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Permeation of estradiol through the skin — effect of vehicles

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Summary

The cutaneous permeation characteristics of estradiol were examined to evaluate the effect of 21 different organic solvents, some of which are commonly used in topical formulations. The steady-state permeation rate (F_{ss}) through excised human abdominal skin mounted in open diffusion cells was compared to a reference consisting of estradiol applied in volatile solvent. F_{ss} varied in the range of 0.001–0.215 $\mu g \cdot cm^{-2} \cdot h^{-1}$ where dimethyl sulphoxide and glycols most effectively increased the permeation.

Estradiol solubility was determined in all the vehicles and the magnitude of the vehicle effect on the skin was quantified in terms of relative apparent diffusion coefficient. Mainly the facilitating effect could be ascribed to a change in the nature of the skin barrier. It appeared that the estradiol flux from propylene glycol vehicles was unaffected by occlusion and smaller changes in the applied propylene glycol amount.

Introduction

Potential rate-determining barriers to cutaneous permeation are: (1) dissolution rate of the drug in the vehicle; (2) diffusion rate of dissolved drug in the vehicle; (3) vehicle–stratum corneum interfacial drug transfer; and (4) drug diffusion in stratum corneum. However, it is widely held that the skin permeation rate is limited by the transport across the stratum corneum (Scheuplein and Blank, 1971). A general approach to affect permeation rate is to incorporate solvents which may have two modes of action. One is to favour a high drug concentration in the stratum corneum

by selecting solvents leading to an increase in the thermodynamic activity of the drug in the vehicle and thereby promoting the interfacial drug transfer into the stratum corneum (Higuchi, 1960, 1978; Poulsen et al., 1978; Ostrenga et al., 1971a and b; Coldman et al., 1969a and b). The other is to include solvents that penetrate the stratum corneum themselves and in this way alter the barrier (Allenby et al., 1969a and b; Stoughton and Fritsch, 1964; Chandrasekaran and Shaw, 1978). Thus the importance of the vehicle in determining topical bioavailability is well documented. In recent years growing interest in using the dermal route for administration of drugs for which a persistent systemic and local effect is desirable has strengthened the need for studies of vehicle effect on percutaneous absorption.

In the present study the effect of various pure organic solvents on the permeation of estradiol was investigated. Estradiol was chosen as a model lipophilic drug because of its potential application in systemic percutaneous therapy in the case of hormonal insufficiencies (Lyrenäs, 1981; Lagrova Weill-Hallé, 1977; Whitehead et al., 1980). The range of solvents was chosen with the expectation that their differences in physical–chemical properties or their possible ability to alter the skin barrier can be demonstrated by their effect on estradiol permeation rate.

In the permeation study, excised human skin mounted in open diffusion cells of the same type as those described by Franz (1975) was used. This in vitro technique relates to the non-occluded skin situation in the living system and allows for demonstration of the importance of individual factors concerning the vehicles (Turi et al., 1979; Iyer and Vasavada, 1979; Shahi and Zatz, 1978).

Materials and Methods

Chemicals

Estradiol [1] and [2,4,6,7-^{3}H]estradiol [2] were used as test substances and different organic solvents were used as vehicles: propylene glycol [3], glycerol [3], methyl salicylate [3], Tween 80 [4], dimethyl sulphoxide [4], hexanetriol(1,2,6) [4], butanediol(1,4) [4], pentanediol(1,5) [4], propanediol(1,3) [4], decanol(1) [4], diethylene glycol [4], triethanolamine [4], triethylene glycol [4], dipropylene glycol [4], polyethylene glycol 400 [5], diethanolamine [6], ethylene glycol [7], ethyl oleate [8], betahistine [9], diisoamylamine [10], 2-amino-4-methylhexane [10].

[1] DAK 63.
[2] Radiochemical Center, Amersham, U.K.
[3] Ph.Eur., III.
[4] Merck-Schuchardt "zur Synthese" quality.
[5] Ph.Nord. 63, II.
[6] DLS 82.
[7] Merck, "zur Analyse" quality.
[8] BDH Chemicals, U.K.
[9] 2-[2-methylaminoethyl]-pyridine; ICN Pharmaceuticals, U.S.A.
[10] Koch-Light Laboratories, U.K.

