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LONG-TERM SKIN PERMEATION KINETICS OF ESTRADIOL (I): EFFECT OF DRUG SOLUBILIZER-POLYETHYLENE GLYCOL 400

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ABSTRACT

A skin permeation cell was recently developed to overcome the deficiencies noted in the currently available in vitro diffusion cells, and to provide a cell design which is suitable for studying the long-term drug permeation kinetics through the skin and is also sensitive enough for assessing the mechanisms of skin permeation by a high performance liquid chromatography.

To evaluate the role of drug reservoir concentration in the kinetics of skin permeation as well as to maintain a sink condition in the receptor solution, the water-miscible polyethylene glycol (PEG) 400 was incorporated into the saline solution to act as a

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solubilizer to enhance the aqueous solubility of the relative water-insoluble estradiol. The equilibrium solubility of estradiol at 37°C was observed to increase exponentially as increasing the volume fraction of PEG 400 added.

The rates of permeation of estradiol across the male and female hairless mouse, whole and stripped skins excised freshly from the abdominal region, were measured at various PEG concentrations and the permeability coefficients were determined. The permeability coefficients were found to decrease as increasing the PEG concentration. A linear relationship was established between the permeability coefficients and the skin/solution partition coefficients and the steady-state diffusivity was calculated. Effect of sex was assessed.

The rate of permeation and the permeability coefficient across the stratum corneum were determined, using the multi-laminated diffusional resistance model. Results demonstrated that the stratum corneum acts as the rate-limiting barrier in the skin permeation of estradiol and the incorporation of up to 40% $^{v}/_{v}$ PEG 400 does not influence the barrier properties of stratum corneum, even though PEG 400 has been found to affect the aqueous solubility, permeability coefficient, and skin/solution partition coefficient of estradiol.

INTRODUCTION

Design of a delivery system for the long-term topical administration of a drug represents a challenging opportunity for many biomedical scientists. In the past decades, attention has focused on studying the role of physicochemical parameters in drug absorption from topical formulations like creams or ointments. Solubility of the drug in a vehicle, partition coefficient of the drug between the vehicle and the stratum corneum, pH and nature of the vehicle, and

pka and molecular characteristics of the drug have been consistently identified as the important factors affecting the efficiency of percutaneous absorption (1-3). For instance, vehicles with a relatively high solvent power may produce a preferential retention of the drug in the vehicle.

The literature reports on the influence of vehicles on skin penetration are confusing and sometimes contradictory to one another which could be due to one or a combination of the following facts that (a) different animal species and experimental designs were used in the evaluations, (b) different methodologies were used for the estimation of skin penetration, (c) lack of understanding of any potential drug-vehicle interactions and of the functions of various vehicles (4,5) and (d) lack of consideration of the thermodynamics in the interpretation of results (6,7).

The primary physicochemical factors in relating a drug with a vehicle appear to be the solubility and diffusivity of the drug in the vehicle and the rate of release of the drug from the vehicle. The efficiency of various vehicles in promoting the skin permeation of a drug can be reasonably predicted on the basis of their effects on (a) the hydration of stratum corneum or (b) the activity of the bound water in the stratum corneum, which could modify the stratum corneum/vehicle partition coefficient (8).

This investigation intends to report our findings of the effect of solubilizer, like polyethylene glycol, on the kinetics of skin permeation of a drug with low skin permeability, like estradiol.

EXPERIMENTAL

Materials:

Estradiol[1], polyethylene glycol (PEG) 400[2], and acetonitrile[3]

(distilled-in-glass HPLC grade) were used as obtained. HPLC grade water was prepared freshly in the laboratory[4].

Skin Permeation Cell

An in vitro skin permeation cell was designed (Fig. 1) and constructed[5] for the investigation of long-term skin permeation kinetics. Each cell consisted of two cylindrical half-cells in mirror image. Each of the half-cells was composed of a solution compartment, which was enclosed inside a water-jacket compartment and measured 0.9 cm in diameter and 3.8 cm in length and yielded a cell volume of 3.5 ml. Each half-cell was equipped with one sampling port (∿2.0 cm long), which can be tightly closed with a matched glass stopper, and a depression, which measured 10mm in diameter and 4mm in height, in the solution compartment as the rotating platform for a star-head magnet[6] (8.5mm in diameter; 6mm in height). The magnets stirred the solution in the half-cells at a constant rate of 600 rpm by an external synchronous driving unit[7]. Both donor and receptor compartments were thermostated at constant temperature by circulating 37°C water through the water-jacket compartment by an external circulator[2]. After assembly with skin sample (sandwiched in-between the donor and receptor compartments), the whole skin permeation cell became a totally enclosed system with an effective skin surface for permeation maintained at 0.6362 cm^2.

Skin Preparation

The skin sample used in the present investigations was a full-thickness skin freshly excised from a 5-7 weeks old hairless mouse (HRS/J strain)[8]. The hairless mouse was sacrificed by snapping the spinal cord at the neck. A square section of the abdominal skin (∿3x3cm) was surgically removed from the animal and

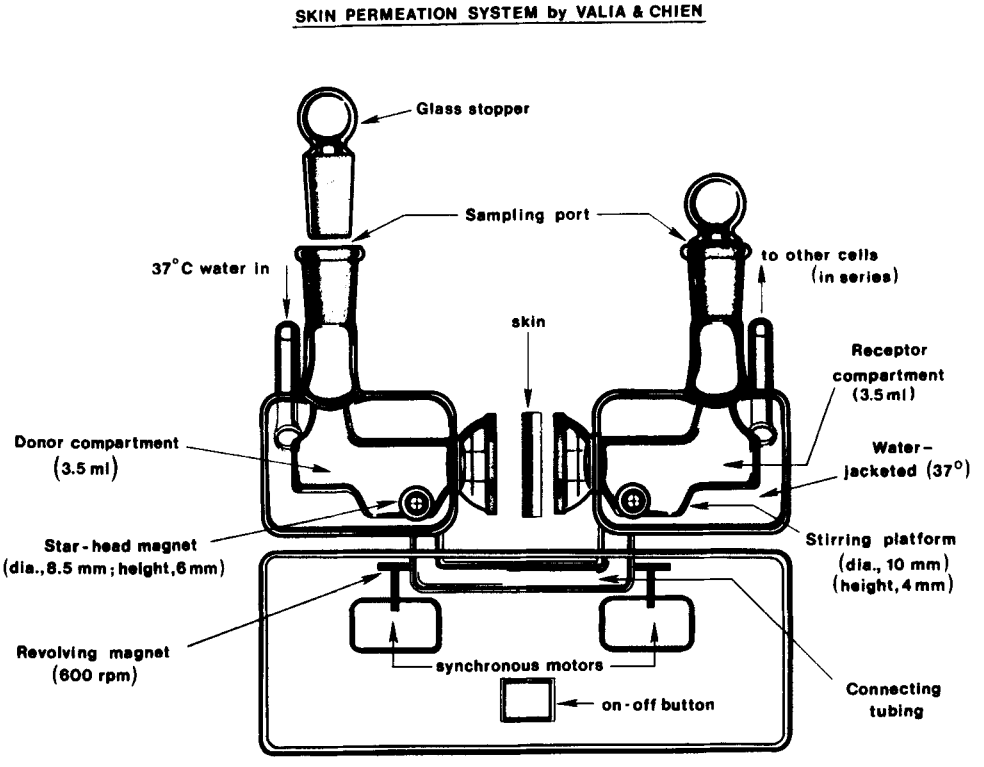

Figure 1: Diagrammatic illustration of the skin permeation cell developed (see the text for detailed description of the design).

the subcutaneous tissue and blood vessels were cleaned (9,10).

