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Use of lipid disperse systems in transdermal drug delivery: Comparative study of flufenamic acid permeation among rat abdominal skin, silicon rubber membrane and stratum corneum sheet isolated from hamster cheek pouch

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Summary

The characteristics of in vitro permeation of flufenamic acid (FA) from lipid disperse systems composed of phosphatidylcholine (PC) and glycosylceramide (GC) were compared among rat abdominal skin, a silicon rubber membrane (Silastic®) and a stratum corneum (SC) sheet isolated from hamster cheek pouch. When a PC dispersion (PCD) containing 20 μmol PC/ml was applied, the permeation of FA through rat skin was enhanced approx. 2.2-fold compared with that from the lipid-free suspension (LFS). Further, a nearly 2-fold enhancement was observed when a GC-containing PCD (10% GC-PCD) was examined. A similar pattern of enhancement could be reproduced when cheek pouch SC was used instead of rat skin, whereas it was not observed in Silastic®. The enhanced permeation in the skin could not be explained on the basis of the incremental increase in the apparent solubilities. A significant correlation was observed between skin permeation and epidermal tissue uptake of FA from LFS and PCDs, although the nearly 2-fold increase found in 10% GC-PCD might be due to mechanisms other than the increase in epidermal tissue uptake. The usefulness of an SC sheet isolated from hamster cheek pouch, a new model membrane without appendages, in studying the direct action of either permeation enhancers or dosage forms designed to enhance the percutaneous permeation of drugs on the SC is discussed.

Introduction

The development of new therapeutic systems providing enhanced percutaneous penetration of a drug is desirable in order to improve the systemic therapeutic efficacy of the drug. For pharmaceuticals, the addition of phosphatidylcholine (PC) to dermal dosage forms has been recently reported to be advantageous for the enhancement of percutaneous absorption (Kato et al., 1987; Natsuki and Takabatake, 1987; Nishihata et al., 1987). We have previously reported that a number of lipid disperse systems containing polar lipids, such as

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PC and glycosylceramide (GC), are useful for increasing the percutaneous permeation of flufenamic acid (FA) through rat abdominal skin both in vitro and in vivo (Kimura et al., 1989). Briefly, maximal enhancement of approx. 3-fold in FA permeation through rat skin was observed in PC dispersions (PCDs) of PC content in the range 10–20 μmol PC/ml. Furthermore, the addition of GC to the PCDs resulted in a further enhancement (approx. 2-fold as compared to the PCDs), particularly when the PC/GC ratio in the dispersion was 9:1 (10% GC-PCD). However, precise details concerning the mechanisms of enhancement remain to be established.

The stratum corneum (SC) is generally regarded as being the rate-limiting barrier to the permeation of most materials through the skin. In previous studies aimed at increasing the degree of absorption of drugs that are absorbed very poorly, experiments were performed with the use of permeation enhancers (Cooper, 1985; Barry, 1987). To clarify the modes of action of enhancers, Morimoto et al. (1986) compared the levels of enhancement by Azone, a percutaneous absorption enhancer, on drug permeation profiles in full-thickness skin with those in stripped skin and discovered indirectly that Azone reduced the barrier properties of the skin SC. However, the demonstration of the direct action of an enhancer on the skin SC is a difficult task to perform, owing to the problem of obtaining a pure skin SC layer whilst maintaining its function as a permeation barrier intact.

We have used hamster cheek pouch mucosa as a model for studying the absorption processes across keratinized oral mucosa without appendages (Kurosaki et al., 1986, 1987, 1988a,b, 1989a,b) and have determined that the in vitro permeability of cheek pouch SC, isolated by trypsin treatment, to drugs was almost equal to that of the full-thickness preparation of cheek pouch mucosa (Kurosaka et al., 1989a).

Here, we have compared the characteristics of permeation of FA from lipid disperse systems through an artificial membrane (Silastic®) and the SC isolated from hamster cheek pouch with those through rat abdominal skin, in order to elucidate the mechanism of enhancement by these systems. Moreover, we discuss the usefulness of cheek pouch SC as a model membrane for pure, intact SC membranes without appendages for permeation studies not only in investigation of the mechanisms of action of skin permeation enhancers on the SC layer but also in the development of transdermal drug delivery systems.

