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Research Article

The Role of Protein and Lipid Domains in the Uptake of Solutes by Human Stratum Corneum

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The uptake of a series of hydrocortisone esters varying in lipophilicity from water into untreated and delipidized human stratum corneum has been determined. The partition coefficients of solutes into fully hydrated stratum corneum are postulated to represent the separate contributions of three structurally distinct domains—the extractable lipids, protein, and the solvent domain. The solvent domain was assumed to have the properties of bulk water. The relative affinities of the protein and lipid domains of stratum corneum for solutes varying in structure were determined by comparing solute uptake in untreated and delipidized stratum corneum. Partitioning into the extracted lipids was also examined. Solute uptake into stratum corneum may be governed by the protein domain, the lipid domain, or a combination of the two, depending on solute lipophilicity. Due to differences in the selectivity of the two domains, a change in uptake mechanism occurs with increasing solute lipophilicity from protein-dominated uptake for hydrophilic solutes to lipid domain-dominated uptake for lipophilic solutes. The stratum corneum lipid content, which varies dramatically from individual to individual (3–46% in this study), is an important determinant of the affinity of the stratum corneum for highly lipophilic solutes but has no effect on the uptake of hydrophilic solutes.

KEY WORDS: stratum corneum; partition coefficients; hydrocortisone esters; lipids, protein; uptake; water of hydration.

INTRODUCTION

The stratum corneum, generally regarded to be the rate-limiting barrier for transport of most solutes of pharmaceutical interest across the skin (1–3), consists of a mosaic of cornified cells containing cross-linked keratin filaments and intercellular lipid-containing regions (4). In spite of this well-documented heterogeneity, most studies of drug transport through the skin treat the stratum corneum as a homogeneous membrane. Thus, solute fluxes are assumed to be directly proportional to stratum corneum/water partition coefficients and diffusivities and inversely proportional to the macroscopic thickness of the stratum corneum.

Some recent experimental observations appear to conflict with predictions arising from the assumption of homogeneity. For example, rates of percutaneous transport across human skin are not influenced by the number of cell layers or the thickness of the stratum corneum but, instead, correlate inversely with the lipid content (5). These stratum corneum lipids may be pooled in the intercellular spaces, forming broad, multilamellar sheets which constitute the barrier to diffusion (6). Similarly, in reaggregated stratum corneum cell systems, the effectiveness of the barrier function is directly proportional to the lipid content (7) rather than the barrier thickness. Stratum corneum/water (vehicle) partition coefficients obtained in equilibrium experiments may also have limited value in predicting relative permeabilities if the domain probed in partitioning studies is not the rate-limiting domain for transport. A molecular-level understanding of the nature of the transport barrier, its selectivity to solutes varying in chemical structure, and its modification by penetration enhancers, occlusion, and other treatments requires methods which probe the properties of the actual barrier microenvironment and not the stratum corneum as a whole.

The knowledge that systematic increases in solute lipophilicity lead to corresponding increases in permeability (1,8) for most solutes of pharmaceutical interest and that lipid extraction destroys the barrier properties of the stratum corneum (9) supports the idea that a lipid pathway is involved. Recent studies suggest that lipids in the intercellular spaces organized as bilayer membranes constitute the rate-limiting barrier for solutes which permeate via the lipid pathway (8). Solute partition coefficients into the lipid regions of the stratum corneum may therefore be more relevant quantities for predicting relative permeabilities.

In this paper an approach is developed to measure solute partition coefficients into the lipid and protein domains of stratum corneum. The separation of solute partitioning into the protein and lipid domains represents the first level of complexity beyond treating the stratum corneum as a homogeneous membrane. Uptake studies have been conducted as a function of solute lipophilicity using a series of 21-esters of hydrocortisone varying in acyl chain length and

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terminal substitution to characterize the solvent properties of these distinct domains.

MATERIALS AND METHODS

Full-thickness human skin was obtained from cadavers at autopsy or from elective abdominoplastic surgery (Department of Dermatology, School of Medicine, University of Utah, Salt Lake City). The source of skin with respect to body region, age, sex, etc., was not controlled but such information was recorded for each sample. Skin samples were stored 7–10 days in RPMI-1640 with 10% bovine serum at 4°C prior to isolation of the stratum corneum. Sunburn stratum corneum samples were obtained from volunteers and were used without further treatment.

Reagents used for chemical synthesis are described in the following section. All other chemicals were of analytical grade, obtained commercially, and were used as received.

All melting points (mp), uncorrected, were obtained using a Mel-Temp capillary melting point apparatus (Laboratory Devices, Cambridge, Mass.). Proton nuclear magnetic resonance (NMR) spectra and elemental analyses were determined by 3M Corporate Research Center, St. Paul, Minnesota. Ultraviolet (UV) spectra were determined using a Perkin–Elmer Lambda 7 UV/visible spectrophotometer. Fourier transform infrared spectrometry (FTIR) studies were conducted using a Beckman 2100 spectrometer equipped with a triglycine sulfate detector. Spectra were obtained at 2-cm^{-1} resolution and 64 scans. Radioactivity was counted on a Beckman Model LS 1801 liquid scintillation counter.

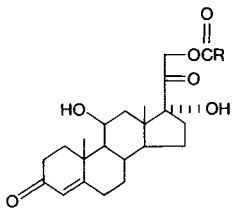
Liquid Chromatographic Analyses

A modular high-performance liquid chromatographic (HPLC) system consisting of an automated sample injector (Wisp Model 710B; Waters Associates, Milford, Mass.), a dual-wavelength absorbance detector (Model 441; Waters Associates) operated at 245 nm, an integrator (Model 740; Waters Associates), a constant-flow pump (Model M-45; Waters Associates) operated at 1 to 1.5 ml/min, and a reversed-phase column packed with 5-μm Spheri-5 RP-18 (NOVA PAK C_{18}; Waters Associates) was used. The mobile phase was altered depending on the compound used, but the mobile-phase composition was generally 30 to 75% acetonitrile in water.

Solute concentrations were determined using peak heights against external standards. The peak height response versus the concentration of standards was linear and the test samples were appropriately diluted to bring the solute concentrations within this linear range.

General Procedure for Hydrocortisone Ester Synthesis (1a–c, 1e–j)

Esters of hydrocortisone varying in chain length and terminal substituents (Scheme I) were prepared using previously published techniques (10) either (i) via displacement of the 21-mesylate by the appropriately substituted carboxylic acid or (ii) via initial synthesis of the appropriate dicarboxylic acid hemiester followed by amide formation at the terminal carboxyl. Product formation was monitored by HPLC.



1a–k

COMPOUND	R
1a	$-CH_2CH_2CONH_2$
1b	$-CH_2CH_2CON(CH_3)_2$
1c	$-CH_2CH_2COOCH_3$
1d	$-CH_2CH_2COOH$
1e	$-(CH_2)_4CH_2COOH$
1f	$-(CH_2)_4CH_2CONH_2$
1g	$-(CH_2)_4CH_2OH$
1h	$-CH_2CH_3$
1i	$-(CH_2)_4CH_2COOCH_3$
1j	$-(CH_2)_4CH_3$
1k	$-(CH_2)_6CH_3$

Scheme I

The products were purified by using classical pH-controlled extraction techniques with ethyl acetate or isobutyl alcohol as solvent. Further purification, when necessary, was accomplished by preparative reversed-phase liquid chromatography (Hibar Lichrosorb RP-18 semipreparative column, E. Merck, Darmstadt, West Germany). As a final step, the compounds were recrystallized in an appropriate organic solvent and dried *in vacuo*.