Analytical Methods

A liquid chromatograph[9] equipped with a reciprocating pump (model 6000A), injector (model U6K), UV detector (model 440, cell volume 15.6 µl), a reverse-phase µBondapak C_{18} column with a guard column containing 37-50 µm Bondapak C_{18}/Corasil packing material, and an Omniscribe recorder[10] was used in this investigation. A combination of acetonitrile and water, at a ratio of 50:50, was used as the mobile phase. At ambient condition, a flow rate of 1.5 ml/min was used, yielding an operating pressure of 2,000 psi. The UV de-

tector was operated at the wavelength of 280 nm at a sensitivity of 0.005 AUFS. Another UV detector[11] (model 773, cell volume 12 µl) was also used at 205 nm and 0.010 AUFS. Determination of estradiol concentration in the sample solution was carried out by first measuring the peak height of estradiol peak at a retention time of 4.9 minutes and then computing the concentration (µg/ml) from the calibration curve constructed from standard solutions.

Determination of Drug Solubility

An excess amount of drug was equilibrated with 10 ml of a saline solution containing various volume fractions of PEG 400 at 37° for 24 hours with constant shaking in a shaking waterbath[2]. The saturated drug solution was then quickly filtered through a Teflon filter[12]. The drug concentration in the filtered drug solution was then determined, after proper dilution, by HPLC.

Determination of Skin/Solution Partition Coefficient

Skin preparations of known weight (∼100-200 mg) were equilibrated with estradiol solutions (∼1.5 µg/ml in 3 ml saline solution containing 0-40% $^{v}/_{v}$ of PEG 400) in screw-capped test tubes shaken for 24 hours in a water bath[2] at 37°C. The initial and equilibrium drug concentrations in the solutions were assayed by HPLC. The volume of skin preparations was calculated from the skin weight before equilibration and its average density reported in the literature (1.04 gm/ml) (11).

By definition, the skin/solution partition coefficient ($K_{s/w}$) can be determined from the following relationship:

$$K_{s/w} = \frac{C_s}{C_w} = \frac{M_s/V_s}{M_w/V_w} \qquad \text{(Eq. 1)}$$

where, C_s and C_w are the drug concentrations in the skin and in the aqueous solution, respectively, at equilibrium; M_s and M_w are the masses of estradiol in the skin and in the aqueous solution, respectively, at equilibrium; V_s and V_w are the volumes of the skin and the aqueous solution, respectively. The M_s may be expressed in terms of M_w and M_w^o, the total mass of drug initially present in the aqueous solution:

$$M_s = M_w^o - M_w \qquad \text{(Eq. 2)}$$

Therefore, Eq. 1 may be rearranged to give:

$$K_{s/w} = \frac{(M_w^o - M_w)}{M_w}\frac{(V_w)}{(V_s)} = \frac{C_w^o - C_w}{C_w} \qquad \text{(Eq. 3)}$$

where C_w^o is the initial concentration of drug in the aqueous phase. By assaying C_w^o and C_w, $K_{s/w}$ may be determined by Eq. 3.

Measurement of Drug Permeation Profiles

The skin sample was mounted between the two half-cells of the skin permeation cell immediately after excise. A drug solution of known concentration or a suspension of known loading dose in a given combination of PEG 400 and saline was filled into the donor compartment, and a same or a different combination of PEG 400 and saline (without the drug) was added into the receptor compartment. At each of the predetermined intervals, a 50 µl sample was withdrawn from the receptor solution and analyzed immediately by HPLC for estradiol concentration in the sample solution. The concentration of estradiol in the donor solution was also determined in the same way at the end of each experiment by first filtering through a Teflon filter[12]. The drug permeation measurement was so designed that the drug concentration in the receptor compartment always remained under sink condition

(12), except those solutions containing low concentration of PEG 400. Each experiment was carried out in triplicate.

Effect of Skin Stripping

Immediately following sacrifice by cervical dislocation, the abdominal surface of the hairless mouse was stripped with a cellophane tape[13] for 25 times. It was carried out by securing the animal on a table and the abdominal skin was stripped by placing the tape on the stratum corneum surface and moving the thumb back and forth a few times, with a pressure as uniform as was possible (13). A fresh piece of the tape was used for each stripping.

RESULTS AND DISCUSSION

Estradiol was selected for investigation in the present skin permeation study, since this naturally-occurring therapeutically-active hormone has been known to be inactivated by extensive hepatic first-pass elimination following oral administration (14). On the other hand, estradiol was reported to be absorbed through the skin, though its rate of skin permeation was extremely low (15).

Solubilization of Estradiol

The aqueous solubility of estradiol is extremely low (3 μg/ml) (15), which could be improved by addition of a water-miscible hydrophilic polymer like polyethylene glycol into the aqueous solution as the solubilizer for estradiol (12) and, thus, the reservoir drug concentration in the donor solution can be varied while a sink condition in the receptor solution can be maintained. By doing so, only a small solution volume will be needed in the studies and a high analytical sensitivity can be achieved. PEG 400 was reported to be an excellent solubilizer for many steriods (12, 16). In a solution containing 100% PEG 400 at 37°C, estradiol has achieved

a solubility of 116 mg/ml (16). In the present investigation, it was observed that the aqueous solubility of estradiol increases exponentially as increasing the volume fraction of PEG 400 in the saline solution (12). The results are illustrated in Figure 2.

Effect of Solubilizer on Skin Permeation of Estradiol

The cumulative amount of drug permeating through a unit surface area of skin (Q_s) can be expressed mathematically by the following relationship (1):

$$Q_s = P_s (C_D - C_R) t \qquad \text{(Eq. 4)}$$

where P_s is the skin permeability coefficient; and C_D and C_R are the drug concentrations in the donor (D) and the receptor (R) solutions, respectively.

