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LONG-TERM PERMEATION KINETICS OF ESTRADIOL:
(V) DEVELOPMENT AND EVALUATION OF TRANSDERMAL BIOACTIVATED HORMONE DELIVERY SYSTEM

Yie W. Chien*, Kirti H. Valia, and Uday B. Doshi

Controlled Drug Delivery Research Center

Rutgers University

College of Pharmacy

Piscataway, New Jersey 08854

ABSTRACT

Based on the results of simultaneous skin permeation and bioconversion profiles, a Transdermal Bioactivated Hormone Delivery (TBHD) System was developed from the microreservoir partition-controlled drug delivery technology for the transdermal controlled delivery of estradiol prodrugs.

Using the *in vitro* skin permeation apparatus developed earlier, the kinetics of release and skin permeation of estradiol prodrugs from the TBHD system and the regeneration of estradiol were simultaneously studied. Results indicated that all prodrugs are totally converted by the esterase to estradiol during the course of skin permeation.

The release rate of estradiol was found to be first enhanced by the esterification of the OH groups at 3- and 17-position and then decreased as the alkyl chain length increased.

The rate of regeneration of estradiol from the prodrugs was found to follow the order of: diacetate > valerate > heptanoate > acetate > cypionate.

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INTRODUCTION

Prodrug has been described as a compound which undergoes a chemical transformation in the body prior to exhibiting their pharmacologic actions (1). The prodrug approach in dermal drug delivery has been in its infancy. Only a few series of prodrugs are well studied and reported in literature.

The main purpose of this study was to investigate the feasibility of developing a Transdermal Bioactivated Hormone System to deliver a series of lipophilic prodrugs of estradiol at controlled rates for better skin permeation. In the viable skin layers, the prodrugs will be quickly metabolized to regenerate the biologically active estradiol. As demonstrated in earlier reports of this series of investigations (2, 3) that the lipophilic prodrugs of estradiol are absorbed by the skin at a rate greater than estradiol itself and are metabolized by the esterases in the skin to regenerate the bioactive estradiol.

"What are the factors controlling the rate of skin absorption of a drug?" The conscientious response must include a careful appraisal of the physicochemical properties of the penetrant species, of the delivery system from which the penetrant molecules are delivered to the skin, and the structure and function of the skin. Following is a sequence of transport events which are believed to be taken by a drug molecule when delivered by a transdermal delivery system to become available in the systemic circulation: a) drug dissolution and diffusion within the delivery system, b) drug release from the delivery system and partitioning onto the stratum corneum, c) drug transport across the stratum corneum into the viable epidermal tissue, d) drug movement through the viable tissue, e) drug uptake by the cutaneous microcapillary network for subsequent systemic distribution.

Several approaches can be utilized to effectively control the release of systemically-active drugs for permeation at a programmed rate through the skin tissue: a) membrane-moderated drug delivery, b) matrix diffusion-

controlled drug delivery and c) microreservoir partition-controlled drug delivery (4, 5).

Recently, optimization of estrogen substitution in postmenopausal women was achieved by an estradiol-releasing transdermal delivery system developed from the membrane-moderated drug delivery system and designed to provide continuous transdermal delivery of 17β-estradiol at a rate sufficient to maintain physiological levels of estrogen in the systemic circulation similar to those in the early follicular phase of the menstrual cycle (6).

The present investigation describes the development of a Transdermal Bioactivated Hormone Delivery System from the microreservoir partition-controlled drug delivery system. It is capable of releasing natural estradiol and its lipophilic prodrugs at programmed rates for controlled percutaneous absorption through intact skin. During the course of skin permeation the prodrug is metabolized by the enzyme, such as esterase, to regenerate the biologically-active estradiol. It was designed with the objective of hybridizing the constant drug release mechanism of microreservoir partition-controlled drug delivery system with the concept of cutaneous enzymatic bioactivation of prodrugs.

In this investigation, we intend to report our findings on the kinetics of release of a series of estradiol-17-esters from the newly developed transdermal delivery system and their skin permeation, using the female hairless mouse skin as the animal model, and also the kinetics of bioconversion of 17-esters to regenerate the natural estradiol.