Preparation of skin membranes

Unselected human abdominal skin obtained at autopsy was separated from the subcutaneous fat. Until use the skin was stored at −18°C for a period not exceeding 2 weeks. A section of skin derived from a single donor yielded 6–8 pieces and thus made it possible to evaluate several vehicles on the same donor simultaneously.

Diffusion cells assembly

The skin membranes were mounted in open glass diffusion cells having an available diffusion area of 1.8 cm^2. The epidermal side of the skin was exposed to ambient laboratory conditions while the dermal side was bathed with receptor medium consisting of 7.5 ml of 0.05 M isotonic phosphate buffer, pH 7.4, with 0.01% mercury chloride. Each cell was placed on a magnetic stirrer in a 37°C incubator. In our laboratory this procedure has been used earlier in studying skin permeation of linoleic acid (Hoelgaard and Møllgaard, 1982) and 5-fluorouracil (Møllgaard et al., 1982).

Preparation of test solutions

To 350 μl of an 0.1% ethanolic solution of estradiol was added 20 μCi of [^{3}H]estradiol. After evaporation to dryness by inert gas the radioactively labelled estradiol was redissolved in 300 μl of a 10% ethanolic solution of the vehicle. The test solutions thus consisting of 0.12% estradiol in vehicle–ethanol solutions (1 : 10) were analyzed for radioactivity.

Permeation procedure

Before application of the test solution the skin was allowed to equilibrate in the cells for one hour. 100 μl of the test solution (e.g. 117 μg estradiol) was spread across the entire surface and the ethanol was allowed to evaporate within few minutes leaving a uniform thin layer of the drug–vehicle solution on the skin surface. At appropriate intervals samples were withdrawn and replaced by fresh receptor medium keeping an infinite sink. The permeation studies of each vehicle were done in duplicate, the data obtained being reproducible within ±20% for skin from the donor.

Radiochemical assays

Tritium quench correction curves were conducted by means of the external standard ratio method using tritium standard kits (Instand; Lumac) and chloroform as a quenching agent. The receptor phase samples were analyzed in a Beckman LS-250 liquid scintillation system after addition of aqueous scintillation fluid (Picoflour 15, Packard).

The test solutions were analyzed in the same way but after dilution with ethanol and evaporation to dryness.

Solubility studies

The solubility of estradiol in the organic solvents was determined at 22°C by adding an excess of estradiol to the solvents in screw-capped test tubes. The tubes

TABLE 1

EFFECT OF SOLVENTS ON THE CUTANEOUS PERMEATION OF ESTRADIOL THROUGH HUMAN SKIN IN VITRO

Vehicle	Flux ($\mu g \cdot cm^{-2} \cdot h^{-1}$)	Lag time (h)	Skin section
Ethylene glycol	0.22	13	A
Propylene glycol	0.12	18	B
Dimethyl sulphoxide	0.14	0	B
Diethylene glycol	0.12	53	C
Propanediol(1,3)	0.11	39	D
Triethylene glycol	0.087	57	A
Dipropylene glycol	0.047	54	A
Glycerol	0.037	26	D
Betahistine	0.028	8	E
Methyl salicylate	0.027	11	F
Ethyl oleate	0.025	14	G
Diisoamylamine	0.019	12	E
2-amino-4-methylhexane	0.018	11	E
Butanediol(1,4)	0.017	43	C
Decanol(1)	0.014	18	G
Triethanolamine	0.010	57	I
Hexanetriol(1,2,6)	0.008	28	D
Diethanolamine	0.007	5	G
Pentanediol(1,5)	0.005	28	C
Polyethylene glycol 400	0.001	–	F
Tween 80	0.001	–	H

were rotated for 3 days, preliminary studies having shown that this period of time was adequate to obtain equilibrium in the most viscous vehicles. The concentration of the saturated solutions was determined spectrophotometrically in a Perkin Elmer 124 spectrophotometer at 281 nm after appropriate dilution with ethanol.

The solubility of estradiol in water and in vehicles, which interfere with the spectrophotometric method, was determined by using tritium-labelled estradiol. This was prepared by crystallization from a 60°C hot solution of 5 g of estradiol and 100 μCi [^{3}H]estradiol in ethanol leading to an activity of about 50,000 dpm/mg.

Results and Discussion

Estradiol permeation

The permeation data obtained for the different test solutions are visualized by plotting the amount of estradiol permeated per cm^2 against time (Figs. 1–4). All runs were in duplicate and regression analyses of the mean values against time, within the steady-state time interval yielded two parameters, steady-state flux and lag time (Table 1).

