J. Pharm. Pharmacol. 1997, 49: 397–402
Received December 10, 1996
Accepted January 3, 1997

Characterization of the Influence of some Cyclodextrins on the Stratum Corneum from the Hairless Mouse

M. VITÓRIA L. B. BENTLEY, RENATA F. VIANNA, SUSAN WILSON* AND JOHN H. COLLETT*

*Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av do Café s/n, 14040–903, Ribeirão Preto – SP, Brasil and *Department of Pharmacy and of Biological Sciences, University of Manchester, Oxford Road, Manchester, M13 9PL, UK*

Abstract

Differential scanning calorimetry (DSC), Fourier-transform infrared (FTIR) spectroscopy and transmission electron microscopy (TEM) have been used to determine the influence of β-cyclodextrin (β-CyD), hydroxypropyl-β-CyD (HP-β-CyD) and γ-CyD on the structural properties of the stratum corneum from the hairless mouse.

Some modest changes in the stratum corneum lipid transition temperature were induced by HP-β-CyD and blue shifts were observed in the FTIR spectra of the C–H asymmetric and symmetric stretching of the lipids from the stratum corneum. Results from TEM studies indicated that HP-β-CyD caused removal and possible disorganization of the lipid matrix that envelopes the corneocytes of the stratum corneum, whereas no effect was seen after treatment of the samples with β-CyD and γ-CyD.

These results suggest that HP-β-CyD can increase the permeability of the stratum corneum possibly as a result of extraction of lipids, and might thus enhance drug permeation through the skin.

The stratum corneum is the rate-limiting barrier to percutaneous delivery for most drugs and investigators have attempted to increase its permeability. One approach has been to employ penetration enhancers, which partition into and interact with the constituents of the stratum corneum, thereby reducing the barrier function and thus facilitating drug diffusion through the skin. Cyclodextrins (CyDs) are cyclic oligosaccharides consisting of 6, 7 or 8 glucopyranose units, usually referred to as α-, β- or γ-CyD, respectively. The aqueous solubility of lipophilic substances can be increased by complexation into the hydrophobic cavity of cyclodextrins. Some cyclodextrin derivatives might function as effective penetration enhancers by influencing the distribution and partitioning of the drug in the skin, or by changing the structure of the impermeable barrier (Vollmer et al 1994; Kim & Chien 1995).

The process of penetration enhancement has been studied using physical techniques (Cornwell & Barry 1993). Using differential scanning calorimetry (DSC), Van Duzee (1975) observed four endothermic transitions in the stratum corneum from man. Changes of these transition temperatures are a possible indication of interaction between compounds and the stratum corneum (Leopold & Lippold 1995). Fourier-transform infrared (FTIR) spectroscopy can be helpful in the elucidation of the molecular structure of the stratum corneum and has been used in studies of mechanism of action of penetration enhancers (Clancy et al 1994).

Methods such as DSC and FTIR spectroscopy provide independent yet complementary information about lipid-lipid and lipid-protein interactions in the stratum corneum. Morphological methods for visualization of the structures of stratum corneum, such as scanning electron microscopy (SEM), environmental scanning electron microscopy (ESEM) and transmission electron microscopy (TEM) enable the determination of the structural basis of the effect of different types of penetration enhancer and the impact of different types of vehicle on stratum corneum membrane structure (Elias 1991; Ohara et al 1994).

Correspondence: M. V. L. B. Bentley, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av do Café s/n, 14040–903, Ribeirão Preto – SP, Brasil.

Our purpose in this work was to investigated the influence of β-CyD, γ-CyD and a derivative, hydroxypropyl-β-cyclodextrin (HP-β-CyD) on the stratum corneum from hairless mouse using calorimetric and spectroscopic measurement. In addition, the morphological characteristics of the stratum corneum were analysed by TEM.

Materials and Methods

Materials

β-CyD and HP-β-CyD from Roquette (Lestrem, France) and γ-CyD and trypsin type III from Sigma (St Louis, MO) were used as received. All other chemicals were BDH reagent grade.

