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Use of the Stratum Corneum Reservoir for the Prediction of Skin Penetration

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ABSTRACT

A simple and quick method based on the transient diffusion theory for predicting the steady state rate of penetration of a drug after transdermal drug administration was proposed. The amount of drug entering the stratum corneum was determined by 20 strippings with an adhesive tape. From the profile of the amount of drug as a function of the number of strippings, the quantity of drug on the surface of stratum corneum was extrapolated. Based on the amounts of drug entering the stratum corneum during two time intervals (t_1 and t_2) within 1 hour after the application, the diffusion and partition coefficient were determined. Once the diffusion coefficient of the drug in the stratum corneum and the partition coefficient (stratum corneum/vehicle) were determined from the present approach, the steady-state flux of penetration across the stratum corneum was calculated. The steady-state rates of penetration of ascorbic acid and estradiol across hairless mouse skin were evaluated from this approach and compared with those obtained from *in vitro* penetration experiment using excised hairless mouse skin. The data confirmed that the proposed method can predict the steady-state rate of penetration of these drugs across the stratum corneum

ABBREVIATIONS

DPM : disintegrations per minute
h : stratum corneum thickness
H : total skin thickness
J : steady-state flux
k : ratio of stripping times
K : partition coefficient
Q : cumulative amount of drug penetrated
t : time
x : distance from skin surface

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1. INTRODUCTION

There is considerable evidence to suggest that chemicals penetrating percutaneously are being withheld by various skin layers, foremost in the stratum corneum. The mean thickness of the stratum corneum of human skin ranged 8.7-12.9 μm and the cell layer number ranged 15.6-22.8 layers.[1] Kligman has vividly illuminated that epidermal barrier is located in the stratum corneum by demonstrating: A sheet of isolated stratum corneum tied over the orifice of an inverted vial effectively prevented evaporation of the water within. However, there still existed controversy concerning the specific location of the barrier within this epidermal compartment. On the basis of carefully performed Scotch tape-stripping experiments, Kligman made the first suggestion that all the layers of the stratum corneum contribute to its barrier function.[2] Recent investigations have suggested that the spaces between the layers of horney cells are filled with multiple lipid bilayer, consists principally of ceramides (40%), cholesterol (25%), palmitic acid (25%), and cholesteryl sulfate (10%). However the presence of lipid bilayers in the stratum corneum is not in itself sufficient to account for the water impermeability of the stratum corneum. The key to water barrier is in the array of hydrophobic chains that make up the interior of the extracellular bilayers. These hydrocarbon chains are straight and almost entirely saturated. This lack of structural perturbation among the hydrophobic chains provides the opportunity for the formation of bilayers with closely packed interiors, and such highly ordered bilayers are ideally suited for water impermeability.[3] Vickers first demonstrated the reservoir function of stratum corneum: topically applied steroids forced into stratum corneum by occlusion for a few hours with plastic wrap remained for up to two weeks.[4] Retention of chemicals by cutaneous tissue does not necessarily mean that it is bound in the tissue. Some chemicals by virtue of their solubility parameters are partitioned into several skin strata or sebum. On the other hand, some chemicals retained by cutaneous layers possess a specific affinity of reversible binding nature.[5]

In transdermal drug delivery, drug molecules released from the delivery system first partitioned toward the surface of stratum corneum and then permeate into its lower layer by passive diffusion. Because of the barrier function of the stratum corneum, the diffusion coefficient in the stratum corneum is usually extremely low (10^{-10}-10^{-12} cm^2/sec) for most drugs.[6,7] Due to such slow diffusion, a skin penetration study requires a long duration, usually 24-48 hr, in order to observe the steady-state rate of penetration of a drug in *in vitro* experiments. Under *in vivo* conditions, on the other hand, the amount of drug excreted from the body must be monitored for serveral days after the transdermal application to evaluate the fraction of absorption.[8] Recently, Dupuis *et al.*,[9] reported a simple method to predict the stratum corneum reservoir function *in vivo*. They found a linear relationship existed between the amounts of substance present in the stratum corneum at the end of application (30 min) and the total amounts penetrated in 4 days. However, at this stage of research on transdermal drug delivery, no *in vivo* approach has emerged for predicting the steady-state rate of penetration under *in vivo* conditions.