When the drug concentration in the donor solution (C_D) is maintained at a level greater than the equilibrium solubility (i.e., $C_D > C_e$) and the drug concentration in the receptor solution (C_R) is maintained under the sink condition (i.e., $C_R << C_e$), Equation (4) can be simplified to:

$$Q_s = P_s C_e t \qquad \text{(Eq. 5)}$$

and a constant skin permeation profile should be yielded. The rate of skin permeation is then defined by:

$$\frac{Q_s}{t} = P_s C_e \qquad \text{(Eq. 6)}$$

As expected from Equation (5), when the estradiol concentration in the donor solution was maintained at a level which was greater than its equilibrium solubility, a constant skin permeation profile was achieved (Figure 3). The rate of skin permeation (Q_s/t), which was measured from the slope of Q_s vs. t plots (Equation 5), was

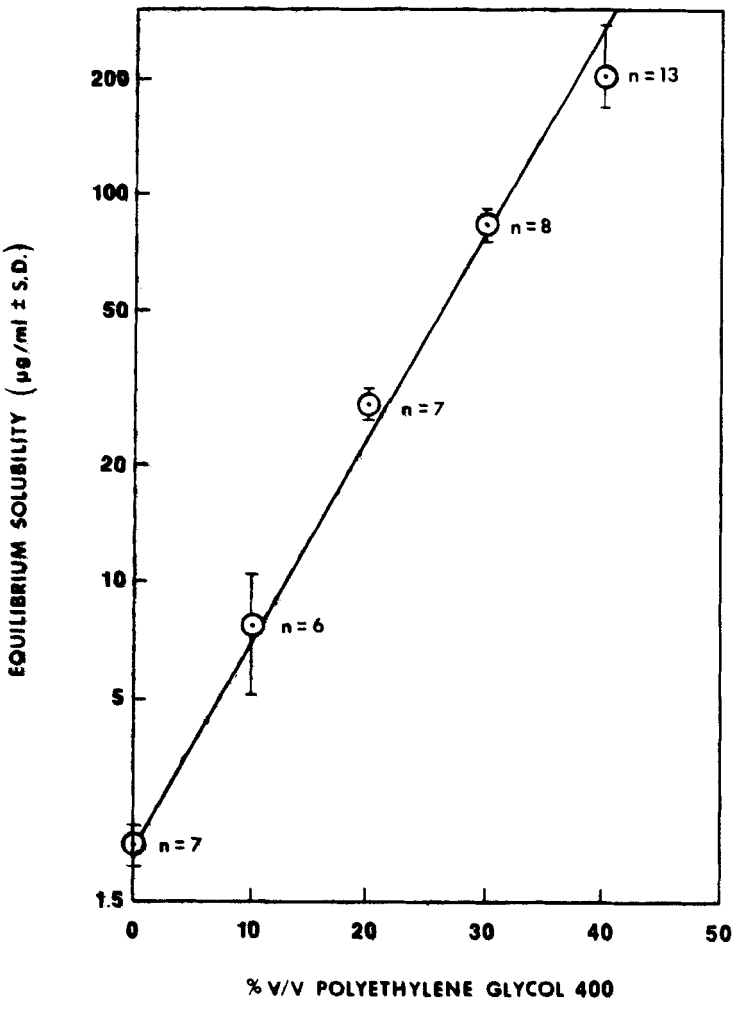

Figure 2: Semilogarithmic relationship between the equilibrium aqueous solubility of estradiol and the volume fraction of PEG 400 incorporated into the saline solution (n designates the number of determinations).

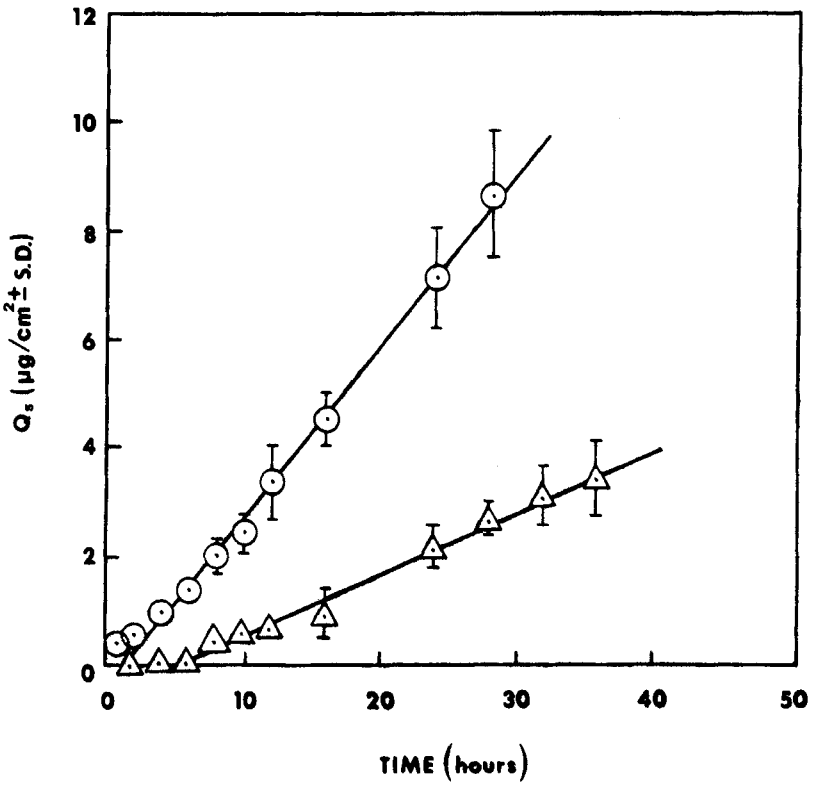

Figure 3: Permeation profile of estradiol across male hairless mouse skin at constant reservoir concentration (in donor solution) Keys: Δ saline solution, ○ saline solution containing 40% $^{v}/_{v}$ PEG 400. Each data point represents the mean and one standard deviation of 3 determinations.

found to increase with the addition of 40% $^{v}/_{v}$ PEG 400 in the saline solution. As expected from Equation (6), the increase in the skin permeation rate (Q_s/t) was observed to be dependent upon the equilibrium solubility (C_e) of estradiol in the PEG 400/saline solutions (Figure 4), where a linear relationship was established for the data generated in 20 to 40% $^{v}/_{v}$ PEG 400 solutions. On the other hand, the rate of skin permeation of estradiol across the fe-

