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LONG-TERM PERMEATION KINETICS OF ESTRADIOL: (III) KINETIC ANALYSES OF THE SIMULTANEOUS SKIN PERMEATION AND BIOCONVERSION OF ESTRADIOL ESTERS+

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Abstract ☐ The skin permeation system developed earlier in this laboratory was utilized to study the kinetics of the simultaneous skin permeation and bioconversion of 5 estradiol esters. The equilibrium solubility of estradiol esters in the lipophilic silicone fluid and in hydrophilic PEG 400/saline solution was found to be dependent upon the alkyl chain length of the esters. Estradiol-3,17-diacetate had a greater solubility in silicone fluid and a lower solubility in PEG 400/saline solution than estradiol-17-acetate. The (skin/silicone fluid) partition coefficients were observed to decrease as the alkyl chain increased

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in length. During the course of skin permeation, the estradiol esters were metabolized by esterase to regenerate estradiol. The rate of appearance of estradiol from the estradiol esters was observed to be dependent upon the ester concentration on stratum corneum surface and to follow the order of: diacetate > valerate > heptanoate > cypionate > acetate. From the dermal uptake and metabolism studies of estradiol esters the first-order rate constants for the metabolism of estradiol esters were determined. The rate constant for the metabolism of estradiol-3,17-diacetate to form estradiol acetate was observed to be about 22 times faster than the rate constant for the metabolism of estradiol acetate to generate estradiol. The enzymatic hydrolysis of the ester group at 17th position was found also to follow a first-order kinetic process and the rate constants varied with the variation in alkyl chain length.

INTRODUCTION

The movement of a drug into and through the skin is a diffusion process. Therefore, the thermodynamic activity of a drug in the delivery phase or in a vehicle is important, as is the intrinsic ability of a drug species partitioning into and diffusing through the skin.

The application of prodrug approach in transdermal drug delivery can be considered as the alterations of skin permeability via the physical or chemical modification of the penetrating

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drug molecule to enhance its rate of percutaneous absorption. The prodrug approach is, in effect, a manipulation of the drug-skin and drug-vehicle interactions.

Prodrugs of a poorly absorbed drug may be synthesized to improve its transport characteristics. By the metabolic processes within the skin, the prodrugs can be transformed back to the active parent drug. In other words, if an active drug has a very low affinity toward the skin and will, therefore, not easily partition into it to any great extent. The partition behavior of this drug can be improved by simple chemical modification to form a lipophilic prodrug, so, the transport of the drug into the skin is substantially enhanced. In the skin, the prodrug is rapidly metabolized to regenerate the active parent drug.

Studies which may be considered as paving the foundation for prodrug development have been conducted on homologous series of compounds. They are exemplified by the in vitro percutaneous absorption studies of straight-chain low-molecular-weight alkanols (1) and steroid homologs (2). Earlier, Wurster and Kramer (3) conducted the in vivo percutaneous absorption of three salicylate ester homologs in humans.

Bodor et al. (4-6) have studied extensively the transdermal delivery of prodrugs of steroids, theophylline and cromolyn. Hydrocortisone-21-diethylsuccinamate was reported to almost double the rate of delivery of hydrocortisone through hairless mouse skin, while at the same time it caused significantly less local

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toxicity than hydrocortisone. Selected acyloxymethyl prodrugs as well as 7-(hydroxymethyl) derivative were found to be effective in increasing the delivery of theophylline through skin by 3.5 to 5 times. It was concluded that the prodrug approach seems to be promising for the topical delivery of such highly polar compounds as cromolyn (4).

The feasibility of pro-drug approach in the dermal delivery of 5-fluorouracil through human skin was studied by Mollgaard et al.(7). The 1-buytryloxymethyl derivative of 5-fluorouracil was reported to permeate more readily then 5-fluorouracil. Bundgaard et al. (8) investigated the leaching of hydrolytic enzymes from human skin in the cutaneous permeation studies. They emphasized that receptor phase metabolism due to the leached enzymes may be of significance in assessing the concurrent transport and metabolism of prodrugs when using human skin in permeation studies.

Recently, permeation of hydrocortisone and its 21-ester homologs through hairless mouse skin was investigated by Smith (9). It was observed that the enzymes have a facilitating effect on the rate of transport of hydrophobic esters across the skin. The *in vitro* rates of transport of hydrocortisone esters was observed to be parallel with the *in vivo* topical anti-inflammatory activity, thus suggesting that topical activity of these esters is significantly controlled by permeation process.

Poulson et al. (10) developed the concept of using combinations of steroidal prodrugs to improve dermal absorption.

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An increase in total drug penetration was demonstrated. The principle behind this approach is the independent partitioning and solubility behavior of a mixture of compounds that are incorporated into a dermal vehicle. Since the flux of skin penetration depends upon the physicochemical parameters of the drugs in the mixture, the flux resulting from the use of mixtures is significantly greater than that of any individual compound.

In fertile women, estradiol is normally secreted from the ovaries and the ratio of estradiol to estrone in the serum is about 1 or greater. With the onset of menopause at the age around 50, ovarian secretion of estrogen completely ceases and the ratio of estradiol to estrone becomes less than one (11, 12). This induces some profound metabolic and trophic changes. These postmenopausal disorders are often treated by estrogen administration, which is referred to as the hormonal replacement therapy (13).

In the past, hormonal replacement therapy was administered mostly by oral route; conjugated estrogens and micronized estradiol were reportedly effective in the treatment or in the prevention of menopausal changes with a daily dose in the milligram range. On its passage through the liver, a major fraction of the oral estrogen dose was often metabolized to estrone and estriol, which were then conjugated to produce a highly unphysiologic pattern of estrogen metabolites and an increase in the liver proteins, like renin substrate (13, 14).

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The present study describes the results of a comprehensive investigation dealing with the delivery of systemically active estrogens across the skin, with special emphasis on the simultaneous transport and bioconversion of estradiol prodrugs to estradiol.

In this investigation, we intend to report our findings on the kinetics of permeation of 17 β -estradiol and its 17-esters through female hairless mouse skin and the kinetics of bioconversion of estradiol-17-esters to regenerate estradiol. One of the objectives for the study is to evaluate the possible relationship between the chemical structure of estradiol-17-esters and the rate of skin permeation and/or the rate of skin metabolism.

EXPERIMENTAL

Materials

Estradiol[1](E), estradiol-17 β -acetate[2](EA), estradiol-17β -valerate[3](EV), estradiol-17 β -heptanoate[3](EH) (or estradiol enanthate), estradiol-17 β -cypionate[2](EC), estradiol-3, 17-diacetate[2](ED), estrone[2], silicone medical fluid[4] (20 cps), 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 water was prepared freshly in the laboratory[8].

Analytical Methods

A liquid chromatograph equipped with a reciprocating pump[9] (model 6000A), an injector[9] (model U6K), an UV detector[10] (model

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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 205nm to detect estradiol, estrone, and estradiol esters. Different combinations of acetonitrile and water (40/60, 50/50, 55/45, 70/30, and 75/25) at a flow rate of 2 ml/min were used as the mobile phase for the elution and separation of estradiol from various estradiol esters (Figure 1). Ambient condition was used.

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

Skin Permeation Cell

The same *in vitro* skin permeation system[12] developed earlier for other studies (15-17) was used in this investigation.

Skin Preparation

For this study, a full-thickness skin sample was freshly excised from a 5-7 weeks old hairless mouse (HRS/J strain)[13]. The hairless mouse was sacrificed just prior to the experiment by cervical dislocation. A square section of the abdominal skin (3 cm x 3 cm) was surgically removed and its dermal surface was carefully cleaned (17).

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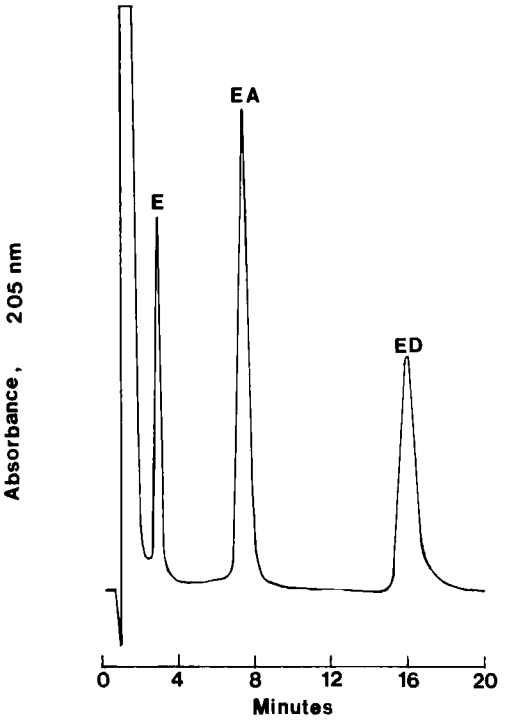

Figure 1: Separation of estradiol (E), estradiol acetate (EA), and estradiol diacetate (ED). Column: μBondapak C_{18}; mobile phase: acetonitrile - water (55:45).

Determination of Drug Solubility

An excess amount of drug was equilibrated with 40% v/v PEG 400/saline solution or with silicone fluid for 24 hr with constant shaking in a shaking waterbath[5] at 37°C. The saturated drug solution was then quickly filtered through a HAWP filter[14]. The drug concentration in 40% v/v PEG 400-saline solution was then determined directly by HPLC. On the other hand, the drug concentration in silicone fluid was first extracted with methanol (1 to 10 dilution, 24 hr, ambient temperature) and then determined by HPLC.

