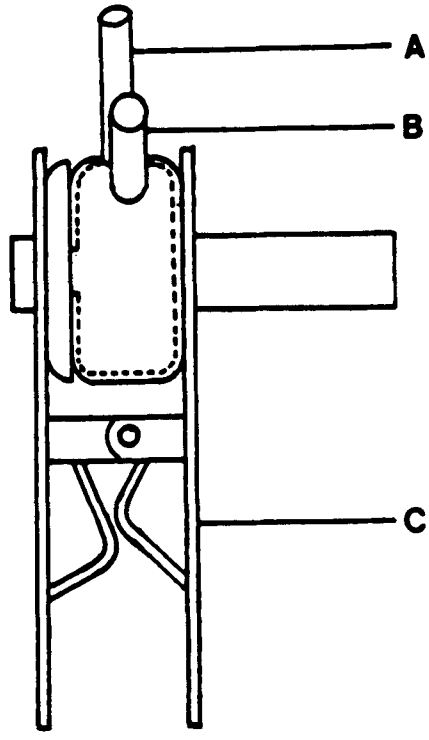

Figure 4—*Diffusion cell for the in-and-out experiment. Key: A, stirrer port; B, sampling port; and C, spring clamp.*

varied from 50 to 150 μm due to the different pressures applied by hand from one preparation to another. After planing, the remaining dermis was excised from the animal and mounted between half-cells as before. The dermis thickness was measured with a micrometer before and after the experiment by sandwiching the membrane between two glass slides.

An epidermal membrane was prepared by a heat separation method (3). A sacrificed mouse was water bathed at 60° for 30 sec, and the body was dried with cleaning tissue. The abdominal skin was marked as usual. The edge of a square, 3 cm in each dimension, was cut to the required depth by a surgical scalpel. A disposable tip of an Eppendorf pipet was employed to separate the epidermal membrane. Due to the thinness of the membrane, the procedure was carried out with care to prevent holes. The separated membrane was spread out on the surface of a saline solution and examined for holes. The membrane was then mounted between half-cells for an experimental run.

Stripped skin was obtained by stripping the stratum corneum off with tape[16] 25 times. The stripped skin was then excised from the animal and was ready for an experimental run.

Preparations of full-thickness skin were used for the in-and-out experiment to determine the enzyme reaction rate constants. The dermis was employed in the go-through experiment for both the determination of the enzyme reaction rate constants and the diffusivity calculation. For the permeability studies, all kinds of skin preparations were used.

TLC—Precoated silica gel TLC plates[17] were used to isolate I–III. The eluent was the lower layer of a mixture of 3% acetic acid–methanol–chloroform (1:2:3). With a disposable micropipet[18], a 3-μl aliquot of the bulk solution (or the receiver chamber solution in a go-through experiment) was spotted on a TLC plate, followed by an equal volume of the nonradiolabeled compound solution for visualization. The plates were developed in a closed chamber for 2–3 hr until the solvent front reached 15 cm from the origin.

After residual solvent evaporation, the spots were localized by UV light. A square, ~2.5 cm in each dimension with the spot in the center, was scraped off and collected in a scintillation vial. To each vial, 5 ml of the scintillation counter cocktail was added. After overnight equilibration in the dark, the samples were counted in a liquid scintillation counter[19].

[16] Scotch.
[17] Silica gel 60 F-254, Brinkmann Instruments, Westbury, NY 11590.
[18] Unopette, A. H. Thomas Co., Philadelphia, PA 19105.
[19] Beckman LS-200, Beckman Instruments, Fullerton, Calif.

Table II—Permeability Coefficients and Diffusivities of I–III in the Dermis Membrane Preparations

Mouse	Thickness of Dermis, μm	Permeability Coefficients[a], × 10^5 cm/sec			Diffusivities[b], × 10^6 cm^2/sec		
		I	II	III	I	II	III
703153[c]	320	4.43	4.48	4.06	1.79	1.77	—
703153 after 30 hr of soaking in saline	320	4.76	—	—	1.93	—	—
611032	400	1.87	—	1.30	0.95	—	—
703154	400	2.59	1.96	—	1.31	0.98	—
706081	400	—	—	—	—	—	0.97[d]
Average					1.50	1.38	0.97

[a] Calculated from steady-state fluxes using Eq. 7, where P_{aq} was 1.05×10^{-3} cm/sec. [b] Determined by using Eq. 8, where $(K_{m/w})_i$ values were the average values shown in Table IX. [c] Raw data shown in Fig. 10. [d] From a rigorous determination (Table XIII) as discussed in the text.

Liquid Scintillation Counting—Samples from a given experiment always were counted along with a blank control. For samples from TLC plates, the control was prepared from a blank plate that had been eluted along with the sample plates. For solution samples, the blank was prepared from a saline solution.

Vials containing silica gel were counted on the 2nd day after the addition of the cocktail, while those containing no silica gel were counted on the same day. The quenching effect of the silica gel was a function of time for the first 3 hr after the cocktail addition and then reached a stable state corresponding to ~50% quenching. Accordingly, the TLC samples were counted on the 2nd day after the cocktail addition. The counts per minute value was converted into percentage concentration on the basis of the prevailing mass balance in an in-and-out experiment.

Leaching of Enzymes from Dermis Side—A full-thickness skin preparation was mounted in the in-and-out cell as described. A 1.0-ml aliquot of saline was introduced into the chamber and stirred. A 100-μl aliquot of the bulk solution was withdrawn and transferred into a small vial at predetermined times, and 100 μl of fresh saline was added to the bulk solution immediately after each withdrawal to keep a constant volume. Necessary corrections for the dilution were made accordingly.

Except when a sample was taken, the sampling port was covered to avoid evaporation. The aliquots withdrawn were incubated with 1 μCi each of ^{3}H-I or ^{3}H-III to determine the enzyme activity. The initial enzyme reaction rate was taken as a measure of the enzyme concentration.

Aqueous Permeability Coefficients—For dissolution rate determinations, benzoic acid[20] pellets were prepared by directly compressing 100 mg of the material in a die, 1.3 cm (0.5 in.) i.d., under a force of 1362 kg (3000 lb) using a laboratory press[21]. The pellet was mounted on the opening of the in-and-out cell and secured firmly by coating the "pellet and cell" setup with melted paraffin. One milliliter of 0.01 *N* HCl, equilibrated at 37°, was introduced into the chamber at zero time. The stirring speed was maintained at 150 rpm during dissolution. To measure the dissolution rate, the amount of benzoic acid dissolved in the solvent was analyzed against time.