To account for the variation of skin from different donors a reference, consisting

TABLE 2

VARIATION OF ESTRADIOL PERMEATION THROUGH HUMAN SKIN FROM 9 DONORS BY THE USE OF OPEN DIFFUSION CELLS

Skin section	Flux ($\mu g \cdot cm^{-2} \cdot h^{-1}$)	Lag time (h)	Skin specification		
			Sex	Age	Storing time (days)
A	0.017	15	F	34	10
B	0.018	27	F	38	4
C	0.009	27	M	22	4
D	0.008	14	F	32	0
E	0.006	14	M	45	3
F	0.005	19	F	19	12
G	0.006	12	F	41	0
H	0.008	25	F	34	10
I	0.011	18	F	64	4
Mean ± S.D.	0.011 ± 0.004	19 ± 6			

F, female; M, male. 65 μg estradiol per cm^2 applied in ethanol which was allowed to evaporate (references).

of estradiol applied in pure ethanol, was investigated simultaneously with the test solution. The reference corresponds to a non-vehicle system, since the ethanol evaporates within a few minutes and leaves the estradiol spread across the skin surface as a thin film without microscopically detectable crystals. In Table 2 the data from 9 duplicate measurements are listed giving a mean flux of 0.011 $\mu g \cdot cm^{-2} \cdot h^{-1}$

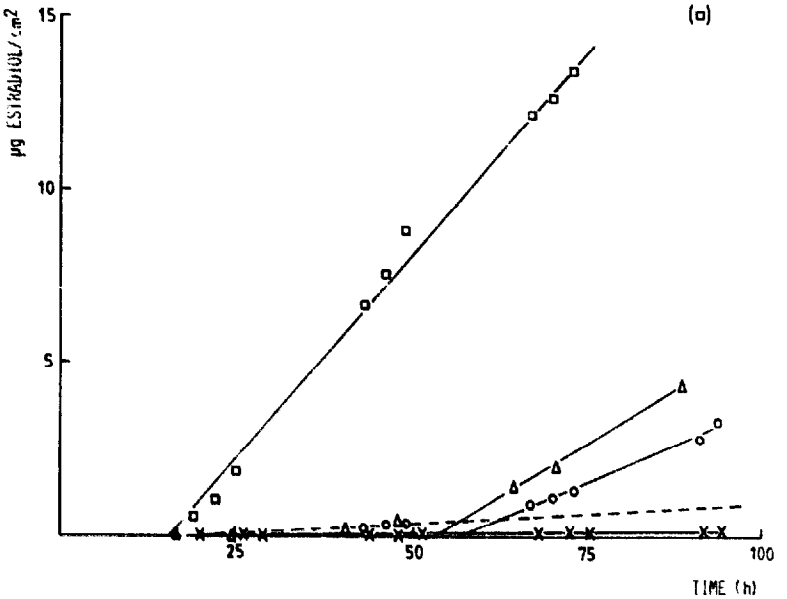

Fig. 1. The effect of different solvents on the permeation of estradiol through human skin in vitro. Key: □, ethylene glycol; △, diethylene glycol; ○, triethylene glycol; ×, polyethylene glycol 400. The dotted line represents the mean flux of the references (Table 2).

