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LONG-TERM PERMEATION KINETICS OF ESTRADIOL: (IV) A THEORETICAL APPROACH TO THE SIMULTANEOUS SKIN PERMEATION AND BIOCONVERSION OF ESTRADIOL ESTERS

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ABSTRACT

A theoretical non-steady-state treatment was developed to analyze the kinetics of metabolism during the course of dermal uptake or skin permeation of estradiol esters across the hairless mouse skin. The first-order rate constants for the metabolism reaction of estradiol acetate → estradiol and estradiol diacetate → estradiol acetate → estradiol were determined. The theoretical

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drug concentration profile calculated from the present model was found to be agreed reasonably well with the experimental data determined in the early stage of skin uptake/metabolism studies (<24 hr). For the skin permeation of estradiol acetate and diacetate and their concurrent metabolism, the experimental Q vs. t profiles were also observed to agree well with the theoretical results for a period of up to 28 hr. A deviation was observed at later phase of experiments, which can be attributed to the reduction in enzyme activity during the permeation studies, possibly due to the result of skin aging.

INTRODUCTION

To gain some understanding of a penetrant's unidirectional transport mechanism within and through a biological membrane, it is necessary to study the penetrant's transient (non-steady-state) and steady-state behavior. Study of the non-steady-state profiles can disclose the degree of interactions between skin composition and penetrant molecules, while the investigation of steady-state permeation profiles will exhibit the penetrant's ultimate flux behavior. The time-lag is a unique parameter which links these two states of transport and provides a relative measure of the time required for the diffusion process to attain steady state.

Baller (1) and Crank (2, 3) were able to derive time-lag expressions for a variety of systems provided that Fick's first and second laws of diffusion apply and that the diffusion coefficient of the penetrant is constant. For systems complicated

by the presence of enzymatic or chemical reaction and/or the partial or complete immobilization of fractions of the penetrant population, expressions for the time-lag have also been derived by various authors (4-6).

Recently, Ludolph et al (6) derived an expression for analyzing the time-lag in a system influenced by linear irreversible reaction and simple penetrant immobilization. This derivation was corrected and improved later by Leypoldt and Gough (7) for the case in which the penetrant reacts catalytically within the membrane following a first-order kinetics.

In all the cases reported earlier (8), the permeability across the stratum corneum was observed to be the dominant factor affecting the levels of prodrug and parent drug in the skin and in the dermal solution. From the standpoint of overall maximization of transdermal estradiol bioavailability, the lipophilicity of ester-type prodrug should be optimized to improve the permeability across stratum corneum, but the easiness to the cleavage by esterase is also important.

In this investigation, a theoretical approach was developed to provide a rigorous analysis of the concurrent permeation and metabolism of estradiol esters in the hairless mouse skin.

THEORETICAL ANALYSIS

1. Dermal Uptake/Metabolism of Estradiol Esters

Metabolism of estradiol esters in the skin was investigated using a two-compartment model. The model considers the system

as consisting of a skin compartment and a solution compartment. An estradiol ester in the solution compartment at a concentration of C_0 permeates into the skin compartment from dermis side and reacts with active enzymes in the viable skin layer. The estradiol formed by metabolism then diffuses back into the solution compartment after a lapse of time. In the present study, the following assumptions were made:

a) Estradiol ester and estradiol molecules in the solution compartment are uniformly distributed, except in the diffusion boundary layer which exists on the dermal surface.

b) Concentration gradient of drugs in the skin compartment can be neglected, since the thickness of skin layer is very thin as compared to that of solution compartment.

c) The reaction kinetics is first-order dependent of drug concentration in the skin compartment or in the solution compartment:

$$A \underset{k_3}{\overset{k_1}{\rightleftarrows}} B \xrightarrow{k_2} C \qquad \text{or} \qquad B \xrightarrow{k_2} C$$

Where

A: Estradiol diacetate (ED),

B: Estradiol acetate (EA),

C: Estradiol (E),

k_1: Rate constant for A → B reaction in the skin compartment,

k_2: Rate constant for B→C reaction in the skin compartment,

k_3: Rate constant for A→B reaction in the solution compartment,

k_4: Rate constant for B→C reaction in the solution compartment.

d) A small amount of enzyme may leach into the solution compartment from the skin ($k_3 \neq 0$, $k_4 \neq 0$).

e) The mass transfer coefficient in the diffusion boundary layer on skin surface is constant.

f) The effect of stratum corneum on the rate of metabolism can be neglected.

g) The enzyme, esterase, is uniformly distributed in the viable skin.

