Human stratum corneum lipids: characterization and regional variations

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Abstract The lipids of mammalian stratum corneum are known to be important regulators of skin permeability. Since the human stratum corneum displays remarkable regional variations in skin permeability, we assessed the total lipid concentration, the distribution of all major lipid species, and the fatty acid composition in Bligh-Dyer extracts from four skin sites (abdomen, leg, face, and sole) that are known to display widely disparate permeability. Statistically significant differences in lipid weight were found at the four sites that were inversely proportional to their known permeability. In all four sites, among the polar lipids, the stratum corneum contained negligible phospholipids, but substantially more cholesterol sulfate (1–7%) than previously appreciated. As in the stratum corneum from other mammals, the bulk of the lipids consisted of neutral (60–80%) and sphingolipids (15–35%). Of the neutral lipids, free sterols (4- to 5-times greater than esterified sterols), free fatty acids, triglycerides, and highly nonpolar species (n-alkanes and squalene) predominated. n-Alkanes, which were present in greater quantities than previously appreciated, comprised a homologous series of odd- and even-chained compounds ranging from C19 to C34. The sphingolipids comprised over 80% ceramides vs. lesser quantities of glycosphingolipids. In all four sites, the sphingolipids were the major repository of long-chain, saturated fatty acids. The neutral lipid:sphingolipid ratio generally was proportional to the known permeability of each site: higher neutral lipids and lower sphingolipids generally were associated with superior barrier properties. These studies provide: *1*) the first detailed, quantitative analysis of human stratum corneum lipids and *2*) information about the variability in lipid composition at four skin sites with known differences in permeability. The latter results suggest that variations in neutral lipids, rather than sphingolipids, may underlie local variations in skin permeability.—**Lampe, M. A., A. L. Burlingame, J. Whitney, M. L. Williams, B. E. Brown, E. Roitman, and P. M. Elias.** Human stratum corneum lipids: characterization and regional variations. *J. Lipid Res.* 1983. **24:** 120–130.

Supplementary key words epidermis • stratum corneum • permeability • sphingolipids • n-alkanes • cholesterol sulfate

The primary goal of the differentiation process in mammalian epidermis is to generate a relatively impermeable, cornified outer layer. This layer, the stratum corneum, consists of lipid-depleted cells embedded in a neutral lipid-rich interstitium (reviewed in reference 1). While the functions of stratum corneum lipids are still not fully understood, abundant evidence supports a critical role for lipids in the modulation of skin permeability (2) as well as a less well-established link to the regulation of stratum corneum cohesion and desquamation (3, 4). Although there are several published studies of human stratum corneum lipid composition, many of these earlier studies examined skin surface lipids (e.g., ref. 5), which comprise a mixture of pilosebaceous- and keratinocyte-derived substances, preventing assignment of specific lipid fractions to either compartment of origin. Nevertheless, it has been well established that cornification is accompanied by disappearance of phospholipids and accumulation of both free and esterified sterols (6–8). More recently, the stratum corneum of several mammals has been found to contain abundant sphingolipids, as well (9–13).

However, except for the pig (10) and neonatal mouse (11), published descriptions of mammalian stratum corneum lipids have been limited to only certain classes of lipids. Thus, comparable data on the broad spectrum of human stratum corneum lipids are not yet available. Considering the presumed importance of these lipids for our understanding of normal and diseased skin function, this represents a considerable void. This study was undertaken in order to: *1*) provide detailed, quantitative analytical information about the full spectrum of human stratum corneum lipids; *2*) to study the topographic variations in the lipid composition of human skin; and, *3*) to relate these differences to established regional variations in skin permeability. This effort has been spurred largely by our recent finding of a strong correlation between the concentration of lipids at different skin regions and the water permeability of the stratum corneum over the same sites (14).

Abbreviations: SC, stratum corneum; PBS, phosphate-buffered saline; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; FAME, fatty acid methyl esters; GSL, glycosphingolipids; SG, stratum granulosum; MS, mass spectrometry.