Typically, in transdermal drug delivery, the drug molecules requires 1 hr or more to traverse the entire thickness of the stratum corneum; the drug concentration near the boundary between the stratum corneum and viable skin remains zero or at a very low level. Under such conditions, the amount of drug entering the stratum corneum is controlled by the physicochemical properties of the drug and of the stratum corneum. In the present study, we propose a simple and quick method based on the transient diffu-

sion theory for predicting the steady-state rate of penetration of a drug *in vivo* after transdermal drug administration. Based on the amounts of drug entering the stratum corneum during two time intervals (t_1 and t_2) within 1 hr after the application, the diffusion and partition coefficient were determined. The steady-state rate of penetration was then evaluated for a given donor concentration. The steady-state rate of penetration predicted was compared with that obtained from an *in vitro* skin permeation experiment using an excised hairless mouse skin. Ascorbic acid and estradiol were used as model drugs because these drugs are stable and bind negligibly in hairless mouse skin.

2. THEORY

Tojo *et al.*,[10] studied the effect of stepwise skin stripping by cellophane tape on the rate of drug permeation through hairless mouse skin. As shown in Table I, the steady state rate of drug permeation was markedly enhanced initially with the increase in the number of strippings, then suddenly reached the plateau at about ten times stripping. The stratum corneum appeared to be completely removed by about 10 consecutive strippings. This finding implies that the boundary between the stratum corneum and the viable epidermis is fairly distinguishable; in other words, a clear interface may probably exist between these two skin layers. This observation supports the idea of using partitioning mechanism rather than space-dependent diffusion mechanism for transdermal drug permeation analysis near the boundary of stratum corneum and viable epidermis. Based on above observation, we assumed that skin consists of the stratum corneum and the viable epidermis, two-layer membrane (Fig. 1). Neither binding nor enzymatic reaction takes place in the skin. The concentration profile of the drug is given as the solution of the following governing equation; equation 1[11]

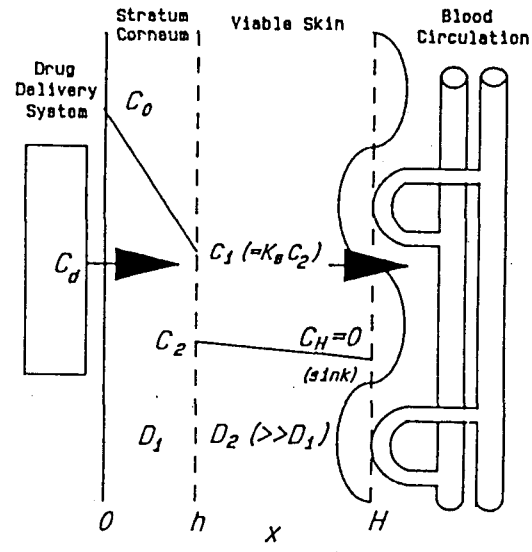

Figure 1—Steady-state concentration profile in the two-layer skin. Neither binding nor metabolism is assumed in the skin.

Table I—Effect of Stripping on the Steady-State Rate of Permeation

Number of stripping	steady-state permeation rate (μg/cm²-day± S.D.)		
	Drug I	Drug II	Drug III
0 (whole skin)	24± 3.0	0.78± 0.04	9.1± 0.8
2	25± 2.2	0.88± 0.09	11± 1.4
4	36± 3.4	0.86± 0.11	–
5	–	–	17± 2.8
6	39± 3.9	1.15± 0.23	–
7	–	–	22± 3.7
8	51± 2.9	1.28± 0.06	–
10	74± 8.0	–	99± 11
12	71± 2.3	1.75± 0.24	–
16	–	1.76± 0.09	–
20	73± 4.8	1.87± 0.37	–
25	72± 5.5	–	117± 14

Saturated concentration in donor compartment, Cd: 50 mg/ml for drug I, 0.21 mg/ml for Drug II, and 5.2 mg/ml for Drug III.

subject to the boundary and initial conditions; equations 2-5:

$$\partial C/\partial t = \partial/\partial x(D\partial C/\partial x) \quad (1)$$