Based on the above assumptions, the mass balance of drugs A, B and C in each compartment is described by the following linear differential equations:

$$V_1(dC_1/dt) = -k_m a(C_1 - K_1C_2) - k_3C_1V_1 \quad (1)$$

$$V_2(dC_2/dt) = k_m a(C_1 - K_1C_2) - k_1C_2V_2 \quad (2)$$

$$V_1(dC_3/dt) = -k_m a(C_3 - K_2C_4) + k_3C_1V_1 - k_4V_1C_3 \quad (3)$$

$$V_2(dC_4/dt) = k_m a(C_3 - K_2C_4) + k_1C_2V_2 - k_2C_4V_2 \quad (4)$$

$$V_1(dC_5/dt) = -k_m a(C_5 - K_3C_6) + k_4V_1C_3 \quad (5)$$

$$V_2(dC_6/dt) = k_m a(C_5 - K_3C_6) + k_2C_4V_2 \quad (6)$$

where: a = skin surface area (=0.64 cm^2)

C_1 = concentration of A in the solution compartment

C_2 = concentration of A in the skin

C_3 = concentration of B in the solution compartment

C_4 = concentration of B in the skin

C_5 = concentration of C in the solution compartment

C_6 = concentration of C in the skin

k_m = mass transfer coefficient

k_i = rate constant

K_i = partition coefficient (K_1=0.033; K_2=0.76; K_3=21.3)

V_1 = volume of the solution compartment (=3.5 cm^3)

V_2 = volume of the skin compartment (=0.024 cm^3)

The appropriate initial conditions are:

$$t = 0; \quad C_1 = C_0$$
$$C_2 = C_3 = C_4 = C_5 = C_6 = 0$$

In the present studies, a numerical method (Runge-Kutta-Gill) was used to solve Equation 1 through 6. To test its accuracy, the numerical solution was compared with the analytical solution, Eq. (7), of the first two equations, Eqs. 1 and 2. The relative error of numerical solution was found to be less than 0.002%.

$$C_1(t) = C_{10}\left[\frac{(\alpha+b')e^{-\alpha t} - (\beta+b')e^{-\beta t}}{\alpha - \beta}\right] \tag{7}$$

where α, β (>0) are the roots of:

$$\gamma^2 + (a + b')\gamma - (-ab' + a'b) = 0 \tag{8}$$

$$a = -\left(\frac{k_m A}{V_1} + k_3\right), \qquad b = k_m A \frac{K_1}{V_1}$$

$$a' = \frac{k_m A}{V_2}, \qquad b' = -\left(\frac{k_m A\, K_1}{V_2} + k_1\right)$$

Parameters employed:

(i) Mass transfer coefficient

The mass transfer coefficient k_m can be calculated from the following correlation equation (9):

$$\frac{k_m d}{D} = 0.0157 \left(\frac{nd^2\rho}{\mu}\right)^{1.03} \left(\frac{\mu}{\rho D}\right)^{0.33} \tag{9}$$

where, d = diameter of the stirring magnet used in the skin permeation system (= 0.8 cm)

n = number of rotation speed of the stirring magnet

D = diffusivity of the drug in the solution (0.98×10^{-6} cm^2/s)

μ = viscosity of the solution ($=4.6 \times 10^{-3}$ Pas)

ρ = density of the solution ($=1.047$ g/cm^3)

In this study, the mass transfer coefficient obtained for the 40% PEG 400 solution in the Valia-Chien skin permeation system is 1.31×10^{-4} cm/sec.