METHODS

Sources of skin samples

Abdomen and leg skin samples were obtained from fresh autopsy and amputation cases, respectively. Facial skin was obtained from facelifts, while plantar stratum corneum was obtained from pared normal feet. In the case of leg and abdominal skin, the donors were all male, with a median age of 50. Most of the facial and plantar skin samples were from males with a similar median age. All samples were grossly normal (i.e., no scars, abrasions, etc.), and previously untreated by history, and only relatively hairless portions of the samples were used.

After scraping off the subcutaneous fat with a #15 blade, the full-thickness skin was floated dermis-side-downward on Weymouth's MB752/1 medium containing L-glutamine, amphotericin (25 μg/ml), penicillin (100 U/ml), streptomycin (100 mg/ml), and partially purified (15) staphylococcal epidermolytic toxin (20 mg/ml). After overnight incubations at 4°C, intact sheets of stratum corneum (SC) and stratum granulosum (SG) were peeled off the underlying skin (15). Care was taken to avoid contamination from subcutaneous fat. The stratum corneum was cleaned further of residual granular cells by incubation of the sheets with the stratum granulosum-side-downward on filter paper, soaked with 0.5% trypsin in phosphate-buffered saline (PBS), pH 7.2, for 2–3 hr at 37°C (10, 12). Loosely adherent granular cells could then be removed from intact stratum corneum by three washes in PBS and vortexing. Ultrastructural analysis of these preparations has revealed them repeatedly to be completely free of nucleated cells (12).

Lipid extraction

The stratum corneum sheets were extracted by the Bligh-Dyer method (16), filtered (Whatman #43), and split into aqueous and organic phases. The organic phases were dried under nitrogen gas and stored in benzene at −20°C until used; the aqueous phases, which contained substantial amounts of cholesterol sulfate but no other lipids (17), also were dried, stored, and saved for further analysis.

Thin-layer chromatography

The whole lipid from each of the stratum corneum samples was separated quantitatively on pre-cleaned high performance thin-layer plates (HP-TLC, Merck, Darmstadt, West Germany). The following sequence of one-dimensional thin-layer chromatography (TLC) solvent systems (**Fig. 1**) was used for each sample. Solvent system I: tetrahydrofuran–methylal–methanol–4 M ammonium hydroxide 60:30:10:4 (v/v) separated cholesterol sulfate from all neutral lipids and phospholipids. The major neutral lipid species were separated in solvent system IIa: petroleum ether–diethyl ether–glacial acetic acid 80:20:1 (v/v), followed by absolute petroleum ether (solvent system IIb), which fractionated squalene and n-alkanes from the sterol and wax esters. Ceramides and glycosphingolipids were fractionated in solvent system IIIa: chloroform–methanol–distilled water 90:10:1 (v/v), followed by solvent system IIIb: petroleum ether–diethyl ether–glacial acetic acid 70:50:1 (v/v). Solvent system IV: chloroform–methanol–distilled water–glacial acetic acid 60:35:4.5:0.5 (v/v) fractionated the major phospholipid species.

After development in the above solvents, the TLC plates were briefly air-dried, sprayed with 0.2% aqueous 8-anilino-1-napthalene sulfonic acid, and photographed under black light fluorescence. Assignment of individual fractions was based upon: *1*) co-chromatography against known standards in at least three different solvent systems, *2*) biochemical analysis, *3*) gas–liquid chromatography (GLC), and/or *4*) glass capillary GLC–mass spectrometry (GLC–MS). The lipid bands were visualized by spraying plates with an 0.1% aqueous solution of 8-anilino-1-sulfonic acid, excised and the lipid-containing gel was placed in screw-cap test tubes with 7.6 ml of Bligh-Dyer solvent, extracted, dried, and weighed, as described previously (12).

Isolation of cholesterol sulfate from the TLC plates required addition of 0.1 M KCl, to the Bligh-Dyer solvents, which displaces cholesterol sulfate from the aqueous into the organic phase (17). Both the aqueous phases from the initial lipid extractions, and the cholesterol sulfate fractions from solvent system I were analyzed for cholesterol sulfate by the Franey-Amador method (18).