Hydrocortisone, hexanoic acid, monomethyl succinate, succinamic acid, propionic acid, pimelic acid, 6-bromohexanoic acid, hydrocortisone-21-hemisuccinate (**1d**), and hydrocortisone-21-octanoate (**1k**) were obtained commercially (Sigma Chemical Company) and used as supplied.

The monoamide and mono-*N,N*-dimethyl amide of pimelic acid were prepared by substituting the dicyclohexyl carbodiimide derivative of pimelic acid with ammonium hydroxide and dimethylamine, respectively. 6-Hydroxyhexanoic acid was prepared by reacting 6-bromohexanoic acid with aqueous NaOH at 70°C.

Hydrocortisone-21-mesylate was prepared from the reaction of 1.1 equivalents of methanesulfonyl chloride with 1 equivalent of hydrocortisone in tetrahydrofuran (THF) containing 1.1 equivalents of triethylamine. Hydrocortisone-21-iodide was prepared by reacting 1 equivalent of 21-mesylate with 1.6 equivalents of sodium iodide in acetone at 50°C.

21-[(4-Amino-1,4-dioxobutyl)oxy]-11,17-dihydroxypregn-4-ene-3,20-dione (1a). To a solution of 1.32 g (2.89 mmol) hydrocortisone mesylate in *N,N*-dimethylformamide (DMF; 30 ml) was added 2.52 ml (14.45 mmol) diisopropylethylamine and 1.69 g (14.43 mmol) succinamic acid. After heating overnight at 60°C, excess deionized water was added, resulting in a white precipitate which was recrystallized from DMF/water and DMF/butyl chloride. Yield, 97%; mp 226–228°C; UV: λ_{max} 242 nm (ϵ = 16,077); purity >98.5% by HPLC; ^1H NMR (ME_2SO-d_6): δ 0.78 (s, 3, 18-CH_3), 1.35 (s, 3, 19-CH_3), 4.27 (br s, 1, 11-H), 4.9 (AB, 2, 21-CH_2), 5.7 (s, 1, 4-H), and 7.1 (d, 2, -$CONH_2$). Anal. ($C_{25}H_{35}N_1O_7$) C, H, N.

21-[(4-Dimethylamino-1,4-dioxobutyl)oxy]-11,17-dihy-

droxypregn-4-ene-3,20-dione (1b). To a solution of 1 g (2.16 mmol) hydrocortisone hemisuccinate in THF (20 ml) which was immersed in a dry ice/acetone bath and flushed with dry nitrogen was added 0.36 ml (2.6 mmol) triethylamine and 0.36 ml (2.6 mmol) isobutyl chloroformate. The reaction mixture was warmed to room temperature and within 10 min 0.56 ml (3.2 mmol) dimethylamine was added. The product which precipitated out was redissolved in DMF/butanol, purified by extraction, and recrystallized in DMF/butanol/water. Yield, 66%; mp, 223–224°C, lit 228–233°C (11); UV: λ_{max} 242 nm (ϵ-16,285); purity >99% by HPLC; ^{1}H NMR (Me_2SO-d_6): δ 0.75 (s, 3, 18-CH_3), 1.36 (s, 3, 19-CH_3), 2.9 [d, 6, -$N(CH_3)_2$, 4.28 (br s, 1, 11-H), 4.9 (AB, 2, 21-CH_2), and 5.6 (s, 1, 4-H). Anal. ($C_{27}H_{39}N_1O_7$) C, H, N.

21-[(4-Methoxy-1,4-dioxobutyl)oxy]-11,17-dihydroxypregn-4-ene-3,20-dione (1c). To a solution of 1.28 g (2.8 mmol) hydrocortisone mesylate in DMF (25 ml) was added 2.52 ml (14.45 mmol) diisopropylethylamine and 1.19 g (14.45 mmol) monomethyl succinate. The mixture was heated at 60°C overnight. Following extractive purification (ethyl acetate/water) and solvent evaporation the product was dissolved in acetonitrile. Evaporation of acetonitrile yielded a white solid mass which was triturated in water. Yield, 76%; mp 142–145°C; UV: λ_{max} 242 nm (ϵ = 16,241); purity >98.5% by HPLC; ^{1}H NMR ($CDCl_3$): δ 0.92 (s, 3, 18-CH_3), 1.4 (s, 3, 19-CH_3), 3.7 (s, 3, -OCH_3), 4.46 (br, s, 1, 11-H), 4.95 (AB, 2, 21-CH_2), and 5.7 (s, 1, 4-H). Anal. ($C_{26}H_{36}O_8$) C, H.

21-[(1-Oxo-6-carboxyhexyl)oxy]-11,17-dihydroxypregn-4-ene-3,20-dione (1e). To a solution of 3 g (6.81 mmol) hydrocortisone 21-mesylate in DMF (60 ml) was added 14.6 ml (83.69 mmol) diisopropylethylamine followed by 6.63 g (14.39 mmol) pimelic acid. The mixture was heated at 60°C for 2 hr. Following solvent removal and purification by silica gel column chromatography (ethyl acetate/butyl chloride) and evaporation of ethyl acetate, a 76% yield of an amorphous solid was obtained from the syrupy residue after sitting at room temperature for 2 days. Further purification by liquid chromatography, extraction (ethyl acetate/butyl chloride/water), and acetonitrile recrystallization gave a white solid product: mp 111–114°C; UV: λ_{max} 242 nm (ϵ = 15,300); purity >97% by HPLC; ^{1}H NMR (Me_2SO-d_6): δ 0.75 (s, 3, 18-CH_3), 1.35 (s, 3, 19-CH_3), 4.25 (br s, 1, 11-H), 4.9 (AB, 2, 21-CH2), and 5.6 (s, 1, 4-H). Anal. ($C_{28}H_{40}O_8$) C, H.

21-[(7-Amino-1,7-dioxoheptyl)oxy]-11,17-dihydroxypregn-4-ene-3,20-dione (1f). To a solution of 1.1 g (2.5 mmol) hydrocortisone 21-mesylate in DMF (10 ml) was added 0.54 ml (3.1 mmol) diisopropylethylamine followed by 0.4 g (2.51 mmol) pimelic amide and 0.113 g (0.75 mmol) sodium iodide in acetone. The mixture was heated at 50°C overnight. Following extractive purification (ethyl acetate/water) and evaporation of ethyl acetate, the product was recrystallized in acetonitrile and triturated in butyl chloride to yield 46% white solid. Two additional recrystallizations in acetonitrile and a liquid chromatographic purification gave a white solid product: mp 184–186°C; UV: λ_{max} 242 nm (ϵ = 17.073); purity >99% by HPLC: ^{1}H NMR (Me_2SO-d_6): δ 0.8 (s, 3, 18-CH_3), 1.4 (s, 3, 19-CH_3), 4.28 (br s, 1, 11-H), 4.9 (AB, 2, 21-CH_2), 5.6 (s, 1, 4-H), and 7.1 (d, 2, -$CONH_2$). Anal. ($C_{28}H_{41}N_1O_7$) C, H, N.