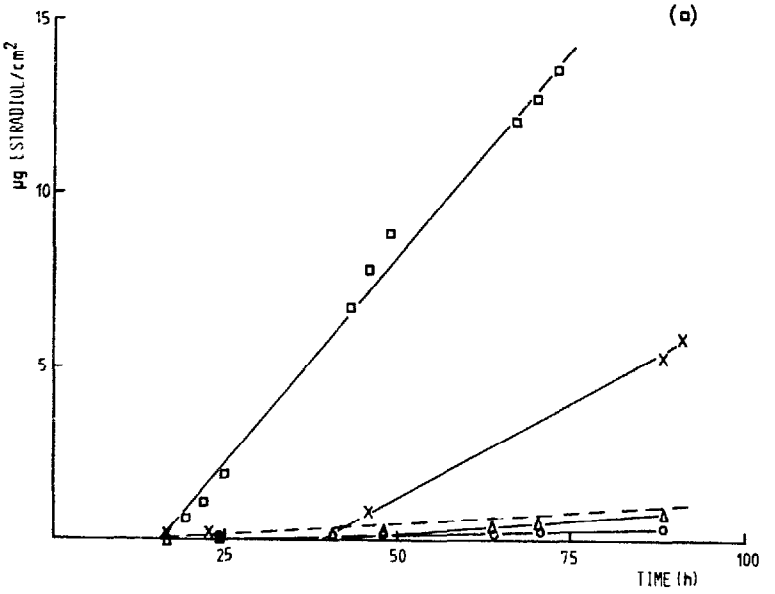

Fig. 2. The effect of different solvents on the permeation of estradiol through human skin in vitro. Key: □, ethanediol(1,2) (ethylene glycol); ×, propanediol(1,3); △, butanediol(1,4); ○, pentanediol(1,5). The dotted line represents the mean flux of the references (Table 2).

and a mean lag time of 19 h. The results demonstrate that estradiol is a slowly permeating substance which is in accordance with previous findings of Michaels et al. (1975). Comparing the flux with the skin specifications (Table 2) it is obviously

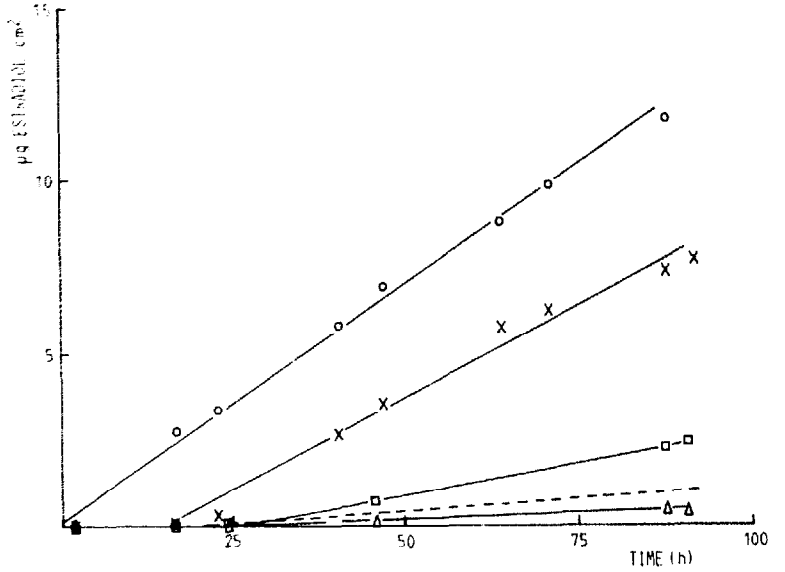

Fig. 3. The effect of different solvents on the permeation of estradiol through human skin in vitro. Key: ○, dimethyl sulphoxide; ×, propylene glycol; □, glycerol; △, hexanetriol(1,2,6). The dotted line represents the mean flux of the references (Table 2).

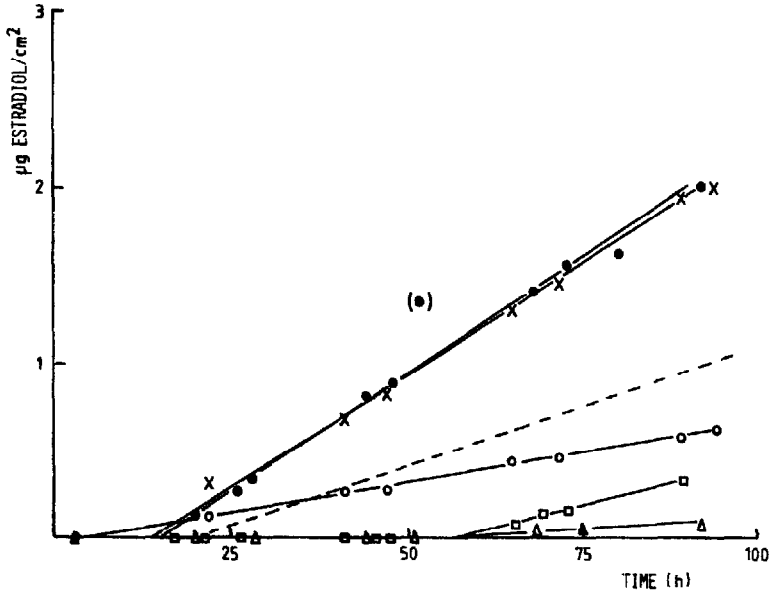

Fig. 4. The effect of different solvents on the permeation of estradiol through human skin in vitro. Key: ●, methyl salicylate; ×, ethyl oleate; ○, diethanolamine; □, triethanolamine; △, Tween 80. The dotted line represents the mean flux of the references (Table 2). Note the change in the ordinate axis.

not possible to demonstrate any correlation between the skin permeability and sex or age of the donor, or storage time.

