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Report

Analysis of Drug Penetration Through the Skin by the Two-Layer Skin Model

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A diffusion model for the skin penetration of drug in the finite-dose system was developed considering the skin to be composed of two layers, the outermost layer (stratum corneum) and the lower layer (viable epidermis and dermis). Based on this skin model, the Laplace transforms of the equations for the drug amounts in the receptor, the vehicle, and the skin were derived. The penetration profiles of 6-mercaptopurine (6-MP) through the intact and stripped guinea pig skin were obtained from *in vitro* diffusion experiments. The computer fitting of those profiles to the Laplace-transformed equations by a nonlinear least-squares program based on a fast inverse Laplace transform algorithm [MULTI(FILT)] gave parameters such as diffusion coefficients of 6-MP and thicknesses of both layers. The mean transit time (MTT) for each diffusion process was defined based on statistical moment concept and calculated using the obtained parameters. Under the present condition, the process to move from the vehicle to the stratum corneum is demonstrated to have the longest mean time in overall processes of 6-MP penetration.

KEY WORDS: percutaneous absorption; diffusion model; two-layer skin model; Laplace transform; fast inversion of Laplace transform (FILT); statistical moment.

INTRODUCTION

Drug penetration through the skin is considered to be a passive diffusion process, and mathematical analyses based on the diffusion theory serve to correlate drug movement with its physicochemical properties. The simplest model for *in vitro* drug penetration considers the skin as a homogeneous plane sheet and assumes a constant drug concentration in the vehicle (infinite dose) and sink conditions in the receptor. According to this model, a diffusion equation in the real-time scale is derived from Fick's second law of diffusion (1). The most convenient approach to analyze drug penetration with this model is the lag time method (2), which utilizes only the penetration data after a steady-state condition has been reached. One can determine the diffusion coefficient and partition coefficient of a penetrant from steady-state flux and lag time of diffusion. The computer fitting of the diffusion data to the diffusion equation with the nonlinear least-squares program allows one to obtain these parameters from the whole penetration profile without any judgment on the linearity of the penetration profile (3,4).

In most cases, however, the assumption of infinite dose is invalid, and we should consider the diffusion model in which the drug concentration in the vehicle decreases (finite dose system). In a previous report, we derived the Laplace transforms of the equations for drug penetration in the finite-dose system based on a one-layer skin model (5). The analytic inversions of these equations are rather complicated so that the analyses of the penetration profiles were carried out using the Laplace-transformed equations by the aid of MULTI(FILT) (6), which is a nonlinear least-squares computer program based on a fast inverse Laplace transform algorithm (7). Computer fitting of the penetration profile of 6-mercaptopurine (6-MP) through an excised guinea pig skin to the Laplace-transformed equation gave two parameters corresponding to drug diffusion and partitioning. The effect of a series of percutaneous absorption enhancers was thus characterized in terms of drug diffusivity and partitioning.

The model considering skin as a homogeneous plane membrane has been successfully employed in many studies. However, skin is composed of several layers with physiologically and physicochemically different properties. The outermost layer, stratum corneum, is known as the most impermeable layer to most drugs, whereas viable epidermis and dermis exhibit their barrier function only for lipophilic penetrants (8). Percutaneous penetration enhancers such as azone (9) and oleic acid (10) reduce the barrier properties of the stratum corneum but have little effect on viable epidermis or dermis. Therefore, both the stratum corneum and the lower layer should be considered for elucidating the mechanism of drug penetration or the action of enhancers. Recently, Tojo *et al.* proposed a method for the evaluation of diffusivity and solubility in both these layers from the lag times and steady-state rates of permeation across the intact skin and the stripped skin (11).

In the present study, a two-layer skin model for the finite-dose system was developed and Laplace-transformed equations describing percutaneous penetration were de-

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rived. We demonstrate here a new method for analyzing the percutaneous penetration process based on this model. The mean transit time (MTT) is also calculated for each penetration process with the use of statistical moment concepts (12).

THEORETICAL

The diffusion models employed in this study are shown in Figs. 1A and B. These models are adopted for the *in vitro* diffusion experiment using a flow-through-type diffusion cell, and a well-stirred finite-dose condition and sink condition are assumed in the donor and receptor, respectively.

Diffusion Model for Intact Skin (Two-Layer Skin Model)

Figure 1A shows the diffusion model for the intact skin composed of stratum corneum (volume, V_2; and thickness, L_2) and the lower layer (epidermis and dermis) (volume, V_3; and thickness, L_3).