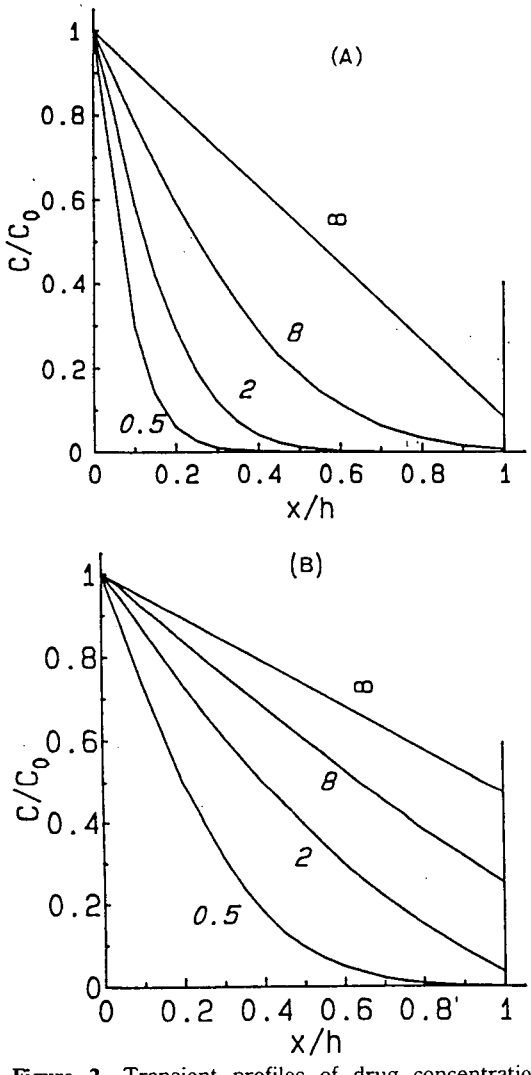

Figure 2—Transient profiles of drug concentration in the stratum corneum calculated from equation 1 subject to equations 2-5. The numbers with the lines are the values of time (h) after the drug application on the surface of the stratum corneum.
h=20 μm, H=200 μm, K_s=0, $D_2/D_1=10^3$, (A) $D_1=10^{-11}$ cm^2/s, (B)=10^{-10} cm^2s.

$$C_1=K_sC_2 \quad \text{at } x=h \quad (2)$$

$$C_H=0 \quad \text{at } x=H \text{ (sink)} \quad (3)$$

$$C=KC_d=C_o \text{ (constant) at } x=0 \quad (t>0) \quad (4)$$

$$C=0 \quad \text{at } 0\leq x\leq H \quad (t=0) \quad (5)$$

Where the diffusion coefficient D is a function of the distance x from the surface of the skin and is assumed to be constant in each skin layer; D_1 in the stratum corneum and D_2 ($>D_1$) in the viable epidermis. Equation 1 was solved by the method of Lines procedure.[12] An IBM PC/AT computer was employed to numerically intergrate the differential equations. Microsoft Fortran 77 complier (v.4) was used in this study. The transient profiles of drug concentration in the stratum corneum were calculated with two different diffusion coefficients and are plotted on Fig. 2 as a parameter of time. This figure simulates the drug concentration in the human stratum corneum (about 20 μm thick). The concentration changes discontinuously on the boundary between the stratum corneum and viable epidermis (x=h) due to partitioning, although this is not shown in Fig. 2. The concentration on the lower surface of the stratum corneum increases gradually with time until the steady-state profile is developed. Because the diffusion across the stratum corneum is very slow, as can be expected from the value of the diffusion coefficient, the concentration on the lower boundary of the stratum corneum remains negligible within the initial 1-hr period after the transdermal administration of drug. Fig. 2 indicates that the drug molecules do not reach the lower surface 1 hr after the oneset of the transdermal drug application. Under such transient conditions, the boundary condition (equation 2) is simplified to $C_1=0$ (at x=h), and the total amount of drug which has entered the stratum corneum during the period of time t is given by.[11]

$$\frac{m}{m_0}=1-\sum_{n=0}^{\infty}[8/\{(2n+1)^2\pi^2\} \exp\{-D(2n+1)^2\pi^2t/h^2\}] \quad (6)$$