Materials and Methods

Chemicals

FA was supplied by Sankyo Co. (Tokyo, Japan), ketoprofen by Rhône-Poulenc Japan (Tokyo, Japan), and soybean PC by Nippon Shoji Co. (Osaka, Japan). GC, extracted from grain, of 88% purity according to GLC determination (major impurities: free sterols) was supplied by Dr H. Komatsu (Pola Kasei Co., Yokohama, Japan) and was used without further purification. Silastic®, a silicon-rubber membrane, was purchased from Dow Corning Corp. (Midland, MI). Trypsin (Type IX, purified from porcine pancreas) was purchased from Sigma (St. Louis, MO). All other chemicals were reagent grade products obtained commercially.

Preparation of rat abdominal skin membrane

Shaved abdominal skin without subcutaneous tissue was excised with care from male Wistar rats (200–260 g) as described previously (Kimura et al., 1989).

Preparation of SC sheet isolated from hamster cheek pouch

SC sheet was isolated from cheek pouch mucosa of male golden hamsters (110–160 g) by the trypsin-treatment method reported previously (Kurosaki et al., 1989a).

Tissue staining

A tissue specimen of hamster cheek pouch was removed under urethane anesthesia. The specimen was rinsed in saline and immediately fixed in 40% formaldehyde for 12 h. After 20 min rinse in 0.1 M phosphate buffer (pH 7.4), the tissue was bound in Tissue Tec O.C.T. Compound (Miles Laboratories Inc., Elkhart, IN) and rapidly frozen by spray-

TABLE 1

Composition of lipid disperse system

System	PC (μmol)	GC (μmol)	FA (μmol)
LFS	0	0	10
20-PCD	20	0	10
40-PCD	40	0	10
60-PCD	60	0	10
10% GC-PCD	18	2	10

Compositions are represented by the amounts in 1 ml of each preparation. PC, phosphatidylcholine; GC, glycosylceramide; FA, flufenamic acid.

ing with dichlorodifluoromethane (Freeze®, Structure Probe Inc., West Chester, PA). Then the tissue was sectioned into slices of 6 μm thickness and stained with hematoxylin and eosin. Isolated SC was also treated in the same manner.

Test preparations

The compositions of lipid disperse systems examined in this study are listed in Table 1. The lipid disperse systems were prepared by ultrasonic agitation of an evaporated mixture of FA and the lipids in isotonic buffer solution (citric acid-Na_2HPO_4, pH 3.0) as described previously (Kimura et al., 1989). The lipid-free suspension (LFS) was prepared in the same manner without the lipids. FA content in each preparation was 10 μmol/ml. The pH of each preparation was adjusted to 3.0. As the pK_a value of FA is 3.9 (McDougall et al., 1988), the apparent solubility of FA was quite low (Table 3) and the majority of FA was suspended in each preparation. Accordingly, the thermodynamic activity of FA in each preparation appeared to remain constant during the permeation study (Kimura et al., 1989).

In vitro permeation study

In vitro permeation studies with rat abdominal skin, hamster cheek pouch SC and Silastic® were performed using Franz-type cells with effective permeation areas of 1.13 cm^2 as described previously (Kimura et al., 1989). In the case of cheek pouch SC, a glass filter (GA-100, Toyo Roshi, Tokyo, Japan), which does not affect the permeation rate of drugs through the SC, was clamped under the SC as a support (Kurosaki et al., 1989a). 2 ml of the test preparation was applied to the donor side. The donor cell was closed to the atmosphere with Parafilm® (American Can Co., Greenwich, CT). The receptor of the cell was filled with 11.4 ml of an isotonic phosphate buffer solution (pH 7.4) and was maintained at 37 °C using a water jacket. The receptor phase was stirred constantly at 600 rpm with a magnetic bar. An aliquot of the receptor fluid was withdrawn periodically and replaced with the same volume of fresh buffer solution. The concentration of FA in the receptor was determined by HPLC and the cumulative amounts of FA transferred into the receptor side were calculated. The results were expressed as percentages of the dose.

Tissue uptake of FA from test preparations

A tissue specimen (12 mm diameter) of the shaved abdominal skin, including both the epidermal and the dermal parts, of the rat was incubated with 4 ml of a test preparation at 37 °C for 12 h. After washing out the entire surface with saline, the skin was blotted onto a filter paper and was boiled in a water bath with 3 ml of 0.5 N NaOH solution for 1 h. After cooling, 1 ml of 0.1 mM ketoprofen, an internal standard, dissolved in 0.5 N NaOH solution was added to the tissue solution. Here, some impurities in the resultant solution were extracted with chloroform. The remaining aqueous phase was made acidic with concentrated HCl and FA was extracted with chloroform. Then, an aliquot of the organic phase was evaporated and the residue was dissolved in the mobile phase used for HPLC. The concentration of FA was determined by HPLC and the amount of FA in the skin was calculated. Appropriate amounts of FA were added to another skin tissue and the same procedures were carried out in order to construct a calibration curve for estimation of FA amounts in the sample tissue.