Preparation of hairless mice stratum corneum samples

Abdominal full-thickness skin was excized from hairless mice (male, one month old, strain CD1 nude, Charles River, Manston, Kent, UK) and the subcutaneous fat and connective tissue were removed using forceps. The stratum corneum was separated from the epidermis after 'floating' for 14 h on a solution of trypsin type III (0·1% w/v) and sodium bicarbonate (0·5% w/v) at room temperature. The enzyme digests the nucleated epidermal tissue, enabling the remnants to be removed by gentle rubbing with cotton wool. The stratum corneum sheets were rinsed repeatedly with distilled water, gently pressed between paper tissue, spread on filter paper and subsequently dried by storage in a desiccator over silica-gel for a maximum of two weeks before use. Delipidized stratum corneum samples

were obtained by exhaustive extraction of dried stratum corneum sheets with a mixture of chloroform and methanol (2 : 1, v/v) for 24 h, then soaking in acetone for 4 h, hexane for 24 h and ethanol-diethyl ether (8 : 92, v/v) for 24 h (Anigbogu et al 1995). Lipid extraction was performed at room temperature with constant stirring. At the end of the procedure the delipidized stratum corneum sheets were dried at room temperature and stored as described above.

Preparation of hairless mouse stratum corneum samples for DSC and spectroscopic measurements

Dried stratum corneum samples of known weight were incubated for 12 h with aqueous solutions of β-CyD (1·8% w/v), HP-β-CyD (20·0% w/v) and γ-CyD (6·0% w/v) at room temperature. The use of different concentrations of the cyclodextrin solutions was a consequence of their different aqueous solubilities. At the end of the incubation period the samples were rinsed with distilled water, dried for several hours over silica-gel and re-weighed. All samples were than hydrated to 30–60% w/w by storage for 3 days in a chamber at constant relative humidity (97% at 25°C) containing a saturated potassium sulphate solution. The hydration level was defined as: [(weight of the hydrated sample – weight of the dry sample)/weight of the dry sample] × 100. Untreated and delipidized stratum corneum samples served as controls. Samples of stratum corneum of the same origin were used for treatment with the different cyclodextrin solutions.

DSC measurements

The DSC studies were performed using a Seiko 2400 (Seiko Instruments, Tokyo, Japan) linked to a Seiko 2100 data analysis station. Stratum corneum samples (20–25 mg) equilibrated to 30–60% hydration were hermetically sealed in steel pans. The samples were scanned over the temperature range 0–120°C at a heating rate of 5° min^{-1}.

Thermograms were examined for phase transition enthalpies (peak areas), lipid-phase transition temperatures and transition temperature endotherms. The data obtained from stratum corneum alone were compared with those obtained after treatment with the cyclodextrins studied.

FTIR measurements

FTIR studies of the stratum corneum were performed using an ATI Mattson Genesis Series FTIR spectrometer. Stratum corneum samples approximately 1 cm in diameter at 30–60% hydration were sealed between two zinc sulphide IR-transparent windows using a Perkin-Elmer solution cell as a support. The ensemble was mounted in the path of the IR beam. All spectra were recorded at ambient temperature (20–25°C) in the frequency range 4000–400 cm^{-1} (4 cm^{-1} resolution, representing the average of 100 scans).

Transmission electron microscopy

Skin samples (1 cm^2) freshly excized from the abdominal surface of hairless mice, were incubated in aqueous solutions of β-CyD (1·8% w/v), HP-β-CyD (20·0% w/v) and γ-CyD (6·0% w/v) for 12 h at ambient temperature. At the end of the incubation the samples were rinsed with distilled water and dried with tissue paper. Layers of stratum corneum were removed from the skin samples by use of adhesive tape (Sellotape), fixed with an aqueous solution of ruthenium tetroxide (0·3% w/v) for 10 min, then rinsed well in distilled water and left to dry. The adhesive tape was removed and the remaining adhesive and stratum corneum layers were coated with agar (1% w/v) and left to dry. This procedure enabled easier handling of the samples during the rest of the processing schedule. The samples coated with agar were dehydrated by successive immersion in a series of aqueous ethanol solutions containing increasing concentrations of ethanol, then embedded in Spurr resin (Polyscience, Warrington, PA) and polymerized at 60°C overnight. Ultra-thin transverse sections (40–60 μm) were cut by use of a diamond knife, collected on formvar-coated grids, stained with uranyl acetate and lead citrate and examined in a Philips 400 transmission electron microscope.