(ii) Solubility of drugs in the skin

The equilibrium solubilities of estradiol diacetate (A), estradiol acetate (B) and estradiol (C) in the skin were employed as the concentrations for drugs A, B and C, respectively. These were measured previously (8) using the whole skin. Since the enzymatic reaction usually takes place in the viable skin, in which the solubility of drugs is much less than that in the stratum

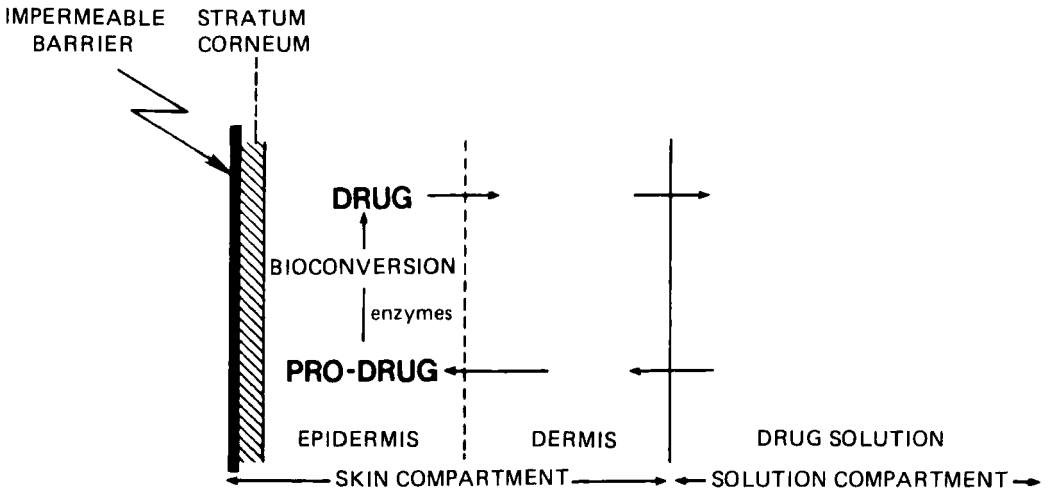

Figure 1

Diagrammatic illustration of the two-compartment model for the uptake/metabolism of prodrugs from dermal solution compartment.

corneum; so, the drug solubility in the viable skin calculated from the data generated with the whole skin may be considerably overestimated. However, the steady-state concentration profiles in the skin for these steroids would be analogical because the concentration ratio at the boundary between the stratum corneum and viable skin is mainly influenced by the steroid diffusivities across each skin layer. The diffusivities of steroids can be assumed to be the same, because of the similarity in their molecular weights and structure. The estradiol solubility in the viable skin was previously found to be about 20% of that in the whole skin (10). In the present calculation, the solubility of each drug was, therefore, assumed to be 1/5 of that measured in the whole skin.

(iii) Rate constant of metabolism reaction

In spite of the fact that the principal metabolism reaction takes place in the skin, a small amount of drugs may also be metabolized in the solution compartment by the enzyme leaching out of the skin. The rate constants in Equations 1 to 6 were determined in such way that the calculated concentration profiles in the solution compartment fit best with the experimental data for both reaction mechanisms of ED → EA → E (Figure 2) and EA → E (Figure 3). The rate constants obtained are listed in Table 1.

The theoretical drug concentration profile was compared with the experimental results in Figures 2 and 3 for the reactions: ED → EA → E and EA → E, respectively. It can be seen that the experimental data points fit reasonably well with the predicted concentration profile during the early stage of experiment ($t < 24$ hr). However, the experimental data beyond 24 hours were noted to deviate increasingly from the predicted result as increasing the duration of experiment. This increasing deviation could be due to the decrease in enzyme activity in the viable skin as the result of skin aging.