The equations of Fick's second law of diffusion for the stratum corneum (drug concentration, C_2) and the lower layer (drug concentration, C_3) are expressed as follows, respectively (distance, x):

$$\frac{\partial C_2}{\partial t} = D_2 \frac{\partial^2 C_2}{\partial x^2} \tag{1}$$

$$\frac{\partial C_2}{\partial t} = D_3 \frac{\partial^2 C_3}{\partial x^2} \tag{2}$$

A penetrant partitions from donor solution to the surface of stratum corneum with a partition coefficient K_{12} and diffuses in stratum corneum with a diffusion coefficient D_2. At the end of stratum corneum, the penetrant partitions to the surface of the lower layer according to its partition coefficient (K_{23}) between the lower layer and the stratum corneum and diffuses in the lower layer with a diffusion coefficient D_3. The boundary conditions are as follows:

$$K_{12}C_1 = C_2 \qquad (x = -L_2) \tag{3}$$

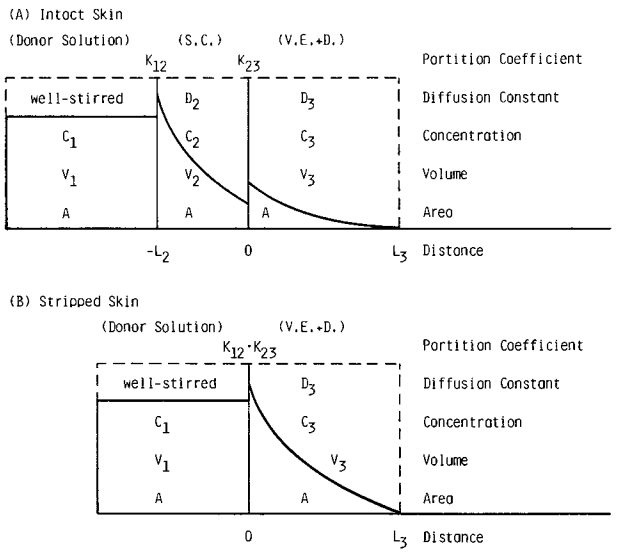

Fig. 1. The two-layer skin model (A) and the one-layer skin model (B). S.C., stratum corneum; V.E. + D., the combination of viable epidermis and dermis. Note that $K_{12}K_{23} = K_{13}$.

$$V_1 \frac{\partial C_1}{\partial t} = D_2 A \frac{\partial C_2}{\partial x} \qquad (x = -L_2) \tag{4}$$

$$K_{23}C_2 = C_3 \qquad (x = 0) \tag{5}$$

$$D_2 \frac{\partial C_2}{\partial x} = D_3 \frac{\partial C_3}{\partial x} \qquad (x = 0) \tag{6}$$

$$C_3 = 0 \qquad (x = L_3) \tag{7}$$

The initial conditions are (applied amount of a penetrant, X_0)

$$C_1 = C_0 \quad \text{or} \quad V_1C_1 = X_0 \tag{8}$$

$$C_2 = C_3 = 0 \tag{9}$$

According to this model, the Laplace transforms of the equations for the drug amounts penetrating into the receptor medium ($\overline{Q}_A$) and remaining in the vehicle ($\overline{X}_A$) and in the skin ($\overline{M}_A$) are expressed, respectively, as (see Appendix A)

$$\overline{Q}_A = K_{12}K_{23}V_2V_3X_0/s/k(s) \tag{10}$$

$$\overline{X}_A = V_1X_0\{V_2d_3\cosh(d_2)\sinh(d_3) + K_{23}V_3d_2\sinh(d_2)\cosh(d_3)\}/s/k(s) \tag{11}$$

$$\overline{M}_A = \overline{M}_{A,2} + \overline{M}_{A,3} \tag{12}$$

$$\overline{M}_{A,2} = K_{12}V_2X_0\{d_3/d_2V_2\sinh(d_2)\sinh(d_3) + K_{23}V_3\cosh(d_3)[\cosh(d_2) - 1]\}/s/k(s) \tag{12a}$$

$$\overline{M}_{A,3} = K_{12}V_2X_0K_{23}V_3[\cosh(d_3) - 1]/s/k(s) \tag{12b}$$

where s is the Laplace variable with respect to time, $\overline{M}_{A,2}$ and $\overline{M}_{A,3}$ are the Laplace transforms for the drug amounts in the stratum corneum and the lower layer, respectively, and

$$d_2 = L_2(s/D_2)^{1/2} \tag{13}$$

$$d_3 = L_3(s/D_3)^{1/2} \tag{14}$$

$$k(s) = V_2d_3\sinh(d_3)\{V_1\cosh(d_2) + K_{12}/d_2V_2\sinh(d_2)\} + K_{23}V_3\cosh(d_3)\{V_1d_2\sinh(d_2) + K_{12}V_2\cosh(d_2)\} \tag{15}$$