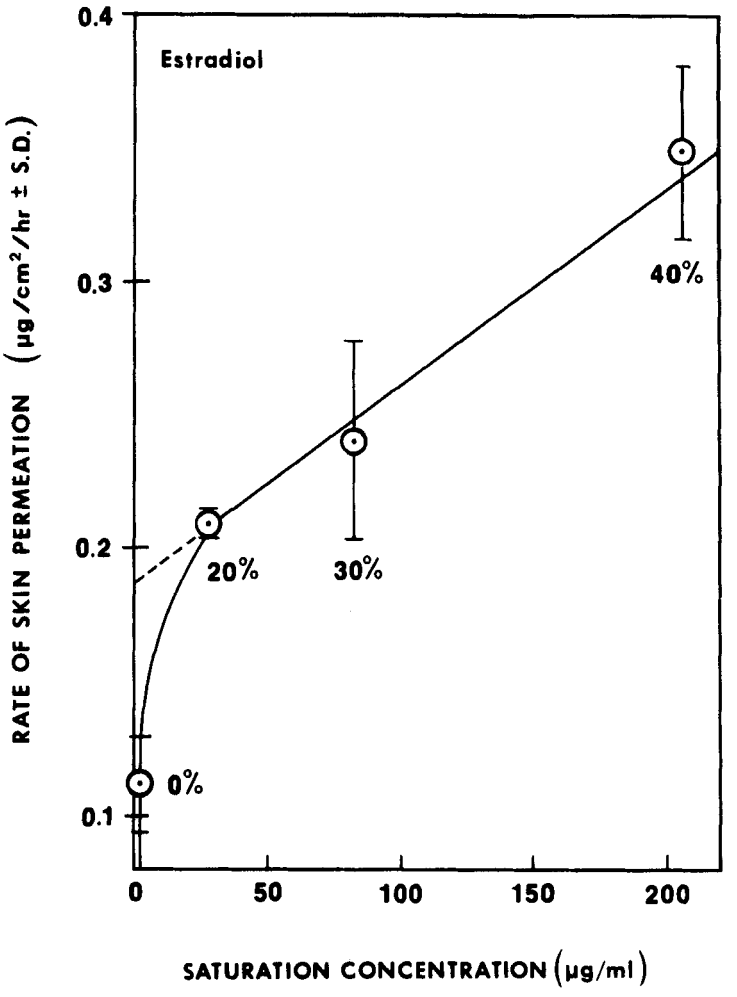

Figure 4: Dependency of the skin permeation rate of estradiol across the male hairless mouse skin on the saturation concentration of estradiol in the donor solution. Each data point represents the mean and one standard deviation of 3 determinations where the % under the data points represents the volume fraction of PEG 400 used.

male hairless mouse skin showed no dependency on the equilibrium solubility of estradiol in the same PEG 400 concentration range (20 - 40% $^{v}/_{v}$) (Figure 5).

The low rate of skin permeation of estradiol observed in the saline solutions containing less than 20% PEG 400 (Figures 4 & 5) could be attributed to the fact that the sink condition is difficult to maintain in these solutions.

Effect of PEG 400 on the permeability coefficient (P_s) of estradiol across male and female hairless mouse skin can be determined by the use of Equation (7):

$$P_s = \frac{Q_{s/t}}{C_e} \qquad \text{(Eq. 7)}$$

The results (Table 1) indicated that the skin permeability coefficient (P_s) decreases as increasing the volume fraction of PEG 400 in the saline solution.

The primary requirements in topical medication are that a drug incorporated in a vehicle should be capable of release and should reach the skin surface at a rate adequate for absorption, so a therapeutic dose can be administered. There are two general approaches which can be applied in the development of a vehicle which may enhance the skin penetration of drug: one is to incorporate an agent in the vehicle that could affect the barrier function of the stratum corneum so as to promote the penetration of a therapeutic compound (17, 18); and another approach is to alter the physicochemical characteristics of the vehicle so as to affect the delivery of the drug from the vehicle to the skin (19, 20). The major factors in the second approach are the solubility and diffusivity of the drug in the vehicle and the rate of interfacial partitioning

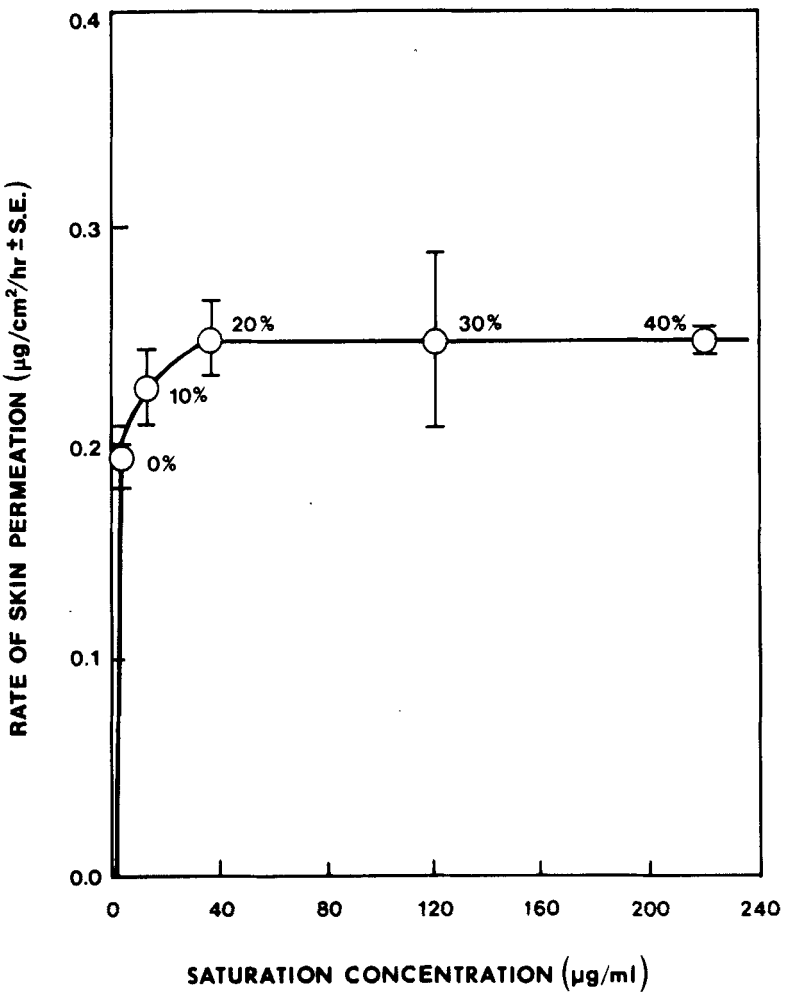

Figure 5: Dependency of the skin permeation rate of estradiol across the female hairless mouse skin on the saturation concentration of estradiol in the donor solution. Each data point represents the mean and one standard error of 3 determinations where the % under the data points represents the volume fraction of PEG 400 used.

TABLE 1

Effect of PEG 400 on Permeability Coefficient (P_s) of Estradiol Across Hairless Mouse Skin

PEG 400 (% v/v)	P_s (cm/hrx10^3 ± S.D.) Male	Female
0	62.96 ± 10.12	80.91 ± 19.67
10	19.90 ± 5.05	17.45 ± 3.83
20	7.12 ± 0.11	6.85 ± 0.99
30	2.81 ± 0.50	2.22 ± 1.03
40	1.86 ± 0.32	1.11 ± 0.06

of the drug molecules from the vehicle onto the skin (2, 19). Incorporation of PEG 400 into the donor solution is expected not only to enhance the equilibrium solubility of estradiol in the vehicle, which leads to a reduction of the skin/solution partition coefficient, but also to reduce the solution diffusivity due to the increase in viscosity. The combination of these effects may result in the increase or the decrease of the rate of skin permeation of estradiol or produce no effect at all.