21-[1-Oxo-6-hydroxyhexyl)oxy]-11,17-dihydroxypregn-4-ene-3,20-dione (1g). To a solution of 1.49 g (3.15 mmol) hydrocortisone 21-iodide in DMF (6.3 ml) was added 0.61 ml (3.5 mmol) diisopropylethylamine followed by 0.42 g (3.18 mmol) 6-hydroxyhexanoic acid in DMF. The mixture was heated at 60°C for 2 hr. Following pH-controlled extractive purification (ethyl acetate/water) and evaporation of ethyl acetate, the product was recrystallized from acetonitrile/butyl chloride. Two additional purifications by liquid chromatography gave 12% of a white solid product: mp 143–145°C; UV: λ_{max} 242 nm (ϵ = 16,098); purity >99% by HPLC: ^{1}H NMR ($CDC1_3$): δ 0.92 (s, 3, 18-CH_3), 1.46 (s, 3, 19-CH_3), 3.65 (t, 3, -CH_2OH), 4.46 (br s, 1, 11-H), 4.9 (AB, 2, 21-CH_2), and 5.7 (s, 1, 4-H). Anal. ($C_{27}H_{40}O_7$) C. H.

21-[(1-Oxopropyl)oxy]-11,17-dihydroxypregn-4-ene-3,20-dione (1h). To a solution of 1.25 g (2.74 mmol) hydrocortisone 21-mesylate in DMF (50 ml) was added 2.39 ml (13.7 mmol) diisopropylethylamine followed by 1.02 ml (13.7 mmol) propionic acid. The mixture was heated at 60°C for 1–2 days. Following solvent removal and extractive purification (ethyl acetate/water) the product was recrystallized in acetonitrile/butyl chloride and acetonitrile to give a white crystalline product. Yield, 84%; mp 196–197°C; UV: λ_{max} 242 nm (ϵ = 16,770); purity >98% by HPLC; ^{1}H NMR ($CDC1_3$): δ 0.92 (s, 3, 18-CH_3), 1.2 (t, 3, 24-CH_3), 1.42 (s, 3, 19-CH_3), 4.46 (br s, 1, 11-H), 4.95 (AB, 2, 21-CH_3), and 5.7 (s, 1, 4-H). Anal. ($C_{24}H_{34}O_6$) C, H.

21-[(7-Methoxy-1,7-dioxoheptyl)oxy]-11,17-dihydroxypregn-4-ene-3,20-dione (1i). Eleven drops of sulfuric acid in methanol (prepared by adding 2 drops of pure sulfuric acid to 80 ml methanol) were added to 0.75 g (1.5 mmol) hydrocortisone 21-pimelate (1e) in 45 ml methanol. The mixture was heated at 50°C for 3 hr. Following termination of the reaction by neutralization and liquid chromatographic purification, a 43% yield of white amorphous solid was obtained. Trituration in butyl chloride for 2 days and two additional purifications by liquid chromatography gave a solid product: mp 142–143°C; UV: λ_{max} 242 nm (ϵ = 16,855); purity 98.7% by HPLC; ^{1}H NMR ($CDC1_3$): δ 0.92 (s, 3, 18-CH_3), 1.4 (s, 3, 19-CH_3), 3.7 (s, 3, -OCH_3), 4.46 (br s, 1, 11-H), 4.9 (AB, 2, 21-CH_2), and 5.7 (s, 1, 4-H). Anal. ($C_{29}H_{42}O_8$) C, H.

21-[(1-Oxohexyl)oxy]-11,17-dihydroxypregn-4-ene-3,20 dione (1j). To a solution of 1.32 g (2.89 mmol) hydrocortisone mesylate in 30 ml dry *N,N*-dimethylformamide were added 2.52 ml (14.45 mmol) diisopropylethylamine and 1.81 ml (14.45 mmol) hexanoic acid. The mixture was heated overnight at 60°C. Following solvent removal and extractive purification (ethyl acetate/water) and evaporation of ethyl acetate, the product was recrystallized twice from acetonitrile/water. Yield, 78%; mp 152–153°C, lit. 146–154°C (12); UV: λ_{max} 242 nm [ϵ = 16,000, lit. ϵ = 15210 (12)], purity >99% by HPLC; ^{1}H NMR ($CDC1_3$): δ 0.92 (s, 3, 18-CH_3), 1.4 (s, 3, 19-CH_3), 4.46 (br s, 1, 11-H), 4.95 (AB, 2, 21-CH_2), and 5.7 (s, 1, 4-H). Anal. ($C_{27}H_{40}O_6$) C, H.

Isolation of Human Stratum Corneum

A slight modification of a previously published epidermal separation technique was used (13). The dermal side of the whole skin was placed on a filter paper saturated with 0.75% EDTA solution (EDTA, tetrasodium salt trihydrate,

98%), Aldrich Chemical Company, Milwaukee, Wis.) and kept at 37°C in a sealed petri dish for 2 hr. At the end of this period the entire intact layer of the epidermis was carefully removed from the rest of the skin. The thin sheet of epidermis was then placed dermal side down on a filter paper saturated with 0.0001% trypsin solution (Sigma Chemical Company., St. Louis, Mo.) in carbonate buffer at pH 8.0 and digested overnight at 37°C in a sealed petri dish. After digestion of the viable epidermis the dermal side of the stratum corneum was gently swabbed with a moist Q-tip, rinsed thoroughly with deionized water, and dried overnight.

Delipidization

Preweighed dry stratum corneum samples were placed in 10-ml screw-cap glass tubes containing a 2:1 chloroform/methanol mixture and gently agitated for 20 hr at room temperature using a Hematology/Chemistry mixer (Fisher Scientific Products, Springfield, N.J.). At the end of this period the delipidized stratum corneum samples were removed, rinsed twice with fresh chloroform/methanol solvent, and dried to a constant weight. Selected samples were further subjected to the same treatment a second and third time to ensure complete removal of extractable lipids.

Stratum corneum lipid content was determined by two methods—by the change in weight of the stratum corneum after solvent extraction and by the weight of the lipid residue obtained after filtering the solvent extract in preweighed class vials and evaporating the solvent. The time required for removal of chloroform–methanol-extractable lipids was examined by analyzing lipid content in selected samples after extraction for up to 19 days. In some cases after 1 day of extraction, the same sample was reextracted with fresh solvent for several days.

FTIR spectra were also obtained on untreated and delipidized stratum corneum (after 1 and 19 days of extraction) to examine the completeness of removal of extractable lipids.

Octanol/Water Partition Coefficients

Octanol/water partition coefficients were determined by the shake flask method (14). After vigorous mixing and centrifugation, the combined phases were allowed to stand at 37°C for at least 24 hr. The aqueous phase was appropriately diluted and assayed by HPLC. Aliquots from the organic phase were evaporated and the residues were redissolved in the mobile phase before analyzing by HPLC. The apparent partition coefficient was calculated as the ratio of peak heights of the solute in each of the two phases.