Determination of apparent solubilities of FA in lipid disperse systems

The apparent solubility of FA in each test preparation was determined by ultrafiltration using a Micropartition System (MPS-1; Amicon

Co., Tokyo, Japan) at 20°C as described previously (Kimura et al., 1989).

Analytical method

For determining the concentration of FA by HPLC, a high-pressure liquid chromatograph (LC-5A; Shimadzu, Kyoto, Japan) equipped with a UV detector (SPD-2A; Shimadzu) operating at 290 nm was used in reversed-phase mode with a Nucleosil $5C_{18}$ column (4.6 mm i.d. × 150 mm). A mixture of acetonitrile and 0.025% phosphoric acid aqueous solution (6 : 4 by vol.) was employed as the mobile phase at a flow rate of 0.9 ml/min.

Statistical analysis

The results were expressed as the mean ± standard error. Statistical analysis was carried out by using Student's *t*-test.

Results

In vitro permeation profiles of FA from lipid disperse systems through model membranes

Abdominal rat skin

Fig. 1 shows the permeation profiles of FA from lipid disperse systems through rat abdominal skin in vitro. The penetration of FA from 20-PCD was enhanced by approx. 2.2-times compared with that from LFS. Further, the addition of GC to the PCD (10%GC-PCD) nearly doubled the enhancement. However, when the preparations with higher PC contents were applied, the enhancement was completely lacking and the permeation was retarded as the PC content increased further (60-PCD).

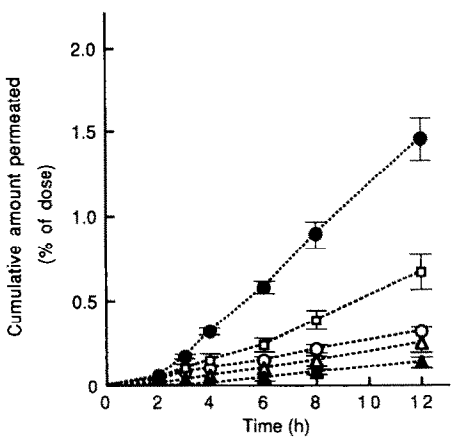

Fig. 1. Permeation profiles of FA from lipid disperse systems through excised rat abdominal skin. Systems: LFS (○); 20-PCD (□); 40-PCD (△); 60-PCD (▲); 10%GC-PCD (●). Results are expressed as the mean ± S.E. of 3–5 experiments.

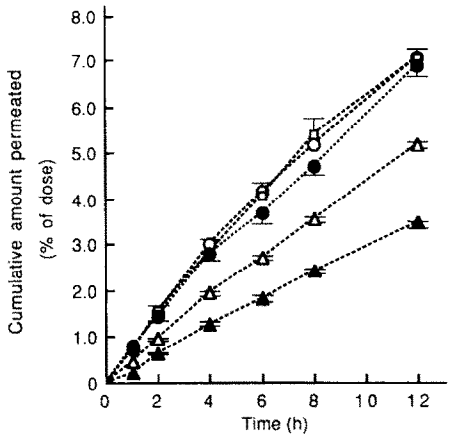

Fig. 2. Permeation profiles of FA from lipid disperse systems through Silastic® membrane. Systems: LFS (○); 20-PCD (□); 40-PCD (△); 60-PCD (▲); 10%GC-PCD (●). Results are expressed as the mean ± S.E. of 3–7 experiments.

Silicon-rubber membrane (Silastic®)

Fig. 2 shows the permeation profiles of FA from lipid disperse systems through an artificial membrane (Silastic®). There was no significant difference among the permeation profiles of FA from LFS, 20-PCD and 10%GC-PCD. However, retarded permeation was observed when 40-PCD or 60-PCD was applied. The magnitude of the retardation was related to the PC content in the preparation.