Diffusion Model for Stripped Skin (One-Layer Skin Model)

We regard the stripped skin, i.e., viable epidermis and dermis, as a homogeneous plane membrane and employed the previously reported diffusion model (5) for the analysis (Fig. 1B). The product of K_{12} and K_{23} corresponds to the partition coefficient of a penetrant between stripped skin and donor solution ($K_{13} = K_{12}K_{23}$). The Laplace transforms of the equations for the drug amounts penetrating into the receptor ($\overline{Q}_B$) and remaining in the vehicle ($\overline{X}_B$) and in the skin ($\overline{M}_B$) are expressed, respectively, as

$$\overline{Q}_B = K_{12}K_{23}V_3X_0/s/g(s) \tag{16}$$

$$\overline{X}_B = V_1X_0d_3\sinh(d_3)/s/g(s) \tag{17}$$

$$\overline{M}_B = K_{12}K_{23}V_3X_0\{\cosh(d_3) - 1\}/s/g(s) \tag{18}$$

where

$$g(s) = V_1d_3\sinh(d_3) + K_{12}K_{23}V_3\cosh(d_3) \tag{19}$$

Mean Transit Time (MTT)

MTT for an applied penetrant to diffuse from the vehicle to the receptor (MTT_A) is given from Eq. *(10)* as follows (see Appendix B) for the two-layer skin model:

$$MTT_A = \frac{V_1/K_{12}}{D_2'V_2} + \frac{V_1/K_{12}K_{23}}{D_3'V_3} + \frac{1}{2D_2'} + \frac{V_2/K_{23}}{D_3'V_3} + \frac{1}{2D_3'} \tag{20}$$

where D_2' and D_3' are the diffusion parameters (4):

$$D_2' = D_2/L_2^2 \quad (21)$$
$$D_3' = D_3/L_3^2 \quad (22)$$

MTTs for an applied penetrant to traverse the boundary at $x = -L_2$, between the donor and the stratum corneum ($MTT_{A,12}$), and that at $x = 0$, between the stratum corneum and the lower layer ($MTT_{A,23}$), are given, respectively, as follows (see Appendix B):

$$MTT_{A,12} = \frac{V_1/K_{12}}{D_2'V_2} + \frac{V_1/K_{12}K_{23}}{D_3'V_3} \quad (23)$$

$$MTT_{A,23} = MTT_{A,12} + \frac{1}{2D_2'} + \frac{V_2/K_{23}}{D_3'V_3} \quad (24)$$

The last two terms in Eq. *(24)* may be divided into the two terms, one of which is determined only by the nature of the stratum corneum ($1/2D_2'$) and the other affected by the nature of the lower layer. The comparison of Eqs. *(20)* and *(24)* reveals that the $1/2D_3'$ is the MTT for the penetrant to diffuse in the lower layer. Figure 2A shows the MTT for each diffusion process. The MTTs for each process can be calculated directly from Eqs. *(11)*, *(12a)*, and *(12b)* (see Appendix B).

MTT for an applied penetrant to diffuse from the vehicle to the receptor (MTT_B) is given as follows for the one-layer skin model (5):

$$MTT_B = \frac{V_1/K_{12}K_{23}}{D_3'V_3} + \frac{1}{2D_3'} \quad (25)$$

MTT for the applied penetrant to traverse the boundary at $x = 0$, between the vehicle and the stripped skin ($MTT_{B,13}$), is

