Development of a Model Membrane System Using Stratum Corneum Lipids for Estimation of Drug Skin Permeability

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Several model membrane systems have been reported to predict the skin permeability of drugs, but model membranes using stratum corneum (SC) lipids have never been reported. Thus, we developed a model membrane system for drug permeation study by fixing liposomes composed of SC lipids (ceramides, palmitic acid, cholesterol, and cholesterol-3-sulfate) onto a supporting filter, Biodyne B. The permeability of several drugs with different lipophilicities was investigated. Permeability increased with drug lipophilicity, estimated from the octanol/buffer solubility ratio of the drug. For relatively polar drugs, however, the permeability was almost constant, and very close to the value of a K^+ ion, suggesting the membrane has both lipidic and aqueous pathways. Drug permeability through our system was compared with that through guinea pig skin. A good corelation ($r=0.880$) was observed, although the former was one order of magnitude greater than the latter. Our model system will be useful not only for parctical application, but also for basic studies, such as the elucidation of the relationships between SC lipid composition and drug permeability.

Keywords model membrane; stratum corneum lipid; permeability; lipophilicity; skin

Introduction

Recently, the percutaneous absorption of drugs has been extensively investigated as a drug delivery system. The rate-limiting barrier for the transport of most solutes is the stratum corneum (SC).[1] The structure of SC is heterogeneous, consisting of cornified cells embedded in a matrix of lipid lamellae.[2] The SC lipid bilayers play an important role in the percutaneous absorption of drugs. Studies on relationships between drug permeability and lipophilicity demonstrated that the intercellular lipid membranes constitute a barrier for the absorption of hydrophilic drugs.[3,4] Several absorption enhancers have been considered to fluidize the SC lipid bilayers, facilitating drug permeation.[5] Furthermore, the SC lipid content may account for individual[3] or species[6] difference in skin permeability.

Several model membrane systems have been used for the estimation of percutaneous absorption,[4,7-9] but satisfactory model membranes using SC lipids have never been reported. The investigation of the drug permeability of the SC lipid bilayers *per se* is significant from both basic and practical points of view. Downing's group found that a mixture of major components of the SC lipids forms liposomes under appropriate conditions,[10] and they fixed the vesicles onto a membrane filter to evaluate the permeability of water vapor, not drugs.[11] Firestone and Guy[12] developed a model system using a filter disc impregnated with SC lipids and preliminarily reported a correlation between drug lipophilicity and membrane permeability, but they neither included hydrophilic drugs nor carried out a systematic comparison with skin permeability.

Thus, for the estimation of drug permeability, we developed a model membrane system by sandwiching liposomes composed of the SC lipid mixture between two supporting filters. The preparation method and properties will be reported.

Experimental

Materials Ceramides from bovine brain (type IV, approx. 99%), palmitic acid (sodium salt, approx. 99%), cholesterol (>99%), and cholesterol-3-sulfate (sodium salt) were purchased from Sigma. Perdeutrated palmitic acid (d_{31}) was a product of Cambridge Isotope Laboratories. Aminopyrine (AP), ibuprofen (IB), lidocaine (LC), indomethacin (ID), and ketoprofen (KP) were obtained from Wako. Flurbiprofen (FL) and cyclobarbital (CB) were manufactured by Sigma and Tokyo Kasei, respectively. Membrane filters tested were Pall Biodyne B (pore size, 0.45 μm), Nucleopore (0.1 μm), and Millipore GS (0.22 μm). All other chemicals from Wako were of special grade. Buffers were prepared with water twice distilled from a glass still.

Preparation of Liposomes A mixture of lipids (ceramides/palmitic acid/cholesterol/cholesterol-3-sulfate = 3.25/4.5/3.25/1, mole ratio)[10] was dissolved in a chloroform/methanol (2/1 or 1/1, v/v) solution. After evaporation of the solvent, the residual film was vacuum-dried overnight. The film was hydrated with a 5 mM Tris–HCl/1 mM EDTA buffer (pH 7.5) and then vortexed at 80—85 °C. The gel to liquid-crystalline phase transition temperature of the liposomes was determined by the Fourier transform IR (FTIR) technique. The IR transmission spectra (a CaF_2 cell of 100 μm path length) of liposomes prepared with perdeutrated palmitic acid instead of palmitic acid were recorded at various temperatures on a Nicolet 205 FTIR spectrophotometer (4 cm^{-1}, 256 scans).