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Determination of (Skin/Silicone Fluid) Partition Coefficient

Skin samples of known weight (100-200 mg) were equilibrated with drug solution (3 ml) in silicone fluid in screw-capped test tubes shaken for 24 hours in a waterbath[5] at 37°C. The initial and equilibrium drug concentrations in the silicone fluid were determined, after first extraction with methanol (1 to 10 dilution, 24 hr, ambient temperature), by HPLC. The volume of skin samples was calculated from the skin weight before equilibrium and its average density (1.04 gm/ml) (18).

The (skin/silicone fluid) partition coefficient ($K_{s/v}$) was determined by the following relationship:

$$K_{s/v} = \left(\frac{C_v^o - C_v^e}{C_v^e}\right)\left(\frac{V_v}{V_s}\right) \quad (1)$$

where C_v^o and C_v^e are the initial and equilibrium concentrations of the drug, respectively; V_v is the volume of silicone fluid, and V_s is the volume of skin.

Measurement of Drug Permeation/Metabolism Profiles

The skin sample was mounted between the two half-cells of the skin permeation cell immediately after excise. A drug suspension with a known loading dose in silicone fluid was filled into the donor compartment, and the 40% v/v PEG 400/saline solution (without drug) was added into the receptor compartment. The PEG 400 was added to achieve a sink condition for estradiol, estradiol esters, and its metabolites. At each of the predetermined intervals, a 50 µl sample was withdrawn from the

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receptor solution and analyzed immediately by HPLC, using a given composition of mobile phase for estradiol and estradiol esters. At the end of the experiment, the drug concentration in the donor solution (silicone fluid) was determined by first filtering the sample through a HAWP filter[14], extracting the filtrate by methanol (1 to 10 dilution, 24 hr, ambient temperature), and then assaying the extracts by HPLC. Each experiment was carried out in triplicate.

Kinetic Analysis of Esterase Reaction with Estradiol Valerate and Estradiol Diacetate

After mounting a full-thickness skin sample between the two compartments of the skin permeation cell, a drug solution of known concentration (1, ½, ¼ and 1/8 of the saturated solubility) in silicone fluid was filled into the donor compartment and 40% v/v PEG 400/saline solution was added into the receptor compartment. The concentrations of estradiol esters and estradiol in the receptor solution were determined by HPLC. Each experiment was carried out in triplicate.

Dermal uptake/Metabolism Studies

A full-thickness skin sample was mounted between the two compartments of the skin permeation cell in such a way that its dermal side facing a bulk of drug solution, while its stratum corneum side was protected with an impermeable aluminum foil (17). A saturated solution of estradiol ester in 40% v/v PEG 400/saline (3.5 ml) was introduced into the compartment facing

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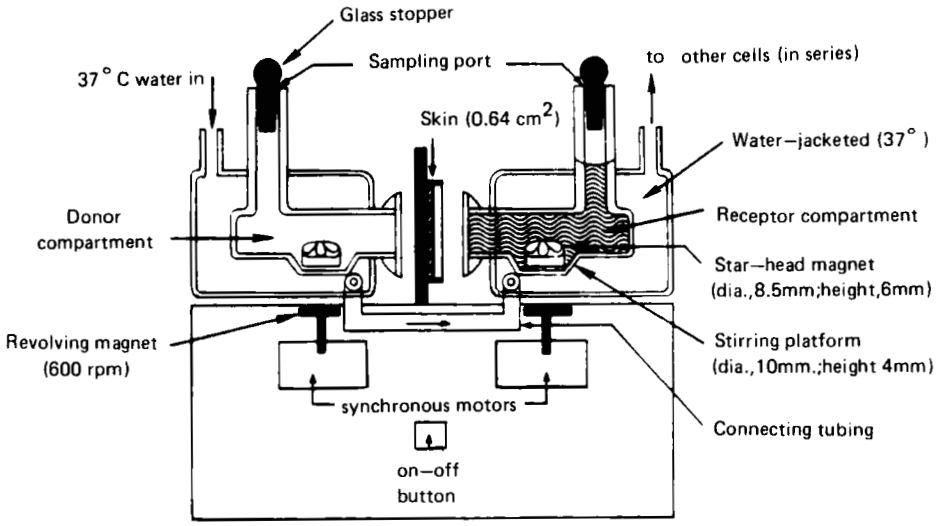

Figure 2: Diagrammatic illustration of the setup for the skin uptake/metabolism studies on the dermal side.

the dermal side, while the other compartment remained empty (Figure 2). A 50 µl sample was withdrawn from the drug solution at each predetermined time interval and assayed for estradiol ester and possible metabolites by HPLC method. The first order rate constants for the bioconversion of estradiol ester to estradiol were determined.

RESULTS AND DISCUSSION

The reaction scheme for the bioconversion of prodrug to active drug is a simple one, yet the prodrug concept presents a complex domain of possibilities for overcoming the barrier effect of skin to the transdermal delivery of drugs. A prodrug may be defined as a bioreversible derivative of a pharmacologically

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active compound. The prodrug approach can be employed to improve the properties of a drug ranging from the physical (e.g., solubility, lipophilicity) and chemical (e.g., stability) to the pharmacodynamic (e.g., prolongation of action) and organoleptic (e.g., taste, smell) properties.

Effect of Ester Chain Length on Solubility and Partition Coefficient

To study the simultaneous transport and metabolism, estradiol and the prodrug-type esters of estradiol were used (Figure 3). Table I summarizes the variation in solubilities and partition coefficients of these estradiol derivatives. Some general trends may be noted as follows: the aqueous solubility (in 40% v/v PEG 400/saline) of esters decreases rapidly as increasing the ester chain length from acetate to heptanoate (Table I). The aqueous solubility of 3,17-diacetate was further decreased as compared to 17-acetate. Estradiol cypionate, which is the cyclopentylpropionate derivative, was least soluble among all the esters studied. The rapid decrease in aqueous solubility in the series could be attributed to the increase in hydrophobicity as the result of the increase in the length of hydrocarbon chains.

On the other hand, the solubility of estradiol esters in the lipophilic silicone fluid was found to increase from acetate to heptanoate (Table I). The solubility of cypionate is between valerate and acetate. Estradiol-3, 17-diacetate solubility was about 500 times higher than estradiol and 70 times greater than

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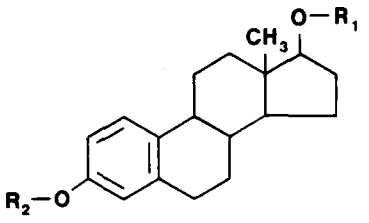

	R_1	R_2
Estradiol - 17 - β	-H	-H
Estradiol - 17 - Acetate	$-C(=O)-CH_3$	-H
Estradiol - 3,17 - Diacetate	$-C(=O)-CH_3$	$-C(=O)-CH_3$
Estradiol - 17 - Valerate	$-C(=O)-(CH_2)_3-CH_3$	-H
Estradiol - 17 - Heptanoate	$-C(=O)-(CH_2)_5-CH_3$	-H
Estradiol - 17 - Cypionate	$-C(=O)-CH_2-CH_2-$ cyclopentyl	-H

Figure 3: Chemical structure of estradiol and prodrug-type esters of estradiol.

estradiol-17-acetate. The increase in the lipophilicity of this series of estradiol esters enhances the solubility in the "like" solvents e.g., silicone fluid, and decreases the solubility in the "dislike" solvents, e.g., PEG 400/saline solution. The lipophilicity of these compounds, which was also reflected in the magnitude of (skin/silicone fluid) partition coefficients, was observed to decrease with the increase in the alkyl chain length (Table I).

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Table I - Solubility and (Skin/Silicone Fluid) Partition Coefficient of Estradiol and Estradiol Esters:

Drugs	Solubility (μg/ml ± S.D.) 40% PEG 400/saline	Silicone Fluid	Partition Coefficient (mean ± S.D.)
Estradiol (E)	220.15 ± 9.32	3.08 ± 0.03	20.15 ± 3.76
Estradiol Acetate (EA)	26.14 ± 1.48	23.40 ± 0.16	7.67 ± 0.77
Estradiol Valerate (EV)	9.33 ± 0.22	131.58 ± 3.32	2.84 ± 0.39
Estradiol Heptanoate (EH)	3.07 ± 0.31	322.27 ± 4.76	1.09 ± 0.08
Estradiol Cypionate (EC)	1.11 ± 0.07	91.30 ± 2.12	2.81 ± 0.43
Estradiol Diacetate (ED)	18.11 ± 0.45	1567.18 ± 76.72	1.76 ± 0.58

The partition coefficients for estradiol esters in the system of skin/40% PEG 400-saline solution could not be determined since the estradiol esters were found to be metabolized by the esterase in the skin, partly or completely, in the time period (24 hrs) required for partitioning studies. Because of this complication, the (skin/40% PEG 400-saline) partition coefficients are not reported here to avoid any misleading.

Skin Permeation of Estradiol

When the estradiol concentration in the donor solution (silicone fluid) was maintained at a level which was greater than its equilibrium solubility, a constant skin permeation profile was obtained (Figure 4). The rate of skin permeation (dQ/dt), which was measured from the slope of Q vs. t plot, was calculated to be 7.87 (±0.73) x 10^{-8} μmoles/cm^2/sec.