The effect of the variation in PEG 400 concentration in the receptor solution on the rate of skin permeation was also examined. When the volume fraction of PEG 400 in the donor solution was kept at constant (40% v/v) to provide a constant reservoir concentration of estradiol while the volume fraction of PEG 400 in the receptor

solution varied from 10 to 40% $^{v}/v$ to see the effect on the rate of skin permeation. The results indicated that a constant skin permeation profile is also achieved; however, the rate of skin permeation of estradiol was observed to increase as increasing the concentration of PEG 400 in the receptor solution (Figure 6). The increases could be rationalized as due to the increase in the rate of interfacial partitioning of estradiol from the dermis to the receptor solution as increasing the PEG concentration.

Effect of Solubilizer on Skin/Solution Partition Coefficient and Skin Diffusivity

The partition coefficient of estradiol from PEG/saline solution to skin was found to reduce as increasing the concentration of PEG 400 in the saline solution (Table 2); it is the results of the increase in the aqueous solubility of estradiol following the addition of PEG 400 in the solution (Figure 2) against a constant solubility in the skin tissue.

The effect of PEG 400 on the skin/solution partition coefficient was observed to occur on both the abdominal and dorsal skins, though the mean values of partition coefficient toward the abdominal skin were frequently greater than those toward the dorsal skin (Table 2).

It is interesting to observe that a linear relationship (Figure 7) exists between the skin permeability coefficient (P_s) and the skin/solution partition coefficient ($K_{s/w}$) as expected from the following relationship:

$$P_s = \frac{D_{ss}}{h_s} K_{s/w} \qquad \text{(Eq. 8)}$$

where D_{ss} is the steady-state diffusivity and h_s is the thickness of the skin.

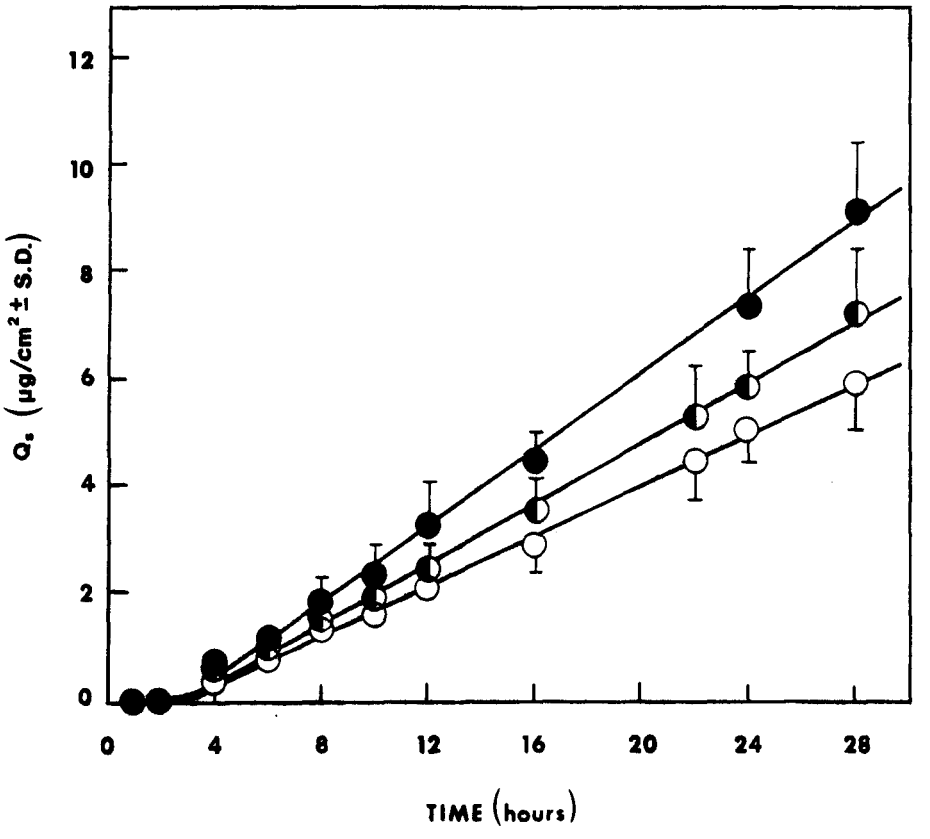

Figure 6: Effect of the variation in PEG 400 concentration in receptor solution on the rate of skin permeation across the male hairless mouse skin. Keys: ○ 10%, ◐ 30%, and ● 40% $^{v}/_{v}$ PEG 400. (PEG 400 concentration in the donor solution was maintained at 40%). Each data point represents the mean and one standard deviation of 3 determinations.

TABLE 2

Effect of PEG 400 on the Skin/Solution Partition Coefficient of Estradiol

PEG 400 (% v/v)	Partition Coefficient[1] (Mean ± S.D.)			
	Abdominal Skin[2]		Dorsal Skin[2]	
	Male	Female	Male	Female
0	58.90 ± 3.95	85.56 ± 2.10	36.01 ± 21.29	65.54 ± 4.33
10	15.20 ± 1.63	19.13 ± 7.88	9.97 ± 1.15	16.90 ± 3.80
20	9.02 ± 0.83	6.90 ± 0.76	5.66 ± 1.77	8.81 ± 0.98
30	6.84 ± 0.50	5.37 ± 0.72	3.60 ± 1.48	6.13 ± 2.36
40	2.53 ± 0.19	2.15 ± 0.17	1.56 ± 1.70	4.98 ± 0.89

1) Triplicate determinations

2) Hairless mouse, 5-7 week old

If h_s is known or predetermined, the steady-state diffusivity (D_{ss}) may be calculated from the slope of the P_s vs. $K_{s/w}$ plots as follows:

$$D_{ss} = \text{slope} \times h_s \qquad \text{(Eq. 9)}$$

The results in Figure 7 indicate that the linear relationship between P_s and $K_{s/w}$ has a slope value of 1.143×10^{-3} and 0.963×10^{-3} cm/hr, respectively, for male and female hairless mouse skin. Taking the literature h_s value of 0.0379 cm (11), the steady-state diffusivity (D_{ss}) can be determined from the slope as 1.204×10^{-8} and 1.013×10^{-8} cm^2/sec, respectively, for male and female hairless mice. The observation suggested that the steady-state diffusivity of estradiol across the hairless mouse skin is fairly constant and basically independent of the sex difference.