SC sheet isolated from hamster cheek pouch

Fig. 3 shows the permeation profiles of FA from lipid disperse systems through SC isolated from hamster cheek pouch by trypsin treatment. The permeation of FA from 20-PCD was enhanced by approx. 1.5-times compared with that from LFS. Likewise in the case of rat abdominal skin shown in Fig. 1, further enhancement could be observed when 10%GC-PCD was applied. In

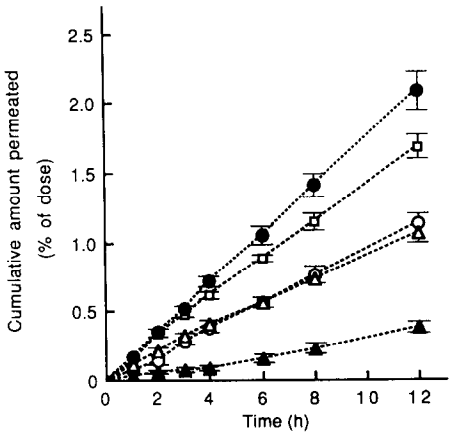

Fig. 3. Permeation profiles of FA from lipid disperse systems through stratum corneum sheet isolated from hamster cheek pouch. Systems: LFS (○); 20-PCD (□); 40-PCD (△); 60-PCD (▲); 10%GC-PCD (●). Results are expressed as the mean ± S.E. of 3–5 experiments.

addition, the lack of the enhancement and the retardation of the permeation could also be observed on application of 40-PCD and 60-PCD, respectively.

Tissue uptake of FA from lipid disperse systems

Table 2 summarizes the effects of lipid disperse systems on in vitro uptake of FA by rat abdominal skin during 12 h incubation at 37 °C. When FA was applied as 20-PCD or 10%GC-PCD, the amounts of FA transferred to the tissue increased approx. 3-times in comparison with LFS. However, there was no significant difference in the tissue uptake of FA between LFS and 40-PCD. In addition, the FA uptake from 60-PCD was reduced to one-half of that from LFS.

TABLE 2

Effect of lipid disperse systems on FA uptake into epidermal tissue in vitro

System	Tissue uptake (μmol/g)
LFS	14.34 ± 1.15
20-PCD	48.91 ± 1.77 [b]
40-PCD	16.69 ± 1.04
60-PCD	6.98 ± 1.01 [a]
10%GC-PCD	47.67 ± 4.71 [a]

Results are expressed as the mean ± S.E. of 3 experiments.
[a] $p < 0.01$; [b] $p < 0.001$, compared with LFS.

TABLE 3

Apparent solubility of FA in preparations

System	Solubility (μM)
LFS	0.87 ± 0.20
20-PCD	4.60 ± 0.14 [b]
40-PCD	3.94 ± 0.48 [a]
60-PCD	2.10 ± 0.21 [a]
10%GC-PCD	5.00 ± 0.26 [b]

Ultrafiltration was carried out using a Micropartition System (Amicon MPS-1). Results are expressed as the mean ± S.E. of 4 experiments.
[a] $p < 0.01$; [b] $p < 0.001$, compared with LFS.

Apparent solubilities of FA in lipid disperse systems

To determine the apparent solubilities of FA in test preparations, ultrafiltration experiments were carried out using a Micropartition System at 20 °C. Table 3 summarizes the FA concentrations in the filtrates of the preparations examined. The FA concentration in each preparation significantly increased in comparison with LFS and the order of incremental increase was 10%GC-PCD ≥ 20-PCD > 40-PCD > 60-PCD (≫ LFS).

Photomicrographs of hamster cheek pouch mucosa and SC sheet

Fig. 4 shows the photomicrographs of (a) hamster cheek pouch mucosa and (b) SC isolated from the mucosa by the trypsin-treatment method. Hamster cheek pouch mucosa has two distinct layers of tissue, a stratified squamous epithelium and a lamina propria, and the surface of the epithelium is covered with a few keratinized layers (Fig. 4a). The thicknesses of the SC and the epithelium (except for SC) were approx. 7 and 35 μm, respectively. Isolated SC has no appendages and was swollen to some extent during trypsin treatment. The apparent thickness of the isolated SC shown in Fig. 4b was approx. 10 μm.