^{14}C-Sucrose Uptake and Recovery Studies

Accurately weighed (10- to 15-mg) stratum corneum samples (untreated and delipidized) were placed in glass vials containing an aqueous solution of carbon-14-labeled sucrose (New England Nuclear, Boston, Mass.) of a known specific activity and equilibrated at 37°C for 72 hr. At the end of this period, the stratum corneum samples were gently blotted to remove excess water, weighed immediately, and placed in glass vials containing known volumes of deionized water, with constant gentle shaking, to extract the radiolabeled sucrose. At 24 and 48 hr of extraction time, aliquots were placed in scintillation vials, mixed with scintillation cocktail (Opti-Fluor, Packard Instrument Company Inc.), and counted.

Stratum Corneum/Water Partition Coefficient Determinations

Known volumes of aqueous solutions of the desired compound in 0.01 ionic strength pH 4 succinate buffer (15) were placed in screw-cap glass vials, securely capped with a Teflon septum, and equilibrated for several hours in a circulating water bath at 37°C. An aliquot from each labeled vial was analyzed by HPLC to obtain the initial concentration of the solute in each of the bathing solutions. Accurately weighed individual dry stratum corneum samples, untreated and delipidized, were then placed in each vial. The vials were capped and placed in a water bath at 37°C with occasional gentle agitation for 48–72 hr. The hydrated stratum corneum samples were then removed, blotted gently, and weighed immediately. The solute concentrations in the bathing solution at equilibrium were then measured by HPLC. The amount of stratum corneum (mg) and the volume of the inital bathing solution were chosen, based on the solute under study, such that at least 20 to 40% depletion of the solute occurred at the end of equilibration. Uptake studies of hydrocortisone succinate and hydrocortisone pimelate were also conducted at pH's higher than 4.0 using 0.01 ionic strength succinate and phosphate buffers (15).

Isolated Stratum Corneum Lipid/Water Partition Coefficient Studies

Partition coefficients were conducted in glass vials containing a known weight of lipid residue (2–4 mg) obtained by evaporating the chloroform–methanol extract from the previously described delipidization procedure. Bathing solution containing the solute of interest was added to these vials and equilibrated for at least 24 hr at 37°C with occasional gentle agitation. Since the lipids were coated to the wall of the glass vial, their dispersion into the aqueous bathing solution did not occur and care was taken not to disperse them. At the end of the equilibration period, the aqueous phase was carefully sampled and assayed by HPLC for solute depletion.

RESULTS

Human Stratum Corneum Lipid Content and Water Uptake

Table I summarizes the data on the lipid content of stratum corneum derived from various sources determined by the change in weight of the sample after extraction. FTIR spectra of an untreated and a delipidized stratum corneum sample which originally contained 17% lipid are shown in Fig. 1. The absorption band at approximately 1740 cm^{-1}, representing the C=O stretching vibration of aliphatic ester functional groups, nearly disappears after a 1-day extraction with chloroform–methanol and shows no further change after an additional 19-day extraction period.

Equilibrium water uptake capacities of both untreated and delipidized stratum corneum at 37°C are also reported in Table I. These determinations were made as described previously by weighing the stratum corneum samples before and after equilibration in the aqueous bathing solution.

Table I. Lipid Content and Equilibrium Water Uptake in Various Samples of Isolated Human Stratum Corneum

Stratum corneum ID No.	Subject[a]	Lipid content (%)[b]	Water uptake at 37°C (mg/mg dry stratum corneum)		
			Untreated[c]	Delipidized[c]	N
SB-1	LEG/40/M/C	6.7 ± 2.2 (3)	1.15 ± 0.24	0.88 ± 0.12	3
SB-2	???/50/M/C	21.1 ± 3.8 (3)	2.09 ± 0.67	0.53 ± 0.043	
SB-3	BCK/18/F/C	28.4 ± 8.8 (3)	1.94 ± 0.42	0.77 ± 0.16	3
SB-4	THI/31/F/C	19.7 ± 2.3 (3)	2.97 ± 0.04	0.69 ± 0.05	3
SC-22	ABD/39/M/C	15.2 ± 0.9 (2)	—	—	
SC-24	HIP/??/?/C	9.9 ± 1.2 (2)	1.55 ± 0.31	0.79 ± 0.07	2
SC-25	HIP/??/?/C	10.5 ± 1.8 (3)	1.46 ± 0.06	1.19 ± 0.05	3
SC-26	BUT/31/M/C	8.3 ± 1.1 (3)	—	—	
SC-27	BUT/??/M/C	9.6 ± 2.9 (3)	—	—	
SC-28	ABD/58/F/C	7.6 ± 2.1 (3)	—	—	
SC-29	BUT/51/M/C	10.8 ± 2.0 (8)	2.83 ± 0.72	1.11 ± 0.23	3
SC-30	BUT/37/M/C	9.2 ± 1.7 (2)	—	—	
SC-31	CHT/59/M/C	13.5 ± 1.7 (2)	3.91 ± 1.63	1.34 ± 0.4	3
SC-32	LEG/59/M/C	11.4 ± 1.8 (2)	2.86 ± 0.52	1.15 ± 0.09	3
SC-33	BUT/37/M/C	9.1 ± 1.4 (3)	2.02 ± 0.43	1.21 ± 0.10	3
SC-34	ARM/26/M/C	17.2 ± 0.1 (2)	4.70 ± 0.60	1.74 ± 0.3	3
SC-35	???/??/?/C	10.8 ± 0.8 (2)	2.42 ± 0.30	1.28 ± 0.13	3
SC-36	ARM/31/M/C	8.6 ± 0.1 (3)	2.28 ± 0.30	1.26 ± 0.10	3
SC-39	ABD/37/F/C	12.2 ± 0.6 (2)	4.36 ± 0.16	1.11 ± 0.14	3
SC-40	BUT/26/M/C	4.9 ± 1.5 (2)	—	—	
SC-42	ABD/44/F/C	37.2 ± 1.8 (2)	2.41 ± 0.27	2.21[d]	2
SC-43	ABD/60/M/C	17.8 ± 3.0 (3)	2.12 ± 0.50	1.46 ± 0.13	6
SC-44	ARM/35/F/C	8.3 ± 0.9 (2)	2.32 ± 0.06	0.95 ± 0.16	3
SC-45	THI/38/F/C	27.7 ± 0.3 (2)	—	—	
SC-46	???/??/?/C	10.5 ± 2.2 (3)	—	—	
SC-47	BUT/62/M/?	22.1 ± 1.3 (2)	3.11 ± 1.00	1.28 ± 0.12	4
SC-48	ARM/42/F/C	5.5 ± 2.5 (2)	2.34 ± 0.70	1.23 ± 0.07	2
SC-49	CHT/22/M/C	3.2 ± 0.2 (2)	3.19 ± 0.60	1.82 ± 0.21	6
SC-50	CHT/61/M/C	8.5 ± 0.1 (2)	6.38	3.30	
SC-51	ABD/52/F/C	46.2 ± 1.3 (3)	4.76 ± 0.62	1.46 ± 0.60	6
SC-59	???/39/F/C	18.6 ± 1.9 (2)	—	—	
SC-62	ABD/49/F/C	24.3 ± 0.9 (3)	2.56 ± 0.54	1.39 ± 0.14	3
SC-65	ABD/49/F/C	36 ± 2.2 (2)	—	—	
SC-66	ABD/49/F/C	37.1 ± 1.4 (3)	3.99 ± 0.16	1.47 ± 0.08	3
SC-68	ABD/35/F/C	20.7 ± 1.6 (2)	—	—	
Mean ± SE		16 ± 2 (N = 35)	2.91 ± 0.25 (N = 24)	1.32 ± 0.12 (N = 24)	

[a] Listed in the order of site/age/sex/race. BCK, back; THI, thigh; ABD, abdomen; BUT, buttock; CHT, chest; ?, unknown.
[b] Expressed as mean ± SD of number of determinations shown in parentheses.
[c] Expressed as mean ± SD of number of determinations (N).
[d] Single determination.