$$MTT_{B,13} = \frac{V_1/K_{12}K_{23}}{D_3'V_3} \quad (26)$$

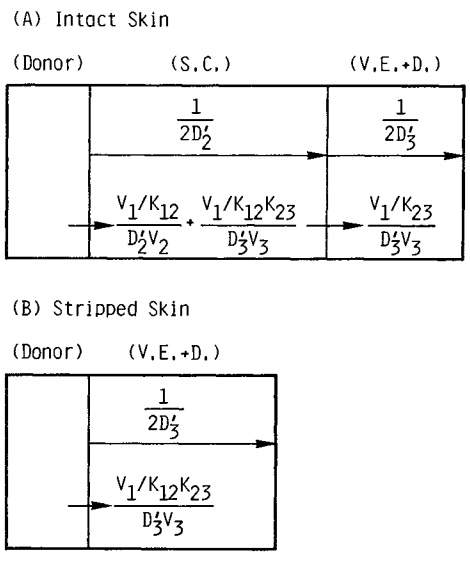

Fig. 2. Mean transit time for each process of drug penetration through the intact skin (A) and the stripped skin (B). S.C., stratum corneum; V.E. + D., the combination of viable epidermis and dermis. Note that $K_{12}K_{23} = K_{13}$.

From Eqs. *(25)* and *(26)*, the term of $1/2D_3'$ is MTT for the drug diffusion in the stripped skin (Fig. 2B).

MATERIALS AND METHODS

In Vitro Skin Penetration Experiment

The diffusion experiment was carried out using a flow-through-type diffusion cell as described previously (3). After the removal of hair, with electric clippers, and adipose tissue, the excised dorsal skin of a guinea pig (Hartley strain, weighing 260–280 g) was mounted on the diffusion cell with the dermis side facing to the receptor cell (diffusion area: A, 3.14 cm^2). Four pieces of skin were obtained from each animal, and two were tape-stripped. Tape stripping was repeated 10 to 15 times until the skin surface glittered. The mounted skin was pretreated with saline for 6 hr at 37°C to stabilize its temperature and condition of hydration. After removal of the saline, a 1-ml aliquot of a 1 m*M* solution of 6-MP (Nacalai Tesque, Japan) in saline containing 0.3 μCi of [^{14}C]6-MP (65 mCi/mmol; Commissariat A L'Energie Atomique, France) was applied. The dermal side of the skin was continuously washed with saline containing streptomycin sulfate (50 mg/liter; Sigma Chemical Co.) and penicillin G potassium salt (30 mg/liter; gift from Toyo Jozo, Japan) at a flow rate of 6 ml/hr. The receptor fluid was collected every 90 min for 24 hr. The donor cell was capped with a silicon stopper and the diffusion cell was thermostated at 37°C in a water bath. At the end of the 24-hr experiment, the amounts of 6-MP remaining in the donor and in the skin were recovered as described previously (4). The determination of 6-MP in the receptor fluid, the donor solution, and the skin was carried out by liquid scintillation counting (Model LSC-903, Aloka) as described previously (4).

Data Analysis

The penetration profiles of 6-MP through the intact and the stripped skin were fitted to Eqs. *(10)* and *(16)* using MULTI(FILT), which is written in MS-FORTRAN and run on personal computer PC-9801 VX, NEC, Japan.

Partitioning Experiment

A sheet of stratum corneum was prepared according to the method described by Kligman and Christophers (13). The skin of an excised guinea pig was removed of its hair. Then it was placed, dermis side down, on a filter paper saturated with 0.1% trypsin (trypsin, 1:250; Difco Laboratories, Detroit, Mich.) solution in Tris/HCl buffer at pH 7.90 in a sealed petri dish. After 16 hr of digestion, the dermal side of the stratum corneum was gently swabbed with tweezers, rinsed with water, and dried.

A piece of stripped skin (about 67 mg) or stratum corneum (about 4 mg) was shaken for 24 hr at 37°C with 1 ml of a radiolabeled 6-MP solution (1 m*M*) in saline. Each sample was blotted on tissue paper, weighed, and dissolved in 1 ml Soluene-350 (Packard Instrument). The saline sample was also weighed. The partition coefficient of 6-MP (K_{12} or K_{13}) between tissue and saline was calculated as follows:

$$K_{12} \text{ or } K_{13} = \frac{\text{(radioactivity in tissue)/(weight of tissue)}}{\text{(radioactivity in saline)/(weight of saline)}}$$

RESULTS

The penetration profiles of 6-MP through the intact and stripped skin are shown in Fig. 3. Tape-stripping drastically increased the 6-MP penetration, and the amount of 6-MP penetrating through the stripped skin within 24 hr was about 40 times that through the intact skin, suggesting the greater barrier function of stratum corneum. The amounts of 6-MP remaining in the donor solution and in the skin were also significantly different between the intact and the stripped skins (Table I).