Skin Permeation Profile from Unsaturated Estradiol Solution

When the PEG 400/saline solutions contained a constant concentration of estradiol at a level below its saturation solubility in the pure saline solution (2 μg/ml), a constant rate of skin permeation was still obtained as observed earlier in the case of saturated solution (Figure 3), but the rate of skin permeation of estradiol was lower and observed to decrease as increasing the volume fraction of PEG 400 (Table 3). This behavior was found to be in a reverse trend as compared to that observed with a saturated solution in the donor compartment (Figures 3-5). This observation can be explained by the fact that the partition coefficient for the interfacial partitioning of estradiol from the solution toward the skin decreases when the volume fraction of PEG 400 in the saline solution increases (Table 2). In the case of a saturated solution,

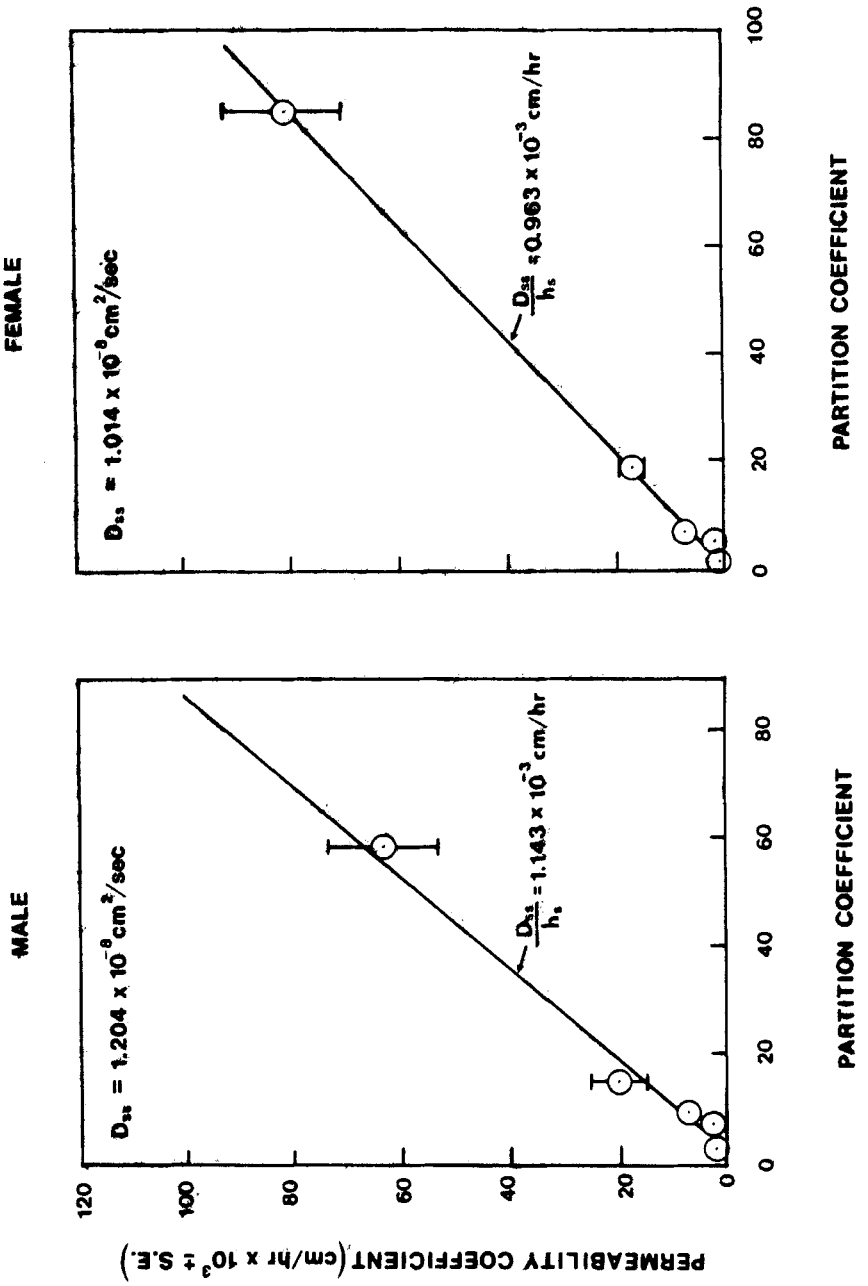

Figure 7: Linear relationship between the skin permeability coefficient (P_s) of estradiol across the male and female hairless mouse skin and the partition coefficient ($K_{s/w}$) of estradiol from PEG/saline solution to the intact abdominal skin. Each data point represents the mean and one standard deviation of 3 determinations.

TABLE 3

Effect of PEG 400 on the Rate of Permeation[1] of Estradiol from Unsaturated Solution[2]

PEG 400[3] (% v/v)	Rate of Skin Permeation ($\mu g/cm^2/hr \times 10^2 \pm$ S.D.)
0	4.91 ± 0.20
20	1.87 ± 0.71
30	1.21 ± 0.60
40	1.00 ± 0.03

1) Male hairless mouse abdominal skin

2) Estradiol concentration in the donor solution = 2 μg/ml

3) Both donor and receptor solutions contain the same PEG 400 concentration

the effect of decreasing partition coefficient on skin permeation was compensated by the increase in estradiol concentration by PEG solubilization (Figure 2), while in the case of a non-saturated solution a constant estradiol concentration (2 μg/ml) was maintained in the donor solution.

Skin Permeation Profile of Estradiol Across a Stripped Skin

The effect of stratum corneum on the skin permeability of estradiol was evaluated by studying the skin permeation across a stripped skin from the hairless mouse. Results indicated that the skin permeation profiles of estradiol across the stripped skin (no stratum corneum) also follow the same linear relationship, as de-

fined by Equation (5), as does the whole skin (with stratum corneum) (Figures 3 and 8). Compared to the data generated earlier in the whole skin (Table 4), stripping appears to promote substantially the skin permeability of the rather impermeable estradiol by elimination of the rate-limiting stratum corneum. In contrast to the behavior observed with the whole skin, the removal of stratum corneum made the skin lose its inherent barrier properties and the skin permeation rate of estradiol became sensitive to the variation of PEG concentration in the donor solution (Table 4). The rate of permeation of estradiol across the stripped skin was observed to increase proportionally as increasing the saturation concentration of estradiol in the donor solution, while the rate of permeation across the whole skin stayed very much independent of the increase in the saturation estradiol concentration applied (Figure 9). The observation further suggests that the stratum corneum presents a great diffusional resistance to the permeation of estradiol across the skin.

The skin/solution partition coefficient of estradiol was also determined with stripped skin at various PEG 400/saline combinations. Results indicated that there is no difference in partitioning behavior between whole skin and stripped skin.