It was reported earlier (17) that estradiol is metabolized to estrone by the enzyme, dehydrogenase, when 0.9% saline solution

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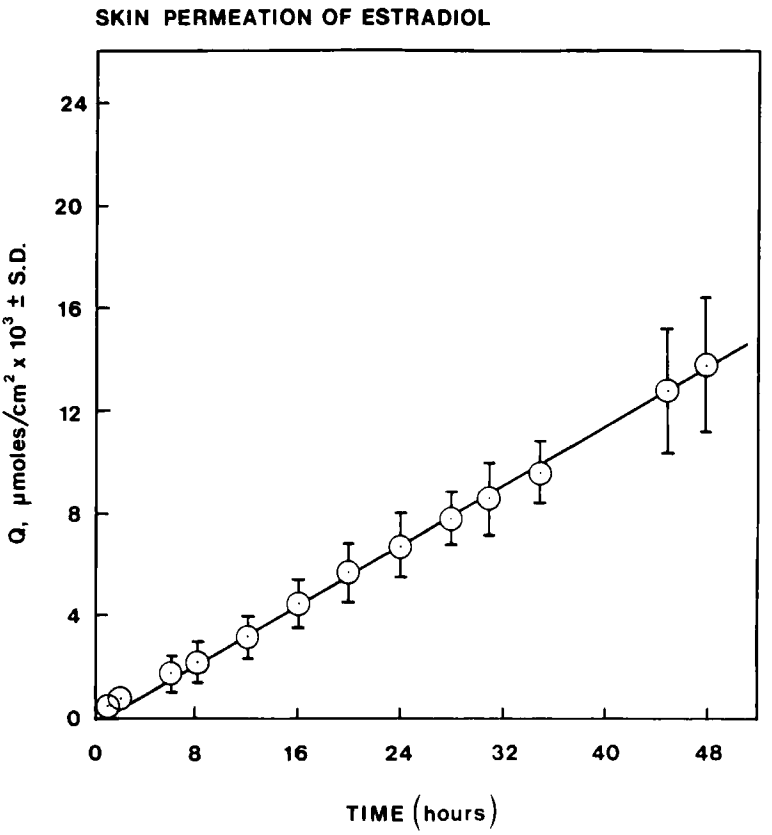

Figure 4: Skin permeation of estradiol across female hairless mouse at constant 17 β-estradiol concentration in silicone fluid (donor solution). No estrone was formed during the permeation process.

was used. In this investigation no estrone was detected in the receptor solution since the dermis was bathing in the saline solution which contained 40% PEG 400 to maintain the sink condition required. It may be related to the observations reported by Baillie et al. (19) that hydroxysteroid dehydrogenase is inhibited by the polyols, like propylene glycol. This observation was

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further confirmed in our laboratory by carrying out the dermal uptake/metabolism experiment with estradiol dissolved in saline solution with and without 40% PEG 400. It was conducted by bathing the dermis in estradiol solution, while the stratum corneum was protected by aluminum foil. The estradiol concentration remained unchanged when the drug was contained in the saline solution having 40% PEG 400 compared to 38% reduction in the estradiol concentration when only saline solution was used.

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Skin Permeation and Metabolism of Estradiol Esters

As proposed by Ando et al. (20), the skin could be considered as a two-ply laminate (Figure 5), which is composed of the stratum corneum and the cutaneous tissue (epidermis & dermis), and the relevant enzymes are assumed to be homogeneously distributed in the cutaneous tissue. Yu et al. (21) reported that the esterase activity is much higher in the outer half layer (epidermis & some dermis) than in the other half of the skin.

Figure 6 shows the hydrolysis of estradiol-17-ester to estradiol in the presence of esterase, which occurs within the cutaneous tissue during the permeation of the prodrugs.

Figure 7 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 epidermis-dermis layers. Estradiol was formed initially at higher rate and then it seemed to be decreasing with time.

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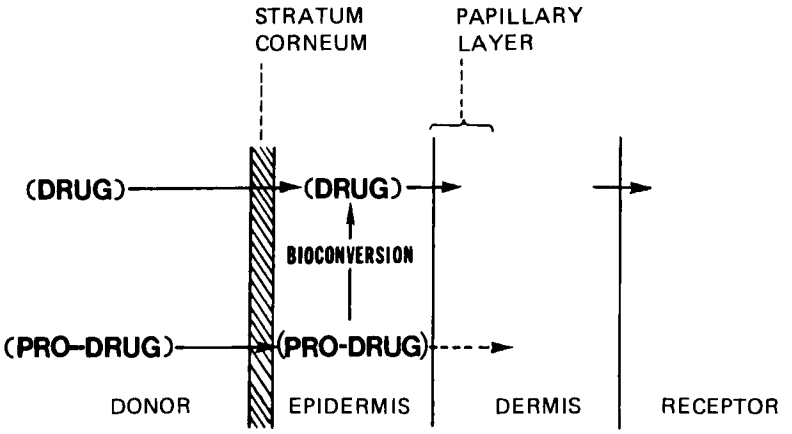

Figure 5: Multilayer model for the simultaneous skin permeation and metabolism of drug and prodrug.

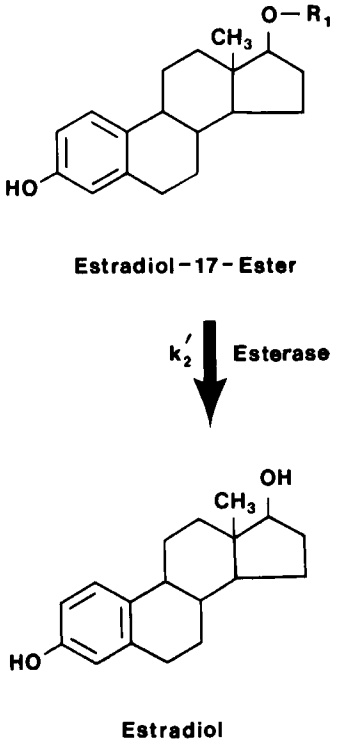

Figure 6: The enzymatic reaction for the metabolism of estradiol-17-esters by esterase in the cutaneous tissue is irreversible with a first-order rate constant of k_2'.

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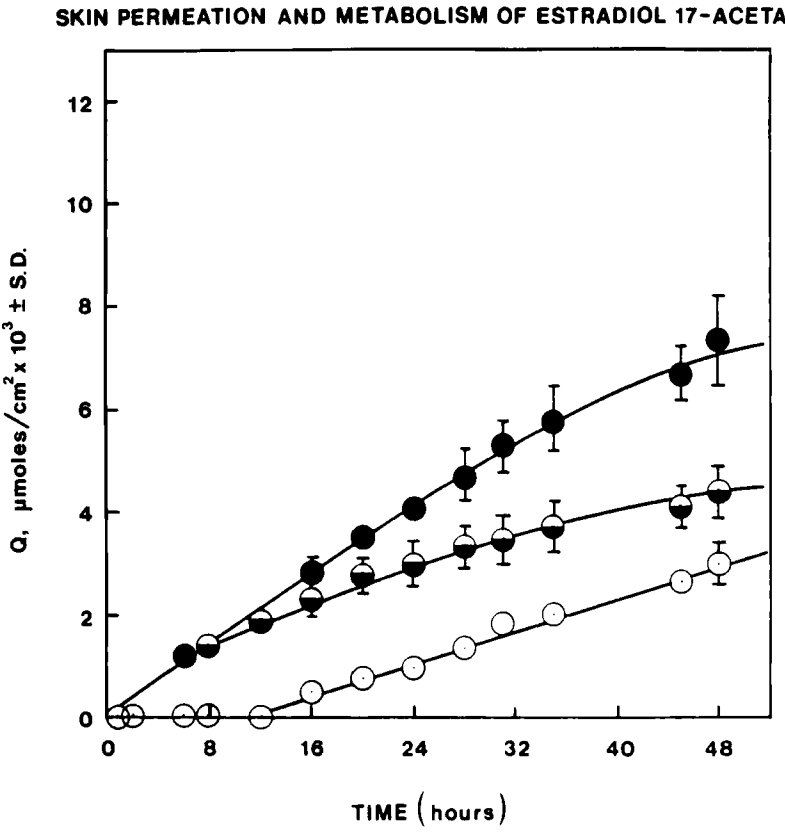

Figure 7: The time course for the skin permeation of estradiol-17-acetate (EA) and its metabolism to estradiol (E). Key: ○ , EA; ◒ , E; and ● , EA + E (sum).

After a lag-time of 12 hr, EA began to appear in the receptor solution at a constant rate of 2.05 (±0.35) x 10^{-8} μmoles/cm^2/sec.