EXPERIMENTAL

Materials:

17β-Estradiol[1] (E), estradiol-17β-acetate[2] (EA), estradiol-17β-valerate[3] (EV), estradiol-17β-heptanoate[3] (EH) (or estradiol enanthate), estradiol-17β-cypionate[2] (EC), estradiol-3,17-diacetate[2] (ED), silicone medical fluid[4]

(DC-360) (20 cps), silastic 382 medical-grade elastomer[4], stannous octanoate[4] (catalyst M), polyethylene glycol (PEG) 400[5], sodium chloride[6], acetonitrile[7] and methanol[7] (both are distilled-in-glass HPLC grade) were used as obtained. HPLC-grade distilled water was prepared freshly in the laboratory[8].

Analytical Method:

A liquid chromatograph equipped with a reciprocating pump[9] (model 6000A), an injector[9] (model U6K), a UV detector[10] (model 773, with a cell volume of 11 µl), a reverse-phase µ-Bondapak C_{18} column[9] (with a guard column containing 37-50 µm Bondapak C_{18}/Corasil packing material), and an Omniscribe recorder[11] was used in this investigation. The UV detector was operated at the wavelength of 205 nm to detect both estradiol and estradiol esters. Different combinations of acetonitrile: water (40:60, 50:50, 55:45, 70:30, and 75:25) were used as the mobile phase for the elution and separation of estradiol from various estradiol esters. At ambient condition, a flow rate of 2 ml/min was used.

Determination of drug concentrations in the sample solution was carried out by first measuring the peak height of drugs and then computing the concentration (µg/ml) from the calibration curves constructed from a series of standard solutions.

Skin Permeation Cell:

The same *in vitro* skin permeation system[12,13] reported earlier (7) was used in this investigation.

Skin Preparation:

For this study, a full-thickness skin sample was freshly excised from a 5- to 7-week old hairless mouse (HRS/J strain)[14]. The hairless mouse was sacrificed just prior to the experiment by the same method outlined in the previous report (2, 3). A square section (~3 x 3 cm) of the abdominal skin was surgically removed from the animal and the subcutaneous tissue and blood vessels were cleaned.

Determination of Drug Solubility:

The solubility of estradiol and estradiol esters in saline-PEG 400 solution (60/40) and in silicone fluid were carried out as described earlier (2).

Determination of (Skin/silicone Fluid) Partition Coefficient:

The partition coefficient (skin/silicone fluid) of estradiol and estradiol esters were determined in the same manner as described earlier (2).

Fabrication of Transdermal Bioactivated Hormone Delivery System:

The methodology outlined earlier (8-11) was utilized in this investigation for the fabrication of Transdermal Bioactivated Hormone Delivery (TBHD) System. Briefly, in a disposable polyethylene beaker[5] (25 ml), 0.5 gm of estradiol or an estradiol ester was dispersed well in one ml of 40% PEG 400 in saline solution using a high torque laboratory stirrer[15] at 1000 rpm (setting 6) for 2 min. to form drug-PEG paste. Then, 8 gm of silastic elastomer 382 was added to the drug-PEG paste and mixed well by the stirrer at 1000 rpm for 5 min. To reduce the viscosity, 0.5 gm of silicone fluid was added and mixed for 2 min. again at 1000 rpm. The dispersion was deaerated under vacuum (at 20 guage) for 20 min. Then, 2 drops of stannous octanoate (catalyst M) was added, as the crosslinking agent, and mixed well for 1 min., and deaerated for another 2 min. Immediately, the dispersion was poured onto a stainless-steel device maker and pressed to a thickness of about 250 μm. The whole device maker was then kept in a constant temperature oven[16] at 60°C for 1 hr. The TBHD sheet formed was removed and then cut into disc-shaped device (2 cm in diameter). Each device was examined for any entrapped air.