Wax esters, which co-migrated with sterol esters in solvent system III, were identified by hydrolysis in 12% boron trichloride in methanol for 30 min at 90°C. The hydrolysate was extracted in hexane, dried, and run in solvent system IIa which separated free sterols, fatty acid methyl esters, and long-chain alcohols (Fig. 1).

Gas–liquid chromatography

The fatty acid composition of the triglycerides (TG), free fatty acids (FFA), sterol/wax esters (SE/WE), and ceramides (CER) were assessed by both packed column and glass capillary GLC. Fatty acid methyl esters (FAME) were prepared in 12% boron trichloride in methanol at 90–100°C, for 1.0–1.5 hr (19). In each case, total hydrolysis of appropriate standards was demonstrated in parallel with the experimental sample. The methyl esters were then extracted in two washes of hex-

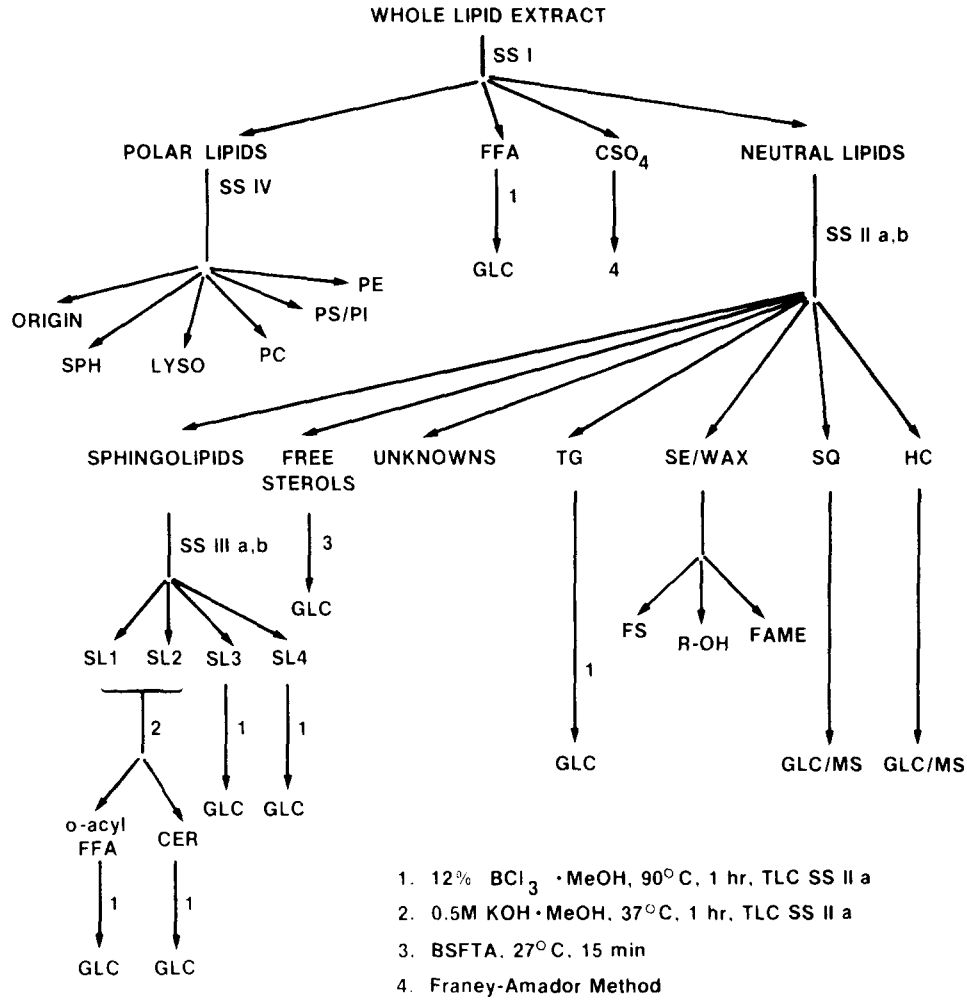

Fig. 1. Flow diagram for sequential, quantitative analysis of human stratum corneum lipids. See Methods for further details. Abbreviations for this and subsequent figures: S, standard; SS, solvent system; FFA, free fatty acids; CS, cholesterol sulfate; SPH, sphingomyelin; LYSO, lysolecithin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; GLC, gas–liquid chromatography; TG, triglycerides; SE, sterol esters, WAX or WE, wax esters; SQ squalene; HC, hydrocarbons; ALK, n-alkanes; SL, sphingolipids; FS, free sterols; R-OH, alcohol; MS, mass spectrometry; TLC, thin-layer chromatography; BSFTA, bis (trimethylsilyl) trifluoroacetamide.

ane, dried under nitrogen, and re-chromatographed in solvent system IIa to recover purified FAME.