In this experiment, the parameters K_{12} and K_{13} were directly determined by the partitioning experiments (Table II). Then the penetration profile of 6-MP through the stripped skin was fitted to Eq. *(16)* by MULTI(FILT) and two parameters, D_3 and V_3, were determined. Finally, the parameters for the stratum corneum, D_2 and V_2, were determined by the computer fitting of the penetration profile of 6-MP through the intact skin to Eq. *(10)* to which the obtained parameters K_{12}, K_{23}, D_3, and V_3 had been substituted. The obtained parameters are listed in Table II.

Partition coefficients, K_{12} and K_{13}, were almost the same, suggesting the nearly equal affinity of 6-MP to the stratum corneum and the lower layer. The diffusion constant of 6-MP in the lower layer was about 650 times larger than that in the stratum corneum. The thickness of stripped skin (L_3) was estimated to be about 0.8 mm, which was very close to that measured by a vernier caliper (0.80–0.85 mm). The calculated thickness of the stratum corneum, 24 μm, is acceptable compared with the hydrated human stratum corneum, 10–60 μm (14).

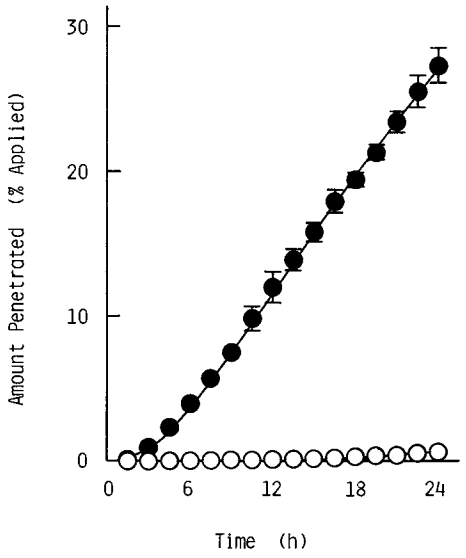

Fig. 3. Percutaneous penetration of 6-MP through intact (○) and stripped (●) guinea pig skin. Each point represents the mean value of three or four experiments and the vertical bars indicate standard deviations. The connected lines were calculated for the parameters listed in Table II.

The amounts of 6-MP remaining in the donor and in the skin at the end of the 24-hr experiment were estimated by Eqs. *(11)*, *(12)*, *(17)*, and *(18)* and are listed in parentheses in Table I. These values agreed well with the experimental values.

Figures 4A and B show the mean time for each process of 6-MP penetration through the intact skin and the stripped skin. Although the diffusion coefficient in the stratum corneum was much smaller than that in the stripped skin, the mean time for diffusion through the stratum corneum (7.50 hr) was comparable to the diffusion through the lower layer (12.55 hr). In the present experiment, the total MTT for the penetration of 6-MP through the intact skin was 26 times that through the stripped skin because of the large value of MTT for the process to move from the vehicle to the stratum corneum.

DISCUSSION

The two-layer skin model employed in this study includes six unknown parameters, K_{12}, K_{23} (or $K_{13} = K_{12}K_{23}$), D_2, D_3, V_2 (or L_2), and V_3 (or L_3), to describe the percutaneous penetration. In this study, we directly determined the K_{12} and K_{13} values by the drug partitioning experiments between skin and saline and estimated the other parameters by the computer fitting of the penetration profiles to the Laplace-transformed diffusion equations. This procedure seems to work well since the L_2 and L_3 values and the drug amounts in the donor and skin were well estimated. When the physiological values of L_2 and L_3 are previously obtained, the K_{12} and K_{23} value can be estimated by the computer fittings even if the direct determination of the partition coefficients is difficult. In order to obtain accurate results, the number of unknown parameters should be decreased.

The partition coefficient and diffusion coefficient are the basic parameters for describing the percutaneous penetration. The combination of them determines the penetrability of a drug in each process or, in other words, the rate-limiting step of the total penetration. The concept of MTT is helpful for understanding the character of each process in the percutaneous penetration. In the present discussion, MTT is given separately for the traversing process across the boundaries of neighboring layers and the diffusion processes through barriers (Figs. 2A and B). The removal of the stratum corneum enhanced the penetration of 6-MP, indicating its great barrier function. The calculated diffusion coefficient of 6-MP in the stratum corneum was much smaller than that in the lower layer; however, the MTT for diffusing through the stratum corneum is about three-fifths of the lower layer because of its shorter diffusion length.