Measurement of Drug Release, Permeation and Metabolism Profiles

The TBHD system, which contains 2 drug-releasing surfaces, was applied onto a skin specimen and then mounted between the donor and receptor compartments of the skin permeation cell (Fig. 1A). The saline/PEG 400 (60/40) solution (3 ml) was added into each of the donor and receptor

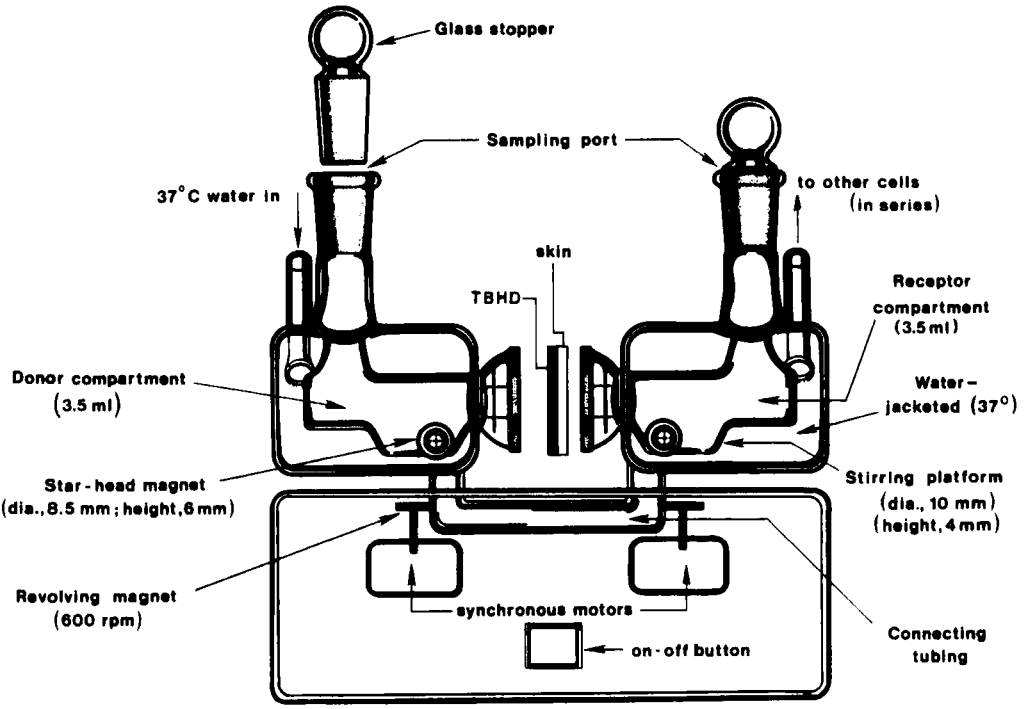


Figure 1A

Schematic illustration of the experimental setup for the simultaneous study of the controlled release and the skin permeation of estradiol and esters from transdermal bioactivated hormone delivery (TBHD) system.

compartments. Using this arrangement, drug release into the donor solution and permeation across the skin tissue were investigated simultaneously. At each of the predetermined intervals, a 50 µl sample was withdrawn from the receptor solution and analyzed immediately by HPLC for estradiol and estradiol esters using a given mobile phase composition. To maintain a sink condition in the donor solution, the whole volume of the donor solution was totally withdrawn and 50 µl of which was sampled and assayed for the drug by HPLC. Immediately, the donor compartment was refilled with the same volume of drug-free elution solution. Each experiment was carried out in triplicate.