Results and Discussion

DSC and IR spectroscopy detect thermally induced structural transitions and molecular modifications, respectively. Transitions in the DSC thermal profile reflect the 'melting' of extended structural domains of the lipids; this results in changes in the heat capacity. Changes in the IR spectrum, on the other hand, reflect changes in vibrational modes and thus provide information at the molecular level. Together, these methods provide complementary information about the structure of the stratum corneum.

Fig. 1 shows the DSC thermograms of hydrated stratum corneum from hairless mouse skin. Three major transitions in the DSC profiles of untreated stratum corneum were observed at approximately 73, 87 and 104°C. The lipid phase transition at approximately 30°C was not observed with all the samples examined. Other workers have indicated that this transition is not easily detected (Potts et al 1991). For this reason it was not possible to determine the effect of the treatment with cyclodextrins on this transition which does not seem be of major importance when investigating the mechanism of action of penetration enhancers (Cornwell & Barry 1993). The transition at approximately 104°C is considered to represent the denaturation of protein (Van Duzee 1975). The enthalpy of this transition was very sensitive to changes in the water content of the stratum corneum. When the water content was below 20% w/w this transition could be small or non existent. In this study the transitions at 73 and 87°C were regarded as being related to the influence of the cyclodextrins on the stratum corneum. The extraction of lipids and re-heating of untreated samples of hairless mouse stratum corneum were performed to characterize the origin of the transition temperatures. The thermogram obtained upon cooling and re-heating of the sample showed two main thermally induced transitions at approximately 30 and 73°C which remained unchanged upon re-heating the sample. In contrast, the highest temperature transition, at approximately 104°C, was heat-irreversible and was probably a result of the denaturation of proteins. The transition temperature at approximately 87°C was slightly altered after the second heating of the stratum corneum and was not present in the thermogram of delipidized stratum corneum. In the thermal profile of delipidized stratum corneum, the three transitions that normally occurred below 90°C were absent or greatly reduced, whereas the peak at approximately 104°C remained, suggesting that the peaks at approximately 30, 73 and 87°C arise from lipid components. Interpretations of the

Table 1. Influence of aqueous cyclodextrin solutions on the thermal properties of the hairless mouse stratum corneum.

Treatment of the stratum corneum	Transition temperature (°C)	Transition temperature shift (°C)*	Transition enthalpy (mJ mg^{-1})	Endotherm transition (mW)
Untreated	73·3 ± 0·5	–	14·1 ± 1·5	−14·85 ± 1·1
1·8% β-CyD	71·3 ± 0·7	−2·0	20·4 ± 3·5	−12·94 ± 0·9
20% HP-β-CyD	70·1 ± 0·9	−3·2	26·3 ± 2·7	−5·75 ± 0·3
6% γ-CyD	73·0 ± 1·1	−0·3	22·3 ± 2·9	−6·52 ± 0·5

All the data are summarized as mean ± s.e.m., n = 3. *Difference between the average of transition temperatures of the treated stratum corneum and untreated stratum corneum.

significance of the endotherm at approximately 87°C are contradictory. The parameter is considered by several investigators to represent a phase transition of lipids associated with proteins (Knutson et al 1985; Barry 1991), whereas Van Duzee (1975) proposed that this transition reflected characteristics of both lipid and protein.