The time course for the formation of estradiol from various estradiol-17-esters during the course of skin permeation is shown in Figure 8. Interesting enough that no estradiol valerate (EV), estradiol heptanoate (EH) and estradiol cypionate (EC) were

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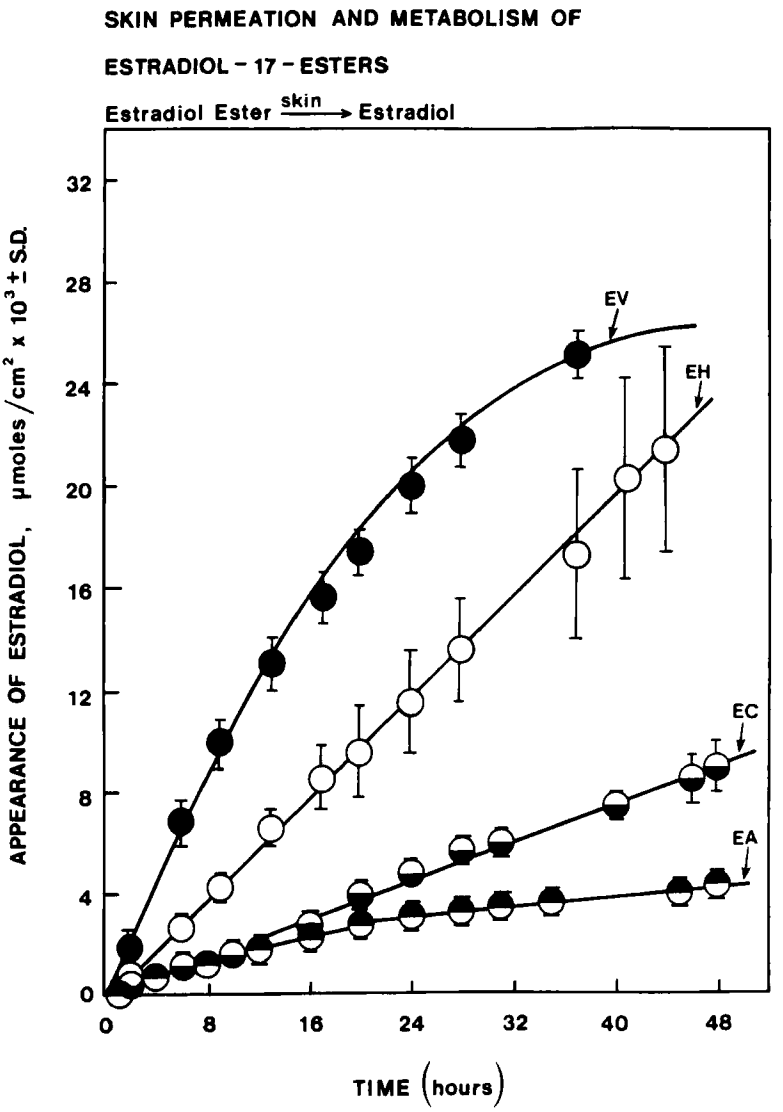

Figure 8: The time course for the formation of estradiol (E) during the skin permeation of various estradiol-17-esters by metabolism. Key: ● , estradiol valerate (EV); ○ ,estradiol heptanoate (EH); ◒ , estradiol cypionate (EC); ◓ , estradiol acetate (EA).

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detected during the course of 48-hr study. From the estradiol appearance data, the rate of skin permeation was calculated to be 22.91 (±0.74) x 10^{-8}, 13.32 (±2.09) x 10^{-8} and 5.97 (±0.38) x 10^{-8} μmoles/cm^2/sec for EV, EH and EC, respectively (Table II).

Estradiol-3,17-diacetate (ED) could be metabolized consecutively by esterase to estradiol-17-acetate (EA) and then to estradiol (E) during its permeation through the skin (Figure 9). The time course for the skin permeation and metabolism of ED is shown in Figure 10. The results indicated that estradiol appears in the receptor solution in less than an hour, while estradiol acetate appears later with a lag-time of 8 hr. The rate of appearance of estradiol seemed to be much faster than that of estradiol acetate initially and then gradually slowed down. And, the formation of estradiol acetate appeared to be at a constant rate of 27.35 (±4.84) x 10^{-8} μmoles/cm^2/sec. Since there was no ED detected in the receptor solution during the 48-hr study period, the initial rate of permeation for estradiol diacetate could be calculated indirectly from the total appearance of E and EA and found to be 81.90 (±8.91) x 10^{-8} μmoles/cm^2/sec.

Scheuplein and his coworkers (1, 22, 23) have thoroughly investigated the permeation of aliphatic alcohols. The effect of polar and nonpolar groups in the alkanol molecule was examined. As the alkyl chain length increased, the steady-state flux increased to a maximum value at n = 6 (hexanol) and then decreased

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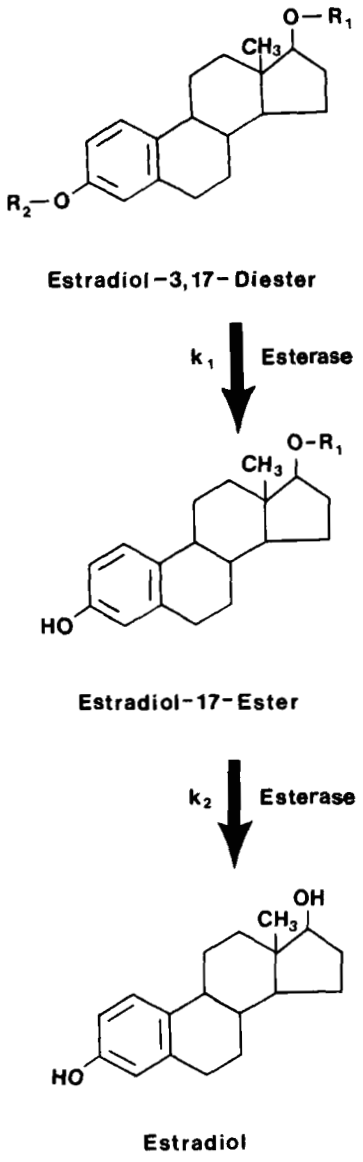

Figure 9: The enzymatic reactions for the metabolism of estradiol-3, 17-diacetate by esterase in the cutaneous tissue are consecutive and irreversible with first-order rate constants of k_1 and k_2.

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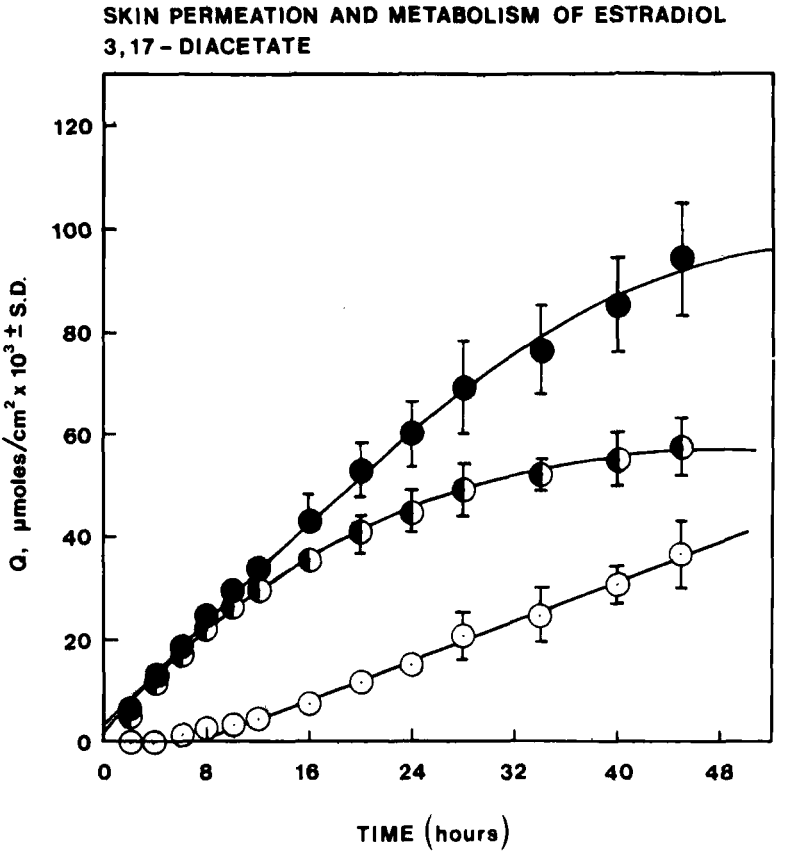

Figure 10: The time course for the skin permeation of estradiol-3, 17-diacetate (ED) and its metabolism to estradiol acetate (EA) and estradiol (E). Key: ○ , EA; ◐ , E; and ● , EA + E (sum). No diacetate was detected in the receptor solution.

when the number (n) of methylene (CH_2) groups was greater than six. Smith (9) recently studied the permeation of hydrocortisone and its 21-ester homologs through hairless mouse skin. It was observed that the skin permeation flux increases with the increase in the alkyl chain length at 21-position and the maximum flux

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is obtained with hydrocortisone hexanoate. In the present investigation with estradiiol-17-esters, the maximum steady-state flux was obtained with estradiol valerate.

The drug concentration in the skin, the rate of permeation, and permeability coefficients of estradiol and various estradiol esters are also summarized in Table II. The rate of appearance of estradiol from the estradiol esters was found to decrease in the order of: diacetate > valerate >heptanoate >cypionate > acetate. There was almost 5-fold increase in the rate of estradiol formation from acetate to valerate, i.e., the increase in the rate of estradiol production with an increase in the alkyl chain length of the esters. But, further increase in the chain length from valerate to heptanoate showed a reverse trend with a decrease in the flux. The fluxes for estradiol valerate and estradiol heptanoate were about 3 and 2 times higher than 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. The flux of estradiol-3,17-diacetate was about 10 times higher than estradiol and about 17 times greater than estradiol-17-acetate.