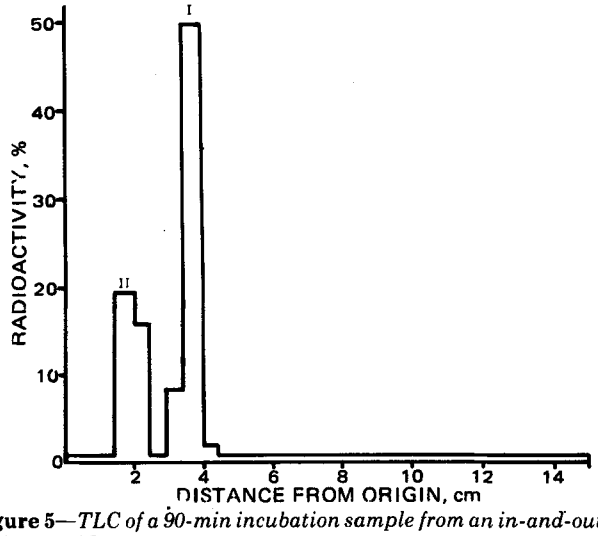

Figure 5—*TLC of a 90-min incubation sample from an in-and-out experiment of I.*

Membrane–Water Partition Coefficient Determinations—Dermis preparations of ~100–200 mg were equilibrated with solutions of ^{3}H-I or ^{3}H-II in vials for 12 hr at 37°. Covidarabine, 5 μg/ml, was included in the solutions to inhibit ^{3}H-I deamination. The initial and equilibrium concentrations of the bulk ^{3}H-I or ^{3}H-II solutions were assayed by scintillation counting. The dermis preparations were weighed before equilibration, and their volumes were calculated from the weights and average density of the skin.

The average skin density was obtained in the following manner. A full-thickness skin of ~400–600 mg was weighed before being transferred into a finely calibrated 10-ml graduated cylinder, which contained 8 ml of a detergent[22] solution. The skin was carefully unfolded with a needle after being submerged in the solution. The detergent helped remove bubbles from the skin surface. To obtain the density, the weight was divided by the volume increment of the detergent solution.

Diffusivity and Permeability Coefficients—The membrane was mounted between the two half-cells immediately after preparation, and appropriate volumes (as specified in a given experiment) of saline solution and covidarabine were introduced into each chamber. Five minutes was allowed for the cell wall, the membrane, and the bulk solution to reach thermal equilibrium with the water bath. Compound ^{3}H-I, ^{3}H-II, or ^{3}H-III was introduced into the chamber, which faced the epidermal side of the membrane (the donor chamber). A 50- or 100-μl sample from the receiver solution was withdrawn at predetermined times and was transferred immediately into a scintillation vial containing 5 ml of the scintillation counter cocktail. The donor solution concentrations were determined in the same way during the 1st min of the run and at the end of the run. All samples were counted together on the liquid scintillation counter.

Go-Through Experiment—The procedure was the same as that for the diffusivity experiment except that instead of obtaining the total counts per minute for each sample, the 3-μl sample solution was subjected to TLC separation before counting to determine the individual fluxes for I, II, or III.

In-and-Out Experiment—A full-thickness skin sample was mounted between the glass plate and the half-cell of the in-and-out cell in such a

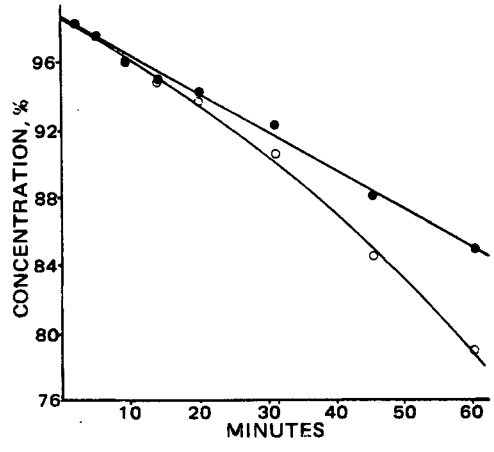

Figure 6—*Correction for the bulk phase metabolism in the in-and-out experiment of I. The slope of the corrected line is −0.222%/min. Numerical data are shown in Table VI. Key: ○, observed; and ●, corrected.*

[20] J. T. Baker Chemical Co., Phillipsburg, N.J.
[21] Model B, Fred Carver Inc., Summit, N.J.
[22] Alconon, Alconox, Inc., New York, N.Y.

Table III—Determinations of k_1 and k_2 from the In-and-Out Experiments

Mouse	Deaminase Slope[a]	Deaminase $k_2 \times 10^2$, sec^{-1}	Esterase Slope[a]	Esterase $k_1 \times 10^2$, sec^{-1}	Correlation Coefficients
702245	—	—	−0.0562	0.104	0.9814
611033[b]	—	—	−0.0558	0.103	0.9626
703011	—	—	−0.0641	0.124	0.9817
608261[c]	−0.222	1.154	—	—	0.9538
611032	−0.224	1.178	—	—	0.9924
611031[d] preleached for 7 hr	−0.203	0.948	—	—	0.9954
Average		1.093		0.110	

[a] Slopes of linear regression lines from concentration–time plots. Steady-state fluxes were obtained from slopes according to flux = (slope)(volume/area), where volume = 1.0 ml and area = 0.317 cm^2. Then k_1 and k_2 were calculated from these fluxes using Eqs. 30 and 31, respectively. [b] Raw data are shown in Fig. 16. [c] Raw data are shown in Fig. 6. [d] Raw data are shown in Fig. 8.

way that the dermis side faced the bulk solution and the stratum corneum side was covered by the glass plate. A 1.0-ml aliquot of saline was introduced into the chamber. For the K_1 determination, 50 μl of the saline solution was replaced by an equal volume of the covidarabine solution.

At zero time, 10 μCi of ^{3}H-I (for the k_2 determination) or ^{3}H-III (for the k_1 determination) was introduced, and 3-μl aliquots were withdrawn at predetermined times. Samples were spotted on TLC plates and localized under UV light. The separated spots were scraped into scintillation vials and counted.

RESULTS AND DISCUSSION

Reproducibility and Sources of Error—The primary purpose of the present studies was to investigate methods for determining parameters of the physical model describing the transport and metabolism of a prodrug in hairless mouse skin. Consequently, some results were obtained for illustrative purposes, and only single determinations were made. In many situations, however, repeat experiments were conducted and the reproducibility of the techniques was investigated.

The reproducibility of the transport experiments was generally good when full-thickness skin preparations (Table I) or when dermis membrane preparations (Table II) were involved. In the permeation experiment, repeat experiments with the same membranes were generally within 20% of each other (Tables I and II). The variations were greater than when membranes from different animals were compared but typically were within a factor of two (Tables I and II).

The repeat experiment studies involving the same membrane indicated that the permeability properties of the full-thickness skin and of the dermis preparation generally remained constant up to 30 hr, and the deaminase and the esterase activities were stable up to at least 7 hr (Table III). Thus, the "viability" of these membrane preparations was good in experiments up to several hours.