Figs. 1–4 demonstrate the influences of various vehicles on estradiol permeation. The dotted line in the plot indicates the mean of the references (Table 2).

The effect of different glycols is shown in Figs. 1 and 2. For ethylene glycol, diethylene glycol, triethylene glycol and polyethylene glycol 400 a marked tendency is seen towards the increased number of ethylene oxide groups in the molecules leading to the steady-state flux becoming smaller and the lag time increasing. Consequently ethylene glycol has the greatest effect while polyethylene glycol 400 with 8–10 ethylene oxide groups has almost a retarding effect. The diols in Fig. 2 show a similar result: as the number of alkyl groups increases, the steady-state flux decreases.

In Figs. 3 and 4 are included data from studies with liquid compounds commonly included in topical formulations. Propylene glycol, often used in corticosteroids preparations as a permeation enhancer (Ostrenga et al., 1971a and b), improves, as expected, permeation of estradiol. The dipolar aprotic solvent, dimethyl sulphoxide, is known to enhance the permeation of various chemical agents by acting directly on the barrier (Chandrasekaran and Shaw, 1978). It is noticeable that dimethyl sulphoxide and propylene glycol have almost the same effect on steady-state flux, but the lag time is almost negligible in the case of dimethyl sulphoxide.

The effect of glycerol is of the same order of magnitude as that of methyl salicylate and ethyl oleate. Hexane-triol(1,2,6), triethanolamine and diethanolamine show no enhancing effect on permeation rate in comparison to the reference. Tween 80 even decreases the permeation of estradiol.

The results from the permeation studies are all summarized in Table 1.

Barrier effect

The experiments in this study have been performed by applying the same amount of drug and vehicle. Therefore the thermodynamic activity varied due to alteration in degree of saturation of the samples. In order to investigate a possible connection between thermodynamic activity and vehicle effect, the solubility of estradiol in the vehicles was determined and the data listed in Table 3.

Obviously it is not possible to demonstrate any correlation between the steady-state flux and the solubility in the different vehicles. For example, the solubility of estradiol in ethylene glycol, diethanolamine and ethyl oleate are almost identical, but only ethylene glycol has a marked effect on the permeation rate. Even for vehicles related chemically, the flux does not highly correlate with the degree of saturation. For example, for the glycols, where the order of increase in permeation is for polyethylene-, triethylene-, diethylene- and ethylene glycol, the estradiol solubility is 105, 19, 84 and 16 mg/g, respectively.

These results establish that some specific vehicle–skin interactions ("barrier effect") are involved in the transport of estradiol into the skin.

TABLE 3

SOLUBILITY OF ESTRADIOL AND EFFECT OF VEHICLE ON THE BARRIER

Vehicle	C_s(vehicle) [a] ($mg \cdot g^{-1}$)	$((D_{app})^2)/D_{ref}$ [b] ($10^{-12} \cdot cm^2 \cdot s^{-1}$)	Barrier [c] effect
Ethylene glycol	16	85	+
Propylene glycol	75	990	+
Dimethyl sulphoxide	> 500	> 62,000	+
Diethylene glycol	84	1400	+
Propanediol(1,3)	55	240	+
Triethylene glycol	19	21	+
Dipropylene glycol	104	180	+
Glycerol	1.5	1.3 [d]	−
Betahistine	> 500	> 1300	+
Methyl salicylate	4.4	0.97 [d]	−
Ethyl oleate	16	0.89	−
Diisoamylamine	95	22	+
2-amino-4-methylhexane	179	69	+
Butanediol(1,4)	36	4.9	−
Decanol(1)	28	0.91	−
Triethanolamine	101	8.9	−
Hexanetriol(1,2,6)	40	0.68	−
Diethanolamine	15	0.065	÷
Pentanediol(1,5)	98	3.1	−
Polyethylene glycol 400	105	0.11	−
Tween 80	36	0.016	÷

[a] The solubility of estradiol at 22°C.

[b] Calculated from Eqn. 5.

[c] Effect based on comparison with diffusion coefficient of 10^{-13}–10^{-11} $cm^2 \cdot s^{-1}$.