Where m_0 is the total amount of drug entered in the stratum corneum after infinite time. The ratio of the amount of the drug at two different time intervals, t_1 and t_2 ($=kt_1$, $k>1$), is then

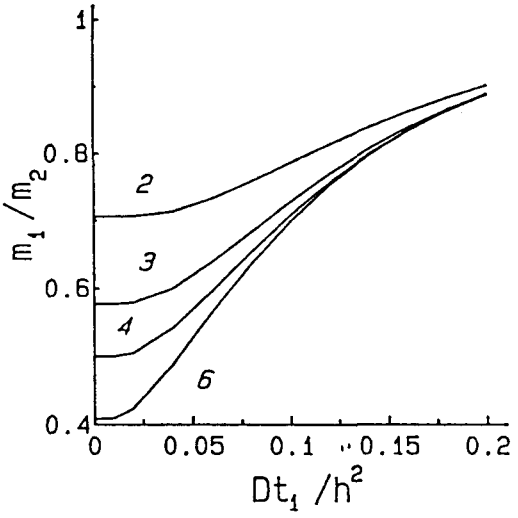

Figure 3—Effect of Dt_1/h^2 on m_1/m_2 (equation 7). The numbers with the curves are the values of k ($=t_2/t_1$).

$$\frac{m_1}{m_2}=\frac{1-\sum_{n=0}^{\infty}[8/\{(2n+1)^2\pi^2\}\exp\{-D(2n+1)^2\pi^2t/h^2\}]}{1-\sum_{n=0}^{\infty}[8/\{(2n+1)^2\pi^2\}\exp\{-kD(2n+1)^2\pi^2t/h^2\}]} \quad (7)$$

The relationship between the dimensionless quantity Dt_1/h_2 and m_1/m_2 is plotted as a parameter of k ($=t_2/t_1$) in Fig. 3. The ratio m_1/m_2 approaches the square root of 1/k and is independent of the diffusion coefficient when Dt_1/h^2 is small ($Dt_1/h^2<0.02$). Beyond this short time period, the ratio m_1/m_2 if the time intervals t_1 and t_2 are suitably selected. Because the concentration on the boundary between the stratum corneum and viable skin depends largely on the thickness of the stratum corneum, the time intervals t_1 and t_2 must be carefully determined. For normal human stratum corneum (about 20 μm thick), 20-30 min and 60-90 min may be used as the time intervals t_1 and t_2, respectively. For hairless mouse skin (about 10 μm thick), however, 10 min and 30 min may be used for most drugs. The total amount of drug which enters the stratum corneum by time t may be determined by stripping using adhesive tape.[9,13] From the profile of the amount of drug as a function of the number of strippings, we can extrapolate the quantity of drug on the surface of stratum corneum. The ratio of the surface concentration thus determined to that of the donor vehicle is defined as the partition coefficient, K. Once the diffusion coefficient of the drug in the stratum corneum, D and the stratum corneum/vehicle partition coefficient are determined from the present *in vivo* approach, the steady-state flux of penetration across the stratum corneum can be calculated by

$$J=dQ/dt=DK_d/h \quad (8)$$

Assuming negligible resistance to drug transport across the viable skin, the time-lag, which is defined at the time intercept of the steady state penetration profile, is given by

$$t_d=h^2/6D \quad (9)$$

From equations 8 and 9, we can predict both the steady-state rate of penetration and the approximate time-course of the cumulative amount of drug penetrated under *in vivo* conditions.

3. MATERIALS AND EXPERIMENTAL METHOD

Ascorbic acid-C^{14}, 10.0 mCi/mM, was obtained from E.I. du Pont (Wilmington, DE). Estradiol-C^{14}, 56 mCi/mM was obtained from Amersham (U.K.). About 30-40 nmol of radiolabelled ascorbic acid and estradiol were sparked in 50 μl of 50% glycerin solution of ascorbic acid (12± 0.1 mg/ml; 5% Cs) and 40% PEG 400 solution of estradiol (220.2± 9.3 μg/ml; Cs), respectively, on the abdominal site of a hairless mouse (Jackson Lab., 5-8 weeks age, 20± 2 g weight, stratum corneum thickness; about 10 μm). Prior to administration, the animals were anesthesized. Each drug was applied to a 3.14 cm^2 area of the abdomen by using an open cell fixed with silicone glue. After 10 or 30 min of applica-