2. Skin Permeation and Metabolism of Estradiol Esters

Metabolism of estradiol esters during the course of skin permeation was investigated using a three-compartment model which considers a system consisting a donor solution compartment, a skin compartment and a receptor solution compartment (Figure 4). A prodrug in the donor solution compartment at a concentration

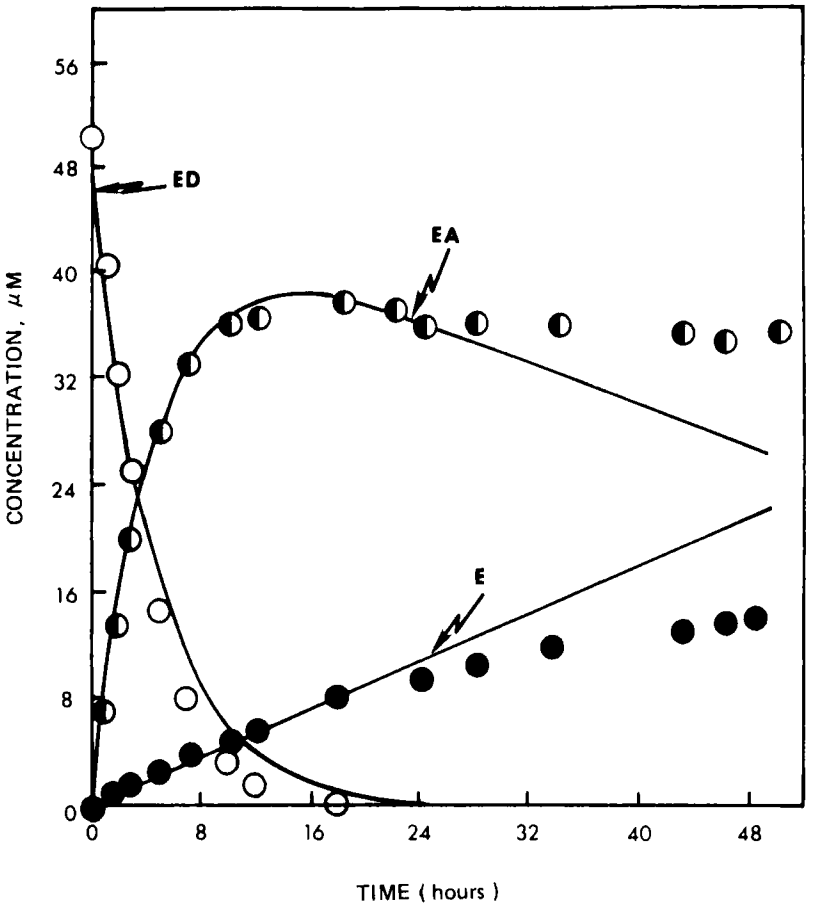

Figure 2

The time course for the theoretical concentration profile of ED → EA → E in comparison with the experimental observations. Key: predicted (——); experimental, (○, ◐ and ●).

of C permeates into the skin compartment, via stratum corneum and reacts with active enzymes in the viable skin layers. The drug formed by metabolism diffuses into the receptor solution compartment after a lapse of time.

In the present study the following assumptions were made:

a) Skin is a unilayer tissue

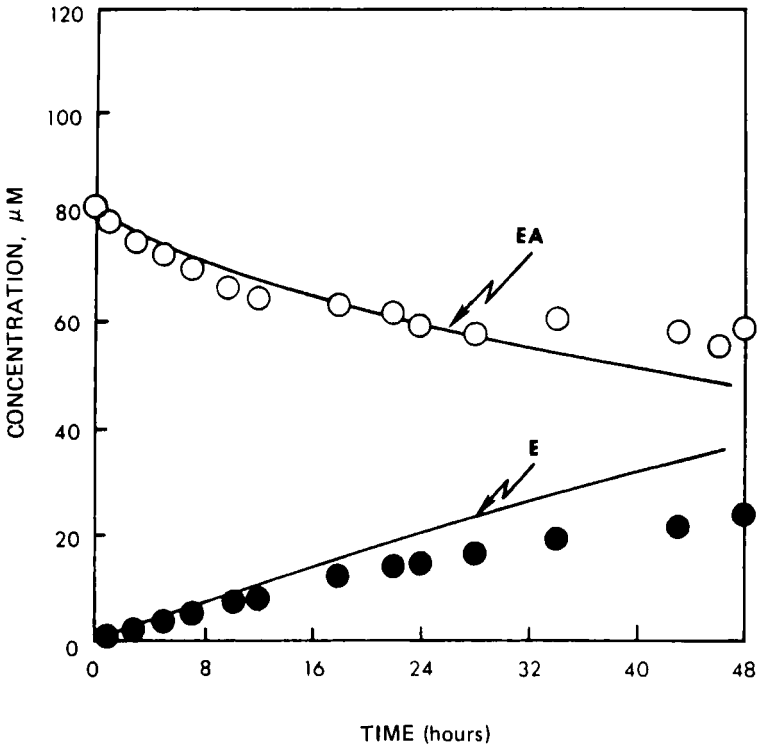

Figure 3

The time course for the theoretical concentration profile of EA → E in comparison with experimental observations. Key: predicted (——); experimental (○ and ●).

b) Surface drug concentration on the skin is constant (C_0).

c) All the kinetics of metabolism are first-order:

$$ED \xrightarrow{k_1} EA \xrightarrow{k_2} E.$$

d) The solubility of drugs and rate constants were determined previously.

Notations used:

C = concentration of the prodrug,

D = diffusivity of the drug in the skin,

TABLE 1

First-Order Rate Constants for Dermal Uptake/

Metabolism of Estradiol-3,17-Diacetate

Rate Constant	ED→EA→E (hr^{-1})	EA→E (hr^{-1})
k_1	8.57	-
k_2	0.51	-
k_3	0.12	0.12
k_4	0.0086	0.0086

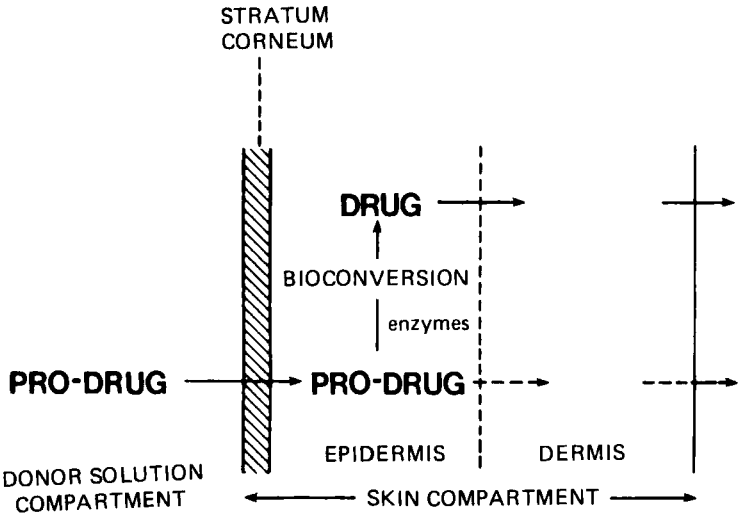

Figure 4

Diagrammatic illustration of the three-compartment model for the skin permeation/metabolism of prodrugs from stratum corneum side (donor solution compartment).

x = distance from the surface of the skin,

k = first-order rate constant,

$\bar{c}$ = dimensionless concentration,

ζ = dimensionless distance,

θ = dimensionless time,

ϕ = modulus,

ℓ = thickness of the skin,

t = time.

η = reduction factor due to reaction,

C_t = total concentration,

Q = cumulative amount,

C_0 = surface concentration on the skin,

n = natural number 1, 2, ...

π = 3.14.