The heat-treated epidermal membrane showed greater preparation-to-preparation and stability with time variabilities (Table IV). Table V shows the results of thickness measurements. These data illustrate the degree of uncertainty in the measurements and variations encountered with typical membrane preparations.

Table IV—Permeability Coefficients of I–III in the Heat-Separated Epidermal Membrane

Mouse	Permeability Coefficients[a], $\times 10^8$ cm/sec I	II	III
801031[b]	98.0	173.0	135.0
801034	342.0	480.0	—
610202	—	—	9.9
705103 after 18 hr of soaking in saline	—	—	5.8
708021 after 48 hr of soaking in saline	40.2	—	—
801035 after 48 hr of soaking in saline	46.8	—	—

[a] Calculated from steady-state fluxes using Eq. 7, where P_{aq} was 1.05×10^{-3} cm/sec. [b] Raw data are shown in Fig. 11.

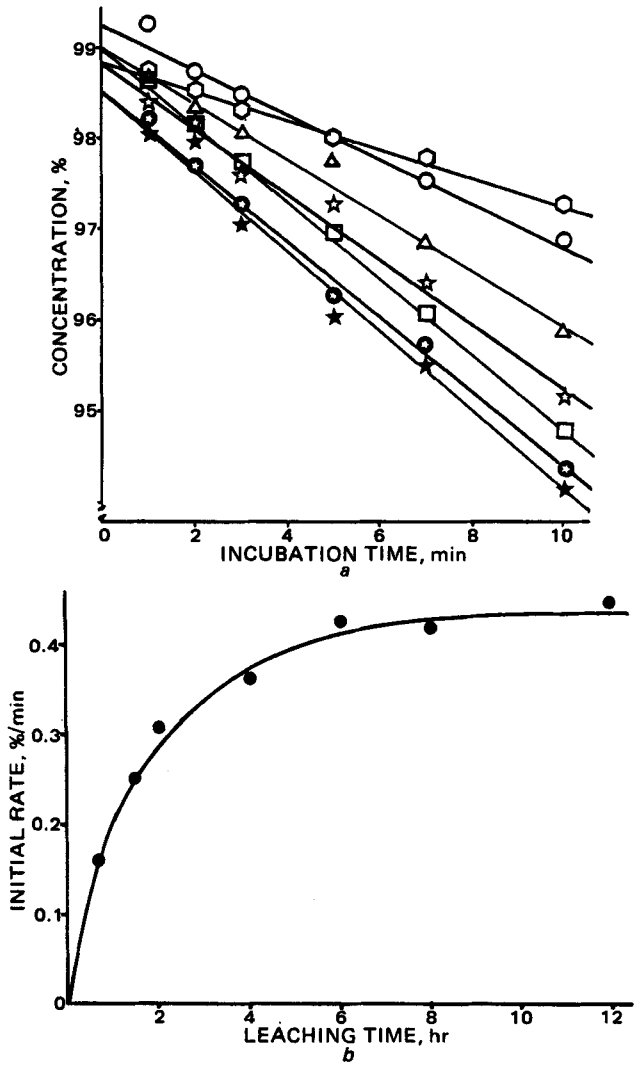

Figure 7—*Deaminase leaching. (a) A 0.01-ml aliquot of the bulk solution, into which the deaminase had leached for the time indicated, was incubated with I at 37°. The initial rates are proportional to the enzyme concentrations at the given times. Key (leaching times): ○, 40 min; ○, 80 min; △, 2 hr; ☆, 4 hr; □, 6 hr; ●, 8 hr; and ★, 12 hr. (b) The initial rates from Fig. 7a were plotted* versus *leaching time. The enzyme leaching rate of the 1st hr, from a linear estimation of the curve, is equal to 3.33 × 10^{-3}%/min^2.*

Metabolites Other than II—The TLC system used was able to isolate many nucleotides and nucleosides (4). Possible metabolites other than II, such as 9-β-D-arabinofuranosylxanthine (IV), which could be the next metabolite after II in the metabolism sequence of purine nucleosides, and the phosphorylation products of I and II were considered; the TLC system was employed for the detection of their presence. The TLC plate on which the 90-min sample of an in-and-out experiment was developed was examined by differential scraping (Fig. 5). No major metabolites other than II were found.

Enzyme Leaching Problem—The deaminase was reported (5) to leach out from the dermis side of the whole skin into the bulk solution, and the bulk solution deaminase activity was found to increase linearly with time over a 2-hr period. Thus, the rate of species concentration change in the bulk solution during an in-and-out experiment reflected not only the cutaneous phase metabolism but also a bulk phase enzyme reaction. Such a combined disappearance rate of I that is not properly corrected for the bulk phase reaction will give an overestimation of k_2.

The correction procedure for this bulk phase metabolism was proposed by Ando *et al.* (5) based on the linear increase of the deaminase activity in the bulk solution with respect to time. The bulk phase kinetics are described by:

$$-\frac{dA_b}{dt} = (k_b t)A_b \qquad \text{(Eq. 32)}$$

Table V—Measurements of Thickness (Micrometers) of Hairless Mouse Skin [a]

	Mouse 801034		Mouse 802141,	Mouse 705103, Abdomen	
	Abdomen, Full-Thickness Skin	Back, Full-Thickness Skin	Back, Full-Thickness Skin	Heat-Separated Epidermal Membrane	Remaining Dermis
Skin preparation plus two glass slides	2328	2405	2405	1914	2396
	2326	2409	2393	1910	2380
	2318	2397	2385	1904	2380
	2314	2393	2380	1900	2382
	2314	2386	2378	1911	2400
	2308	2377	2375	1906	2396
	2305	2374	2360	1904	2386
Mean	2316	2392	2398	1907	2388
Glass slides alone	1938	1938	1941	1864	2048
	1937	1937	1940	1860	2052
	1937	1937	1941	1869	2043
Mean	1937	1937	1941	1864	2047
Net thickness, μm	379	455	457	43	342

[a] Mouse 801034 was 16 weeks old, Mouse 802141 was 11 weeks old, and Mouse 705103 was 18 weeks old.