The O-acyl-linked fatty acids on the orcein-positive glycosphingolipids were removed by alkaline hydrolysis as described previously (20). The sugar-containing lipid was dissolved in 5 ml of chloroform–methanol–10 M sodium hydroxide 2:7:1 (v/v). After incubation for 1 hr at 37°C, 2 M hydrochloric acid was added to lower the pH to 6. Addition of chloroform and water (1:1) forced the O-acyl free fatty acids and glycosyl-ceramides into the lower organic phase, and the salts into the upper aqueous phase. O-acyl fatty acids were then separated from the ceramides by re-chromatography in solvent system III. Derivatives of the amide-linked fatty acids on the ceramides were then prepared with boron trichloride (see above), and separated from long-chain bases by re-chromatography in solvent system IIa (Fig. 1). The ability of alkaline hydrolysis to selectively remove the O-acyl-linked fatty acids without affecting the amide-linked fatty acids was checked with appropriate standards, including natural ceramides, gangliosides, cerebrosides (Sigma, St. Louis, MO), as well as authentic 1-(3′-O-acyl)-glycosyl-N-dihydroxypentatriacontadienoylsphingosine (provided courtesy of Dr. Harold Yardley).

Fatty acid methyl esters of all these compounds were analyzed on a Hewlett Packard 5830 Gas Chromatograph using either a 6-ft 3% SP-2330 on 100/120 Chromosorb W column (Supelco, Bellefonte, PA) or a 15-m SP-1000 glass capillary column (J & W Scientific, Orangevale, CA). Temperature programming was used in both instances: 170–220°C at 2°C/min., 160–180°C