Dermal Uptake/Metabolism of Estradiol Esters

When the estradiol ester solution in 40% PEG 400/saline was added to the compartment facing the dermis (Figure 2), the drug was considered to be taken up first by the dermis, diffused through the epidermis and, during the course of diffusion, it

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Table II - Skin Permeation Parameters for Estradiol and Estradiol Esters

Drug	C_{skin} [a] (μmoles/cm^3 x 10^3)	dQ/dt (μmoles/cm^2/sec x 10^8)	$\frac{dQ/dt}{C_{skin}}$ (cm/sec x 10^8)
E	190.12	7.87 ± 0.73	41.41
EA	546.65	4.79 ± 0.46	8.77
EV	1181.69	22.91 ± 0.74	19.39
EH	987.31	13.32 ± 2.09	13.49
EC	761.84	5.97 ± 0.38	7.83
ED	7825.23	81.90 ± 8.91	10.47

a) $C_{skin} = (C_{sat.\ soln.\ in\ silicone\ fluid}) \times (k_{skin/silicone\ fluid})$

was metabolized by the esterase to form estradiol. The estradiol formed was then diffusing back into the solution.

The time course for the uptake of estradiol-3,17-diacetate by the dermis in the female hairless mouse abdominal skin and for the formation of estradiol-17-acetate and estradiol is shown in Figure 11. The results indicated that the disappearance of estradiol diacetate (ED) and the formation of estradiol acetate (EA) are rather rapid. Within 18 hr, ED was completely disappeared, while EA reached the peak concentration. The appearance of estradiol occurred at a slower rate. Further analysis suggested that the initial disappearance of ED from the dermal solution can be described fairly well by a first-order kinetics with a k_1 value of 155.5 x 10^{-3} hr^{-1} (Figure 12). Similarly, the disappearance of estradiol-17-acetate, after reaching the peak level at 16 hrs (Figure 11), could also be

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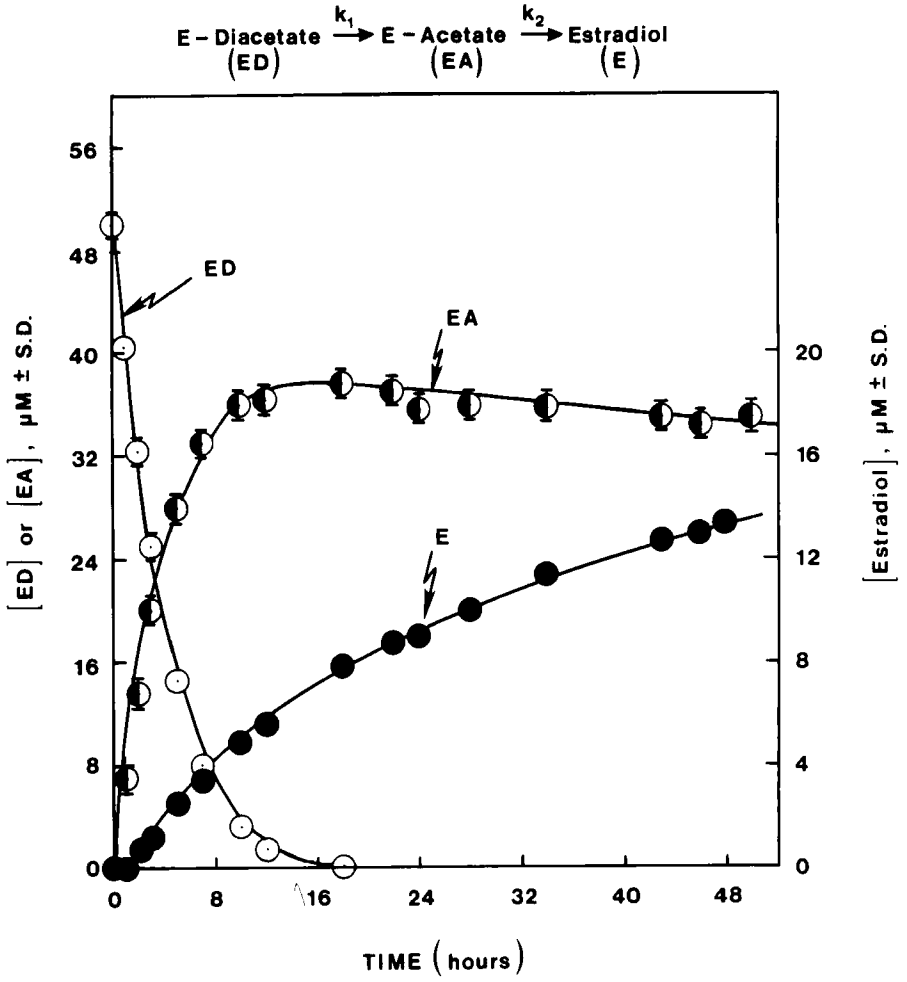

Figure 11: The time course for the uptake of estradiol-3, 17-diacetate (ED) by the dermis and the formation of estradiol acetate (EA) and estradiol (E). Key: ○, ED; ◐, EA; and ●, E.

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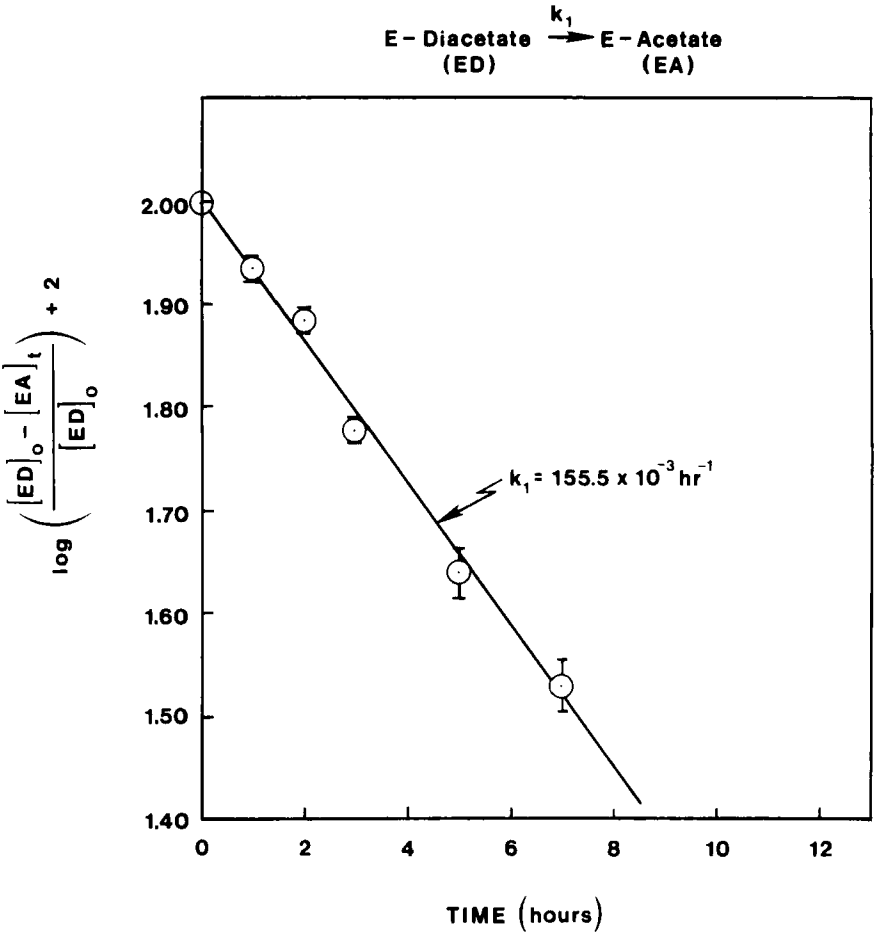

Figure 12: A first-order kinetic plot for the initial phase of the disappearance of estradiol-3, 17-diacetate (ED) from the dermal solution. A first-order rate constant, K_1, of 155.5 x $10^{-3}hr^{-1}$ was determined.

described by a first-order kinetics with k_2 value of 6.88 x 10^{-3} hr^{-1} (Figure 13). The first-order rate constant k_1 (155.5 x 10^{-3} hr^{-1}) was about 22 times greater than k_2 (6.88 x 10^{-3} hr^{-1}), which suggested that the metabolism of estradiol-17-acetate to estradiol appears to be the rate-limiting step in the formation of estradiol from estradiol-3,17-diacetate.

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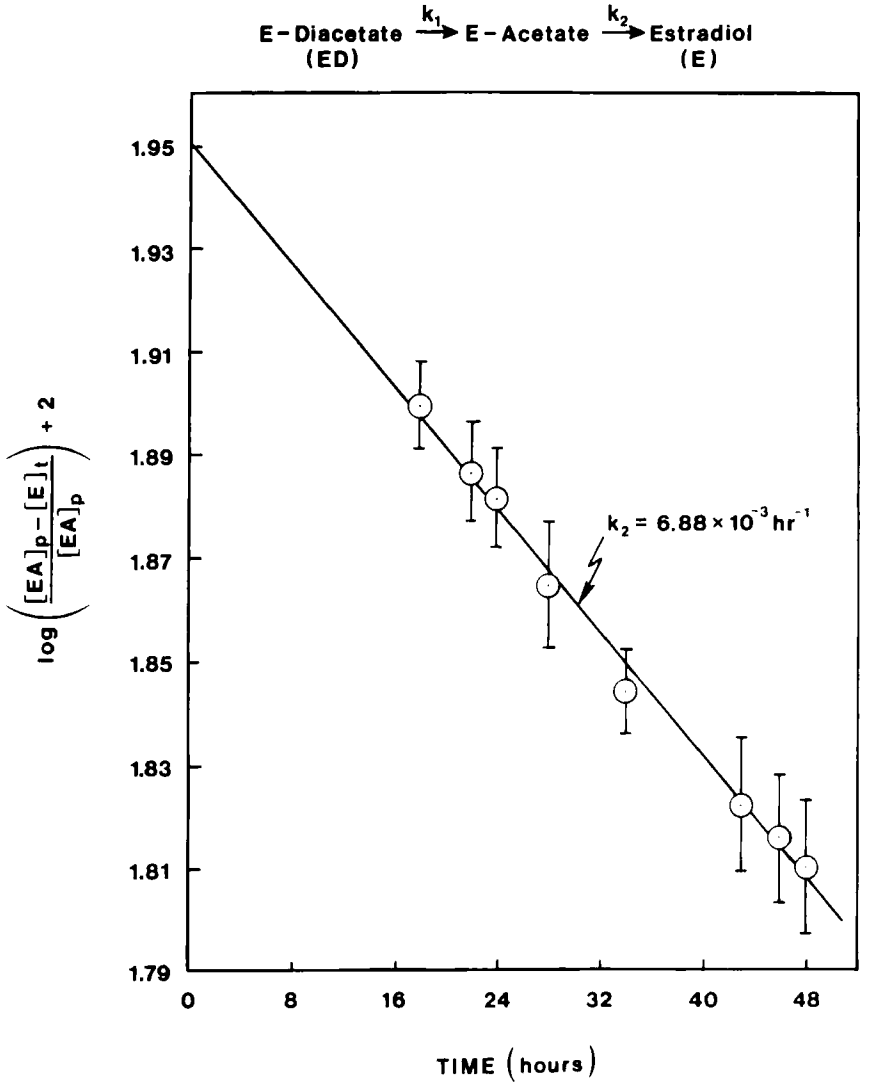