Table 1 shows the values of transitions, transition enthalpies and endotherm obtained from the DSC thermograms of the stratum corneum samples after treatment with aqueous solutions of β-CyD, HP-β-CyD and γ-CyD (Fig. 1). The transition at approximately 87°C was not usually detected in stratum corneum samples treated with cyclodextrin solutions. The transition temperature at approximately 73°C was clearly identified in each thermogram and has been considered as a main parameter for comparing the thermal profile of stratum corneum samples. It can be seen that treatment with γ-CyD did not cause changes in the transition temperature, whereas treatment with β-CyD and HP-β-CyD caused a small shift to a lower temperature. Curiously, the transition enthalpies were increased after treatment of the stratum corneum with cyclodextrins. Yamane et al (1995) noted similar behaviour in a study of the influence of menthone and nerolidol on the stratum corneum in man. Shifting the lipid transition temperatures to lower values suggests disruption or fluidization in the lipid bi-layers, whereas the increase in the transition enthalpies suggests an increase in lipid bi-layer organization. Studies of small-angle X-ray diffraction (Cornwell et al 1994) might, however, be used to account for this apparently contradictory finding. It was suggested that lipid disruption is achieved without reducing bi-layer periodicity, indeed some bi-layer reflections, possibly arising from lateral bi-layer swelling, appeared to be slightly enhanced. This effect might explain the observed increase in the lipid peak enthalpies. In addition, the endotherm of the peak at this transition was reduced in all the thermograms of samples treated with cyclodextrins, indicating less energy absorption by the sample.

The FTIR spectra from 2800–3000 cm^{-1} of hairless mouse stratum corneum at room temperature and approximately 30% hydration are shown in Fig. 2. Of particular interest are the peaks near 2850 and 2920 cm^{-1}, CH_2 and CH_3 symmetric stretching modes, respectively. The major contribution to the C–H stretching peaks of the stratum corneum is the absorbance of hydrocarbon chains of the lipids. It can be seen that the delipidization of the stratum corneum by the sequence of organic solvents dramatically reduced both C–H stretching absorbances. The treatment of stratum corneum with aqueous

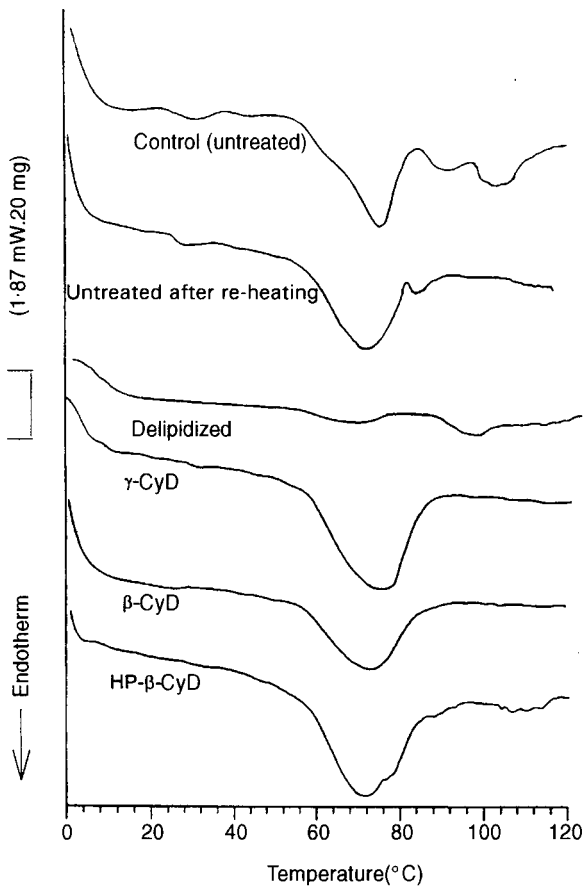

FIG. 1. DSC thermal profiles of hairless mouse stratum corneum hydrated to 30–60% w/w: untreated stratum corneum, untreated stratum corneum after re-heating, stratum corneum delipidized by use of a sequence of organic solvents and stratum corneum after treatment with aqueous solutions of γ-CyD (6·0% w/v), β-CyD (1·8% w/v) and HP-β-CyD (20·0% w/v).

solutions of cyclodextrins reduced the absorbances of the C–H stretching. The effect of HP-β-CyD was greatest in this respect. Others factors, such as pathlength (sample thickness), opacity, etc., can affect the absorbance in a spectrum. These possibilities were not considered, because we used stratum corneum samples (approximately five samples) from the same animal and the pathlength between the transparent windows