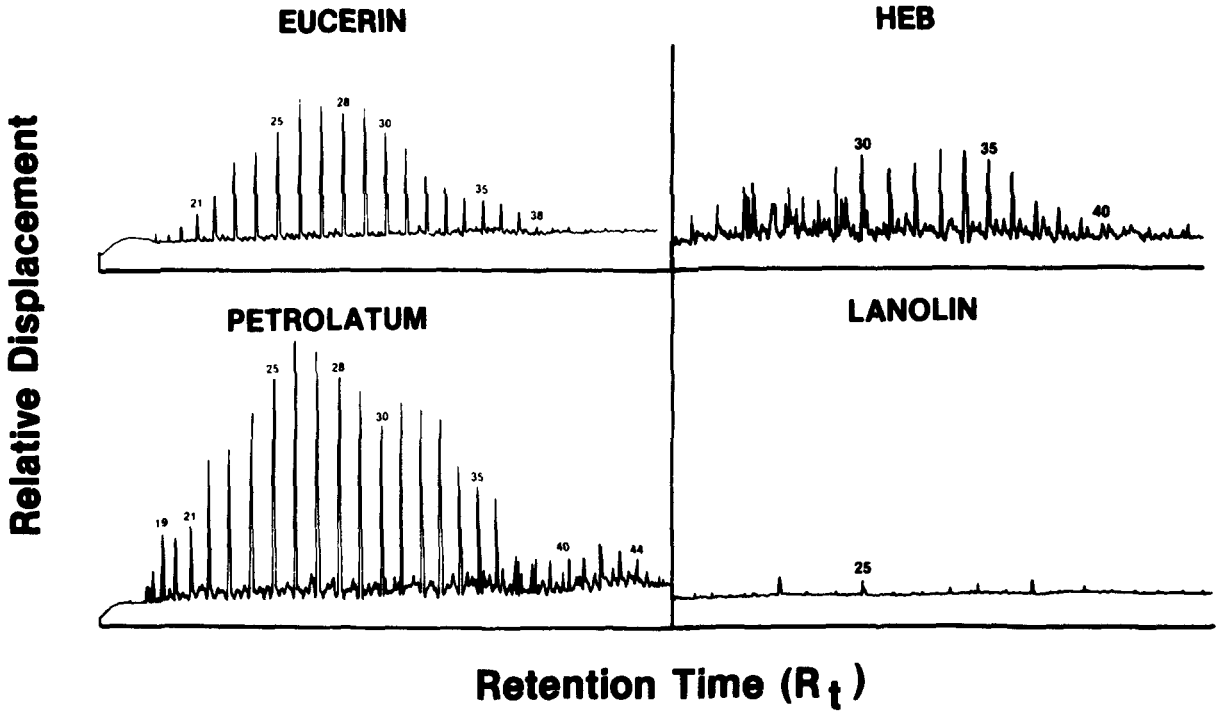

Fig. 2. Glass capillary GLC of four commonly employed skin emollient-vehicles. Each emollient was fractionated in the neutral lipid solvent systems (see Methods) followed by chain-length analysis of the hydrocarbon fraction. Whereas Eucerin and petrolatum possess a similar spectrum of n-alkanes to those in stratum corneum, cf. Fig. 6 below, HEB and lanolin do not present problems in identification. However, both Eucerin and petrolatum display more long-chain n-alkanes than do authentic stratum corneum n-alkanes, cf. Fig. 6 below.

at 5°C/min., respectively. Peaks were identified by comparison of retention time (R_t) values to known standards (**Fig. 2**). Fatty acid analysis was limited to identification of the straight chain unsubstituted fatty acids for several reasons: *a*) unavailability of suitable commercially available standards for branched or hydroxy-fatty acids, and *b*) alternate chemical methods for identification of complex fatty acids required more lipid than was available for this study.

The most nonpolar lipids, comprising squalene and n-alkanes, were analyzed further by glass capillary GLC on a 25-m FS-OV-1 column programmed from 170–340°C at 5°C/min. Assignment of squalene, n-alkanes, and sulfated sterols (pre- and post-solvolysis) was checked by glass capillary GLC–MS (21).

RESULTS

Polar lipids

Phospholipids. The stratum corneum lipids from all four skin sites contained little phospholipid (**Table 1**).

TABLE 1. Regional variations in lipid weight percent and distribution of major lipid species

	Site			
	Abdomen (n = 4)	Leg (n = 4)	Face (n = 3)	Plantar (n = 3)
Lipid weight %	6.5 ± 0.5	4.3 ± 0.8	7.2 ± 0.4	2.0 ± 0.6
Major species				
Polar lipids	4.9 ± 1.6	5.2 ± 1.1	3.3 ± 0.3	3.2 ± 0.89
Cholesterol sulfate[a]	1.5 ± 0.2	6.0 ± 0.9	2.7 ± 0.3	3.4 ± 1.2
Neutral lipids	77.7 ± 5.6	65.7 ± 1.8	66.4 ± 1.4	60.4 ± 0.9
Sphingolipids[b]	18.2 ± 2.8	25.9 ± 1.3	26.5 ± 0.9	34.8 ± 2.1

[a] Significant differences: abdomen vs. leg, $P < 0.01$; leg vs. face, $P < 0.02$; abdomen vs. face, $P < 0.02$; face vs. plantar, $P < 0.01$; abdomen vs. plantar, $P < 0.01$; plantar vs. leg, $P < 0.02$. Cholesterol sulfate: leg > plantar > face > abdomen.

[b] Significant differences: abdomen vs. leg, $P < 0.05$; abdomen vs. face, $P < 0.05$. Sphingolipids: plantar > face > leg > abdomen.