Fixation of Liposomes An SC lipid liposome suspension was filtered on a membrane filter (25 mm diameter) using an Amicon ultrafiltration cell (type 8010) under nitrogen gas pressure of 1—3 kg/cm^2, so that the vesicles were uniformly spread on the filter. The milky suspension became clear after the filtration, indicating that almost all vesicles were trapped onto the filter. After another protecting filter was placed onto the lipid loaded filter, the lipid sandwiched filters were vacuum-dried above P_2O_5 overnight. This procedure seems to facilitate liposomal fusion. The quantities of the lipids loaded were gravimetrically checked.

Scanning Electron Micrograph Small pieces of liposome-coated filters after vacuum drying over P_2O_5 were coated with Pt–Pd in a sputter coater (Hitachi E-102) and analyzed in a Hitachi S-800 scanning electron microscope operating at 20 kV.

Preparation of Skin Male guinea pigs (Hartley, 250—300 g) from Shimizu Laboratory Supplies were shaved with electrical clippers the preceding day. The dorsal skin of the anesthetized animal was excised. After the removal of the subcutaneous fat, the skin was punched out into disks of 18 mm diameter.

Membrane Permeation A model membrane or a piece of skin was mounted on a Valia-Chien type skin permeation cell (3.5 ml capacity, 0.636 cm^2 permeation area) with packings (Fig. 1). The membrane was hydrated with a 10 mM Tris–HCl/150 mM NaCl/1 mM EDTA buffer (pH 7.5) filled in both compartments for 1 h at 37 °C. The donor solution was then replaced with a drug suspension in the buffer. To eliminate any volume changes due to osmotic pressure effects, silicone tubes were connected to both sampling ports. After the drug suspension (donor compartment) or the buffer (acceptor compartment) was filled (6.5 ml per compartment), the tubes were pinched with clips, so that there was no air within the cell. The temperature was maintained at 37 ± 1 °C. At intervals,

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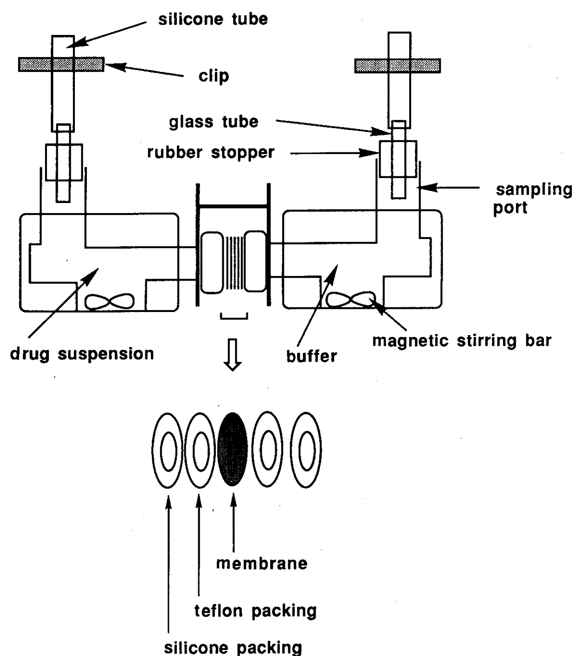

Fig. 1. Schematic Representation of Diffusion Cell

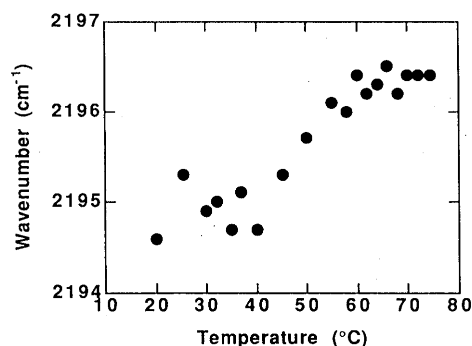

Fig. 2. Phase Transition of SC Lipid Liposomes

The frequency of the C–D antisymmetric stretching vibration of perdeutrated palmitic acid incorporated in SC lipid liposomes plotted as a function of temperature.