Figure 13: A first-order kinetic plot for the disappearance of estradiol acetate (EA) after termination of the metabolism of estradiol-3, 17-diacetate (ED). A first-order rate constant, k_2, of 6.88 x $10^{-3}hr^{-1}$ was determined.

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As the result of the resonance effect in the ring A of estradiol-3,17-diacetate, the acetate group is expected to be less stable at 3rd position than at 17th position (i.e., 3-acetate is more labile for hydrolysis than 17-acetate) and, therefore, estradiol-3,17-diacetate is theoretically expected to be metabolized first to estradiol-17-acetate and then to estradiol (Figure 9). To verify this sequence of kinetic process, the dermal uptake and metabolism of estradiol-17-acetate was also conducted (estradiol-3-acetate is not available commercially). Figure 14 shows the time course for the disappearance of estradiol-17-acetate and the appearance of estradiol. The results indicated that the disappearance of estradiol-17-acetate can be described also by a first-order kinetics (Figure 15) with a first-order rate constant k_2' of 9.25×10^{-3} hr^{-1}. The k_2' ($9.25 \times 10^{-3} hr^{-1}$) agrees fairly well with the k_2 value ($6.88 \times 10^{-3} hr^{-1}$) obtained earlier (Figure 13). The agreement substantiates the reaction scheme outlined in Figure 9. The HPLC data also showed the coincidence of the estradiol acetate peak from the metabolism of estradiol-3,17-diacetate with the peak from the estradiol-17-acetate reference standard.

The first-order rate constants k_2' determined for the dermal uptake/metabolism of various estradiol-17-esters are summarized in Table III. The results suggested that the first-order rate constants for the hydrolysis of the ester from the 17th position on estradiol molecule is decreased first from acetate (9.25×10^{-3} hr^{-1}) to valerate (4.19×10^{-3} hr^{-1}) and then increased

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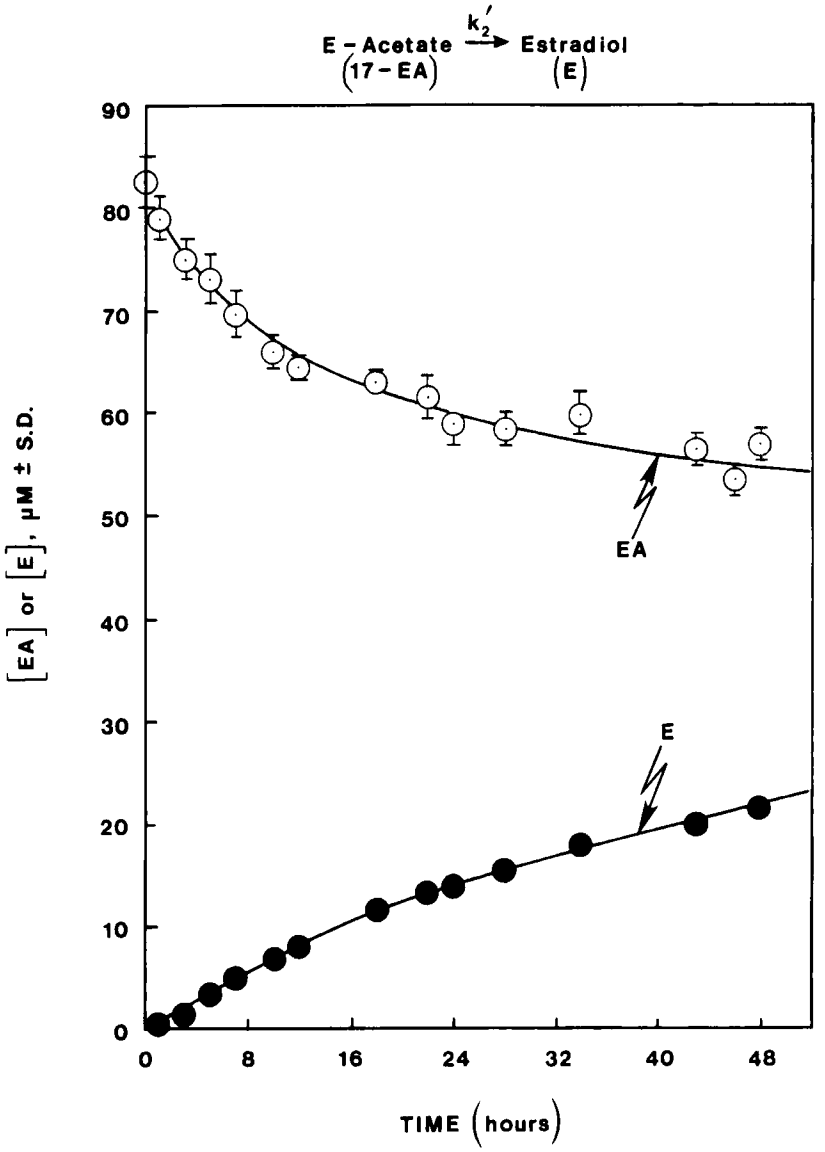

Figure 14: The time course for the uptake of estradiol-17-acetate (EA) by the cutaneous tissue and the formation of estradiol (E). Key: ○, EA and ●, E.

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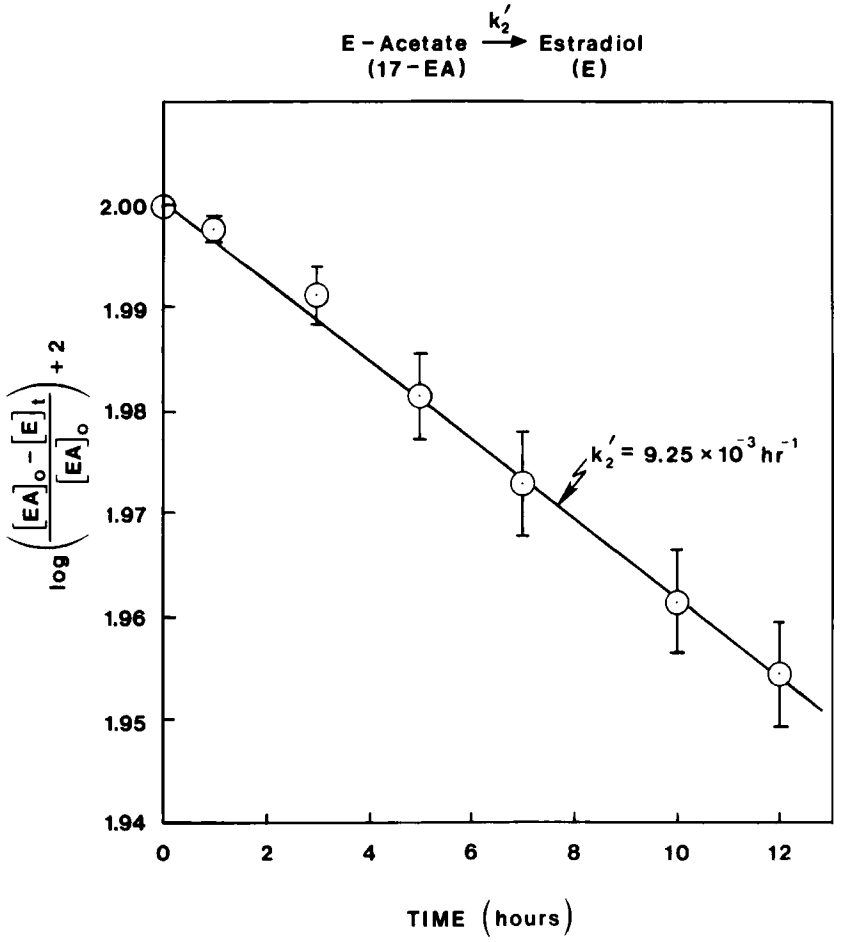

Figure 15: A first-order kinetic plot for the initial disappearance of estradiol-17-acetate (EA) from the dermal solution. A first-order rate constant, k_2, of 9.25 x $10^{-3}hr^{-1}$, was determined.