^{14}C-Sucrose Uptake and Recovery

The sucrose uptake and recovery (by extraction) data, for two different sources of stratum corneum (SC-48 and SC-50), containing differing amounts of lipid (37.2 and 8.5%, respectively), are presented in Table II. Comparisons between the actual amount of water (by weight) taken up by the untreated and delipidized stratum corneum and that calculated from the sucrose recovery data indicate that the two methods are comparable.

Octanol/Water Partition Coefficients

Octanol/water partition coefficient values determined for various hydrocortisone esters at 37°C are listed in Table III. The hydrocortisone octanoate (1k) value was estimated from Hansch π values (14) and experimentally measured values of hydrocortisone propionate (1h) and hydrocortisone hexanoate (1j).

Solute Partition Coefficients into Lipid and Protein Domains

A representative plot of the uptake of hydrocortisone octanoate, 1k, versus the bathing solution concentration is shown in Fig. 2 for two samples of stratum corneum differing in lipid content. Both polar and nonpolar solutes exhibited linear uptake versus solution concentration profiles in both untreated and delipidized stratum corneum. However, only for relatively nonpolar solutes such as the octanoate did uptake differ significantly in untreated and delipidized stratum corneum or with samples differing in lipid content, as evident in the difference in slopes of the two lines in Fig. 2. The stratum corneum/water partitioning data obtained for various hydrocortisone esters are presented in

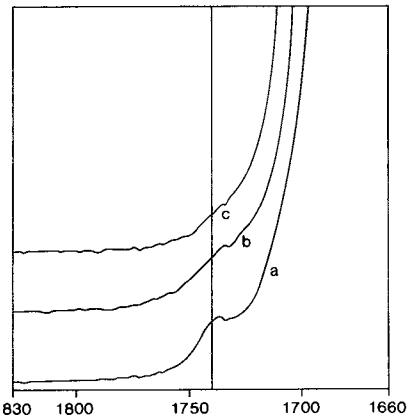


Fig. 1. FTIR spectra of (a) untreated human stratum corneum containing 17% extractable lipids, (b) SC after 1 day of extraction in 2:1 chloroform/methanol, and (c) SC after 19 days of extraction in 2:1 chloroform/methanol.

Table IV. $PC_{intrinsic}$ and $PC_{protein}$ were obtained directly in untreated and delipidized stratum corneum, using Eqs. (1) and (2), respectively, and PC_{lip} was calculated using Eq. (3).

$$PC_{intrinsic} = \frac{\mu g \text{ solute/mg (dry) } SC_u}{\mu g \text{ solute/mg water}} \quad (1)$$

$$PC_{pro} = \frac{\mu g \text{ solute/mg (dry) } SC_d}{\mu g \text{ solute/mg water}} \quad (2)$$

$$PC_{lip} = \frac{PC_{intrinsic} - PC_{pro} \times W_{f(pro)}}{W_{f(lip)}} \quad (3)$$

where SC_u and SC_d refer to the untreated and delipidized stratum corneum, respectively, and $W_{f(pro)}$ and $W_{f(lip)}$ are weight fractions of the protein and lipid domains in the untreated stratum corneum. PC_{lip} values were also obtained for selected solutes by measuring partitioning directly into isolated stratum corneum lipids. For relatively nonpolar solutes, where PC_{lip} could be calculated using either method, the two methods gave similar results.

DISCUSSION

The heterogeneous nature of the stratum corneum and the likely existence of distinct domains within this "barrier layer" of the skin have been noted for many years. The view reflected in the classic paper of Scheuplein and Blank nearly 20 years ago was that keratin filaments in the interior of the cornified epithelial cells were distributed in an amorphous matrix of mainly lipid and nonfibrous protein (1). They argued that "the separation between the lipid and protein components of the tissue is probably increased on hydration," resulting in "a stable two-phase system at the macromolecular level: a continuous, water-rich polar region intermingled with a network of nonpolar lipid." More recent studies suggest that the lipid and protein domains of stratum corneum lie in morphologically distinguishable regions. Histochemical studies show that the keratin-filled stratum corneum cells are virtually devoid of lipid, while almost all of the lipids lie in the intercellular spaces (4–6,16).

This paper addresses a necessary component to the ultimate understanding of the influences of stratum corneum heterogeneity on drug delivery through the skin—the relative affinities of the lipid and protein domains of the stratum corneum toward model permeants and the combined role of these domains in determining the uptake of solutes in the stratum corneum. Our treatment assumes the existence of three independent domains in fully hydrated stratum corneum: (1) the solvent-extractable lipid domain, (2) the residual protein (largely cross-linked keratin) domain, and (3) an aqueous solvent domain. It is further assumed that the solvent domain has the properties of bulk water, that the lipid and protein domains do not interact in a way that affects their solvent nature, and that the protein domain is not altered by solvent extraction of lipids.

In the following discussion, evidence supporting the validity of the above assumptions is first presented. This evidence is not believed to constitute conclusive proof of their validity, however.

The "Sponge" Domain

The solvent domain, which has also been referred to as the sponge domain (17), is comprised of water of hydration as determined by the change in weight of the stratum corneum after equilibration in aqueous bathing solution. This water is assumed to have the properties of bulk water and to carry an amount of solute into the stratum corneum equal to the amount of solute in the same volume of bathing solution. The intrinsic solute uptake values obtained by monitoring solute depletion from the bathing solution therefore do not include uptake due to water of hydration. If this water did

Table II. Comparison of Water Uptake Obtained by Monitoring the Weight Change on Hydration with That Measured by the ^{14}C-Sucrose Uptake Method in Untreated and Delipidized Isolated Human Stratum Corneum at 37°C

SC No.[a]	Lipids (%)	Weight (mg)		Water uptake (mg)		% deviation
		Dry	Wet	By weight	By ^{14}C-sucrose	
42						
U1	37.2	10.20	36.77	26.57	26.23	−1.3
U2		11.71	37.66	26.95	24.59	−8.8
D		2.97	9.53	6.56	7.27	+10.8
50						
U	8.5	7.76	57.29	49.53	48.44	−2.0
D		6.77	29.08	22.31	22.73	+2.0

[a] U, untreated stratum corneum; D, delipidized stratum corneum.