tion, the excess substance on the treated area was quickly washed with cottonballs soaked in 3 m*l* methanol and 3 m*l* water and then dried with cottonballs. The entire washing process was carried out within 1 min. At the end of washing, the stratum corneum was removed by 20 strippings using adhesive tape (Scotch, 3M810). Each stripping tape was put into the scintillation vial which contained 3 m*l* of methylene chloride (solvent for tape) and 5 m*l* of Bioflour. After complete dissolving of the scotch tape, the radioactivity in each stripping tape was then measured by a liquid scintillation counter. The concentration profile as a function of the number of strippings was approximated by a nonlinear exponential equation using the Modified Marquardt method for extrapolating the surface concentration. The amount of drug that entered the stratum corneum during the time intervals (10 or 30 min) was determined by the sum of the radioactivity in the 20 strippings. The diffusion coefficient was then calculated from equation (7) using the Newton-Raphson method. The partition coefficient K was determined by

$$K = \frac{\text{Surface radioactivity per unit volume of stratum corneum}}{\text{Radiocactivity per unit volume of donor solution}} \quad (10)$$

After determining both the diffusion and partition coefficients, the steady state rate of penetration was computed from equation 8 for a given donor (or vehicle) concentration C_d. An *in vitro* permeation experiment was also carried out using a hydrodynamically well calibrated *in vitro* skin permeation system.[14] The details of the experimental procedure were described previously.[15] The *in vitro* penetration profile was compared with the rate predicted from the present *in vivo* method.

4. RESULTS AND DISCUSSION

The concentration profiles (radioactivity) in the stratum corneum of the hairless mouse after 10 min (t_1) and 30 min (t_2) application have been plotted as a function of the number of strippings in Fig. 4. A sharp decrease in quantities of drug was observed with each successive stripping, and after 10 strippings, the drug concentrations remains very low. It is evident that the total amount of the drug entering the stratum corneum within 30 min is greater than that within 10 min. From the total radioactivity at each time interval, the ratio of the amounts m_1/m_2 for ascorbic acid and estradiol was determined to be 0.595 and 0.749, respectively. These

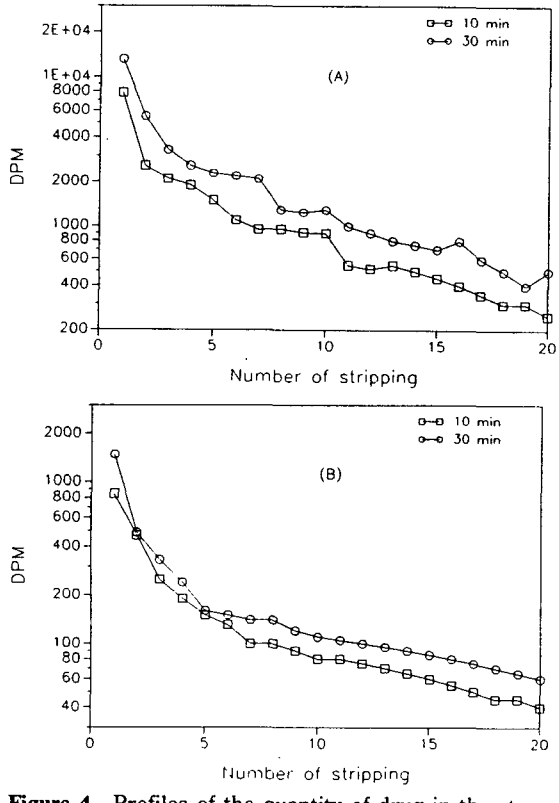

Figure 4—Profiles of the quantity of drug in the stratum corneum of hairless mouse skin as a function of the number of stripping. Key: (○) 30 min, (□) 10 min. Average values of triplicate experiment. (A) ascorbic acid, (B) estradiol.