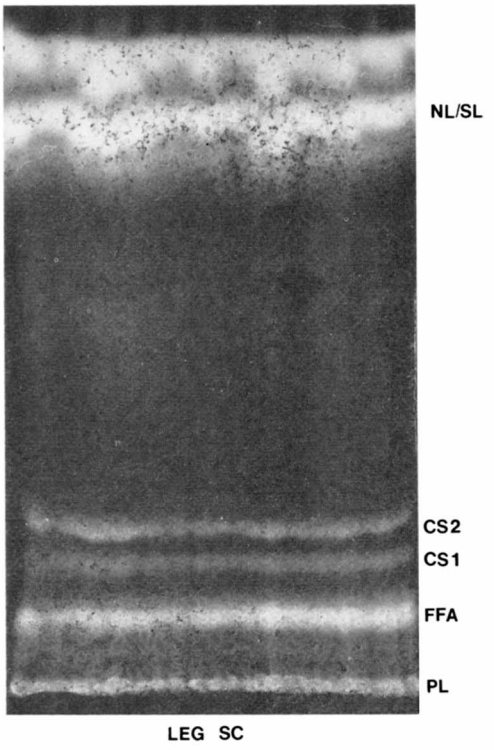

Fig. 3. One-dimensional TLC of Bligh-Dyer extract developed in cholesterol sulfate solvent system. In this system (see Methods), phospholipids remain at the origin and both sphingolipids and neutral lipids migrate to the front. In leg stratum corneum (SC), but not in abdomen, face, or plantar SC, two Franey-Amador-positive, sulfated bands are visible. The upper band (CS2) has been positively identified as cholesterol sulfate by GLC–MS after solvolysis (17), but the lower band (CS 1) has not been completely characterized, and, therefore, was not counted in cholesterol sulfate weight determinations.

Although the fractional weight percent varied from 5.2% (face) to 3.3% (plantar), these differences were not significant. The only polar lipid that persisted into the stratum corneum possessed the mobility of phosphatidylethanolamine on TLC. This fraction was identified as PE by *1*) a positive reaction to molybdenum (22) and *2*) generation of FAME with boron trichloride in methanol.

Cholesterol sulfate. Statistically significant differences in the weight percents of cholesterol sulfate from the four sites were found (Table 1), with more cholesterol sulfate in both leg and plantar stratum corneum than in either abdomen or face samples. In some TLC preparations, two sulfated sterol bands migrated in close proximity to each other after fractionation in solvent system I (**Fig. 3**). In these studies, we derived our weights only from the more mobile upper band, which was present consistently and shown by GLC–MS to comprise cholesterol sulfate (17). On fast atom bombardment (FAB), the upper band showed a quasi-molecular ion at M/Z 465 and an ion representing loss of H_2SO_4 at M/Z 389. After solvolysis, the only free sterol present in either band demonstrated the typical spectrum of cholesterol (M/Z 389). The lower band demonstrates a large ion at M/Z 481, which is consistent with an oxygenated cholesterol sulfate, a conclusion that is supported by the presence of fragments at M/Z 465 (?loss of H_2O) and M/Z 389 (loss of sulfate). After solvolysis this band also demonstrates a predominant ion at M/Z 389, indicative of cholesterol. Neither band stained with orcein, effectively ruling out the presence of a sterol glucoside.

Neutral lipids

The neutral lipids, comprising free sterols, free fatty acids, triglycerides, sterol and wax esters, squalene, and n-alkanes contributed the greatest proportion to the stratum corneum lipids (Table 1, **Fig. 4**). Within this class, the free sterols, nonpolar species, and free fatty acids demonstrated significant regional variations. Plantar stratum corneum contained significantly more free sterols than either leg, face, or abdominal stratum cor-