Table III. Octanol/Water Partition Coefficients (PC) of Various Hydrocortisone 21-Esters (Scheme I) at 37°C

Compound	R	PC[a]	N
1a	$-CH_2CH_2CONH_2$	27 ± 1.6	2
1b	$-CH_2CH_2CON(CH_3)_2$	108	1
1c	$-CH_2CH_2COOCH_3$	380 ± 1.5	2
1d	$-CH_2CH_2COOH$		
	pH 2.5	130 ± 3.3	2
	pH 5.5	13[b]	
	pH 6.1	4[b]	
1e	$-(CH_2)_4CH_2COOH$		
	pH 3.3	1,810	1
	pH 3.4	1,790	1
	pH 3.47	1,710	1
	pH 3.51	1,600	1
	pH 4.0	1,560[b]	
	pH 7.0	16	
1f	$-(CH_2)_4CH_2CONH_2$	202 ± 0	2
1g	$-(CH_2)_4CH_2OH$	610 ± 2	2
1h	$-CH_2CH_3$	990 ± 9.1	2
1i	$-(CH_2)_4CH_2COOCH_3$	5,000 ± 12	4
1j	$-(CH_2)_4CH_3$	30,000 ± 16	2
1k	$-(CH_2)_6CH_3$	310,000[c] ± 3.4	

[a] Expressed as mean ± CV (%) of the number of determinations (*N*) indicated.
[b] Calculated from PCs measured at other pH values.
[c] Estimated by extrapolating from **1h** and **1j** data.

not have the properties of bulk water, a small (generally 1–20%) correction in the intrinsic uptake data would be necessary.

NMR (18) and desorption kinetics experiments (19) suggest that a significant fraction of the water of hydration in stratum corneum may be strongly bound. In view of these findings and the now well-established heterogeneity of the stratum corneum, it is germane to explore the relative contributions of both the lipid and the protein domains to the overall water uptake and the solvent properties of the water in each domain. Data for the uptake of water in untreated and delipidized stratum corneum are shown in Table I. Presuming that extraction of lipids does not appreciably alter the physicochemical nature of the protein phase, both the lipid and the protein domains seem to contribute significantly to the overall water-holding capacity of human stratum corneum. The extractable lipids, which account for only 16% of the total weight of the stratum corneum on average, appear to play a dominant role in water uptake by untreated stratum corneum. An essential role of the intercellular lipids in the water-holding properties of the stratum corneum has recently been reported by others (20). The solvent nature of water bound to bilayer forming lipids is therefore of considerable interest.

Katz and Diamond examined the solvent properties of water enclosed in liposomes of dimyristoyl lecithin (DMPC) using radiolabeled sucrose as a solute due to its negligible affinity for the lipid bilayers themselves and concluded that approximately one-third of the enclosed water is nonsolvent water, unavailable for dissolving sucrose (21). A similar test was applied in this study to the water of hydration in untreated and delipidized stratum corneum using ^{14}C-labeled sucrose. The results are shown in Table II. The overall water uptake determined by weight agrees closely with the calculated from the levels of ^{14}C-sucrose in the stratum corneum at equilibrium, assuming that the concentration of sucrose in the solvent domain is unchanged from that in the bathing solution. This evidence appears to conflict with the studies of Katz and Diamond. However, the lipids of the stratum corneum are largely neutral lipids (5), whereas dimyristoyl lecithin is zwitterionic. The water molecules in primary hydration sheaths surrounding the ionic groups in a zwitterion are likely to be highly ordered and arranged much differently than the molecules in bulk water (22), but the water hydrating the polar but neutral head groups of stratum corneum lipids, or proteins, probably involves largely hydrogen bonding interactions. Thus, "bound" water within stratum corneum may not differ greatly in its solvent properties from bulk water, which is certainly not "free" but also largely hydrogen bonded.

Separation of the Lipid and Protein Domains

The partition coefficient of solute into the stratum corneum, $PC_{intrinsic}$, is assumed to represent the independent contributions of the lipid and protein domains, each multiplied by their respective weight fractions, evident by rearranging Eq. (3). It is assumed in this treatment that solvent extraction removes the lipid domain so that PC_{pro} can be determined by direct measurement using lipid extracted stratum corneum.

PC_{pro} values are reliable only if the solvent extraction technique does not alter the protein's affinity for solute. Furthermore, the values obtained truly reflect the proteinaceous domain only if all the lipids are removed. Presently, there is no evidence to suggest that the keratin of stratum corneum is altered by lipid extraction. DSC transitions attributed to protein denaturation appear to be unchanged by lipid removal and bands attributed to proteins in FTIR spectra appear to be insensitive to lipid removal (23). Also, the intrinsic partition coefficients of solutes which appear to partition primarily into the protein domain are not changed significantly by lipid extraction. This conclusion comes from a comparison of the $PC_{intrinsic}$ values with PC_{pro} values for solutes **1a–g** in Table IV. The uptake of these solutes into untreated stratum corneum, represented by $PC_{intrinsic}$, is not significantly different from the values for PC_{pro}. These observations lend support to the assumption that solvent ex-

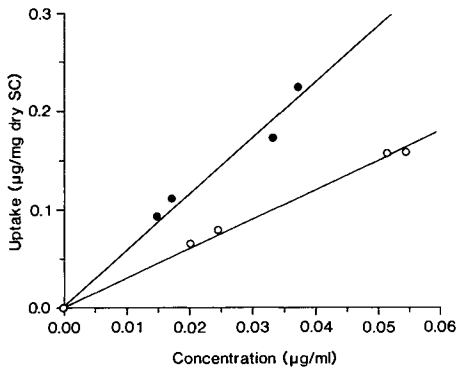


Fig. 2. Equilibrium uptake of **1k** versus **1k** bathing solution concentration in untreated stratum corneum containing 15% lipid (○) and 36% lipid (●).

Table IV. Partition Coefficients[a] of Various Hydrocortisone 21-Esters into Isolated Human Stratum Corneum at 37°C