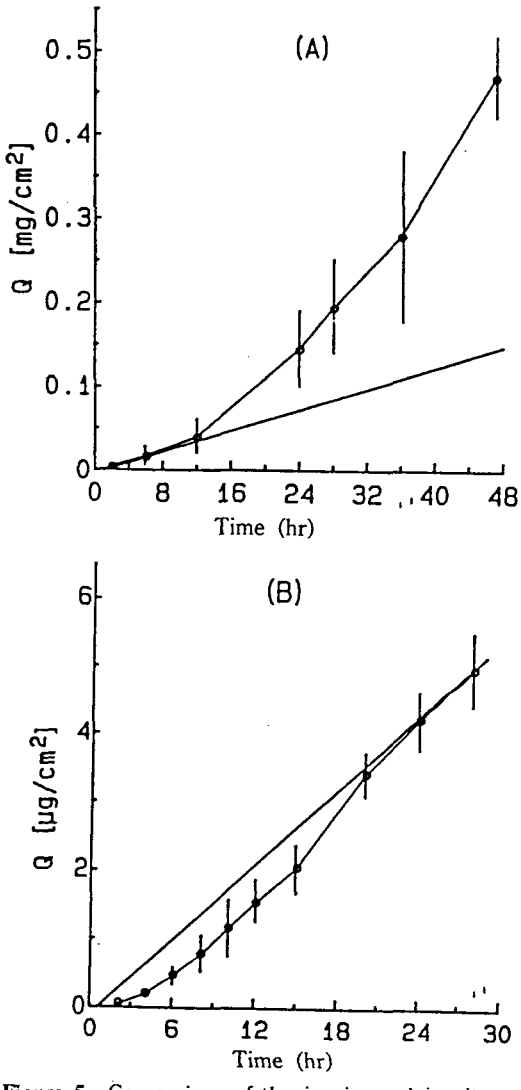

Figure 5—Comparison of the *in vivo* and *in vitro* penetration profiles. Key: (○) *in vivo* penetration experiment, (—) present *in vitro* method. (A) ascorbic acid, (B) estradiol.

diffusion coefficients for ascorbic acid and estradiol were calculated from equation (7) and found to be 6.2×10^{-11} cm²/s and 1.8×10^{-10} cm²/s, respectively. These diffusion coefficients were found to satisfy the condition of $Dt/h^2>0.02$ for both drugs. From the extrapolation of the profiles, the surface quantities were determined as 2.1×10^4 DPM/cm² /μm-thick for ascorbic acid and 2.8×10^3 DPM/cm²/μm-thick for estradiol, respectively.

Table II—Comparison of the Steady-State Rate of Penetration of Ascorbic Acid and Estradiol under *in vivo* and *in vitro* Conditions

Drug	Steady-state rate of permeation	
	In vitro (μg/cm²-hr)	*In vivo* (μg/cm²-hr)
Ascorbic acid	3.43± 0.74 (2-12 hrs)	2.9
	9.75± 3.83 (12-36 hrs)	
Estradiol	0.207± 0.043 (4-28 hrs)	0.18

By assuming a uniform distribution of the donor solution over the applied area (3.14 cm²), the skin partition coefficient were then calculated to be 1.05 for ascorbic acid and 1.23 for estradiol, respectively.

The penetration profiles determined by the present *in vivo* approach were compared with the *in vitro* data in Fig. 5. This figure indicates that the present *in vivo* method pridicts well the *in vitro* penetration profiles. The steady-state rates of penetration obtained by the different approaches are compared in Table II. It is found that the steady-state penetration rates based on the present *in vivo* method are close to but slightly lower than those obtained from the *in vitro* permeation experiments. This is probably due to skin hydration during the long-term *in vitro* permeation experiment using excised hairless mouse skin. The *in vitro* penetration profile of ascorbic acid deviates increasingly from that predicted after about 12 hr. This is due to the skin damage by the acidic donor solution (pH 3.0) in addition to the skin hydration. In general, however, the good agreement between the *in vivo* and *in vitro* penetration rates indicates that the present method is useful for predicting the *in vivo* steady-state rate of penetration across the stratum corneum of normal skin for ascorbic acid and estradiol, and could also be useful

for testing other drugs and formulations. However, if the drug is metabolized and/or binds in the skin during the penetration, the present approach may not be applied directly.

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