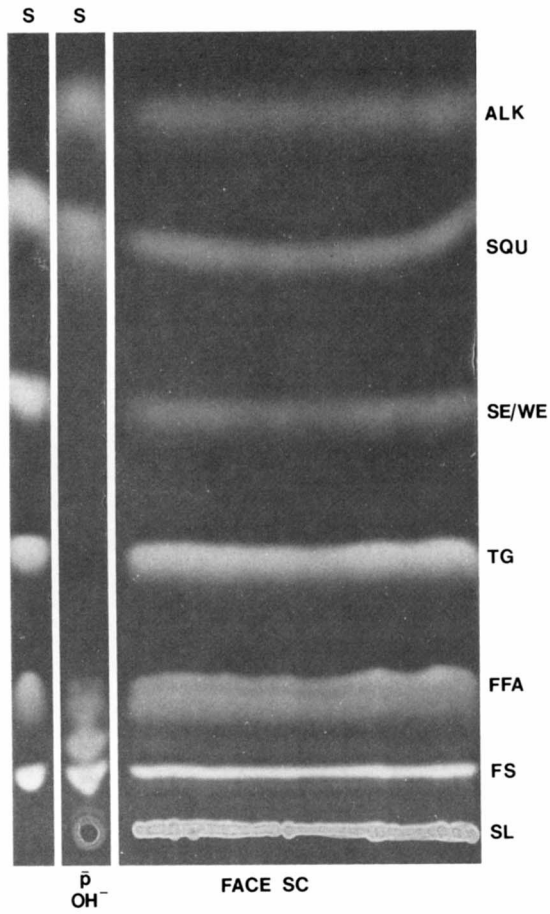

Fig. 4. One-dimensional TLC of neutral lipids fractionated as in Methods. This sequence first separates the sphingolipids (SL), free sterols (FS), free fatty acids (FFA), and triglycerides (TG), followed by separation of the nonpolar species into a sterol/wax ester (SE/WE), squalene (SQU), and n-alkane (ALK) band.

TABLE 2. Distribution of individual neutral lipid and sphingolipid fractions

	Site			
	Abdomen (n = 4)	Leg (n = 4)	Face (n = 3)	Plantar (n = 4)
	lipid weight % ± SEM			
Neutral lipids				
Free sterols[a]	14.0 ± 1.1	20.1 ± 2.0	17.3 ± 0.5	32.8 ± 1.6
Free fatty acids[b]	19.3 ± 3.7	13.9 ± 1.8	19.7 ± 0.6	9.0 ± 1.7
Triglycerides	25.2 ± 4.6	20.1 ± 1.0	13.5 ± 1.0	5.9 ± 0.6
Nonpolar[c]	16.3 ± 6.5	11.1 ± 0.7	15.9 ± 0.9	12.7 ± 2.1
Sterol/wax esters	6.1 ± 0.6	4.6	6.2 ± 0.7	7.1 ± 0.7
Squalene	6.5 ± 2.0	3.6	6.9 ± 0.3	2.9 ± 1.8
n-Alkanes[d]	3.7 ± 0.5	3.0	2.8 ± 0.3	2.9 ± 1.0
Sphingolipids	18.1 ± 2.8	25.9 ± 1.3	26.5 ± 0.9	34.8 ± 2.1
Glycosphingolipids I	0.7	1.2	1.9	3.2
Glycosphingolipids II	1.9	2.2	4.8	3.2
Ceramides/NL I	3.9	4.4	4.4	6.8
Ceramides II	11.6	18.2	15.5	21.6

[a] Abdomen vs. leg, $P < 0.05$; abdomen vs. face, $P < 0.05$; abdomen vs. plantar, $P < 0.01$; plantar vs. face, $P < 0.001$; plantar vs. leg, $P < 0.01$. Free sterols: plantar > leg > face > abdomen.
[b] Abdomen vs. plantar, $P < 0.05$; leg vs. face, $P < 0.01$; plantar vs. face, $P < 0.01$.
[c] Leg vs. face, $P < 0.02$.
[d] Plantar vs. abdomen, $P < 0.05$.

neum ($P < 0.01$). Although free fatty acids were present in almost equal quantities in leg, abdominal, and facial stratum corneum, they occurred in lesser quantities in plantar stratum corneum (**Table 2**).

The greatest regional variations occurred in the triglyceride fraction, particularly in abdominal samples, possibly reflecting some contamination from subcutaneous fat during tissue preparation. Therefore, to insure that the differences noted above were not due only to variations in triglycerides, the statistical analyses were done both with and without inclusion of the triglyceride data. Regardless of the inclusion of triglycerides, the same significant differences were found.