Compound	R	Stratum corneum lipid content (%)	$PC_{intrinsic}$	N	$PC_{protein}$	PC_{lipid} Difference method[b]	PC_{lipid} Direct method[c]
1a	$-CH_2CH_2CONH_2$	—[d]	9 ± 1	14	13 ± 2	—	—
1b	$-CH_2CH_2CON(CH_3)_2$	—[d]	12 ± 1	14	17 ± 2	—	—
1c	$-CH_2CH_2COOCH_3$	—[d]	22 ± 2	14	22 ± 2	—	—
1d	$-CH_2CH_2COOH$						
	pH 5.5	—[d]	11 ± 2	2	14 ± 2	—	—
	pH 6.1		10 ± 1	2	14 ± 0.3	—	—
1e	$-(CH_2)_4CH_2COOH$						
	pH 4.0	—[d]	68 ± 4	2	71 ± 2	—	—
	pH 7.0		33 ± 0.5	2	29 ± 4	—	—
1f	$-(CH_2)_4CH_2CONH_2$	—[d]	25 ± 1	4	28 ± 1	—	—
1g	$-(CH_2)_4CH_2OH$	—[d]	20 ± 0.4	4	23 ± 0.1	—	—
1h	$-CH_2CH_3$	—[d]	30 ± 2	25	22 ± 2	66 ± 4	—
1i	$-(CH_2)_4CH_2COOCH_3$	8	150 ± 20	2	66 ± 6	1,070 ± 140	
		12	110 ± 10	2	90 ± 2	280 ± 190	
		18	140 ± 6	6	41 ± 3	550 ± 40	
					66 ± 14[f]	630 ± 230[g]	420 ± 70 (N = 2)
1j	$-(CH_2)_4CH_3$	3	85 ± 4	2	41 ± 3	1,400 ± 120	
		8	210 ± 10	2	100 ± 9	1,400 ± 130	
		10	150 ± 20	2	35 ± 5	1,200 ± 200	
		11	170 ± 4	2	42 ± 8	1,200 ± 30	
		12	260 ± 20	2	115 ± 5	1,400 ± 170	
		22	170 ± 30	2	35 ± 7	820 ± 140	
		46	410 ± 50	2	—	810 ± 110[e]	
					62 ± 15[f]	1,200 ± 100[g]	2,000 ± 160 (N = 2)
1k	$-(CH_2)_6CH_3$	3	830 ± 190	2	400 ± 110	14,000 ± 6,000	
		5	980 ± 80	2	400 ± 50	11,000 ± 1,500	
		10	1,700 ± 10	4	660 ± 160	11,000 ± 200	
		15	3,100 ± 90	2	390 ± 150	18,000 ± 500	
		19	—	2	780 ± 140	—	
		21	3,800 ± 300	2	650 ± 40	16,000 ± 100	
		24	5,000 ± 500	2	—	19,000 ± 1,900[e]	
		36	6,600 ± 500	2	590 ± 110	17,000 ± 1,300	
		37	7,100 ± 400	2	—	18,000 ± 1,000[e]	
					550 ± 60[f]	16,000 ± 1,000[g]	16,000 ± 1,700 (N = 4)

[a] Expressed as mean ± SE of the number of determinations (N) indicated.
[b] Calculated using $PC_{intrinsic}$ and $PC_{protein}$ values.
[c] Measured directly using the isolated stratum corneum lipids.
[d] Uptake of these solutes is independent of stratum corneum lipid content.
[e] PC_{lipid} values calculated using mean $PC_{protein}$ value.
[f] Mean ± SE of $PC_{protein}$.
[g] Mean ± SE PC_{lipid}.

traction does not alter the uptake characteristics of the protein domain.

The degree to which the lipid domain in the stratum corneum is removed by extraction is uncertain. A crude method of evaluating the chloroform–methanol extraction procedure is via a comparison of the FTIR spectra of untreated and delipidized samples (Fig. 1). Although only qualitative comparisons are warranted, >80–90% of the ester-containing lipids appear to be removed by the extraction. Wertz and Downing have recently suggested that a lipid fraction representing approximately 2% of the total weight of stratum corneum is not removed by chloroform–methanol extraction (24). These lipids are believed to be covalently bound to the cell envelope proteins and are removed only after treatment with strong base. Because such harsh treatment may alter the protein domain, this method was not employed in this study. It is possible, therefore, that the "protein" domain investigated herein may contain a small percentage of unextractable lipid.

A small quantity of residual lipid, if present, would have a discernible effect only on the PC_{pro} value for the most lipophilic compound in the series, hydrocortisone 21-octanoate (**1k**). Indeed, the PC_{pro} value obtained for hydrocortisone octanoate is significantly (fivefold) higher than the value obtained by extrapolation of the remaining PC_{pro} data via linear regression (see Fig. 4). This discrepancy could be accounted for if there were 2–3% residual lipid present having the same affinity for solute as the extracted lipids.

Solute Uptake in Human Stratum Corneum—The Role of Protein and Lipid Domains

A comparison (Table IV) of the partition coefficients of esters of hydrocortisone varying systematically in lipophilicity [as measured by the octanol/water partitioning scale (Table III)] into untreated stratum corneum, from which $PC_{intrinsic}$ data are obtained, and delipidized stratum corneum, from which PC_{pro}'s are derived, is quite revealing. Solutes having an octanol/water partition coefficient of <1000 (compounds **1a–g**) exhibit $PC_{intrinsic}$ values which are not significantly different from PC_{pro}, suggesting that the uptake of these solutes into stratum corneum is accounted for by uptake into the protein domain. With increasing lipophilicity (log PC >3) a divergence is observed between $PC_{intrinsic}$ and PC_{pro}, as shown graphically in Fig. 3, where the difference is plotted versus log PC(octanol/water). *This divergence in $PC_{intrinsic}$ and PC_{pro} suggests that a change in uptake mechanism into stratum corneum occurs with increasing solute lipophilicity.* The uptake of relatively hydrophilic solutes (log PC <3) is governed by the protein domain, while more lipophilic solutes (log PC >3) reside preferentially in the lipid domain.

A change in uptake mechanism can occur only if the selectivities of the protein and lipid domains differ toward solutes varying in lipophilicity. Such a difference is, of course, expected since these environments differ significantly in polarity. Biomembrane selectivities are generally evaluated by comparison of biomembrane/water partition coefficients to bulk solvent/water partition coefficients using linear free-enegery relationships as described by Eq. (4):

$$\log PC_{bio} = \alpha \log PC(\text{octanol/water}) + \beta \qquad (4)$$

where PC_{bio} is the biomembrane/water (or domain/water) partition coefficient. The slope, α, represents the relative selectivity of the biomembrane to lipophilicity compared to the octanol/water partition system. Extensive efforts have been expended to identify a bulk solvent partitioning system which closely mimics biomembrane/water partition coefficients (25), but the question of the appropriate reference solvent for use in such correlations remains a topic of debate. The octanol/water system was selected for this study for experimental convenience, not for any theoretical advantage.

Plots of log PC_{pro} and log PC_{lip} versus log PC(octanol/water), shown in Fig. 4, reflect the sensitivities of the protein and lipid domains to solute lipophilicity. The slopes of α = 0.24 (PC_{pro} data) and α = 0.91 (PC_{lip} data) indicate, as expected, that the protein domain is much more polar than the lipid domain and therefore less sensitive to changes in solute structure. The lipid domain appears to have a polarity similar to octanol. PC_{lip} values were also obtained for the more lipophilic compounds by direct measurement of partitioning into pooled samples of the extracted stratum corneum lipids. As shown in Table IV and Fig. 4, PC_{lip} values obtained by the two methods were similar.

Because a change in uptake mechanism occurs with increasing solute lipophilicity, plots of log $PC_{intrinsic}$ versus log PC (octanol/water) should be nonlinear, reflecting the selectivity of the domain which is predominant in the uptake. Also, $PC_{intrinsic}$ should depend on the stratum corneum lipid content only for those solutes the uptake of which is governed by the lipid domain. These predictions have been confirmed.