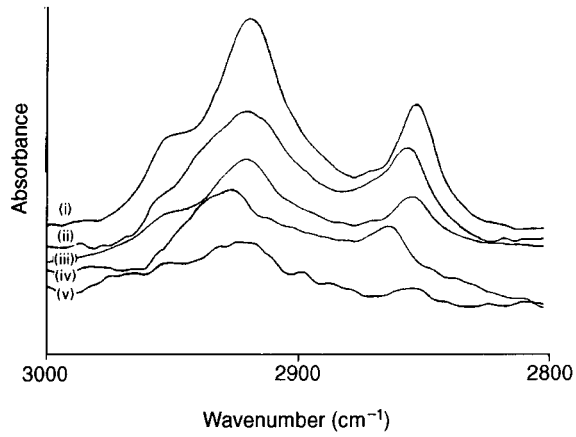

FIG. 2. FTIR spectra of hairless mouse stratum corneum in the C–H stretching region between 2800 and 3000 cm^{-1}: (i) untreated stratum corneum; (ii)–(v) stratum corneum treated with aqueous solution of β-CyD (1·8% w/v), γ-CyD (6·0% w/v), HP-β-CyD (20·0% w/v) and delipidized stratum corneum, respectively.

Table 2. Mean shifts of the CH_2 and CH_3 symmetric stretching frequencies caused by the different treatments.

Treatment	Frequency shift (cm^{-1})	
	CH_2 symmetric stretching	CH_3 symmetric stretching
γ-CyD	1·5 ± 0·2	1·4 ± 0·1
β-CyD	3·6 ± 0·3	4·8 ± 0·4
HP-β-CyD	9·4 ± 0·4	10·9 ± 0.8

was controlled by spacers 0·1 mm thick. Stratum corneum control samples (no treatment) from different animals did not show significant differences in C–H stretching absorbances (Student t-test, probability $< 0{\cdot}05$), probably because of the use of animals of the same age and weight. Similar behaviour was observed for the amide I band (1656 cm^{-1}), which could be used as internal standard. The treatment with HP-β-CyD caused a small blue shift of C–H stretching absorbances, i.e., approximately 9·4 (±0·4) cm^{-1} and 10·9 (±0·8) cm^{-1} to the CH_2 and CH_3 symmetric stretching, respectively (Table 2). Initial interpretation of these results suggests that the HP-β-CyD possibly caused fluidity in the domain of stratum corneum lipids, although the resolution of the equipment (4 cm^{-1}) and results from other work (Legendre et al 1995) do not confirm this possibility.

The unique organization of stratum corneum as a dual-compartment system of lipid-depleted corneocytes embedded in an extracellular matrix of non-polar, lipid-enriched lamellar bi-layers is well established. Fixation with ruthenium tetroxide has been shown to be suitable for characterizing the lipid organization of the corneocyte envelope (Hou et al 1991). Fig. 3 shows the ultra-structure of stratum corneum layers from hairless mouse skin after treatment with cyclodextrin solutions, and of delipidized and untreated stratum corneum. In the untreated stratum corneum the presence of the multiple lamellae filling of intercellular space can be observed. It is clear that the lamellae in the outer stratum corneum are closely opposed to the cell surfaces and there are several layers consisting of alternating electron-dense and electron-lucent bands. In the delipidized stratum corneum the lipids that form the lamellae were removed, and large lucent areas were seen in the intercellular space. Treatment with aqueous solutions of β-CyD and γ-CyD did not cause significant changes in the organization of the lipids in the intercellular spaces, and lamellae were well defined by electron-dense bands. HP-β-CyD, however, significantly changed the lamellar structure, suggesting removal of lipids and possibly disruption of the organized lipid bi-layer structure. These ultra-structural visualizations of the lipid matrix that form the corneocyte envelope confirm the DSC and FTIR studies. The treatment with β- and γ-CyD did not have any effect on the stratum corneum lipid matrix. HP-β-CyD, however, caused extraction of the stratum corneum lipids. In fact, HP-β-CyD could extract approximately 5 to 10% of cholesterol and some proteins from the stratum corneum (Legendre et al 1995), indicating that this cyclodextrin might increase the permeability of the skin.