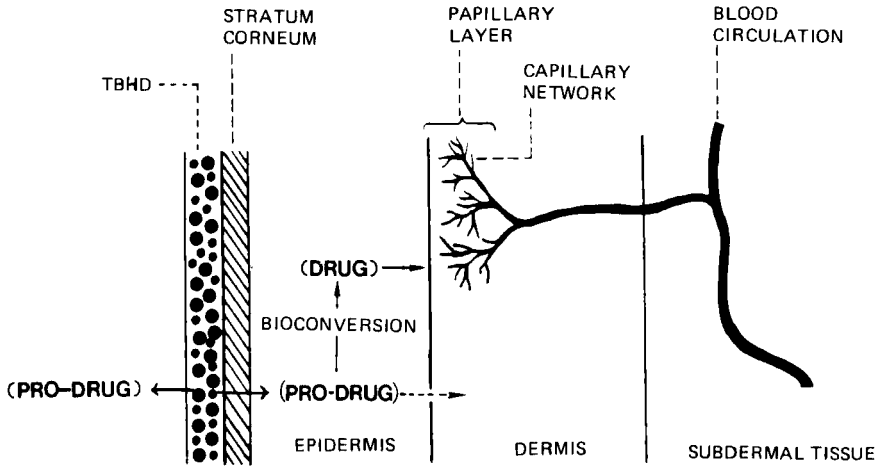


Figure 1B

An expanded diagram of TBHD system/skin tissue combination to illustrate the simultaneous kinetic processes of release and skin permeation of pro-drugs from TBHD system and of regeneration of drug by enzymatic metabolism of prodrugs.

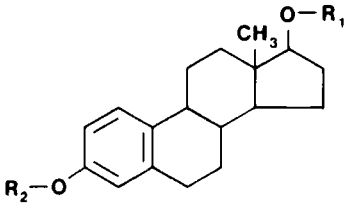
RESULTS AND DISCUSSION

Effect of Ester Chain Length on Partition Coefficient

The application of prodrug concept in transdermal drug delivery can be viewed as the alteration of skin permeability by chemical modifications of the drug structure to enhance its rates of skin uptake and transdermal permeation. In this investigation, the hydrophilic OH groups at 3- and 17-position of estradiol were esterified to form estradiol esters with various alkyl chain lengths (Figure 2). The partition coefficients for the interfacial partitioning into the saline/PEG solution (for drug release studies) and onto the skin (for permeation studies) from the lipophilic silicone polymer were observed to decrease as increasing the alkyl chain length from acetate to heptanoate (Table I). The partition coefficient of 3, 17-diacetate was found to be further reduced from 17-acetate.

Release of Estradiol and Estradiol Esters from TBHD System

A TBHD system is composed of a homogeneous microdispersion of estradiol or estradiol ester suspension (in 40% aqueous PEG 400 solution) in a solid



	R_1	R_2
Estradiol – 17 – β	–H	–H
Estradiol – 17 – Acetate	$-C(=O)-CH_3$	–H
Estradiol – 3,17 – Diacetate	$-C(=O)-CH_3$	$-C(=O)-CH_3$
Estradiol – 17 – Valerate	$-C(=O)-(CH_2)_3-CH_3$	–H
Estradiol – 17 – Heptanoate	$-C(=O)-(CH_2)_5-CH_3$	–H
Estradiol – 17 – Cypionate	$-C(=O)-CH_2-CH_2-$ (cyclopentyl)	–H

Figure 2

Chemical structure of estradiol and prodrug-type esters of estradiol.

silicone polymer matrix. These drug-containing microscopic spheres distribute homogeneously throughout the crosslinked polymer matrix as a discrete, immobilized, unleachable liquid compartment. The molecules of estradiol or estradiol ester can elute out of the TBHD system only by first dissolution in the liquid compartment, partition into and then diffusion through the polymer matrix, and finally partition into the elution solution surrounding the TBHD system (Figure 3). The theoretical analysis suggested that the controlled release of estradiol and estradiol esters from the TBHD system should be zero-order as defined by the linear Q vs. t relationship (11).