A plot of log $PC_{intrinsic}$ versus log PC (octanol/water) is shown in Fig. 5 for the series of hydrocortisone esters examined in stratum corneum samples containing approximately 15% lipid. Superimposed on the data is a theoretical curve based on Eq. (5),

$$PC_{intrinsic} = W_{f(pro)} * \gamma PC_{0/w}^{\delta} + W_{f(lip)} * \epsilon PC_{0/w}^{\zeta} \qquad (5)$$

where the first and second terms represent the contributions of the protein and lipid domains, respectively, to the intrinsic solute uptake. $W_{f(pro)}$ (= 0.85) and $W_{f(lip)}$ (= 0.15) are the weight fractions of protein and lipid, respectively, applicable to Fig. 5. The values of γ (= 7.4), δ (= 0.24), ϵ

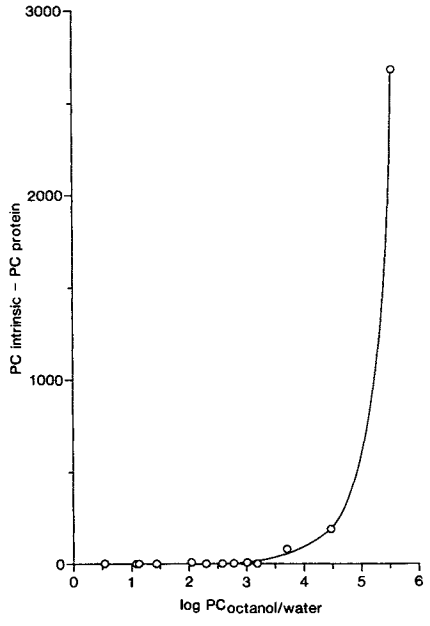


Fig. 3. Difference between partition coefficients in untreated and delipidized human stratum corneum, $PC_{intrinsic} - PC_{protein}$, versus solute lipophilicity ($PC_{octanol/water}$).

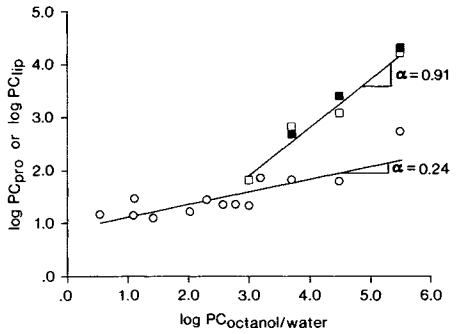


Fig. 4. Log–log plots of solute partition coefficients into the protein (PC_{pro}) and lipid (PC_{lip}) domains of human stratum corneum versus solute lipophilicity ($PC_{octanol/water}$). (○) Partition coefficients into delipidized stratum corneum; (■) partition coefficients into extracted stratum corneum lipids; (□) PC_{lip} values obtained from $PC_{intrinsic}$ and $PC_{protein}$ values using Eq. (3).

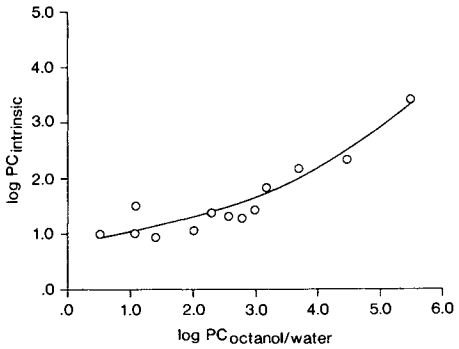


Fig. 5. Log–log plot of solute partition coefficients into human stratum corneum, $PC_{intrinsic}$, versus solute lipophilicity ($PC_{octanol/water}$) for the series of hydrocortisone esters containing approximately 15% lipid. Superimposed on the data is a theoretical curve based on Eq. (5), assuming $W_{f(lip)}$, the weight fraction of lipid, to be 15%.

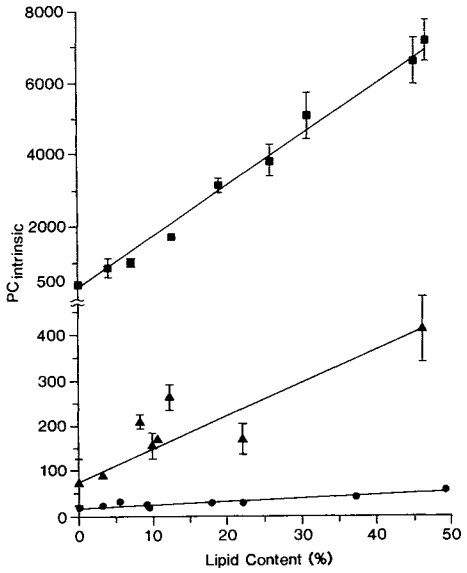


Fig. 6. Plot of solute partition coefficients into human stratum corneum, $PC_{intrinsic}$, versus stratum corneum lipid content for **1h** (●), **1j** (▲), and **1k** (■).

(= 0.15), and ζ (= 0.91) are the β and α values obtained by linear regression of the data in Fig. 4 for PC_{pro} and PC_{lip}, respectively, using Eq. (4).

While some scatter in the data is evident, the experimental points appear to fit this theoretical curve quite well. Previously reported correlations typically assume such plots to be linear (26). Such studies also generally conclude that the stratum corneum has the solvent properties of a quite polar lipid, significantly more polar than octanol. If a straight line were drawn through the data in Fig. 6, the slope of the best fit would be approximately 0.46 but with considerably more scatter of points around the line than for the nonlinear treatment. Thus, these data would also indicate that the stratum corneum is quite polar. We conclude, however, that such uptake data reflect the properties of two domains differing in selectivity toward solutes of varying lipophilicity—the protein domain behaving as a highly polar environment and the lipid domain apparently having properties similar to octanol.

The lipid content in the stratum corneum samples examined was found to vary considerably, ranging from 3 to 46% (Table I). If solute uptake were governed by the lipid domain, one would expect significant variability in partition coefficients depending on lipid content. In the present study, uptake was sensitive to lipid content only for highly lipophilic solutes. This is illustrated in Fig. 6, where $PC_{intrinsic}$ values have been plotted as a function of stratum corneum lipid content for hydrocortisone propionate (**1h**), hydrocortisone hexanoate (**1j**), and hydrocortisone octanoate (**1k**). Uptake of the most lipophilic compound in the series, hydrocortisone octanoate, is highly sensitive to lipid content, varying by approximately one order of magnitude over the range of lipid contents explored. This relationship is approximately linear, consistent with the hypothesis that the uptake of hydrocortisone octanoate is lipid domain dominated. The propionate ester, being on the borderline between protein and lipid domain-controlled uptake, is not highly sensitive to lipid content, while some dependence on lipid content is observed for the more lipophilic hexanoate ester.

We conclude that, depending on the lipophilicity of the solute, its uptake into stratum corneum may be governed by the protein or the lipid domains or a combination of the two. Due to the differences in selectivity of the two domains, log–log plots of stratum corneum uptake versus solute lipophilicity are nonlinear, reflecting a change in mechanism from protein-dominated uptake for hydrophilic solutes to lipid-domain dominated uptake for lipophilic solutes. Stratum corneum lipid content, which varies dramatically from individual to individual, is an important determinant of the affinity of the stratum corneum for highly lipophilic solutes but has no effect on the uptake of hydrophilic solutes. The relationship between the above observations and relative solute permeabilities will be the subject of a future report.

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