On the basis of the capability of cyclodextrins to solubilize lipophilic drugs (Brewster et al 1995) and the presence of free lipids in the stratum corneum (Swartzendruber et al 1987), it might be hypothesized that lipids of the stratum corneum complex with cyclodextrins. Frijlink et al (1991) showed that HP-β-CyD forms stable complexes with endogenous cholesterol in the bloodstream and suggested that this cyclodextrin might be used to reduce the level of cholesterol and lipid-proteins in the blood. So, the mechanism of action of cyclodextrins on the membrane is thought to be via extraction of membrane components, i.e. lipids or proteins or both. The FTIR and most of the TEM results support this hypothesis.

It is interesting to consider that the cyclodextrins can also improve cutaneous drug permeability as a result of an increase in the partition coefficient of the drug into the skin, because its aqueous solubility is increased (Uekama et al 1992). Our previous work (Lopez et al 1996) showed that the permeation (flux) of dexamethasone acetate through hairless mouse skin increased twofold when this drug was complexed with HP-β-CyD. Williams et al (1996), however, have found that the pre-treatment of skin with ointments containing β-CyD or HP-β-CyD resulted in reduced permeation of toluene through the stratum corneum in man. In the case of pre-treatment with cyclodextrins, the drug can have its cutaneous permeability increased only if it does not form an inclusion complex (Legendre et al 1995). It might be suggested that the method used to assess the effect of cyclodextrins might influence the skin–drug permeation profile.

From the results obtained in this work it might be suggested that the HP-β-CyD caused extraction of lipid from the stratum corneum and thus increased permeation of drug through the skin.

Acknowledgements

The authors would like to thank Roquette Freres (Lestrem, France) for their donation of β-CyD and HP-β-CyD, and Mr Maurice Hart (Chemistry Department of University of Manchester for assistance with thermal analyses. This research was supported by a post-doctoral grant from Fundação de Amparo a Pesquisa do Estado de São Paulo, Brasil.