[d] C_v equal to C_s(vehicle).

For diffusion through a homogeneous barrier the steady-state flux (F_{ss}) and the lag time (L) relate to the diffusion coefficient (D) in the following manner:

$$F_{ss} = \frac{D \cdot PC \cdot C_v}{h} \quad (1)$$

$$L = \frac{h^2}{6D} \quad (2)$$

where PC is the partition coefficient of the drug between stratum corneum and the vehicle, h is the barrier thickness, and C_v is the drug concentration in the vehicle.

Since the effective barrier thickness in whole skin is impossible to measure, the lag time of the reference (L_{ref}) is used to characterize the barrier thickness:

$$h = \sqrt{D_{ref} \cdot L_{ref} \cdot 6}$$

where D_{ref} is the estradiol diffusion coefficient when the reference system is applied.

Assuming the barrier thickness is the same in the experiments with the reference and the vehicles, Eqn. 1 can be rewritten as:

$$F_{ss} = \frac{D_{app} \cdot PC \cdot C_v}{\sqrt{D_{ref} \cdot L_{ref} \cdot 6}} \quad (3)$$

where D_{app} is the apparent estradiol diffusion coefficient in the skin when a vehicle system is applied.

The true PC for the diffusing substance is extremely troublesome to determine because of the need of a large amount of stratum corneum material. Since isopropyl myristate is supposed to have similar solvent characteristics as stratum corneum (Poulsen et al., 1963), PC can be estimated by the ratio of drug solubility in isopropyl myristate (IPM) to the solubility in the vehicle:

$$PC = \frac{C_s(IPM)}{C_s(vehicle)} \quad (4)$$

Assuming that Eqns. 3 and 4 apply, the term $(D_{app})^2/D_{ref}$ can be determined from:

$$\frac{(D_{app})^2}{D_{ref}} = \left(\frac{F_{ss} \cdot C_s(vehicle)}{C_v \cdot C_s(IPM)} \right)^2 \cdot 6 \cdot L_{ref} \quad (5)$$

where F_{ss}, L_{ref} and C_s(vehicle) are taken from Tables 1, 2 and 3, C_v = 12 mg/g and C_s(IPM) = 4.9 mg/cm^3. Values of $(D_{app})^2/D_{ref}$ for the vehicles are listed in Table 3.

If no interaction between the barrier and the vehicle occurs, the estradiol diffusion coefficient, D_{app}, is supposed to be unaffected by the vehicle and equiva-

lent to D_{ref} and the term $(D_{app})^2/D_{ref}$ expected to be equal to D_{ref}. On the contrary, if the barrier is affected by exposure to the vehicle resulting in an increase in drug diffusion, the value of the term becomes larger than D_{ref}. From the $(D_{app})^2/D_{ref}$ values given in Table 3 it is seen that the term varies widely ranging from 1.6×10^{-14} cm$^2 \cdot$ s^{-1} to greater than 6×10^{-8} cm$^2 \cdot$ s^{-1}. In the literature the estradiol diffusion coefficient is reported to be 7.24×10^{-12} cm$^2 \cdot$ s^{-1} for fully hydrated stratum corneum (Scheuplein, 1969). Thus it is likely that the diffusion coefficient for the non-hydrated full thickness skin of the reference is in the range of 10^{-13}–10^{-11} cm$^2 \cdot$ s^{-1}. If the value of the term $(D_{app})^2/D_{ref}$ in Table 3 does not differ from 10^{-13}–10^{-11} cm$^2 \cdot$ s^{-1} there is no significance of vehicle effect on the barrier; this situation is marked with (–) in Table 3. However, a larger value than 10^{-11} cm$^2 \cdot$ s^{-1} implies an increase in the apparent diffusion coefficient arising from changes in the barrier.

As expected, dimethyl sulphoxide and the glycols show a positive effect on the estradiol diffusion. These substances are supposed to penetrate skin and this may increase skin permeability to the drug molecules. The amines also show this positive effect, but the fluxes do not increase substantially, even though it has been reported that some amines are supposed to penetrate skin easily (Allenby et al., 1969). The retarding effect of Tween 80 and diethanolamine is somewhat surprising, but may be due to their emulsifying properties by favouring drug affinity to the vehicles.