Table I: Partition Coefficient of Estradiol and Estradiol Esters:

Drugs	Partition Coefficient*	
	K_p	K_s
Estradiol	71.477	20.15
Estradiol Acetate	1.117	7.67
Estradiol Valerate	0.071	2.84
Estradiol Heptanoate	0.010	.09
Estradiol Cypionate	0.012	2.81
Estradiol Diacetate	0.012	1.76

$$*K_p = \frac{\text{Equilibrium drug conc. in saline/PEG 400 solution}}{\text{Equilibrium drug conc. in silicone fluid}}$$

$$K_s = \frac{\text{Equilibrium drug conc. in skin tissue}}{\text{Equilibrium drug conc. in silicone fluid}}$$

As shown in Figure 4, constant (zero-order) release profiles were obtained for the controlled release of estradiol and its esters from TBHD system. The rates of release (Q/t) for estradiol and estradiol esters, as calculated from the slope of the linear Q vs. t relationship, are summarized in Table II. The rate of release for estradiol was found to be 1.826 ± 0.074 $\mu g/cm^2/hr$. Esterification of the 17-OH group in estradiol to form 17-acetate, the release rate was observed to increase more than 2 folds to 4.133 ± 0.525 $\mu g/cm^2/hr$. Esterification of both OH groups at 3- and 17-position to form 3, 17-diacetate further enhanced the release rate by another 2 folds to 9.388 ± 1.160 $\mu g/cm^2/hr$. On the other hand, as the alkyl chain length of 17-ester increased from acetate to cypionate, the rate of release decreased. The observed reduction in release rate in response to the increase in alkyl chain length could be partially explained by the decreasing K_p value (Table I).

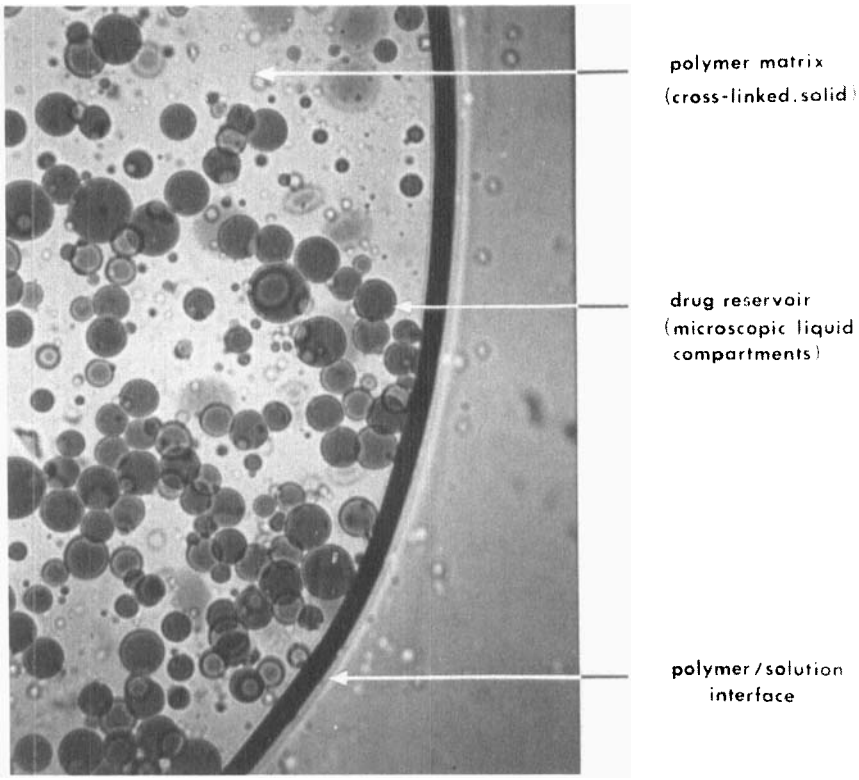


Figure 3

Photomicroscopic view of a cross-section of the transdermal bioactivated hormone delivery (TBHD) system, in which drug reservoir is dispersed as discrete, immobilized, unleachable microscopic spheres in the crosslinked polymer matrix.

Skin Permeation and Metabolism of Estradiol and Estradiol Esters

Permeation of estradiol or estradiol esters through hairless mouse skin, under *in vitro* conditions, following the release from the TBHD systems were also evaluated using the same skin permeation cells maintained at constant body temperature. The drug-releasing surface of TBHD system was placed in intimate contact with the stratum corneum surface of the skin, while the dermal surface of the skin was immersed in saline/PEG solution (Figure 1).