Table VI—Corrections for the Bulk Phase Metabolism

Parameter	Minutes							
	2	5	9	14	20	31	45.5	60
$\%A_{obs}$	97.99	97.36	95.78	94.64	93.64	90.63	84.53	79.04
$\%A_{bulk}$ [a]	0.01	0.05	0.13	0.33	0.66	1.59	3.39	5.82
$\%A_{corr}$ [b]	97.98	97.31	95.65	94.31	92.98	89.04	81.14	73.22

[a] $\%A_{bulk} = 100 - 100 \exp(-0.5K_b t^2)$, where $K_b = 3.33 \times 10^{-3}\%/min^2$. [b] $\%A_{corr} = \%A_{obs} - \%A_{bulk}$.

where k_b is the increment of the bulk phase enzyme activity per unit time and A_b is the bulk concentration of I. Integration gives:

$$\frac{A_b}{A_b^0} = e^{-k_b t^2/2} \quad \text{(Eq. 33)}$$

or:

$$\frac{A_b}{A_2^0}(\%) = 100e^{-k_b t^2/2} \quad \text{(Eq. 34)}$$

On a percentage scale, the remaining I at a given time is A_b/A_b^0 (percent). Therefore, the percentage disappearance of I caused by the bulk phase metabolism at a given time is $100 - (A_b/A_b^0)$ (percent). Subtraction of this value from the observed percentage of I remaining gives the net level of I corresponding to the skin metabolism only. The entire procedure is summarized in Table VI. The observed concentration–time curve for I bent downwards before correction but appeared to be linear with time up to 60 min after the correction (Fig. 6).

The data in Fig. 7*b*, which is a plot of the initial rates from Fig. 7*a* *versus* the leaching times, reveal that Eq. 32 with a constant k_b of 3.33×10^{-3} % *A*/min (*i.e.*, a linear approximation) is actually good only for the first 1 or 2 hr; the enzyme activity in the bulk solution levels off rapidly after this early time period. The plateau value, 0.425% *A*/min/ml, corresponds to about one-third of the total deaminase in the skin before leaching.

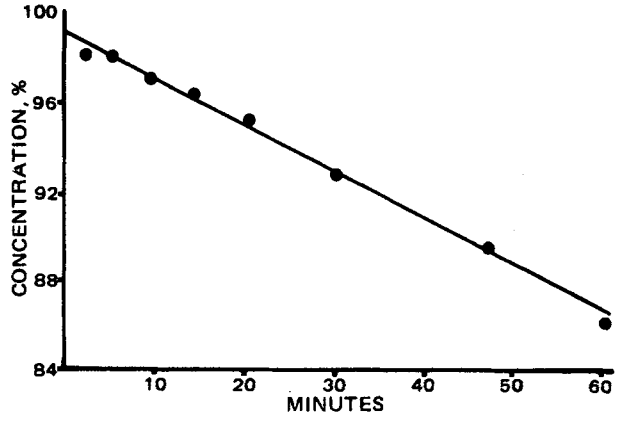

Figure 8—*In-and-out experiment of I with a skin preparation that had been preleached for 7 hr to remove the free enzyme. The slope of the regression line is −0.203%/min.*

An in-and-out experiment conducted with a 7-hr leached skin preparation is shown in Fig. 8. The result of this experiment is in good agreement with the corrected curve of Fig. 6. This finding indicates two things: the stability of the bound enzyme and the reliability of the correction procedure.

Other tests showed that the leached enzyme solution remained stable when stored in a refrigerator (4°). For up to 1 week, no changes in activity were observed.

To see if the esterase also leaches out, two identical aliquots of the bulk phase from a 2-hr leaching experiment were tested for esterase and deaminase activities. The results show a <2% conversion of III to I, in contrast with a 10% conversion of I to II in a 30-min incubation at 37° (Table VII).

Stratum Corneum Permeabilities and Dermal Diffusivities—*Aqueous Permeability Coefficients of I–III*—The thickness of the aqueous diffusion layer for a 150-rpm hydrodynamic system was found to be ~0.010 cm from the benzoic acid dissolution rate experiment (Fig. 9) using Eq. 1. The aqueous permeability coefficients of I–III for this diffusion layer were estimated according to Eqs. 2 and 3 (Table VIII).

Membrane–Water Partition Coefficients—Results of $K_{m/w}$ determinations for I and II are summarized in Table IX. Equation 4 was used in the calculations. The V_m values were obtained by dividing the dermis

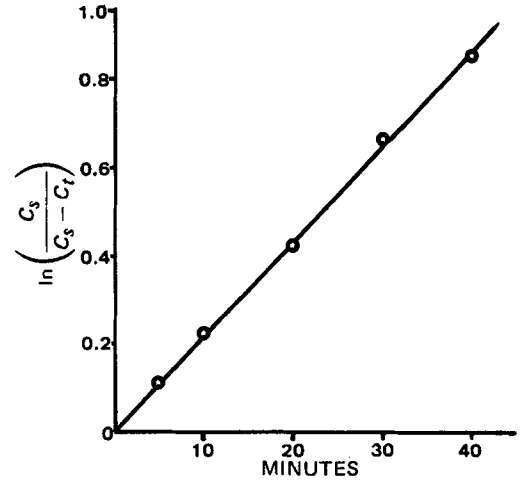

Figure 9—*Benzoic acid dissolution in the in-and-out experimental setup.*

Table VII—Leaching Out of the Enzymes, Deaminase and Esterase

Activity[a]	Percent I	Percent II	Percent III
Esterase	1.82	0.12	98.08
Deaminase	90.01	9.99	—

[a] Identical aliquots of the bulk solution from an in-and-out experimental setup were incubated separately with ^{3}H-I (for deaminase activity) and ^{3}H-III (for esterase activity) at 37° for 30 min.

mass by the average dermis density obtained as shown in Table X. The $K_{m/w}$ of III was undeterminable because an esterase inhibitor was not employed. However, it may not be unreasonable to assume that III has a $K_{m/w}$ value similar to that of I and II.

Dermis Diffusivities of I–III—Figure 10 shows the raw data of permeation of I–III across a dermis membrane. The accumulation of I, II, or III in the receiver chamber increased linearly after a short lag time. The slopes give fluxes of the respective species according to:

$$F_i = [(\text{slopes})_i]\left(\frac{\text{volume}}{\text{area}}\right) \qquad \text{(Eq. 35)}$$

where volume is the volume of the receiver and area is the effective diffusion area. The permeability coefficients were calculated from the resultant fluxes and values of ΔC_i according to Eq. 7. According to Eq. 8, the diffusivities of I and II were calculated from P_i, m, and $(K_{m/w})_i$. Table II summarizes these determinations. The diffusivity of III was undeterminable due to the lack of $K_{m/w}$ data.

Because of the uncertainty in the thickness measurement, a meaningful comparison of magnitudes in permeability or diffusivity of the three species must be done with the same piece of skin preparation. Data shown in Table II are based on such an experimental design.

Diffusivity of II was obtained from a direct flux measurement since the species was inert to the enzymes involved. Diffusivity of I also was obtained directly from the flux because the deaminase was inhibited with covidarabine. For III in the presence of covidarabine, however, the flux gave a measure of mixed diffusivity because an esterase inhibitor was not employed. The receiver solution consisted of 15% of I and 85% of III after 25 min of permeation. The mixed permeability coefficient was not significantly different from the permeability coefficient of I (Table II). Therefore, the permeability coefficient of III is probably of the same magnitude as that of I. A rigorous determination of the diffusivity of III was made possible through the use of Eqs. 18–21, which simultaneously calculate k_1 and D_V.