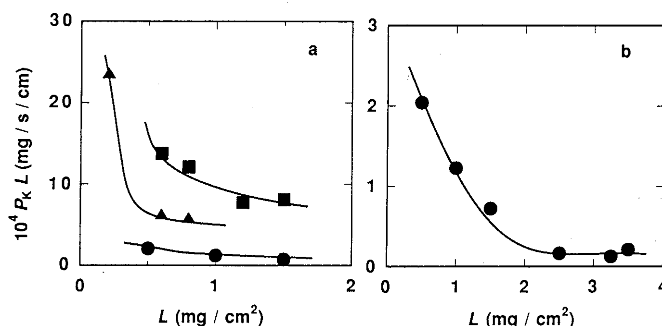

Fig. 3. Barrier Property of SC Lipid Liposomes Fixed onto Supporting Filters

The barrier property is expressed as the permeability coefficient of a K^+ ion, P_K, multiplied by the amount of the lipids, L. Supporting filter: ●, Biodyne B (0.45 μm); ■, Millipore GS (0.22 μm); ▲, Nuclepore (0.1 μm).

aliquots (0.2 ml) of the acceptor solution were sampled and the same volume of the buffer was refilled. The amount of the permeated drug was determined using HPLC.[4] For the permeation of K^+ ions, a 10 mM Tris–HCl/150 mM KCl/1 mM EDTA buffer (pH 7.5) was filled in the donor side, and the concentration of the leaked K^+ ion in the acceptor compartment was measured with a K^+-selective electrode connected to a microprocessor ion analyzer (Orion Research).

The permeability coefficient of a drug or a K^+ ion in the lipid membrane *per se*, P_m (cm/s), is calculated from the observed permeability coefficient, P_{obs}, according to the equation:

$$P_m = P_{obs} \cdot P_{filt} / (P_{filt} - P_{obs})$$

where P_{filt} is the permeability coefficient of the drug through the supporting filters. These permeability coefficients were estimated using a curve fitting procedure, as reported elsewhere.[13] Under our experimental conditions, a sink condition approximately holds, because the drug concentration in the donor compartment was time-independent (equal to the solubility) and that in the acceptor compartment was less than 5% of the donor concentration (see Fig. 4a).

Determination of Solubility Drug powder suspended in octanol or the NaCl containing buffer was shaken overnight at 37 ± 1 °C. The suspension was filtered with a Millipore HV filter (0.45 μm pore size). The drug concentration of the filtrate was determined by HPLC.[4] The octanol/buffer solubility ratio was calculated as a measure of drug hydrophobicity.

Results

Phase Transition of Liposomes Figure 2 shows the frequency of the C–D antisymmetric stretching vibration of perdeutrated palmitic acid in liposomes as a function of temperature. The shift of the frequency to higher values implies an increase in the gauche conformer, *i.e.*, the melting of the acyl chain.[14] A broad phase transition was observed in the temperature range of 40—60 °C, which is lower than the reported temperature range of hydrated human SC lipids (around 65 °C).[15] This discrepancy may be ascribable to the difference in the lipid composition and/or lipid ionization state. For example, human SC contains a considerable amount of longer fatty acids (C_{18}–C_{24}).[16,17] Furthermore, the ionization of fatty acids will reduce the transition temperature (we used the buffer instead of water.[15]). At 37 °C, our experimental temperature, the SC lipid membrane is in the solid state, as in the human SC.[15]

Screening of Filters We used three kinds of hydrophilic membrane filters for supporting the SC lipid membranes; a cellulose ester filter (Millipore GS), a polycarbonate filter (Nuclepore), and a cationized nylon filter (Biobyne B). The barrier property of the lipid membrane was evaluated on the basis of K^+ ion permeability. Since the K^+ ion permeability coefficient through the lipid bilayers, P_K (cm/s), is considered to be inversely proportional to the amount of the lipid, L (mg/cm²), which is proportional to the thickness of the lipid bilayer, the product $P_K \cdot L$ was used as a measure of membrane integrity. As shown in Fig. 3a, the Biodyne B-supported membrane exhibited a maximum barrier property (the lowest plateau $P_K \cdot L$ value). That is, the liposomes were tightly attached to and closely packed on the Biodyne B filter. The barrier ability was constant at L values above 2.5 mg/cm² (Fig. 3b). Thus, we carried out further experiments at an L value of 3.0 mg/cm².