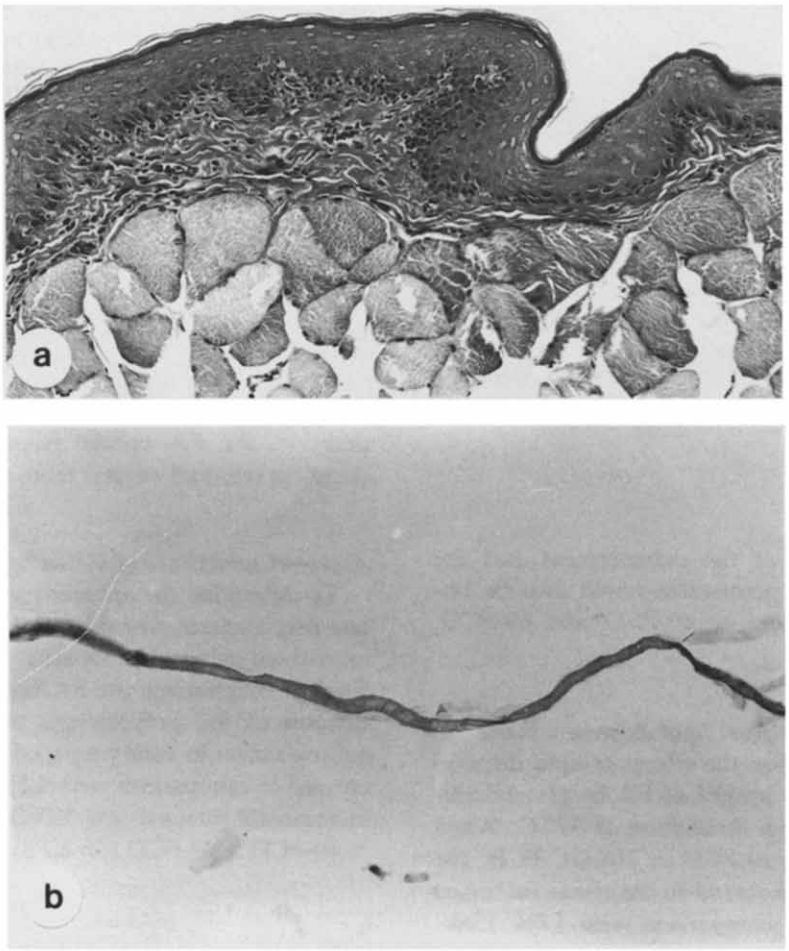

Fig. 4. Photomicrographs of (a) hamster cheek pouch mucosa (×100) and (b) a stratum corneum sheet isolated from hamster cheek pouch by the trypsin-treatment method (×100).

Discussion

The permeability of skin consisting of a keratinized stratified squamous epithelium is significantly inferior to that of a columnar epithelium such as an intestinal wall. Moreover, the permeability of a stratified squamous epithelium is dependent on the degree of keratinization (Squier and Hall, 1985; Nishimura et al., 1989). Recently, the physiological role of epidermal lipids has been elucidated. Intercellular multilamellar lipid bilayers observed by electron microscopy in the SC (Landmann, 1986; Madison et al., 1987) are generally regarded as the practical barrier of the keratinized epithelium (Elias, 1983; Downing et al., 1987). The SC possesses extremely high contents of acylglycosylceramides (AGC) and acylceramides (AC) compared with either the basal layer or the granular layer (Curatolo, 1987). As to the roles of AGC and AC, speculation that these

unusual lipids have a structurally specific function as molecular anchors in the adjacent intercellular lipid bilayers in the SC to provide a highly ordered barrier to water diffusion has been reported (Wertz and Downing, 1982; 1983; Abraham et al., 1988). Wertz et al. (1986) determined the lipid compositions of some epithelia of the pig and reported that the floor and buccal mucosa, non-keratinized and more permeable parts of the oral mucosa, lack AGC, AC and ceramides, instead possessing significant quantities of GC, similar to the brush-border membrane of the intestinal wall. On the other hand, keratinized and less permeable regions, such as the gingiva, palate and the skin, possess significant quantities of AGC, AC and ceramides.

We have been interested in the use of polar lipids especially PC and GC in transdermal dosage forms which provide improved delivery of drugs. In our previous report (Kimura et al., 1989), we were able to demonstrate enhanced percutaneous permeation of FA through rat abdominal skin by some lipid disperse systems including 10%GC-PCD. In the present study, we chose three model membranes, i.e., rat abdominal skin, Silastic® and SC sheet isolated from hamster cheek pouch, and compared the permeation characteristics of FA from LFS, 20-PCD, 40-PCD, 60-PCD and 10%GC-PCD among these membranes to clarify the mechanisms of the enhancement by the lipid disperse systems. The concentrations of FA in the ultrafiltrates of all the lipid disperse systems were higher than that of LFS (Table 3). Since the greater part of the FA in all preparations was still existent as a solid suspension at the end of the permeation experiment, the FA concentrations in the filtrates of the preparations were found to correspond to the saturated or supersaturated state under the present conditions. Table 4 represents the relationship between the apparent solubilities and the degree of the enhancement of the percutaneous permeation of FA in these preparations. The solubility ratios of 20-PCD and 10%GC-PCD are 5.3 and 5.7, respectively. However, there is a significant difference between the enhancement ratios of these two preparations by approx. 2-fold. Further, the enhancement was not recognized in the cases of 40- and 60-PCDs, in spite of the significant increase in solubilities of the FA in these preparations. Therefore, it is suggested that some factors other than the apparent solubility of FA in the preparation are concerned in the enhanced percutaneous permeation of the drug from the lipid disperse systems.