For the purposes of mechanistical analysis, the skin could be considered as a two-ply laminate, which is composed of the stratum corneum and cutaneous

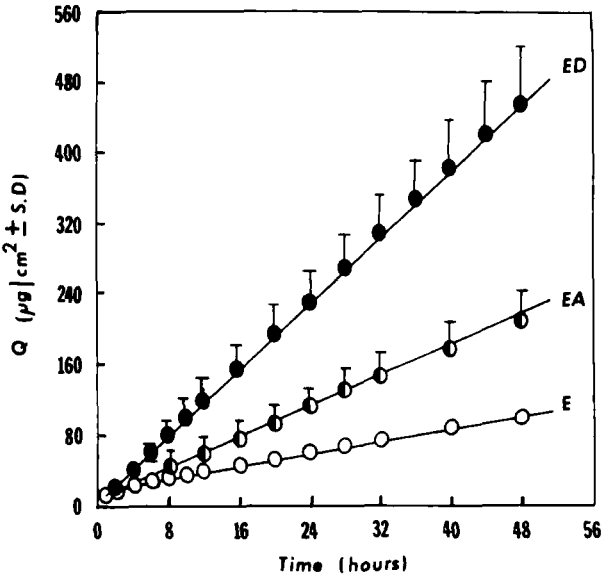


Figure 4

The time course for the cumulative release of estradiol and esters from TBHD system. Key: ○, estradiol; ◐, estradiol-17-acetate (EA); ●, estradiol -3, 17-diacetate.

Table II: Release Rate Profiles of Estradiol and Estradiol Esters from Transdermal Bioactivated Hormone Delivery System

Species	Release Rate ($\mu g/cm^2/hr \pm SD$)
Estradiol	1.826 ± 0.074
Estradiol Esters	
1) Diacetate	9.388 ± 1.160
2) Acetate	4.133 ± 0.525
3) Valerate	3.732 ± 0.662
4) Heptanoate	2.082 ± 0.768
5) Cypionate	0.567 ± 0.189

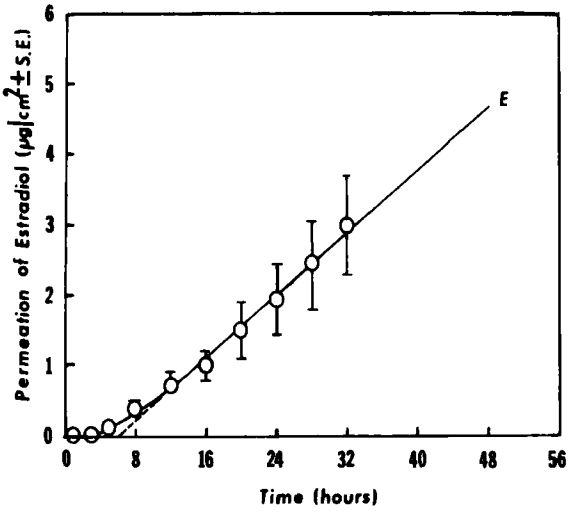


Figure 5

Transdermal controlled administration of estradiol across female hairless mouse from estradiol-releasing TBHD system.

tissue (epidermis and dermis) (Figure 1B); and the relevant enzymes are assumed to be homogeneously distributed in the cutaneous tissue (12).

When an estradiol-releasing TBHD system was applied on the stratum corneum, a constant skin permeation profile was obtained (Figure 5). The rate of skin permeation (dQ/dt), which was measured from the slope of Q vs. t plots, was found to be 11.7×10^{-2} $\mu g/cm^2/hr$.

For this study, it is also assumed that the skin has a homogeneous distribution of enzymes, and the enzymatic reactions occur, for the simplicity of mathematical treatment, at simple first-order rate processes (2, 3).

Figure 6 shows the time course for the skin permeation and metabolism of estradiol-17-acetate (EA). The bioconversion of EA to estradiol (E) appeared to occur during the diffusion through the cutaneous tissue. The rate of appearance of estradiol in the dermal solution was found to be 5.7×10^{-2} $\mu g/cm^2/hr$ (Table III). After a lag-time of approximately 10 hr, EA also began to appear in the dermal solution.