Mass balance for the metabolism of estradiol diacetate by a first-order kinetics can be described by the following relationship:

$$\partial C/\partial t = D(\partial^2 C/\partial x^2) - kC \qquad (10)$$

The boundary (B.C.) and initial conditions (I.C.) are:

B.C.: $x = 0$: $C = C_0$

$x = \ell$: $C = 0$ (sink condition)

I.C.: $t = 0$: $C = 0$

The solution of Equation 10 subjected to the initial and boundary conditions was given by Leypoldt and Gough (7):

$$\bar{c}(\zeta,\theta) = C/C_0 = \sinh[\phi(1-\zeta)]/\sinh(\phi) - 2\sum_{n=1}^{\infty} n\pi/(n^2\pi^2+\phi^2)\exp[-(n^2\pi^2+\phi^2)\theta]\sin(n\pi\zeta) \quad (11)$$

where $\phi = \ell\sqrt{(k/D)}$

$\zeta = x/\ell$

$\theta = Dt/\ell^2$

The rate of permeation is then represented by:

$$dQ/dt = (DC_0/\ell)[\phi/\sinh(\phi)] - 2\sum_{n=1}^{\infty} (n\pi)^2(-1)^n/(n^2\pi^2+\phi^2)\,(DC_0/\ell)\exp[-(n^2\pi^2+\phi^2)Dt/\ell^2] \quad (12)$$

The rate of permeation at steady-state becomes

$$(dQ/dt)_{ss} = [\phi/\sinh(\phi)](DC_0/\ell) = \eta(dQ/dt)_0 \quad (13)$$

where $(dQ/dt)_0$ is the steady-state rate of permeation without any enzymatic reaction and η is defined as a reduction factor for the steady-state rate of permeation with enzymatic metabolism.

The cumulative amount of drug permeated is represented by

$$Q = \int_0^t (dQ/dt)dt = (DC_0/\ell)[\phi/\sinh(\phi)]t - 2C_0\ell\sum_{n=1}^{\infty}\{(n^2\pi^2)(-1)^{(n+1)}\}/(n^2\pi^2+\phi^2)^2\exp[-(n^2\pi^2+\phi^2)Dt/\ell^2] \quad (14)$$

The time-lag, θ_L, is also derived as follows (7):

$$\theta_L = (Dt/\ell^2) = \frac{1}{2}[\coth(\phi)/\phi - 1/\phi^2] \quad (15)$$

The effect of modulus, ϕ, on η (Eq. 13) and $6\theta_L$ (Eq. 15) is shown in Figure 5.