Effect of propylene glycol

Further studies on the permeation characteristics of estradiol from propylene glycol vehicles were performed in an attempt to clarify the mechanism of its

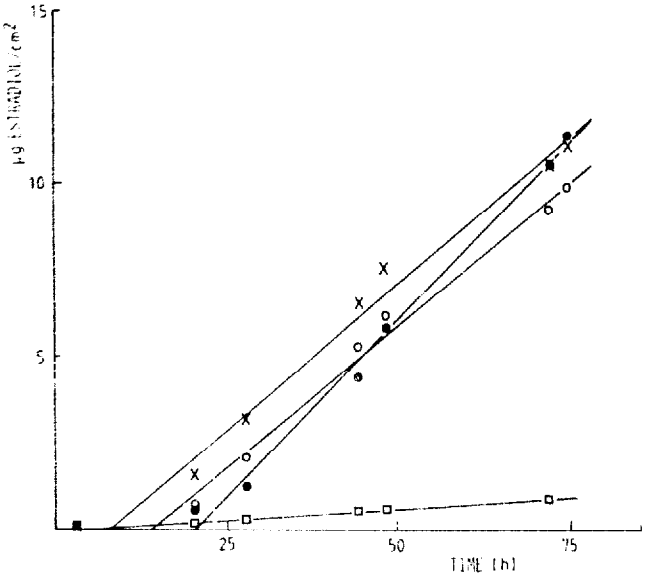

Fig. 5. Effect of varying amounts of propylene glycol on the permeation of estradiol through human skin in vitro. Applied dose of 65 μg estradiol per cm^2. Key: concentration of estradiol in propylene glycol — ×, 7.5%; ○, 2.0%; ●, 1.2%; □, 0.075%.

TABLE 4

EFFECT OF PROPYLENE GLYCOL ON PERMEATION OF ESTRADIOL THROUGH HUMAN SKIN IN VITRO

Vehicle	Flux ($\mu g \cdot cm^{-2} \cdot h^{-1}$)		
	Occluded	Pretreated	Untreated
Propylene glycol	0.12	0.011	0.12
Reference [a]	0.046	0.011	0.011

[a] Estradiol applied in ethanol which was allowed to evaporate.

permeation-enhancing effect. Therefore estradiol permeation was determined after following manipulation of the experimental conditions: (1) applying varying amounts of propylene glycol; (2) occlusion by polyethylene plastic films; and (3) pretreatment of the skin with vehicle.

The permeation profiles of estradiol dissolved in varying amounts of propylene glycol are shown in Fig. 5. By keeping the estradiol dose constant at 65 $\mu g/cm^2$ and increasing the amount of propylene glycol, samples of 0.075%, 1.2%, 2.0% and 7.5% were applied. Since the solubility of estradiol in propylene glycol is 75 mg/g (Table 3), the 7.5% sample corresponds to a saturated system. From Fig. 5 it is seen that the permeation rate is not affected by differences in the propylene glycol amount when the system is 15–100% saturated with estradiol. However, from the highly diluted system of 0.075%, the drug permeates the skin slowly, probably because of less favourable contact between the drug molecules and the stratum corneum.

Test solutions applied under occlusive plastic films increased the reference flux by about 4 times whereas the estradiol permeation rate from the propylene glycol samples was unchanged (Table 4). It is probable that propylene glycol prevents changes in the hydration of the skin by exerting a kind of occlusive effect itself.

The pretreatment was performed by soaking the skin pieces in 20% propylene glycol for 24 h. In order to remove the rest of propylene glycol before the drug was applied, the skin surface was rinsed with ethanol. To prevent propylene glycol from leaking out of the skin during the experiment, 20% propylene glycol was added to the receptor phase. Table 4 shows that this treatment did not have any effect on the drug permeation. Thus it appears that propylene glycol is unable to facilitate permeation unless it is present on the skin surface together with estradiol. A possible explanation of this observation is that estradiol is transported in dissolved state in propylene glycol through the barrier as proposed by Polano and Ponec (1976).

Conclusions

From this systematic study of 21 solvents it is clear that the vehicle subjects the drug to great variation in skin permeation rate as the steady-state flux varies by 200 times. Therefore the significance of the quantitative rate data should be useful for a

prediction of vehicle behaviour in topical formulation. In view of the lack of relationship between degree of saturation of the applied system and the drug permeation rate it is probable that the thermodynamic activity of the drug in the vehicle is only secondary, whereas the drug permeability is mainly directed by a vehicle effect on the barrier function.

Acknowledgement

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