Figure 4A reveals that the rate-limiting step of 6-MP penetration through the intact skin is the traversing process of the boundary between the donor solution and the stratum corneum under the present condition. The comparison between Fig. 4A and Fig. 4B suggests that the faster penetration of 6-MP in the stripped skin is caused by the shortened MTT for traversing the boundary between the donor solution and the tissue layer. Assuming that only the stratum corneum exists as diffusion barrier and sink condition is obtained at the end side of it, MTT for boundary traverse is

Table I. Amounts of 6-Mercaptopurine Recovered at the End of 24-hr Diffusion Experiments[a,b]

Condition of skin	N	Recovery (%)			
		Total	Donor	Skin	Receptor
Intact	4	94.66 ± 2.16 (94.66)	88.40 ± 2.84 (92.52)	5.60 ± 1.36 (1.09)	0.66 ± 0.15 (0.61)
Stripped	3	94.10 ± 2.52 (94.10)	50.07 ± 3.80* (53.46)	16.71 ± 2.60* (13.71)	27.33 ± 1.20* (26.94)

[a] Means ± standard deviations.
[b] Values in parentheses were estimated using parameters obtained by the analyses of 6-MP penetration profiles.
* Significantly different ($P < 0.001$, Student's t test) from the value of intact skin.

calculated to be 1570 hr under the same donor condition. It is clear that the small diffusion coefficient in the stratum corneum results in the low permeability of the intact skin. However, the low permeability is not caused by the diffusion process through the stratum corneum itself but by the process withdrawing 6-MP from the donor solution at the stratum corneum boundary.

Another point of discussion for MTT is that this parameter includes the factor of donor volume which can be controlled by the formulation. MTT for traversing the boundary between the donor solution and the stratum corneum will decrease when the donor volume (V_1) decreases [Eq. *(23)*]. However, the change of V_1 does not affect the MTTs for the other processes (Figs. 2A and B). The effect of applied volume was reported by Takehara *et al.*; the decrease in the donor volume raised a penetration of salicylic acid through rat skin (15). Theoretically the MTT for the 6-MP penetration through the intact skin should be reduced to 20.53 hr if the effective dose can be applied to the surface of the skin as Dirac's delta function. In this case, MTT is calculated as a summation of the last three terms in Eq. *(20)*.

The two-layer skin model and computer analysis with Laplace-transformed equations discussed in this study are useful tools for analyzing the percutaneous penetration of drug. Although it would be difficult for us to discuss the drug penetration based on the Laplace-transformed equations, the concept of MTT enables us to understand intuitively the permeability of a drug.

Table II. Parameters for Percutaneous Penetration of 6-Mercaptopurine

Partition coefficient[a]	Diffusion constant[b] $\times 10^6$ (cm^2/hr)	Volume[b] $\times 10^3$ (cm^3)	Distance[b] $\times 10^3$ (cm)
$K_{12} = 1.257 \pm 0.097$	$D_2 = 0.3900$	$V_2 = 7.596$	$L_2 = 2.418$
$K_{13} = 1.969 \pm 0.293$	$D_3 = 254.8$	$V_3 = 251.2$	$L_3 = 79.97$

[a] K_{12} is a partition coefficient between stratum corneum and vehicle (saline), and K_{13} between stripped skin and vehicle. These values were determined by the partitioning experiments and given as means ± standard deviations of four experiments.
[b] Subscripts 2 and 3 indicate the stratum corneum and the lower layer (viable epidermis and dermis), respectively. These values were obtained by the computer analyses of the penetration profiles.

APPENDIX A: LAPLACE-TRANSFORMED EQUATIONS FOR THE TWO-LAYER SKIN MODEL (FIG. 1A)

The corresponding Laplace transforms of Eqs. *(1)–(7)* are, respectively,

$$s\overline{C}_2 = D_2 \frac{\partial^2 \overline{C}_2}{\partial x^2} \qquad (A1)$$

$$s\overline{C}_3 = D_3 \frac{\partial^2 \overline{C}_3}{\partial x^2} \qquad (A2)$$

$$K_{12}\overline{C}_1 = \overline{C}_2 \qquad (x = -L_2) \qquad (A3)$$

$$sV_1\overline{C}_1 - X_0 = D_2 A \frac{\partial \overline{C}_2}{\partial x} \qquad (x = -L_2) \qquad (A4)$$

$$K_{23}\overline{C}_2 = \overline{C}_3 \qquad (x = 0) \qquad (A5)$$

$$D_2 \frac{\partial \overline{C}_2}{\partial x} = D_3 \frac{\partial \overline{C}_3}{\partial x} \qquad (x = 0) \qquad (A6)$$