TABLE 4

Comparisons of apparent solubility and permeation enhancement of FA among test preparations

System	Solubility ratio [a]	Enhancement ratio [b]
LFS	1.0	1.0
20-PCD	5.3	2.2
40-PCD	4.5	0.80
60-PCD	2.4	0.44
10%GC-PCD	5.7	4.7

[a] Solubility ratio = (S_{app} in preparation)/(S_{app} in LFS).
[b] Enhancement ratio = (A_{12h} from preparation)/(A_{12h} from LFS).
S_{app}, apparent solubility of FA; A_{12h}, cumulative amounts of FA permeated through rat skin in 12 h.

Fig. 5 shows the correlation between skin permeation (Fig. 1) and epidermal tissue uptake (Table 2) of FA from the test preparations. There was a significant correlation ($r = 0.984$) among LFS and PCDs prepared using 20–60 μmol PC/ml. This agrees well with the report of Nishihata et al. (1987), in which a good relationship between the apparent penetration rate and the total amount of

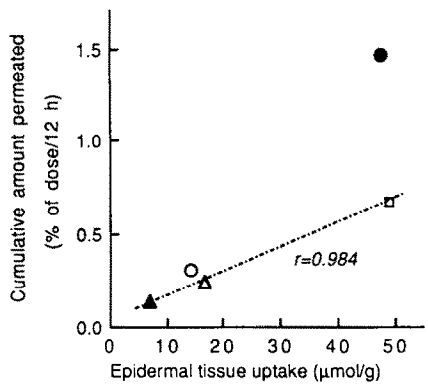

Fig. 5. Correlation between cumulative amounts of FA permeated through rat skin and epidermal tissue uptake of FA in rat skin. Systems: LFS (○); 20-PCD (□); 40-PCD (△); 60-PCD (▲); 10%GC-PCD (●). Each point represents the mean of 3–7 experiments.

diclofenac accumulated in the skin tissue from aqueous gel dosage form containing PC was observed. However, the point corresponding to 10%GC-PCD deviated far above the regression line; i.e., epidermal tissue uptake from 10%GC-PCD was practically identical to that from 20-PCD despite the nearly doubled percutaneous permeation. This means that 10%GC-PCD enhances the permeability of skin further without affecting tissue uptake or partitioning into the SC. One of the possible mechanisms of action of GC might be the enhancement of the permeability characteristics, i.e. diffusibility in SC, although this has not yet been proved experimentally.

Silastic® has been used as a model of lipoidal membranes to evaluate the mechanisms and the kinetics of membrane permeation of lipophilic drugs (Nacht and Yeung, 1985; Ghannam et al., 1986). However, in this study, we could not detect enhanced permeation of FA from 20-PCD or 10%GC-PCD through Silastic® (Fig. 2). These results suggest firstly that the enhancing mechanisms of PCDs are not the increase in partitioning of FA to lipoidal membranes but the direct actions of polar lipids in the PCDs on the skin surface, i.e., the SC, and secondly that lipoidal model membranes such as Silastic® are inadequate for evaluating the enhanced percutaneous permeation of drugs from dosage forms containing some excipients which act on the SC directly. On the other hand, the permeation profiles of FA through isolated SC of hamster cheek pouch from LFS and PCDs corresponded well to those through rat skin. Accordingly, although further investigations are needed to clarify the actions of polar lipids on the SC during drug permeation, isolated cheek pouch SC is a quite adequate model membrane for examination of the mechanisms of enhancement of lipid disperse systems especially actions on the SC.

In conclusion, a new model membrane, the isolated SC sheet of hamster cheek pouch proposed in this paper, is now available for studying the actions of either permeation enhancers or dosage forms designed to enhance the percutaneous permeation of the SC directly. In addition, this enables us to investigate the nature of drug permeation through the SC more precisely than the conventional excised skin method. Some characteristics of permeation enhancers acting on the SC itself will be clarified by this method in the near future.

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