Permeability of Stratum Corneum

The diffusional resistance across the stratum corneum (R_{sc}) can be determined, mathematically, from the diffusional resistance across the whole skin (R_{ws}) and the diffusional resistance across the viable skin (R_{vs}) by the following relation (21):

$$R_{sc} = R_{ws} - R_{vs} \qquad \text{(Eq. 10)}$$

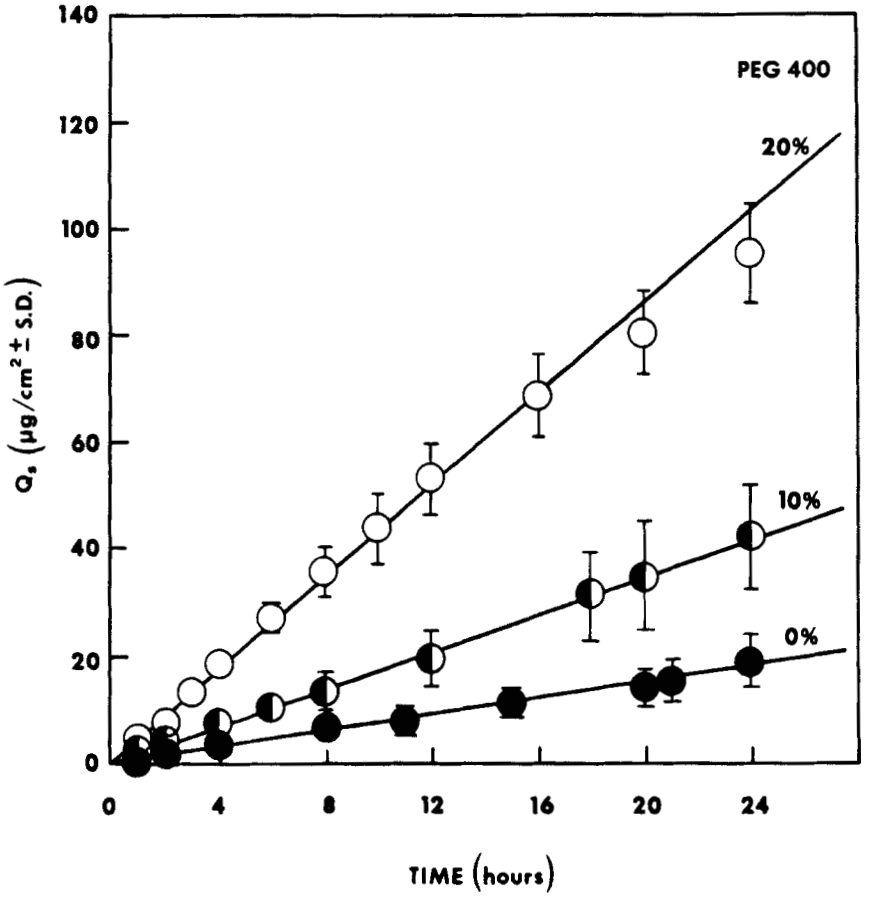

Figure 8: Effect of PEG 400 on the rate of permeation of estradiol through the hairless mouse skin without stratum corneum. Each data point represents the mean and one standard deviation of 3 determinations.

where R_{vs} is the sum of the diffusional resistances across the viable epidermis (without stratum corneum) and dermis. Equation (10) is valid if the permeation across the whole skin or viable skin is the rate-limiting step in the course of skin permeation study. It can be accomplished by maintaining the hydrodynamic diffusion boundary layers on both sides of the skin barrier at a thickness which is

TABLE 4

Effect of PEG 400 on the Rate of Permeation of Estradiol through Hairless Mouse Skin[1)]

PEG 400 (% v/v)	Rate of Skin Permeation ($\mu g/cm^2/hr \pm$ S.D.)	
	Whole Skin[2)]	Viable Skin[3)]
0	0.192 ± 0.025	0.728 ± 0.140
10	0.225 ± 0.032	1.782 ± 0.414
20	0.247 ± 0.032	4.124 ± 0.409
30	0.245 ± 0.103	4.925 ± 0.500
40	0.247 ± 0.008	5.639 ± 0.812

1) Female (5-7 weeks old); abdominal skin

2) Whole skin = stratum corneum + viable skin

3) Stratum corneum was removed by stripping 25 times

negligibly small. This experimental condition has been achieved with the in vitro skin permeation system developed for this investigation (22).

Theoretically, the diffusional resistance of a membrane (R) is related to the reciprocal of the permeability of the membrane (P); so, Equation (10) can be expressed alternatively by:

$$\frac{1}{P_{sc}} = \frac{1}{P_{ws}} - \frac{1}{P_{vs}} \qquad \text{(Eq. 11)}$$

where the P_{sc}, P_{ws}, and P_{vs} stand for the permeability coefficient across the stratum corneum, whole skin and viable skin, respectively.

Using Equation (11), the permeability coefficient across the

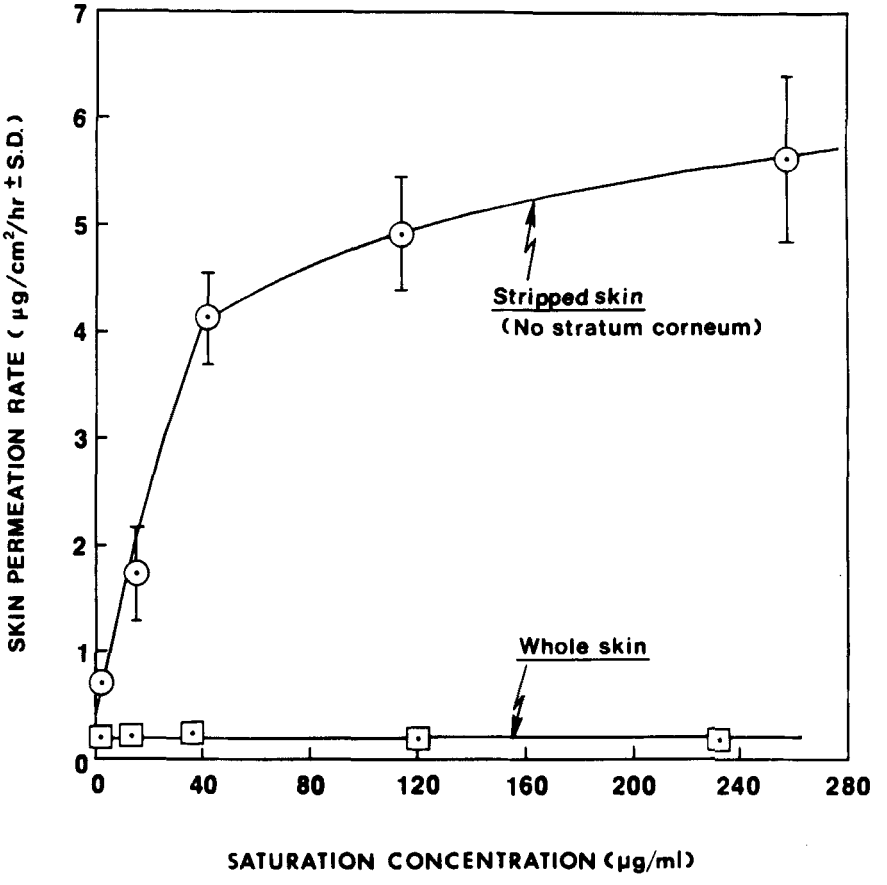

Figure 9: Effect of stratum corneum on the concentration dependency of the skin permeation rate of estradiol through female hairless mouse. Each data point represents the mean and one standard deviation of 3 determinations.