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Table III - First-order Rate Constants for Dermal Uptake/metabolism of Various Estradiol-17-Esters

17-Esters	k_2 ($hr^{-1} \times 10^3$)
Acetate	9.25
Valerate	4.19
Heptanoate	6.84
Cypionate	17.93

as increasing the alkyl chain length (6.84 x 10^{-3} hr^{-1} for heptanoate and 17.93 x 10^{-3} hr^{-1} for cypionate).

The studies on the hydrolysis of steroid esters by an nonspecific carboxylesterase from pig liver microsomes reported the similar observations (24). The investigations aimed to determine whether the hydrolysis of steroid ester was catalyzed by a steroid-specific esterase or by a widely-distributed non-specific esterase, like carboxylesterase. Of those esters studied, which included androgens, corticosteroids, and estrogens, no relationship could be established between the rate of hydrolysis and the chemical structure of steroid esters. However, it was noted that the majority of esters follow Michaelis-Menten kinetics with the Michaelis constants falling in the range of 10^{-5} to 10^{-6}M.

Kinetic Analysis of the Bioconversion of Estradiol Esters

To analyze the kinetics of bioconversion of estradiol esters to estradiol during the course of skin permeation, the skin

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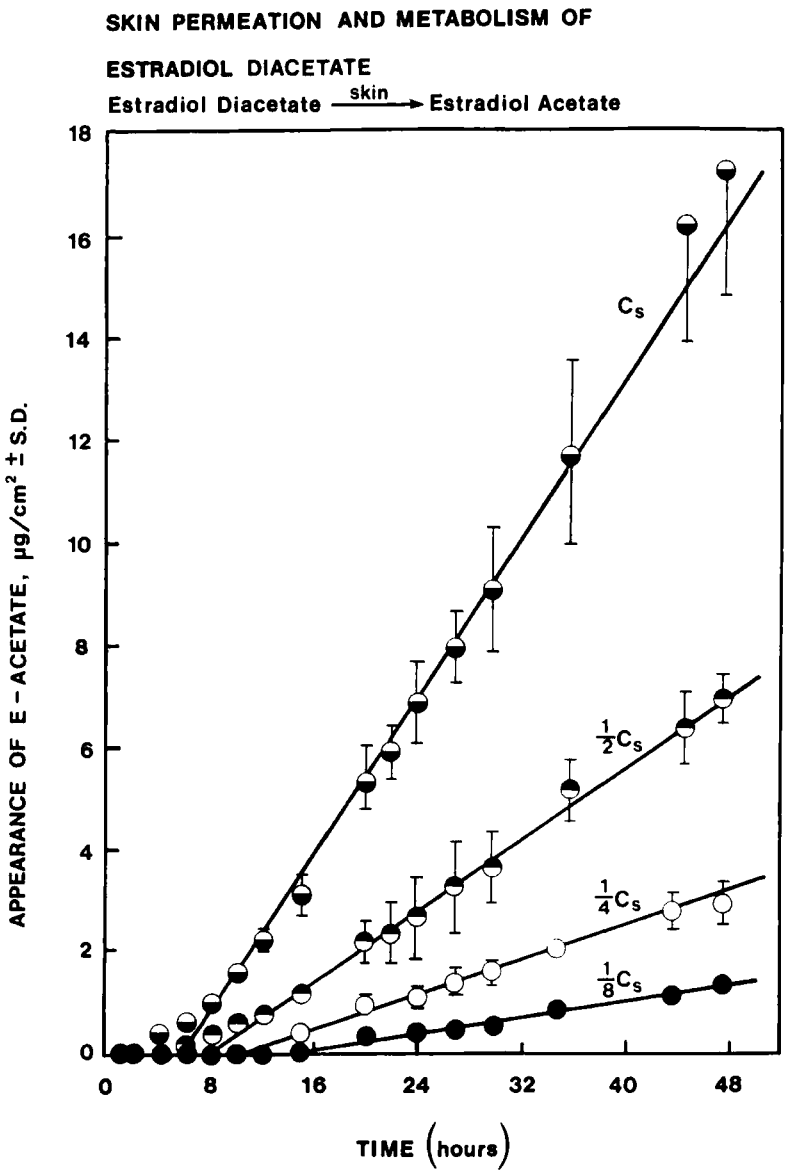

Figure 16: The time course for the appearance of estradiol-17-acetate in the receptor solution at 4 concentration levels of estradiol-3, 17-diacetate (ED).

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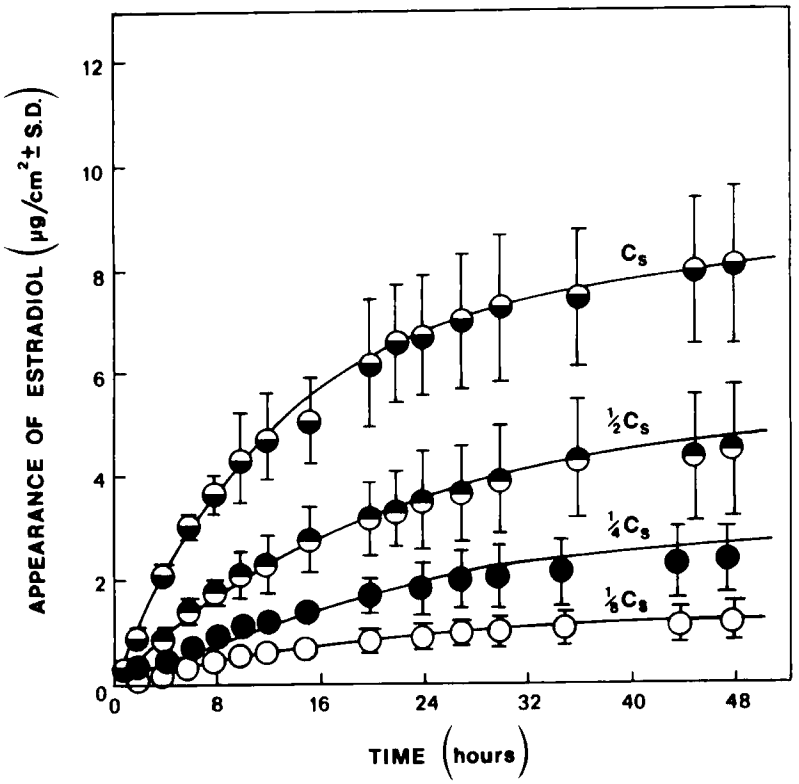

Figure 17: The time course for the appearance of estradiol in the receptor solution at 4 concentration levels of estradiol-3, 17-diacetate.

permeation and metabolism of estradiol-3,17-diacetate and estradiol-17-valerate were studied at four different concentrations. The time course for the appearance of estradiol and estradiol-17-acetate in the receptor solution from estradiol-3,17-diacetate was plotted as a function of the diacetate concentrations in the donor solution at 100, 50, 25, and 12.5% of the saturation solubility (Figures 16 and 17). The results suggested that the formation of estradiol-17-acetate is a linear

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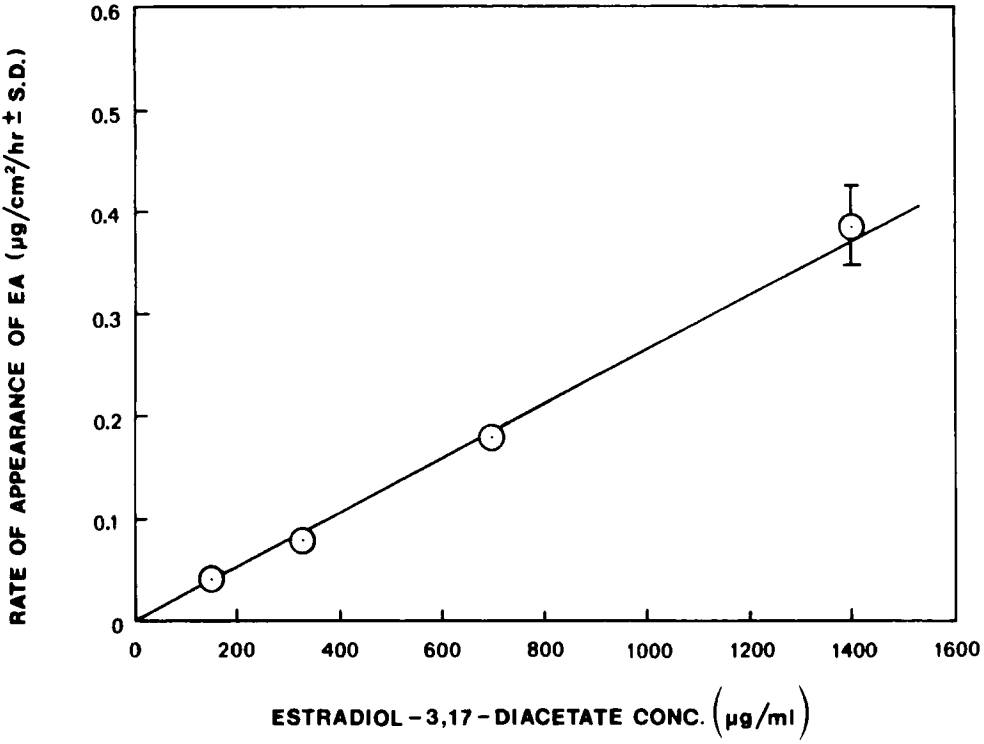

Figure 18: Linear relationship between the rates of appearance of estradiol-17-acetate (EA) in the receptor solution and the concentrations of estradiol-3, 17-diacetate (ED) in the donor solution.

function of the time (Figure 16), while the formation of estradiol is increased with the time, but not at a linear function (Figure 17). In both cases, the bioconversion products were increased in proportional to the concentration of diacetate. The initial rates obtained from the slopes were found to be linearly dependent upon the substrate (ED) concentrations for both estradiol-17-acetate (Figure 18) and estradiol (Figure 19).