The diffusivities for the three species were of the same order of magnitude (Table II). From their molecular sizes and configurations, the similar diffusivities were not unexpected for I–III.

The possible diffusivity variations with respect to position in the membrane are currently under investigation and will be reported later.

Stratum Corneum Permeability Coefficients of I–III—Figure 11 gives the permeation data of I–III across a heat-separated epidermal membrane. The permeability coefficients calculated with Eq. 7 are given in Table IV.

Permeation of I and II (Fig. 12) and III across full-thickness skin was much slower than across the heat-separated epidermal membrane. Permeability coefficients of I–III for full-thickness skin are shown in Table I. These findings contradict the conclusion obtained from studies involving the low carbon number n-alkanols (6, 7) where the heat-separated epidermal membrane retained the major diffusion resistance of the intact full-thickness skin. Apparently, heat treatment has a profound effect on the barrier nature for the transport of I–III.

Valeryl esterification of the 5′-OH gave only a small increase in the permeability across the epidermal membrane and the whole skin. To obtain a substantial increase in the permeability, a longer chain substitution or di- or triester derivatization may be necessary. The effect of the di- or triester derivatization and the chain length effect of the substituting group will be reported later.

Table VIII—Aqueous Permeability Coefficients[a] of I–III

i	$(P_{aq})_2 \times 10^3$, cm/sec	$D_i \times 10^5$, cm^2/sec	M_i
I	1.08	1.08	267.3
II	1.08	1.08	268.3
III	0.98	0.98	351.3
Average	1.05	—	—

[a] An h value of 0.01 cm was determined from the line given in Fig. 9 using Eq. 1, where $D = 1.4 \times 10^{-5}$ cm^2/sec, area = 0.317 cm^2, and volume = 1.2 ml. Then $(P_{aq})_i$ and D_i were calculated from h, D, M, and M_i using Eqs. 2 and 3.

Table IX—Determinations[a] of $(K_{m/w})_i$

i	$(M_m)_i$, g	$(V_m)_i$, ml	$(V_w)_i$, ml	$[C_w^0]_i$, cpm/50 µl	$[C_w]_i$, cpm/50 µl	$(K_{m/w})_i$
I	0.215	0.207	1.000	3489	2899	0.98
	0.147	0.141	0.500	3489	2898	0.60
Average	—	—	—	—	—	0.79
II	0.207	0.199	1.000	6733	5519	0.91
	0.113	0.109	0.500	6733	5655	0.73
	0.207	0.199	2.000	6507	6063	0.74
	0.113	0.199	2.000	6507	6210	0.84
Average	—	—	—	—	—	0.81

[a] Using Eq. 4.

Enzyme Reaction Rate Constants—*Go-Through Experiments with Dermis Membrane and Simultaneous Determination of Enzyme Reaction Rate Constant and Diffusivity*—The go-through experiment together with Eqs. 18–21 provides a novel method for simultaneously determining the diffusivity and the enzyme constant. Figure 13 shows the measurements of the four fluxes that are used with Eqs. 18–21 for the determination of D_V and k_1. According to Eq. 22, the sum (or the difference in absolute values) of the observed fluxes in the donor chamber should be equal to the sum of the observed fluxes in the receiver chamber. For k_1 and D_V determinations, the receiver chamber data were used because the flux calculations based on the donor chamber data always were subject to large errors (Figs. 13–15).

The computer iterations of the fluxes from Eqs. 18 and 19 were done with the predetermined D_A value (Table VIII) by varying k_1 and D_V. The best fit of the calculated fluxes to the measured fluxes then determined a pair of D_V and k_1 values (Table XI). Simultaneous determinations of D_A and k_2 also were carried out similarly with flux data from Figs. 14 and 15 (Tables XII and XIII). The D_A value obtained by this method is consistent with that from the direct measurement (Table VIII).

In-and-Out Experiments with Full-Thickness Skin and Enzyme Reaction Rate Constant Determination—Figures 6, 8, and 16 show the measurements of fluxes in the in-and-out experiments. Since the fluxes are in terms of percentage of total radioactivities, the $V(h)$ and $A(h)$ terms in Eqs. 30 and 31, respectively, take the value of 100%. With Eqs. 30 and 31, k_1 and k_2 were calculated from the respective fluxes, F_V and F_A (Table III).

As a comparison, k_1 and k_2 values obtained from the go-through and in-and-out experiments are listed in Table XIII. The in-and-out method gave lower values for k_1 than did the go-through method. The discrepancy raises the question of validity of the various assumptions made in the models for the system. One assumption is that first-order kinetics obtain for the enzyme reaction. The steady-state approximation and the sink conditions were demonstrated to be valid for the present system, but the constant diffusivity and the homogeneous enzyme distribution assumptions have not been verified.

Studies on the possible variations in the diffusivity and the enzyme rate constants (both the deaminase and the esterase) with respect to position in the viable cutaneous tissue will be reported later.

APPENDIX I: MATHEMATICAL SOLUTIONS TO PERMEATION EXPERIMENT (Fig. 1)

Case I: Go-Through Experiment with III in the Presence of Covidarabine—*Flux of Prodrug across Aqueous Diffusion Layer*—The steady-state flux per unit area of the prodrug, F_V, within the aqueous diffusion layer is given by Fick's law:

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

Table X—Density of the Hairless Mouse Skin

Mouse	Weight, g	Volume[a], ml	Density
705101	0.609	0.59	1.03
704264	0.398	0.38	1.05
704263	0.396	0.38	1.04
Average	—	—	1.04

[a] The method for skin volume measurements is described in the text.

Table XI—Simultaneous Determination of D_V and k_1

	Iteration Parameters[a]		(Flux/Initial Donor Concentration) × 10^5, cm/sec			
			Donor		Receiver	
	$D_V \times 10^6$, cm^2/sec	$k_1 \times 10^3$, sec^{-1}	III	I	III	I
Observed[b]	—	—	—	—	1.33	0.89
Best fit	0.97	1.70	—	—	1.33	0.89

[a] Other parameters used were $m = 420\ \mu m$, $D_A = 1.50 \times 10^{-6}\ cm^2/sec$, and $P_{aq} = 1.08 \times 10^{-3}$ cm/sec. [b] A dermatome-planed dermis (Mouse 706081) was used, and the permeation direction was dermis → epidermis. Raw data are shown in Fig. 13.