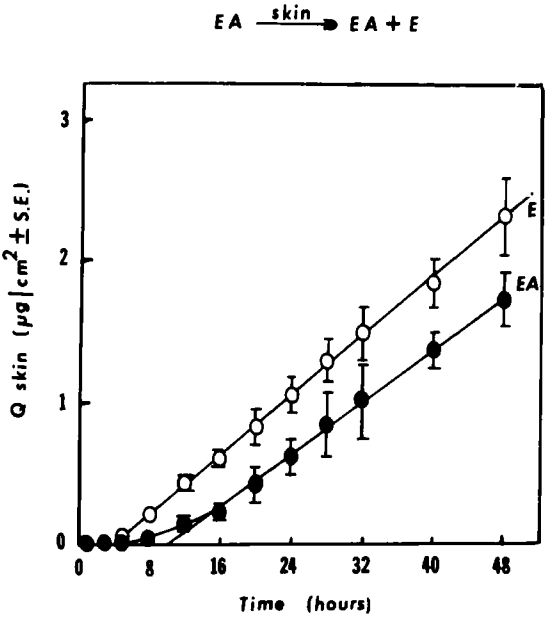


Figure 6

The time course for the transdermal controlled permeation of estradiol-17-acetate (EA) from EA-releasing TBHD system and its metabolism to estradiol (E). Key: ○, E; ●, EA.

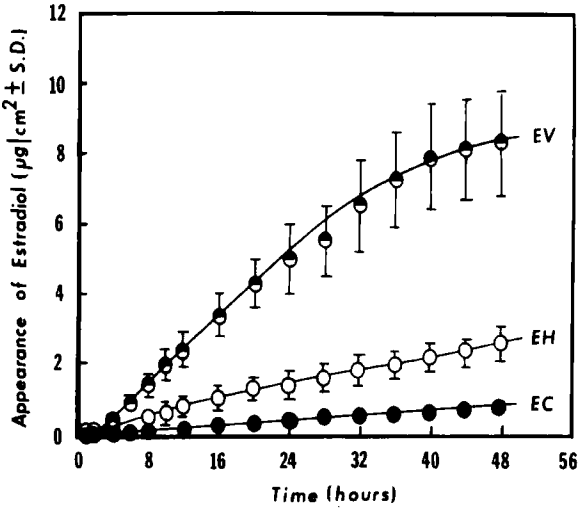


Figure 7

The time course for the regeneration of estradiol (E) during the skin permeation of various estradiol-17-esters by metabolism following transdermal controlled administration from various ester-releasing TBHD systems. Key: ◓ Estradiol-17-valerate (EV); ○, Estradiol-17-heptanoate (EH); ●, Estradiol-17-cypionate (EC). No intact ester was detected.

Table III: Appearance Rate Profiles of Estradiol following Transdermal Controlled Administration of Estradiol and Esters from Transdermal Bioactivated Hormone Delivery System

Species	Rate of Appearance ($\mu g/cm^2/hr \pm SD$)
Estradiol	0.117 ± 0.027
Estradiol Esters	
1) Diacetate	0.490 ± 0.250
2) Acetate	0.057 ± 0.013
3) Valerate	0.227 ± 0.042
4) Heptanoate	0.061 ± 0.013
5) Cypionate	0.016 ± 0.002

The time course for the formation of estradiol from other estradiol-17-esters during the course of skin permeation is shown in Figure 7. Interestingly, no intact estradiol-17-ester was detected in the dermal solution during the course of 48-hr study, indicating the complete bioconversion of ester to estradiol. From the estradiol appearance data, the rate of skin permeation was calculated to be 22.7×10^{-2}, 6.1×10^{-2}, and 1.6×10^{-2} $\mu g/cm^2/hr$ for valerate, heptonoate and cypionate, respectively (Table III). It was previously shown that as the alkyl chain length of alkanols increases, the rate of permeation is enhanced to a maximum value when alkanol has 5 CH_2 units (hexanol) and then decreases as the number of methylene (CH_2) group is greater than five (13-15). Similarly, the results of the present investigation suggested that the maximum rate for the formation of estradiol from permeating esters is reached as the estradiol-17-ester has an alkyl chain with 5 carbons (estradiol valerate) (Table II).