stratum corneum (P_{sc}) at various PEG 400 concentrations can be calculated from the correspondent P_{ws} and P_{vs} values (Table 5). Results indicated that the P_{sc} value decreases as increasing the PEG 400 concentration in the donor solution, as due to the increased solubilization of estradiol in the saline solution (Figure 2). The data show that the P_{sc} values are very much in agreement with the P_{ws}

TABLE 5

Effect of PEG 400 on Permeability Coefficient (P_{sc}) of Estradiol Across Stratum Corneum[1)]

PEG 400 (% v/v)	P_{sc} [2)] (cm/hr x 10^3 ± S.D.)
0	115.06
10	20.53
20	7.37
30	2.34
40	1.17

1) Female hairless mouse
2) Calculated from Equation (11)

values in magnitude at various PEG 400 concentrations (Table 1). The observation further suggests that the stratum corneum is the principal permeability barrier in the course of skin permeation; so, the permeation of estradiol across the stratum corneum has a permeability coefficient very close to the permeability coefficient across the whole skin.

The permeability coefficient of a skin tissue is, theoretically, related to the rate of permeation across the skin tissue (Equation 7); if it is the case, the rate of permeation across the stratum corneum $(Q/t)_{sc}$ can be determined from the following relationship (Equation 12) when the rates of permeation across the whole skin, $(Q/t)_{ws}$, and the viable skin, $(Q/t)_{vs}$, are mea-

TABLE 6

Rate of Permeation of Estradiol Across Stratum Corneum $(Q/t)_{sc}$

PEG 400 (% v/v)	$(Q/t)_{sc}$ [1] ($\mu g/cm^2/hr \pm$ S.D.)
0	0.279
10	0.285
20	0.290
30	0.259
40	0.281
$\bar{X}$	0.279
(± S.D.)	(±0.012)

1) Calculated from Equation (12), using the data in Figure 2 and Table 4.

sured with the same equilibrium drug concentration (C_e):

$$\frac{C_e}{(Q/t)_{sc}} = \frac{C_e}{(Q/t)_{ws}} - \frac{C_e}{(Q/t)_{vs}} \qquad \text{(Eq. 12)}$$

The results are tabulated in Table 6. The data suggest that the rate of permeation of estradiol across the stratum corneum stays very much at a constant value and is invariant with the variation in PEG concentration in the donor solution. This behavior could explain the observation made earlier (Figure 9) that the rate of permeation across the whole skin stays very much at constant while the rate of permeation across the viable skin increases greatly as in-

creasing the saturation estradiol concentration in the applied phase. As expected, the rates of permeation across the stratum corneum, $(Q/t)_{sc}$, are very much smaller than the magnitude of $(Q/t)_{vs}$, the rates of permeation across the viable skin at various PEG concentrations (Table 4); On the other hand, the $(Q/t)_{sc}$ values are approximately the same magnitude as the rates of permeation across the whole skin, $(Q/t)_{ws}$. Again, the results further demonstrate that the stratum corneum acts as the principal barrier in the permeation of estradiol across the skin. This barrier property has not been modified with the addition of up to 40% $^{v}/v$ PEG 400.

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9. Waters Associates, Milford, Massachusetts
10. Houston Instruments, Austin, Texas
11. Kratos Analytical Instruments, Ramsey, New Jersey

12. Millipore Corporation, Bedford, Massachusetts
13. Scotch Brand Cellophane Tape, 3M Company, Minneapolis, Minnesota

REFERENCES

1. Y. W. Chien, "Novel Drug Delivery Systems: Fundamentals, Developmental Concepts and Biomedical Assessments," Marcel Dekker, New York, New York, (1982), pp. 149-217.
2. B. J. Poulsen, "Drug Design", Vol. IV, E. J. Ariens (Ed.), Academic, New York, New York, (1973), pp. 149-192.
3. R. J. Scheuplein and I. H. Blank, Physiol. Rev., 51, 702 (1971).
4. I. H. Blank and R. J. Scheuplein, Brit. J. Dermatol., Suppl., 4, 81, 4 (1969).
5. C. W. Barrett, J. Soc. Cosmet. Chem., 20, 487 (1969).
6. J. G. Wagner, J. Pharm. Sci., 50, 379 (1961).
7. T. Higuchi, J. Soc. Cosmet Chem., 11, 85 (1960).
8. B. Idson, J. Pharm. Sci., 64, 901 (1975).
9. Y. W. Chien, P. R. Keshary, Y. C. Huang, and P. P. Sarpotdar, J. Pharm. Sci., 72, 968 (1983).
10. H. Durrheim, G. L. Flynn, W. I. Higuchi, and C. R. Behl, J. Pharm. Sci., 69, 781 (1980).
11. C. D. Yu, J. L. Fox, N. F. H. Ho, and W. I. Higuchi, J. Pharm. Sci., 68, 1347 (1979).
12. Y. W. Chien and H. J. Lambert, Chem. Pharm. Bull., 23, 1085 (1975).
13. C. R. Behl, E. E. Linn, G. L. Flynn, C. L. Pierson, W. I. Higuchi, and N. F. H. Ho, J. Pharm. Sci., 72, 391 (1983).
14. S. Lyrenas, K. Carlstrom, T. Backstrom and B. Von Schoultz, Br. J. Obstet. Gynaecol., 88, 181 (1981).
15. A. J. Michaels, S. K. Chandrasekaran, and J. E. Shaw, AICHE J., 21, 985 (1975).

16. P. C. Will, R. N. Cartright, and U. Hopfer, J. Pharm. Sci., 69, 747 (1980).

17. R. B. Stoughton, Arch. Dermatol., 91, 657 (1965).

18. R. B. Stoughton and W. Fritsch, Arch. Dermatol., 90, 512 (1964).

19. B. J. Poulsen, E. Young, V. Coquilla, and M. Katz, J. Pharm. Sci., 57, 928 (1968).

20. M. F. Coldman, B. J. Poulsen, and T. Higuchi, J. Pharm. Sci., 58, 1098 (1969).

21. Y. W. Chien, "Novel Drug Delivery Systems: Fundamentals, Developmental Concepts and Biomedical Assessments," Marcel Dekker, New York, New York (1982), pp. 565.

22. Y. W. Chien and K. H. Valia, Development of a Dynamic Skin Permeation System for Long-Term Permeation Studies, Drug Devel. and Indus. Pharmacy, (in press).