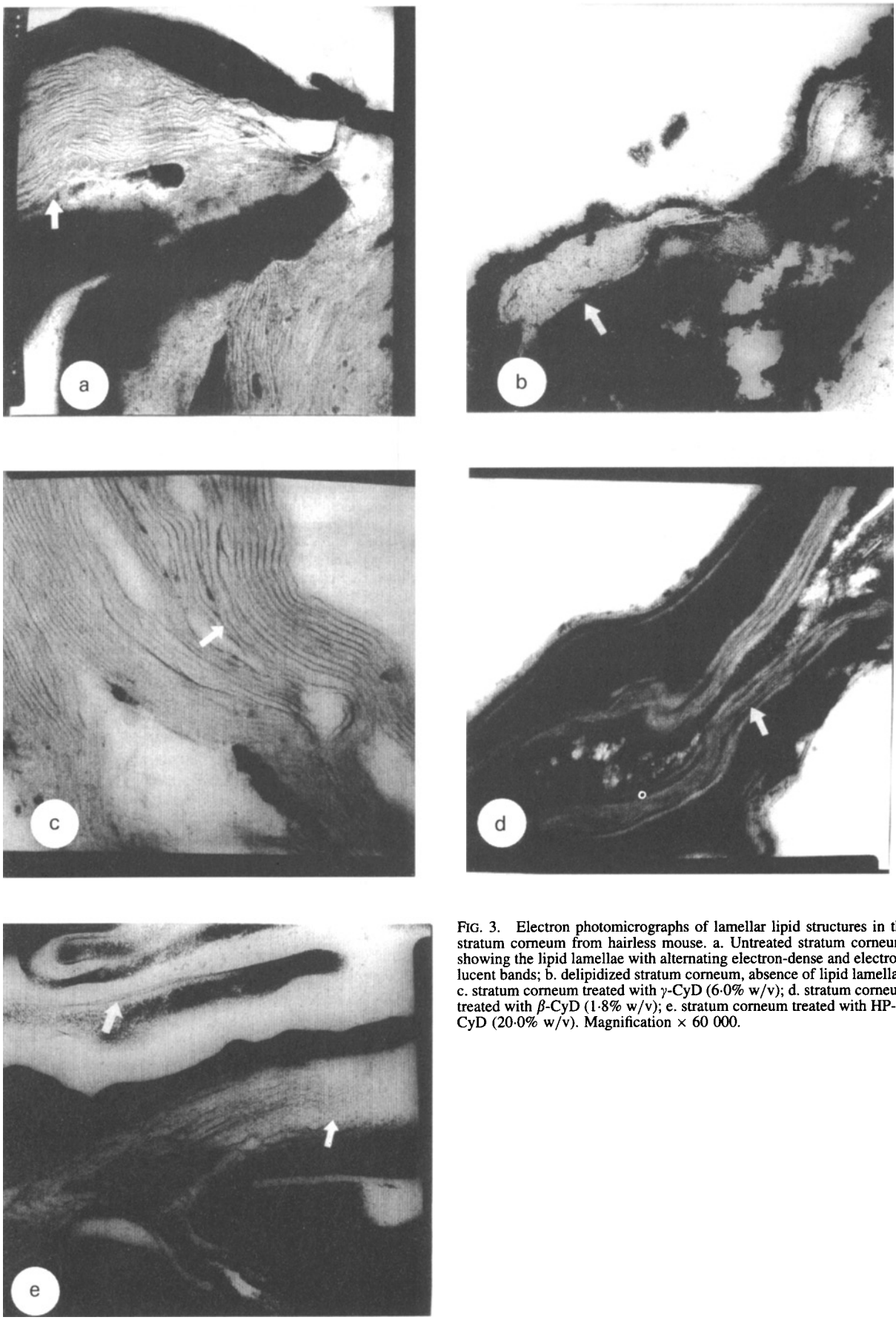

FIG. 3. Electron photomicrographs of lamellar lipid structures in the stratum corneum from hairless mouse. a. Untreated stratum corneum, showing the lipid lamellae with alternating electron-dense and electron-lucent bands; b. delipidized stratum corneum, absence of lipid lamellae; c. stratum corneum treated with γ-CyD (6·0% w/v); d. stratum corneum treated with β-CyD (1·8% w/v); e. stratum corneum treated with HP-β-CyD (20·0% w/v). Magnification × 60 000.

References

Anigbogu, A. N. C., Williams, A. C., Barry, B. W., Edwards, H. G. M. (1995) Fourier transform Raman spectroscopy of interactions between the penetration enhancer dimethyl sulfoxide and human stratum corneum. Int. J. Pharm. 125: 265–282

Barry, B. W. (1991) Lipid-protein-partitioning theory of skin penetration enhancement. J. Contr. Rel. 15: 237–248

Brewster, M. E., Anderson, W. R., Loftsson, T., Huang, M. J., Bodor, N., Pop, E. (1995) Preparation, characterization, and anesthetic properties of 2-hydroxypropyl-β-cyclodextrin complexes of pregnanolone and pregnenolone in rat and mouse. J. Pharm. Sci. 84: 1154–1159

Clancy, M. J., Corish, J., Corrigan, O. I. (1994) A comparison of the effects of electrical current and penetration enhancers on the properties of human skin using spectroscopic (FTIR) and calorimetric (DSC) methods. Int. J. Pharm. 105: 47–56

Cornwell, P. A., Barry, B. W. (1993) The route of penetration of ions and 5- fluorouracil across human skin and the mechanism of action of terpene skin penetration enhancers. Int. J. Pharm. 94: 189–194

Cornwell, P. A., Bouwstra, J. A., Goosis, G. S., Barry, B. W. (1994) Small-angle X-ray diffraction investigations of terpene enhancer actions on the lipid barrier in human skin. In: Brain, K. R., Hadgraft, V. J., Walters, K. A. (eds) Prediction of Percutaneous Penetration. IBC, London, Vol. 3, pp 18–26