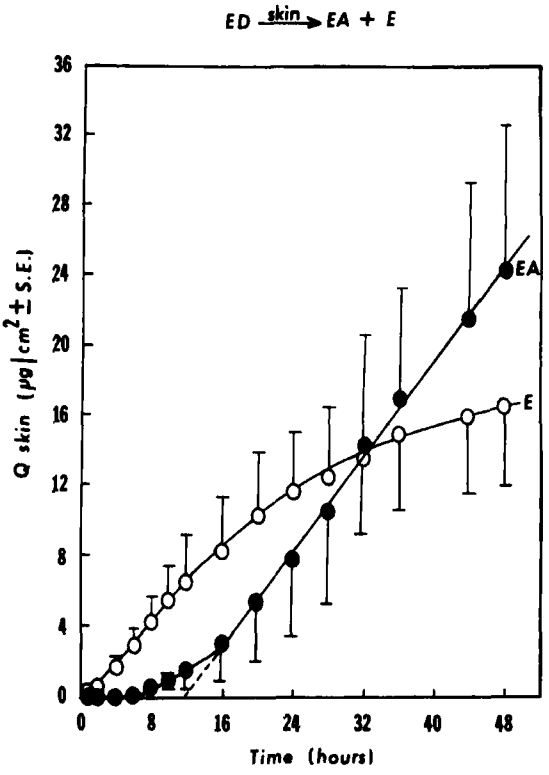


Figure 8

The time course for the skin permeation of estradiol-3, 17-diacetate (ED) and its metabolism to estradiol acetate (EA) and estradiol (E) following the transdermal controlled administration from ED-releasing TBHD system. No intact ED was detected.

Estradiol-3, 17-diacetate (ED) could be metabolized consecutively by esterase first to estradiol-17-acetate (EA) and then to estradiol (E) during the course of permeation through the skin (2, 3). The time course for the skin permeation and metabolism of ED is shown in Figure 8. The rate of appearance of estradiol (E) seemed to be much faster than that of estradiol acetate (EA) initially and then gradually slowed down. The rate of appearance of estradiol in dermal solution was 49.0 x 10^{-2} μg/cm^2/hr (Table III). Again, no ED was detected.

The rate of appearance of estradiol from estradiol esters was found to decrease in the order of: diacetate > valerate > heptanoate > acetate > cypionate. There was almost 4-fold increase in the rate of estradiol

formation as the alkyl chain length increased from acetate to valerate. But, further increase in the chain length from valerate to heptanoate showed a reverse trend with a decrease in the flux. The rate of formation of estradiol from the valerate was found to be 2 times higher than the rate of appearance for estradiol itself, while the rate of estradiol formation from the heptanoate and acetate was only half of that of estradiol. It is interesting to note that by esterifying both OH groups at 3- and 17-positions to form diacetate, the flux was improved substantially. Similar observations were made earlier (2) when the estradiol and its esters was delivered transdermally from a suspension in silicone fluid, which is also a silicone elastomer with similar composition as that used in the TBHD system.

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*All inquires should be directed to:

Professor Yie W. Chien
Controlled Drug Delivery Research Center
College of Pharmacy
Rutgers University
P. O. Box 789, Busch Campus
Piscataway, New Jersey 08854

FOOTNOTES

1. Courtesy of Roussel-UCLAF, Paris, France.
2. Sigma Chemical Company, St. Louis, Missouri.
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13. Caton Industries, Inc., Edison, New Jersey.
14. Jackson Laboratories, Bar Harbor, Maine.
15. Model 4380-00, Cole-Parmer Inc., Chicago, Illinois.
16. Isotemp Oven (Model 126G), Fisher Scientific Co., Fairlawn, New Jersey.

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