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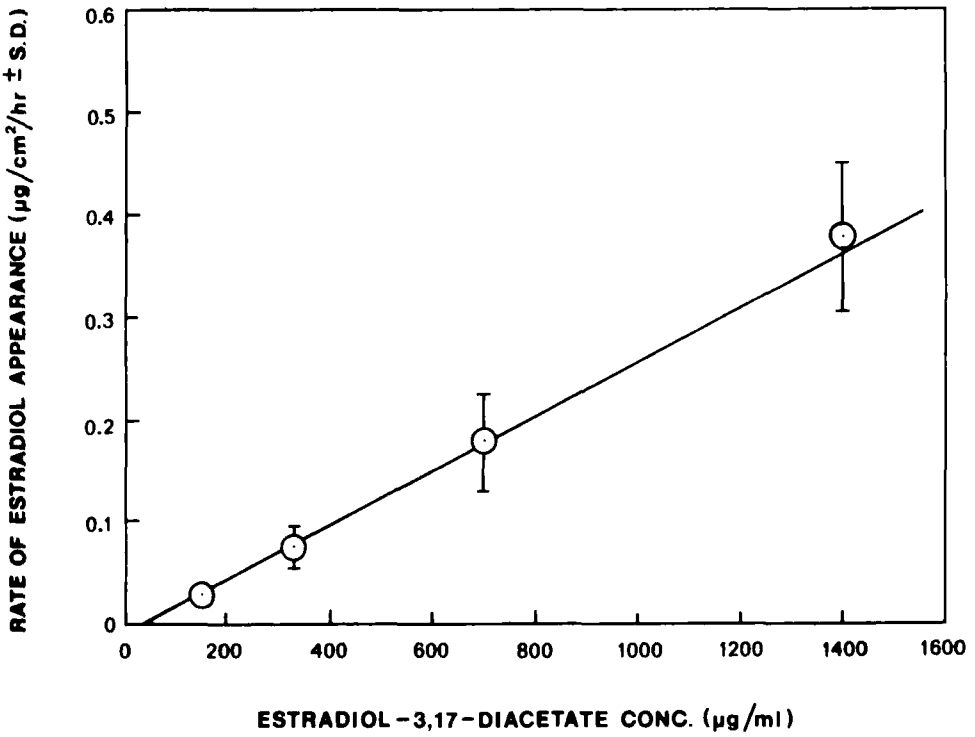

Figure 19: Linear relationship between the rates of appearance of estradiol (E) in the receptor solution and the concentrations of estradiol-3, 17-diacetate (ED) in the donor solution.

Due to the limited solubility of estradiol-17-acetate in the silicone fluid (Table I), the kinetics of bioconversion of the 17-ester of estradiol was investigated using estradiol-17-valerate. As observed earlier in the formation of estradiol from 3, 17-diacetate, the appearance of estradiol from the 17-valerate was also observed not at a linear fashion (Figure 20). Similarly, the initial rate of appearance of

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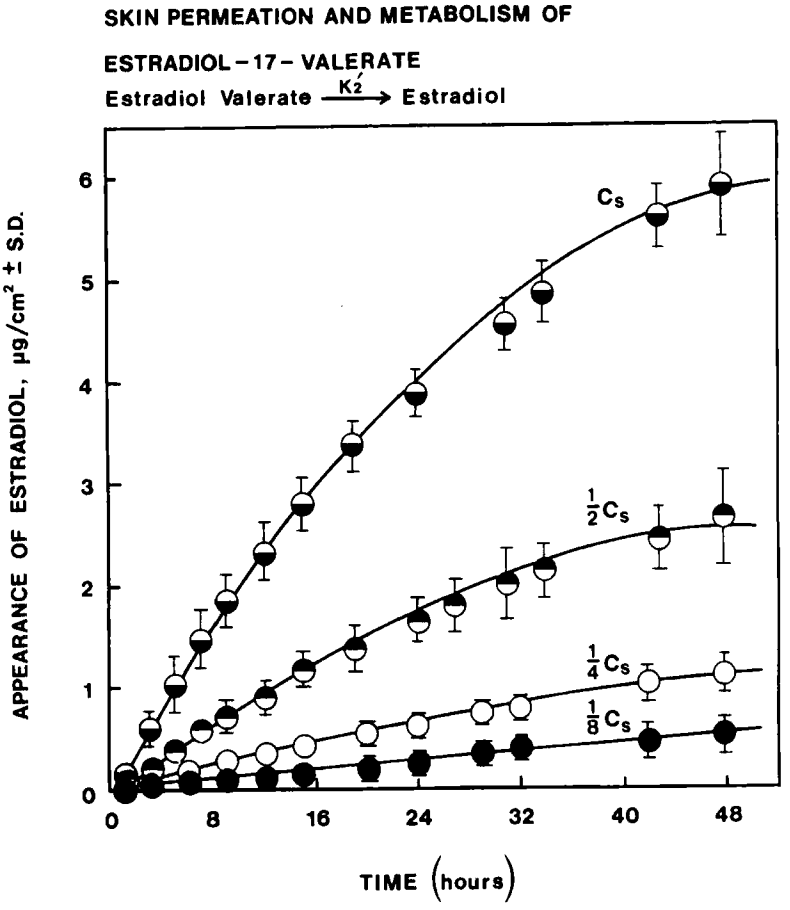

Figure 20: The time course for the appearance of estradiol (E) in the receptor solution at 4 concentration levels of estradiol-17-valerate (EV).

estradiol was also linearly dependent upon the concentration of estradiol-17-valerate (Figure 21).

The kinetics of bioconversion for both 3,17-diacetate and 17-valerate was also submitted for Michaelis-Menten kinetic analysis. Linear relationship was observed. Due to the

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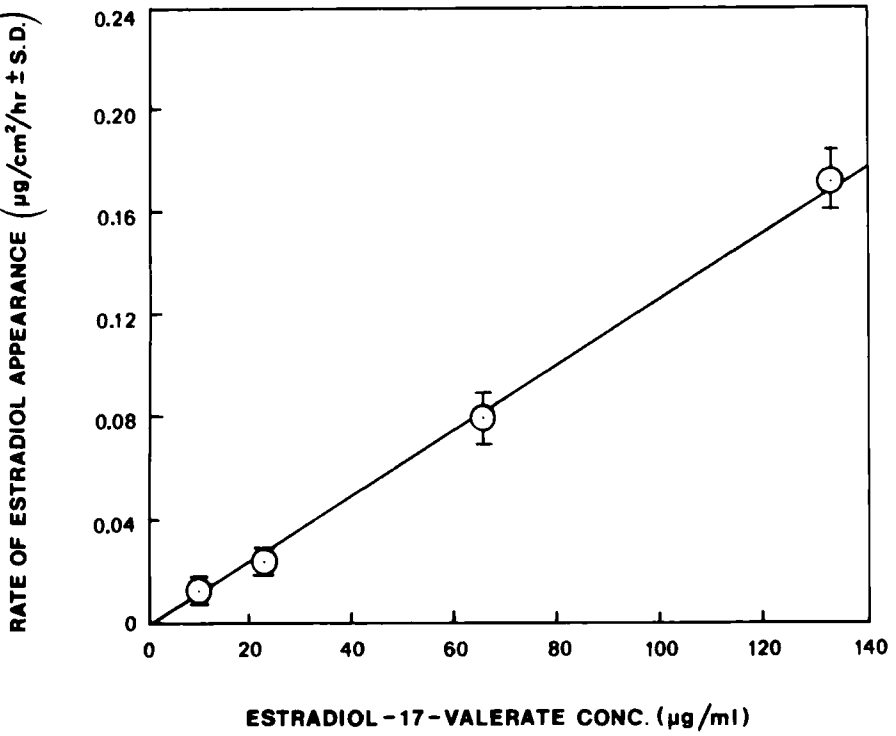

Figure 21: Linear relationship between the rates of appearance of estradiol (E) in the receptor solution and the concentrations of estradiol-17-valerate (EV) in the donor solution.

involvement of permeation, which could well be the rate-limiting step in the course of permeation-metabolism process of estradiol esters, the results will be discussed later after further analysis of the meaning of the data.

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FOOTNOTES

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2. Sigma Chemical Company, St. Louis, Missouri.

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3. Courtesy of E. R. Squibb & Sons, Inc., Princeton, New Jersey 08540.
4. Dow Corning 360 Medical fluid, Dow Corning Corporation, Midland, Michigan 48640.
5. Fisher Scientific Company, Fairlawn, New Jersey.
6. J. T. Baker Chemical Company, Phillipsburg, New Jersey.
7. Burdick & Jackson, Muskegon, Michigan.
8. Nanopure, Sybron/Barnstead, Boston, Massachusetts.
9. Waters Associates, Milford, Massachusetts.
10. Kratos Analytical Instruments, Ramsey, New Jersey.
11. Houston Instruments, Austin, Texas.
12. Crown Glass Company, Somerville, New Jersey.
13. Jackson Laboratories, Bar Harbor, Maine.
14. Millipore Corporation, Bedford, Massachusetts.

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