Table XII—Simultaneous Determination of D_A and k_2 with Dermatome-Planed Dermis

	Iteration Parameters[a]		(Flux/Initial Donor Concentration) × 10^5, cm/sec			
			Donor		Receiver	
Mouse	$D_A \times 10^6$, cm^2/sec	$k_2 \times 10^3$, sec^{-1}	I	II	I	II
703154						
Observed[b]	—	—	—	8.24	1.19	2.18
Best fit	1.46	9.46	—	6.79	1.15	2.72
705245						
Observed[c]	—	—	—	—	1.49	2.75
Best fit	1.62	8.68	—	—	1.49	2.75

[a] Other parameters used were $m = 350\ \mu m$, $D_H = 1.38 \times 10^{-6}\ cm^2/sec$, and $P_{aq} = 1.08 \times 10^{-3}$ cm/sec. [b] The permeation direction was dermis → epidermis. Raw data are shown in Fig. 14. [c] The permeation direction was epidermis → dermis. Raw data are shown in Fig. 15.

or:

$$F_V = \frac{D_{aq}}{h_{aq}}[V_{aq}(m) - V(h)] \qquad \text{(Eq. A2)}$$

for the donor side or the receiver side aqueous diffusion layer as shown in Fig. 1, where the subscript aq refers to the aqueous diffusion region. The other terms are self-explanatory. The membrane–water partition coefficient of the prodrug is:

$$(K_{m/w})_V = \frac{V(0)}{V_{aq}(0)} = \frac{V(m)}{V_{aq}(m)} \qquad \text{(Eq. A3)}$$

Therefore, the fluxes become:

$$F_V = P_{aq}\left[V(-n) - \frac{V(0)}{(K_{m/w})_V}\right] \qquad \text{(Eq. A4)}$$

and:

$$F_V = P_{aq}\left[\frac{V(m)}{(K_{m/w})_V} - V(h)\right] \qquad \text{(Eq. A5)}$$

where the aqueous permeability coefficient is:

$$P_{aq} = \frac{D_{aq}}{h_{aq}} \qquad \text{(Eq. A6)}$$

Concentration–Distance Expressions for Prodrug in Membrane—Equation 12 can be rearranged to give:

$$\frac{d^2V}{dx^2} = (K_1^2)V \qquad \text{(Eq. A7)}$$

with:

$$K_1 = \sqrt{k_1/D_V} \qquad \text{(Eq. A8)}$$

The general solution is:

$$V(x) = C_1 \sinh K_1 x + C_2 \cosh K_1 x \qquad \text{(Eq. A9)}$$

where $0 \le x \le m$ and C_1 and C_2 are the integration constants. By applying the aqueous layer–membrane interphase transfer and sink boundary conditions, *i.e.*:

$$P_{aq}\left[V(-n) - \frac{V(0)}{(K_{m/w})_V}\right] = -D_V \left.\frac{dV}{dx}\right|_{x=0} \qquad \text{(Eq. A10)}$$

and:

$$-D_V \left.\frac{dV}{dx}\right|_{x=m} = P_{aq}\left[\frac{V(m)}{(K_{m/w})_V} - 0\right] \qquad \text{(Eq. A11)}$$

to Eq. A9, the integration constants are found to be:

$$C_1 = \gamma\left[\sinh K_1 m + \frac{P_{aq}}{D_V K_1 (K_{m/w})_V}\cosh K_1 m\right] \qquad \text{(Eq. A12)}$$

$$C_2 = \gamma\left[\cosh K_1 m + \frac{P_{aq}}{D_V K_1 (K_{m/w})_V}\sinh K_1 m\right] \qquad \text{(Eq. A13)}$$

where:

$$\gamma = \frac{-\dfrac{P_{aq}V(-n)}{D_V K_1}}{\left(1 + \dfrac{P_{aq}^2}{D_V^2 K_1^2 (K_{m/w})_V^2}\right)\sinh K_1 m + \dfrac{2P_{aq}}{D_V K_1 (K_{m/w})_V}\cosh K_1 m} \qquad \text{(Eq. A14)}$$

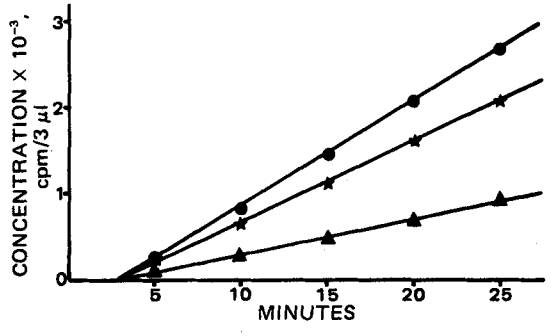

Figure 10—*Permeation of I (●), II (★), and III (▲) across a dermis membrane (320 μm). The three experiments were run concurrently, and the ΔC values were 5.60 × 10⁶ cpm/3 μl for I, 4.95 × 10⁴ cpm/3 μl for II, and 2.38 × 10⁴ cpm/3 μl for III. The fluxes may be calculated from the slopes according to flux = (slope)(volume/area), where volume = 2.5 ml and area = 1.767 cm².*

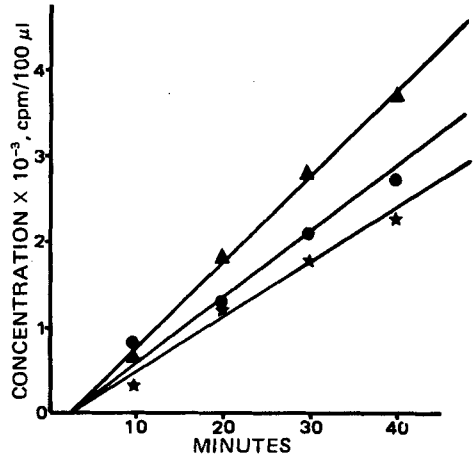

Figure 11—*Permeation of I (●), II (★), and III (▲) across a heat-separated epidermal membrane (57 μm). The three experiments were run concurrently, and the ΔC values were 2.59 × 10⁶ cpm/100 μl for I, 1.15 × 10⁶ cpm/100 μl for II, and 2.61 × 10⁶ cpm/100 μl for III. The fluxes may be calculated from the slopes according to flux = (slope)(volume/area), where volume = 1.5 ml and area = 0.713 cm².*

Table XIII—Comparison of the Enzyme Rate Constants Obtained from the Reflection Boundary and the Permeation Methods

Method	Esterase, $k_1 \times 10^2$, sec^{-1}	Deaminase, $k_2 \times 10^2$, sec^{-1}
In-and-out	0.11[a]	1.09[a]
Go-through	0.17[b]	0.91[c]

[a] From Table III. [b] From Table XI. [c] From Table XII.