Figure 4 illustrates scanning electron micrographs of the lipid-loaded filters in the dehydrated state at various lipid amounts. In the absence of the lipid, the Biodyne filter showed a porous structure (Fig. 4a). At 1.7 mg/cm², most of the filter surface was covered with the liposomes (Fig. 4b). At 2.7 mg/cm³, where the $P_K \cdot L$ value is at a plateau, further loaded liposomes were stacked onto the filter which had been already covered with the vesicles (Fig. 4c). Intervesicular space is expected to narrow upon hydration. Thus, our model membrane system appears to be composed of closely packed liposomes sandwiched between two supporting filters and can be approximately described as a serial array of diffusion resisters in a filter-liposomal

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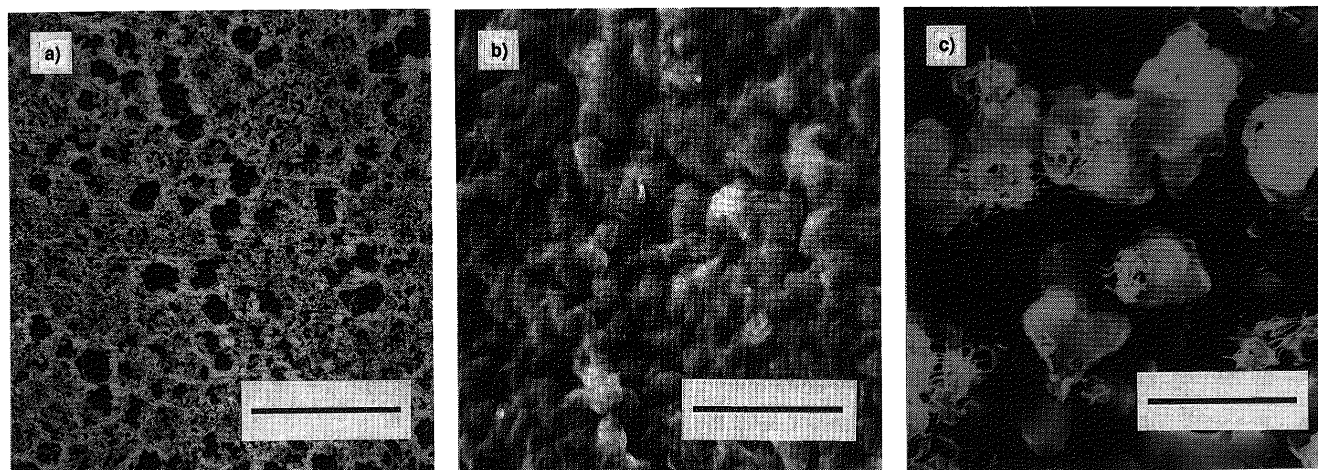

Fig. 4. Scanning Electron Micrographs of Liposome-Loaded Biodyne B Filters in the Dehydrated State

Amount of lipid loaded: (a) 0, (b) 1.7, (c) 2.7 mg/cm². The bar represents 30 μm.

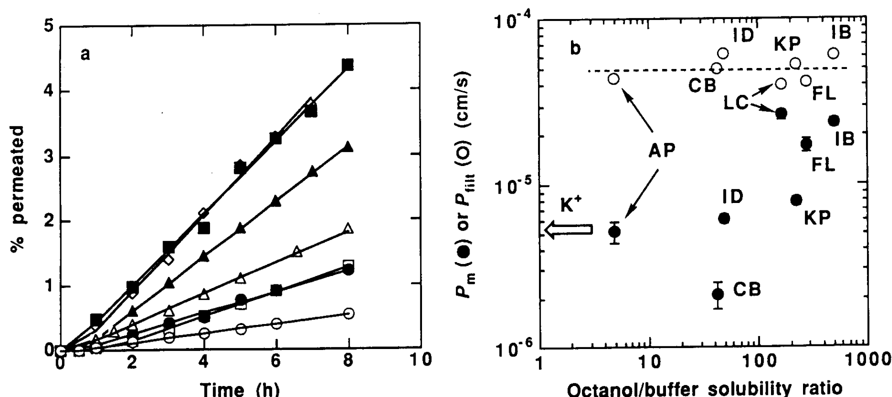

Fig. 5. Permeability of Several Drugs through Biodyne B-Supported SC Lipid Membranes

(a) Time course of drug permeation (average of three preparations). Drug: ○, CB; ●, AP; □, ID; ■, LC; △, KP; ▲, FL; ◇, IB. (b) Permeability coefficients through the filters, P_{filt} (○), and the lipid membranes, P_m (●) are plotted against an octanol/buffer solubility ratio, a measure of the drug's lipophilicity.

layer-filter configuration. Intervesicular aqueous tortuous routes seem to exist in the liposomal layer.