$$\overline{C}_3 = 0 \qquad (x = L_3) \qquad (A7)$$

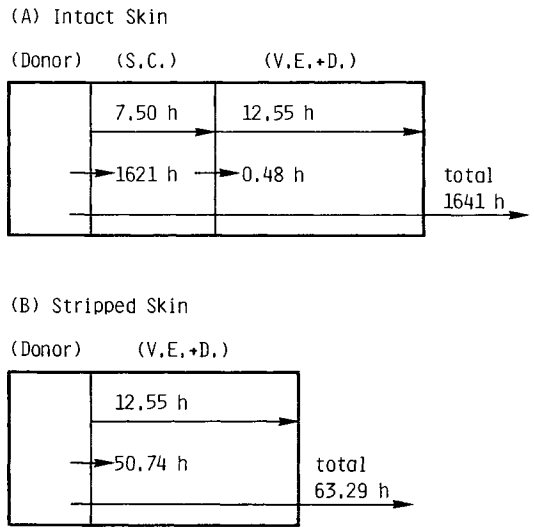

Fig. 4. Calculated mean transit time values for 6-MP penetration through the intact (A) or stripped (B) skin. These values were calculated using the equations in Figs. 2A and B with parameter values listed in Table II. S.C., stratum corneum; V.E. + D., the combination of viable epidermis and dermis.

Equations *(A1)* and *(A2)* have the general solutions, respectively:

$$\overline{C}_2 = A'\cosh(q_2x) + B'\sinh(q_2x) \quad (A8)$$
$$\overline{C}_3 = X'\cosh(q_3x) + Y'\sinh(q_3x) \quad (A9)$$

where

$$q_2 = (s/D_2)^{1/2} \quad (A10)$$
$$q_3 = (s/D_2)^{1/2} \quad (A11)$$

From Eqs. *(A3)–(A11)*, A', B', X' and Y' are determined, respectively, as

$$A' = X'/K_{23} \quad (A12)$$
$$B' = (D_3/D_2)^{1/2}Y' \quad (A13)$$
$$X' = -\tanh(q_3L_3)Y' \quad (A14)$$
$$Y' = \frac{-K_{12}K_{23}X_0}{\tanh(q_3L_3)\alpha + K_3(D_3/D_2)^{1/2}\beta} \quad (A15)$$

where

$$\alpha = sV_1\cosh(q_2L_2) + K_{12}D_2Aq_2\sinh(q_2L_2) \quad (A16)$$
$$\beta = sV_1\sinh(q_2L_2) + K_{12}D_2Aq_2\cosh(q_2L_2) \quad (A17)$$

$\overline{Q}_A$, $\overline{X}_A$, $\overline{M}_{A,2}$, and $\overline{M}_{A,3}$ are calculated, respectively, by

$$\overline{Q}_A = -\frac{D_3A}{s}\left(\frac{\partial\overline{C}_3}{\partial x}\right)_{x=L_3} = -\frac{D_3A}{s} \times q_3\{\sinh(q_3L_3)X' + \cosh(q_3L_3)Y'\} \quad (A18)$$

$$\overline{X}_A = \frac{X_0}{x} + \frac{D_2A}{s}q_2\left(\frac{\partial\overline{C}_2}{\partial x}\right)_{x=-L_2} = \frac{X_0}{s} + \frac{D_2A}{s} \times q_2\{-\sinh(q_2L_2)A' + \cosh(q_2L_2)B'\} \quad (A19)$$

$$\overline{M}_{A,2} = \int_{-L_2}^{0}\overline{C}_2dx = \frac{A}{q_2}\{\sinh(q_2L_2)A' - [\cosh(q_2L_2) - 1]B'\} \quad (A20)$$

$$\overline{M}_{A,3} = A\int_0^{L_3}\overline{C}_3dx = \frac{A}{q_3}\{\sinh(q_3L_3)X' + [\cosh(q_3L_3) - 1]Y'\} \quad (A21)$$

APPENDIX B: CALCULATION OF MEAN TRANSIT TIMES

When the Laplace-transformed equation for the cumulative amount of a drug penetrating a barrier ($\overline{Q}$) is given as a function of the Laplace variable (s) as Eq. *(A22)*,

$$\overline{Q} = \frac{h(s)}{sk(s)} \quad (A22)$$

the area under the curve (AUC) and the area under the first moment curve (AUMC) are obtained as, respectively,

$$\mathrm{AUC} = \lim(s\overline{Q}) = \lim_{s\to0}\left\{\frac{h(s)}{k(s)}\right\} \quad (A23)$$

$$\mathrm{AUMC} = -\lim_{s\to0}\left\{\frac{d}{ds}(s\overline{Q})\right\} = -\lim_{s\to0}\left\{\frac{d}{ds}h(s)/k(s) - h(s)\frac{d}{ds}k(s)/k(s)^2\right\} \quad (A24)$$