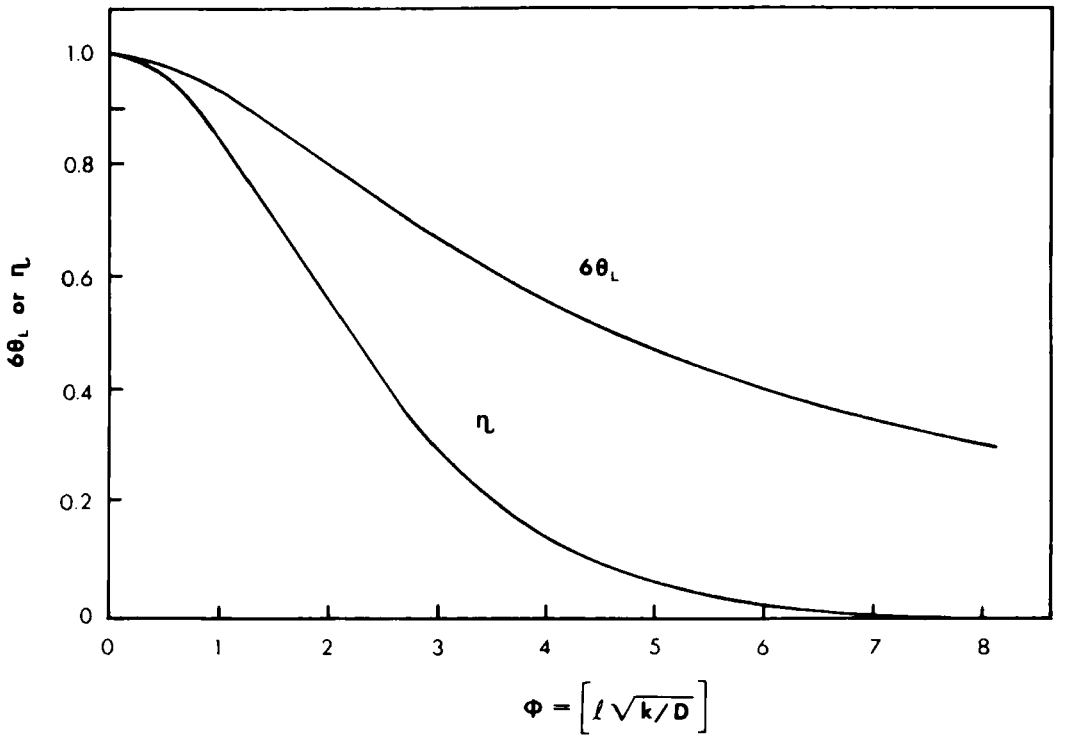

Figure 5

The variation of the dimensionless time-lag ($6\theta_L$) and the reduction factor (η) as a function of modulus (ϕ) according to Equations 13 and 15, respectively.

Total permeation of drugs, estradiol diacetate (ED), estradiol acetate (EA) and estradiol (E), is independent of the rate of first-order enzymatic reactions, because it is an equi-molecular reaction kinetics.

By substituting ϕ = 0 into Eq. (14), the cumulative amount, Q, of the total drug permeated can be evaluated.

The experimental Q versus t profiles obtained for EA → E reaction (8) was compared with the predicted results in Figure

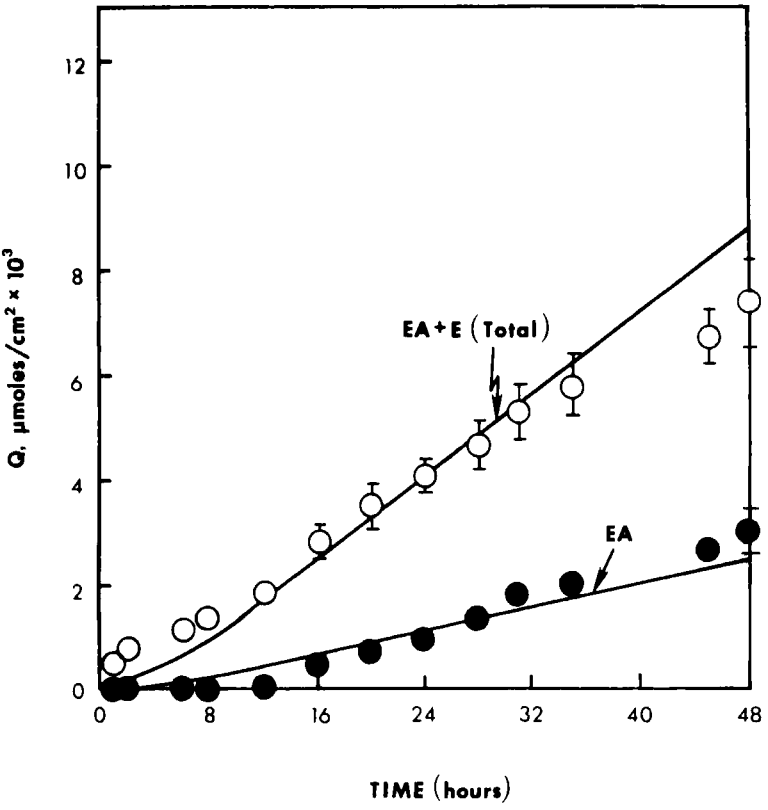

Figure 6

Comparison between the theoretical calculation and experimental results on the permeation profile of estradiol acetate (EA) and the formation of estradiol (E) by metabolism. Key: calculated (——); experimental, (○ and ●).