Permeability in Model Membrane Figure 5a shows the time course of drug permeation through the Biodyne B-supported SC lipid membranes. After lag times of 0.2—1.4 h, the profiles were linear, their slopes being proportional to the permeability coefficients. The linearity up to 8 h guarantees that our model membranes maintain the barrier property without any peeling. IB (open diamonds), the most hydrophobic drug, permeated the membranes eight times faster than relatively hydrophilic CB (open circles). The observed permeability coefficient of each drug, P_{obs}, includes a contribution from the supporting filters, P_{filt}. The permeability coefficient through the SC lipid membranes, P_m, was estimated as described under Experimental, as in the case of P_K determination. However, these corrections were rather small; namely, the P_{obs} and corresponding P_m values differed by less than 15% except for FL and IB, where the discrepancies were around 40%. Therefore, the lipid layer is the rate-limiting barrier. In Fig. 5b, the P_m and P_{filt} values are plotted against the octanol/buffer solubility ratio, a measure of drug hydrophobicity. The solubility ratios were somewhat different from the reported values,[4] because we used the NaCl containing buffer instead of water. The P_{filt} values were almost constant ($4—6 \times 10^{-5}$ cm/s) irrespective of drug hydrophobicity, indicating the Biodyne B filter is a porous membrane. The molecular weights of the drugs[4] are very similar (206—254) except for ID (358). These drugs will differ in their diffusion coefficients by only *ca.* 20% ($(358/206)^{1/3} = 1.2$). The diffusion coefficients through the filters are much larger than those through the SC lipid membranes because no lag times for the drug permeation through the filters could be detected (data not shown). On the other hand, the P_m value increased with drug lipophilicity. For relatively polar drugs (AP, CB, and ID), however, their values were almost constant ($2—6 \times 10^{-6}$ cm/s) and coincided with the P_m value of a K^+ ion (the arrow in Fig. 5b).

Comparison of Model Membrane with Skin Figure 6a shows the permeability coefficients of the drugs through guinea pig whole skin, P_{skin}, as a function of the solubility ratio. The permeabilities of hydrophobic drugs were again higher than those of less hydrophobic drugs (AP, CB, and ID), which were constant (3×10^{-7} cm/s). The permeability of a K^+ ion (the arrow, 6.2×10^{-7} cm/s) was comparable to the latter values. (The larger permeability of a K^+ ion is ascribable to its much smaller size.) Figure 6b compares

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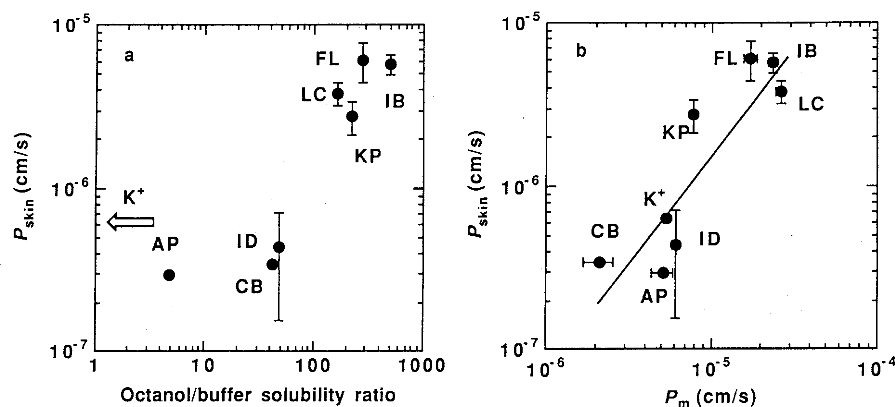

Fig. 6. Permeability Coefficients of Several Drugs through Guinea Pig Skin, P_{skin}

(a) P_{skin} values are plotted as a function of the octanol/buffer solubility ratio. (b) P_{skin} values are compared with P_m values.

the model membrane permeability with the skin permeability. A good correlation ($r=0.880$) was observed, although the P_m values were one order of magnitude greater than the P_{skin} values. The latter appears to be partly ascribable to the larger diffusion coefficients in our model membrane, because the lag times for the model membrane (0.2—1.4 h) were one order of magnitude smaller than those for the skin (2.8—10.1 h, data not shown). Furthermore, the actual epidermis consists of various layers (SC, stratum granulosum, stratum spinosum, and stratum basale) with different lipid compositions. This may partly account for the permeability difference between our model system and the skin.