The MTT is calculated by

$$\mathrm{MTT} = \frac{\mathrm{AUMC}}{\mathrm{AUC}} = \lim_{s\to0}\left\{\frac{d}{ds}k(s)/k(s) - \frac{d}{ds}h(s)/h(s)\right\} \quad (A25)$$

MTT_A is obtained from Eqs. *(10)* and *(A25)*. The Laplace-transformed equations for the total drug amount penetrating the boundary at 0 ($\overline{Q}_{A,12}$) and $-L_2$ ($\overline{Q}_{A,23}$) are derived in the same manner as $\overline{Q}_A$, respectively,

$$\overline{Q}_{A,12} = K_{12}K_{23}V_2V_3X_0\cosh(d_3)/s/k(s) \quad (A26)$$
$$\overline{Q}_{A,23} = K_{12}V_2X_0\{V_2d_3/d_2\sinh(d_2)\sinh(d_3) + K_{23}V_3\cosh(d_2)\cosh(d_3)\}/s/\kappa(s) \quad (A27)$$

$\mathrm{MTT}_{A,12}$ and $\mathrm{MTT}_{A,23}$ are derived from Eqs. *(A26)* and *(A27)* according to Eq. *(A25)*.

MTT is also calculated by the following equation (16):

$$\mathrm{MTT} = \frac{1}{X_0}\int_0^\infty A(t)dt = \lim_{s\to0}\left\{\frac{\overline{A}(s)}{X_0}\right\} \quad (A28)$$

where $A(t)$ is amount of drug in a "compartment" and $\overline{A}(s)$ is the Laplace transform of $A(t)$. Using this relationship, the MTTs for each process can be calculated directly from the equations describing the drug amount in the donor, stratum corneum, and lower layer [Eqs. *(11)*, *(12a)*, and *(12b)*].

$$\lim_{s\to0}\left\{\frac{\overline{X}}{X_0}\right\} = \frac{V_1/K_{12}}{D_2'V_2} + \frac{V_1/K_{12}K_{23}}{D_3'V_3} = \mathrm{MTT}_{A,12} \quad (A29)$$

$$\lim_{s\to0}\left\{\frac{\overline{M}_{A,2}}{X_0}\right\} = \frac{1}{2D_2'} + \frac{V_2/K_{23}}{D_3'V_3} = \mathrm{MTT}_{A,23} - \mathrm{MTT}_{A,12} \quad (A30)$$

$$\lim_{s\to0}\left\{\frac{\overline{M}_{A,3}}{X_0}\right\} = \frac{1}{2D_3'}\mathrm{MTT}_A - \mathrm{MTT}_{A,23} \quad (A31)$$

In this case, the following alternative equation (17) for MTT cannot be employed ($M = C * V$), because first-order elimination is not realized in the present experimental system (16).

$$\mathrm{MTT} = \frac{\int_0^\infty tC(t)dt}{\int_0^\infty C(t)dt} = \frac{\int_0^\infty tM(t)dt}{\int_0^\infty M(t)dt} \quad (A32)$$

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NOMENCLATURE

A	Effective area for diffusion
C	Drug concentration

d	$L/(s/D)^{1/2}$; see Eqs. *(13)* and *(14)*
D	Diffusion constant of drug
D'	Diffusion parameter, D/L^2; see Eqs. *(21)* and *(22)*
K	Partition coefficient of drug
L	Thickness of membrane
M	Drug amount in the skin
MTT	Mean transit time
q	$(s/D)^{1/2}$; see Eqs. *(A10)* and *(A11)*
Q	Total amount of drug penetrating the skin
s	Laplace variable with respect to time
V	Volume of membrane
x	Distance
X	Drug amount in the donor solution

Subscripts

0	Initial condition
1	Donor solution
2	Stratum corneum
3	Stripped skin
12	Boundary between the stratum corneum and the lower layer
13	Boundary between the donor solution and the stripped skin
23	Boundary between the stratum corneum and the lower layer
A	Two-layer skin model
B	One-layer skin model

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