6. The k_2 value in Table 1 (0.51 hr^{-1}) and the diffusivity (2.06 x 10^{-8} cm^2/sec), which was determined by a time-lag method (11, 12), were used in the calculation of predicted results. As can be seen from Figure 6, the experimental data points fit fairly well with the theoretical results.

In Figure 7, the time course for the experimental data points for the simultaneous permeation and metabolism of ED → EA → E were

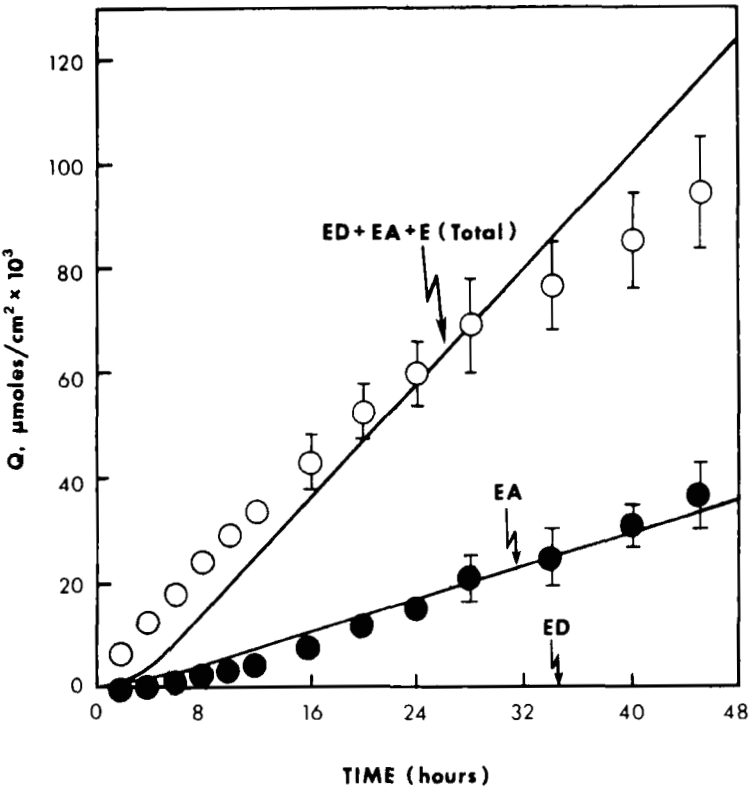

Figure 7

Comparison between the theoretical calculation and experimental results on the permeation profile of estradiol diacetate (ED) and the formation profiles of estradiol acetate (EA) and estradiol (E) by metabolism. Key: calculated (———); experimental, (○ and ●).

compared with the rate profiles calculated by using the rate constants k_1 and k_2 in Table 1. The theoretical line for EA in this figure was computed on the assumption that ED is quickly disappeared in the skin in view of the fact that no ED was detected, the rate constant k_1 is much higher than k_2 (Table 1) and the metabolism of ED→ EA→ E can be approximated by the slowest reaction of EA→E. Results suggested that the experimental

data agreed reasonably well with the calculated results (Fig. 7). It should also be pointed out that the experimental Q data (ED + EA + E) deviate gradually from the predicted line after 28 hr. This finding is consistent with the dermal uptake/metabolism study reported earlier (Figs. 2 and 3). Therefore, this deviation could be attributed to the decreased enzyme activity during the skin permeation experiment, possibly due to the result of skin aging.

CONCLUSION

The enzymatic metabolism of estradiol esters in the hairless mouse skin was analyzed using the non-steady-state approach, and the rate constants for the first-order metabolism reaction were determined.

The simultaneous permeation and metabolism of the estradiol esters were then analyzed theoretically based on the Fick's second law of diffusion accompanied by a first-order chemical reaction. The experimental data were well explained by the present model during the early stage of experiment (<24 hr). A deviation was observed at later phase of experiments, which is probably due to the decrease in enzyme activity caused by skin aging.

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