Elias, P. M. (1991) Epidermal barrier function: intercellular lamellar lipid structures, origin, composition and metabolism. J. Contr. Rel. 15: 199–208

Frijlink, H. W., Eissens, A. C., Hefting, N. R., Poelstra, K., Lerk, C. F., Meirjer, D. K. F. (1991) The effect of parenterally administrated cyclodextrins on cholesterol levels in the rat. Pharm. Res. 8: 9–16

Hou, S. Y. E., Mitra, A. K., White, S. H., Menon, G. K., Ghadially, R., Elias, P. M. (1991) Membrane structures in normal and essential fatty acid-deficient stratum corneum: characterization by ruthenium tetroxide staining and X-ray diffraction. J. Invest. Dermatol. 96: 215–223

Kim, D.-D., Chien, Y. W. (1995) Transdermal delivery of dideoxynucleoside-type anti-HIV drugs. 1. Stability studies for hairless rat skin permeation. J. Pharm. Sci. 84: 1061–1066

Knutson, K., Potts, R. O., Guzek, D. B., Golden, G. M., Mckie, J. E., Lambert, W. I. (1985) Macro- and molecular-physical considerations in understanding drug transport in the stratum corneum. J. Contr. Rel. 2: 67–87

Legendre, J. Y., Rault, I., Luijten, W., Demuynck, I., Horvath, S., Ginot, Y. M., Cuine, A. (1995) Effects of β-cyclodextrins on skin: implications for the transdermal delivery of piribedil and a novel cognition enhancing-drug, S-9977. Eur. J. Pharm. Sci. 3: 311–322

Leopold, C. S., Lippold, B. C. (1995) An attempt to clarify the mechanism of penetration-enhancing effects of lipophilic vesicles with differential scanning calorimetry (DSC). J. Pharm. Pharmacol. 47: 276–281

Lopez, R. F. V., Bentley, M. V. L. B., Carvalho, F. S., Ribeiro, G., Pereira, N. L., Collett, J. H. (1996) Formation of cyclodextrin inclusion complexes with corticosteroids: solubility studies and characterization of the complexes. Pharm. Res. 13: 260

Ohara, N., Takayama, K., Machida, Y., Nagai, T. (1994) Combined effect of D-limonene and temperature on the skin permeation of ketoprofen. Int. J. Pharm. 105: 31–38

Potts, R. O., Golden, G. M., Francoeur, M. L., Mak, V. H. W., Guy, R. H. (1991) Mechanism and enhancement of solute transport across the stratum corneum. J. Contr. Rel. 15: 249–260

Swartzendruber, D. C., Wertz, P. W., Madison, K. C., Downing, D. T. (1987) Evidence that the corneocyte has chemically bound lipid envelope. J. Invest. Dermatol. 88: 709–713

Uekama, K., Adachi, H., Irie, T., Yano, T., Saita, M., Noda, K. (1992) Improved transdermal delivery of prostaglandin E_1 through hairless mouse skin: combined use of carboxymethylethyl-β-cyclodextrin and penetration enhancers. J. Pharm. Pharmacol. 44: 119–121

Van Duzee, B. F. (1975) Thermal analysis of human stratum corneum. J. Invest. Dermatol. 65: 404–408

Vollmer, U., Muller, B. W., Peeters, J., Mesens, J., Wilffert, B., Peters, T. (1994) A study of percutaneous absorption-enhancing effects of cyclodextrin derivatives in rat. J. Pharm. Pharmacol. 46: 19–22

Williams, A. C., Shatri, S. R. S., Barry, B. W. (1996) Transdermal permeation inhibition by cyclodextrins. Pharm. Res. 13: 386

Yamane, M. A., Williams, A. C., Barry, B. W. (1995) Effects of terpenes and oleic acid as skin penetration enhancers towards 5-fluorouracil as assessed with time; permeation, partitioning and differential scanning calorimetry. Int. J. Pharm. 116: 237–251