Thus, the particular solution is:

$$V(x) = -\gamma\left[\cosh K_1(m-x) + \frac{P_{aq}\sinh K_1(m-x)}{D_V K_1 (K_{m/w})_V}\right] \quad \text{(Eq. A15)}$$

Concentration-Distance Expressions for Drug in Membrane—Equation 13 can be arranged to give:

$$\frac{d^2A}{dx^2} = \left(-\frac{k_1}{D_A}\right)V \quad \text{(Eq. A16)}$$

Substitution of Eq. A15 into Eq. A16 gives:

$$\frac{d^2A}{dx^2} = \left(-\frac{k_1}{D_A}\right)(\gamma)\left[\cosh K_1(m-x) + \frac{P_{aq}\sinh K_1(m-x)}{D_V K_1 (K_{m/w})_V}\right] \quad \text{(Eq. A17)}$$

Upon integrating twice, one obtains:

$$A(x) = \frac{\gamma k_1}{D_A K_1^2}\left[\cosh K_1(m-x) + \frac{P_{aq}\sinh K_1(m-x)}{D_V K_1 (K_{m/w})_V}\right] + C_3 x + C_4 \quad \text{(Eq. A18)}$$

whereby the integration constants C_3 and C_4 are evaluated from the following boundary conditions:

$$D_A \left.\frac{dA}{dx}\right|_{x=0} = P_{aq}\left[\frac{A(0)}{(K_{m/w})_A} - 0\right] \quad \text{(Eq. A19)}$$

$$-D_A \left.\frac{dA}{dx}\right|_{x=m} = P_{aq}\left[\frac{A(m)}{(K_{m/w})_A} - 0\right] \quad \text{(Eq. A20)}$$

Accordingly:

$$C_3 = \frac{1}{\dfrac{P_{aq}m}{(K_{m/w})_A} + 2D_A}\left[\frac{\gamma k_1}{K_1}\left(1 + \frac{P_{aq}^2}{K_1^2 D_A D_V (K_{m/w})_A (K_{m/w})_V}\right)\sinh K_1 m + \frac{\gamma k_1 P_{aq}}{K_1^2}\left(\frac{1}{D_V (K_{m/w})_V} + \frac{1}{D_A (K_{m/w})_A}\right)\cosh K_1 m - \frac{\gamma k_1 P_{aq}}{K_1^2}\left(\frac{1}{D_V (K_{m/w})_V} - \frac{1}{D_A (K_{m/w})_A}\right)\right] \quad \text{(Eq. A21)}$$

$$C_4 = \frac{1}{\dfrac{P_{aq}}{(K_{m/w})_A}\left(\dfrac{P_{aq}m}{(K_{m/w})_A} + 2D_A\right)}\left\{\frac{\gamma k_1 D_A P_{aq}}{K_1^2}\left(\frac{1}{D_V (K_{m/w})_V} - \frac{1}{D_A (K_{m/w})_A}\right) - \left(D_A + \frac{P_{aq}m}{(K_{m/w})_A}\right)\left[\frac{\gamma k_1}{K_1}\left(1 + \frac{P_{aq}^2}{K_1^2 D_A D_V (K_{m/w})_A (K_{m/w})_V}\right)\sinh K_1 m + \frac{\gamma k_1 P_{aq}}{K_1^2}\left(\frac{1}{D_V (K_{m/w})_V} + \frac{1}{D_A (K_{m/w})_A}\right)\cosh K_1 m\right]\right\} \quad \text{(Eq. A22)}$$

Finally, the substitution of C_3 and C_4 into Eq. A18 completes the concentration-distance expression for $A(x)$.

Fluxes of Species V *and* A—The fluxes of V and A in both the donor chamber and the receiver chamber are readily found by applying the following relationships:

$$F_{hV} = F_{mV} = -D_V \left.\frac{dV}{dx}\right|_{x=m} \quad \text{(Eq. A23)}$$

$$F_{hA} = F_{mA} = -D_A \left.\frac{dA}{dx}\right|_{x=m} \quad \text{(Eq. A24)}$$

$$F_{-nV} = F_{mV} = -D_V \left.\frac{dV}{dx}\right|_{x=0} \quad \text{(Eq. A25)}$$

$$F_{-nA} = F_{mA} = -D_A \left.\frac{dA}{dx}\right|_{x=0} \quad \text{(Eq. A26)}$$

Without going into the details of the mathematics, the final flux expressions are given in Eqs. 18–21.

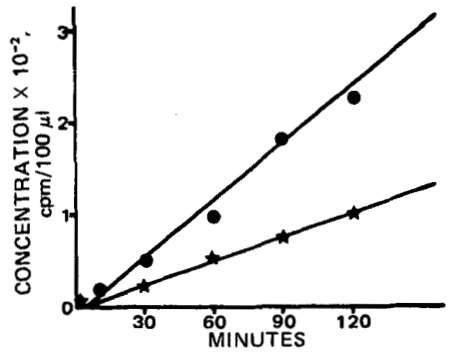

Figure 12—*Permeation of I (●) and II (★) across a full-thickness skin. The two experiments were run concurrently, and the ΔC values were 2.35 × 10⁶ cpm/100 µl for I and 1.82 × 10⁶ cpm/100 µl for II. The fluxes may be calculated from the slopes according to flux = (slope)(volume/area), where volume = 1.5 ml and area = 0.713 cm².*

Case II: Go-Through Experiment with I—In the permeation experiment with I, the mathematics are the same as in the previous case, with species H replacing species A, species A replacing species V, and k_2 replacing k_1. The final flux expressions are given in Eqs. 23–26.

APPENDIX II: MATHEMATICAL SOLUTIONS TO REFLECTION BOUNDARY EXPERIMENT (Fig. 2)

Case I: In-and-Out Experiment with III in the Presence of Covidarabine—*Flux of Prodrug across Aqueous Diffusion Layer*—The steady-state flux per unit area of the prodrug, F_V, within the aqueous diffusion layer is given by Fick's law:

$$F_V = \frac{D_{aq}}{h_{aq}}[V(h) - V_{aq}(m)] \quad \text{(Eq. A27)}$$

where the subscript aq refers to the aqueous diffusion region. The other terms are self-explanatory. The membrane–water partition coefficient of the prodrug is:

$$(K_{m/w})_V = \frac{V(m)}{V_{aq}(m)} \quad \text{(Eq. A28)}$$

Therefore:

$$F_V = P_{aq}\left[V(h) - \frac{V(m)}{(K_{m/w})_V}\right] \quad \text{(Eq. A29)}$$