Discussion

Several model membrane systems have been developed to predict the skin permeabilities of drugs or to understand the transport process through the skin.[4,7-9,12] For example, Hadgraft and Ridout used chemically treated cellulose nitrate membranes saturated with several lipids, such as isopropyl myristate,[7] dipalmitoylphosphatidylcholine, linoleic acid, and tetradecane.[8] These membranes mimic the skin to some extent, but drug permeabilities were 10^2—10^3 times larger than those through the skin. Firestone and Guy[12] preliminarily reported a model system using SC lipids, but they neither examined hydrophilic drugs nor carried out a systematic comparison with skin permeability. Model membranes composed of SC lipids would serve as a useful system not only for practical application, *e.g.* the screening of drug candidates for transdermal delivery, but also for basic studies, such as the elucidation of the relationships between SC lipid composition (including the incorporation of absorption enhancers) and drug permeability. For these purposes, the use of the SC lipids instead of other lipids, *e.g.*, phosphatidylcholine, is inevitable.

Reportedly, the SC lipids can form liposomes under appropriate conditions.[10] Thus, we tried to utilize this membrane for a drug permeation study. Liposomes have limitations for permeability studies. They can trap only rather polar solutes, and their permeability strongly affected by their curvature. Accordingly, we fixed the SC lipid membrane to a supporting filter. The fixation of lipids has been carried out by loading lipids dissolved in organic solvents.[7,8,12,18] We attempted this procedure using several kinds of filters, both hydrophilic and hydrophobic, but we could not obtain a sufficient barrier property. Next, we tried to fix the preformed SC lipid liposomes onto a membrane filter by the dehydration–rehydration procedure. It is well known that this procedure facilitates the fusion of liposomes but never ruins the lipid bilayer structure. The selection of the filter was crucial (Fig. 3). For a Millipore GS filter or a Nuclepore filter, the lipid membranes were often peeled off from the filter upon prolonged hydration, and the barrier property was insufficient. In contrast, a Biodyne B membrane stably held the vesicles, probably because of (1) electrostatic interactions between the positively charged filter and the negatively charged lipids and (2) the negligible shrink-stretch of the filter during the dehydration–rehydration procedure.

The permeability of several drugs through our model membrane depends on drug lipophilicity (Fig. 5b). For relatively polar drugs (AP, CB, and ID), the P_m values were almost constant, and very close to the value of a K^+ ion. These results strongly suggest that an aqueous route is present in our model membrane. That is, our model membrane serves as a porous membrane for hydrophilic solutes, which partition less into the lipid phase. The electron micrograph (Fig. 4c) suggests that water-filled tortuous intervesicular space constitutes the aqueous path. Relatively high permeabilities of polar compounds through skin (Fig. 6a and Refs. 3, 4) have been explained on the basis of the existence of a polar transport route in the skin. For lipophilic drugs, the main transport pathway is considered to be the lipidic phase in both the model membrane and the skin. The drug permeability depends on the solubility ratio. The main permeation barrier in the skin for extremely hydrophobic compounds is the dermis, which acts as an aqueous matrix.[19] The permeation resistance of the dermis ($P=2$—8×10^{-5} cm/s for *n*-alkanols, hairless mouse[19]) corresponds to the P_{filt} parameter (*ca.* 5×10^{-5} cm/s, Fig. 5b) in our model membrane system. Thus, the use of the P_{obs} value instead of the P_m value may be more appropriate, although these values were similar for the drugs we tested. Hatanaka *et al.*[4,9] predicted skin permeability by combining nonpolar (silicone) and porous (poly(2-hydroxyethyl methacrylate)) membranes. In this context, our model membrane closely mimics the skin.

In conclusion, we developed a model membrane system for drug permeation study using a mixture of SC lipids. The membrane has both lipidic and aqueous pathways and

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closely mimics the skin in drug permeability for both hydrophilic and hydrophobic drugs. The permeation resistance is much improved compared to other reported systems, although it is ten times smaller than that of guinea pig skin. We can enhance the barrier property by applying a larger amount of lipids or by modifying the lipid composition. The effects of enhancers on the SC lipid membrane permeability are of a great concern. Our preliminary results show that incorporation of Azone, an enhancer, increased the permeation of CB through the model membrane 3 times (30 mol% incorporation), and through guinea pig skin 5 times (48 μmol/cm^2/application). A systematic study in the presence of several absorption enhancers is in progress.

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