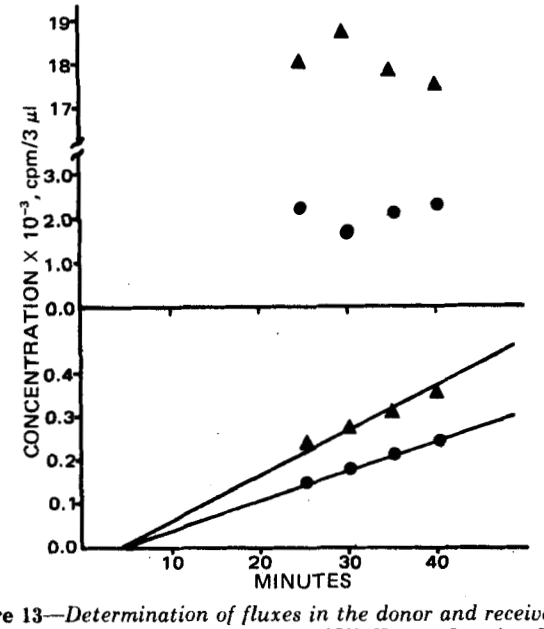

Figure 13—*Determination of fluxes in the donor and receiver compartments of a go-through experiment of III. Key: ●, I; and ▲, III. Flux = (slope)(volume/area), where volume = 3.0 ml and area = 1.767 cm². Covidarabine was employed to inhibit deaminase activity.*

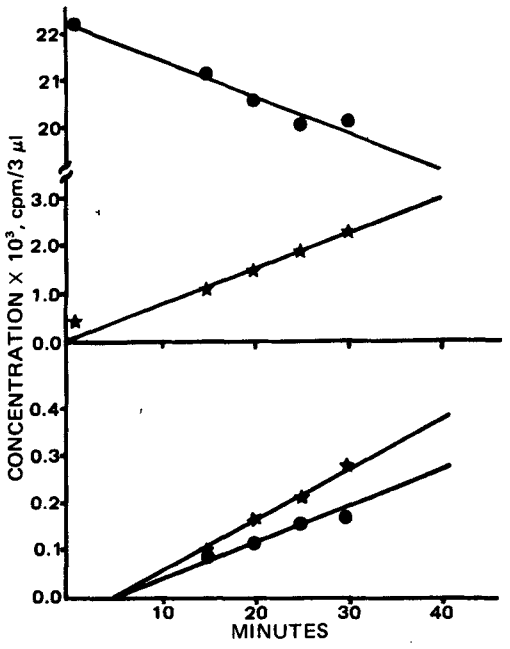

Figure 14—*Determination of fluxes in the donor and receiver compartments of a go-through experiment of I. Key: ●, I; and ★, II. The dermis was preleached for 3 hr. Flux = (slope)(volume/area), where volume = 3.0 ml and area = 1.767 cm².*

where the aqueous permeability coefficient is as given by Eq. A6.

Concentration–Distance Expressions for Prodrug in Membrane—Equation 27 can be rearranged to give Eq. A7 with Eq. A8. The general solution is given by Eq. A9, where $0 \leq x \leq m$ and C_1 and C_2 are the integration constants. By applying the aqueous layer–membrane interphase transfer condition (at $x=m$) and an impermeable boundary condition (at $x=0$), *i.e.*:

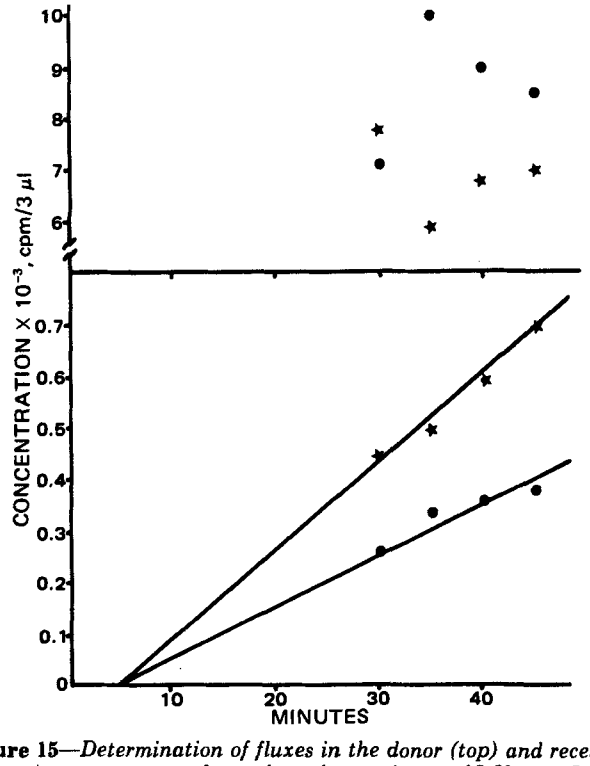

Figure 15—*Determination of fluxes in the donor (top) and receiver (bottom) compartments of a go-through experiment of I. Key: ●, I; and ★, II. The dermis membrane was preleached for 3 hr. Flux = (slope)(volume/area), where volume = 3.0 ml and area = 1.767 cm².*

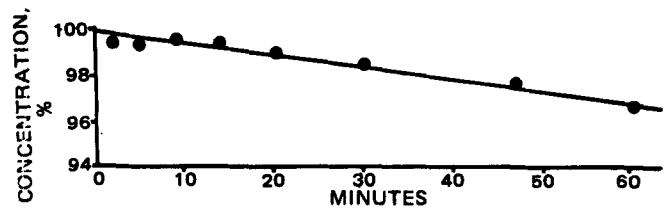

Figure 16—*In-and-out experiment of III. Slope = −0.0558%/min.*

$$P_{aq}\left[V(h) - \frac{V(m)}{(K_{m/w})_V}\right] = D_V \left.\frac{dV}{dx}\right|_{x=m} \quad \text{(Eq. A30)}$$

and:

$$D_V \left.\frac{dV}{dx}\right|_{x=0} = 0 \quad \text{(Eq. A31)}$$

to Eq. A7, the integration constants are found to be:

$$C_1 = 0 \quad \text{(Eq. A32)}$$

$$C_2 = \frac{P_{aq} V(h)}{\frac{P_{aq}}{(K_{m/w})_V}\cosh K_1 m + D_V K_1 \sinh K_1 m} \quad \text{(Eq. A33)}$$

Thus, the particular solution is:

$$V(x) = \frac{P_{aq} V(h) \cosh K_1 x}{\frac{P_{aq}}{(K_{m/w})_V}\cosh K_1 m + D_V K_1 \sinh K_1 m} \quad \text{(Eq. A34)}$$

Flux of Prodrug Entering Membrane—The flux expression for the prodrug entering the membrane is readily obtained by applying:

$$F_{hV} = F_{mV} = -D \left.\frac{dV}{dx}\right|_{x=m} \quad \text{(Eq. A35)}$$

After rearrangement, the final flux equation gives Eq. 30.

Case II: In-and-Out Experiment with I—In the reflection boundary experiment with I, the mathematics are the same as the previous case, with species H replacing species A, species A replacing species V, and k_2 replacing k_1. The final flux expression is given in Eq. 31.

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