The highly nonpolar neutral lipids, fractionated in solvent system IIIa, revealed three distinct compounds after rechromatography in solvent system IIIb. After acid hydrolysis of the putative sterol ester fraction, we found free alcohol, free sterol, and fatty acid methyl esters indicating that the fraction contained both sterol and wax esters. The other two fractions were shown to be squalene and n-alkanes both by glass capillary GLC and by GLC–MS (see below). While both squalene and wax esters are considered to be exclusively of sebaceous origin, it is doubtful that either band is exclusively of sebaceous origin since plantar stratum corneum also exhibited some squalene and wax esters (Table 2).

Sphingolipids

Sequential fractionation of the most polar neutral lipids isolated from solvent system III, revealed four major classes of sphingolipids in solvent systems IVa and IVb (Table 2). The five most polar bands, designated GSL-1 and GSL-2 were identified as glycosphingolipids by orcein staining, although identification of specific sugar moieties was not undertaken. Of the glycosphingolipids, GSL-1 was a single species, which possessed the mobility of the O-acyl-glycosyl-ceramide species identified by Gray, White, and Mayer (20). GSL-2 was a group of four faint bands, which were taken together because there were no other distinguishing characteristics and only small quantities of each were present. Despite the great interest attracted by these compounds, GSL-1 and GSL-2 together comprised only 4–7% of human stratum corneum lipids (Table 2). The ceramide II fraction consisted of four orcein-negative bands with the mobility of ceramides, yielding fatty acid methyl esters and four long-chain bases, with the mobility of sphingosine, when subjected to acid hydrolysis (**Fig. 5**). Ceramides I, the most mobile class of sphingolipids, also contained trace quantities of neutral lipids (Fig. 5). Of the four sites studied, plantar stratum corneum contained significantly more sphingolipid than the other three sites ($P < 0.02$), while abdominal stratum corneum contained significantly less sphingolipid than either leg or facial stratum corneum ($P < 0.05$).

Analysis of fatty acid methyl esters and n-alkanes

Regardless of the site of origin (leg, abdomen, face, or plantar), each of the major lipid species demonstrated a characteristic fatty acid profile (**Table 3**). The fatty acid chain lengths ranged from C12 to C24 with C16 and C18 predominating. Both saturated and unsaturated fatty acids were encountered in all fractions. However, unsaturated fatty acids predominated over satu-

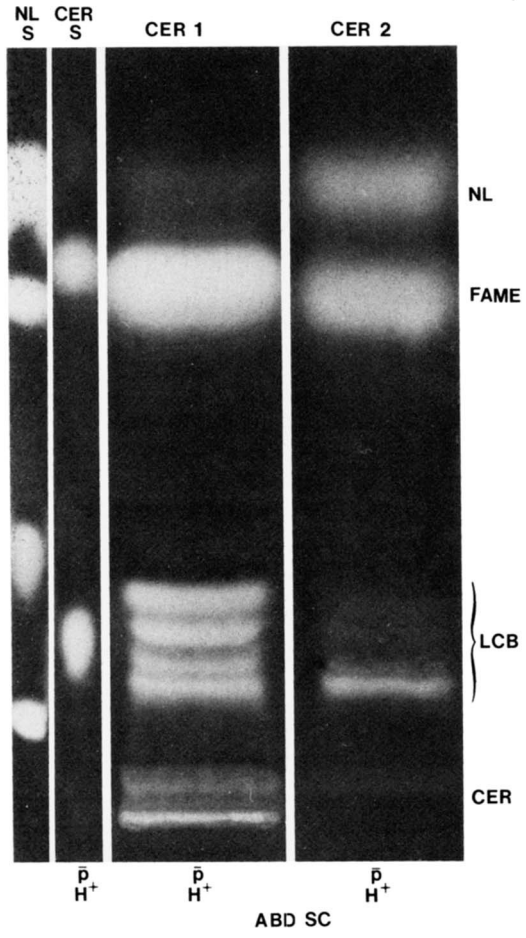

Fig. 5. One-dimensional TLC of the two major ceramide regions (Cer 1 and Cer 2) in the neutral lipid system before and after hydrolysis. Whereas unhydrolyzed sphingolipids remain near the origin, long-chain bases (LCB) migrate in close proximity to each other, and fatty acid methyl esters (FAME) and neutral lipids (NL) migrate toward the front. As can be seen, Cer 2 contains some neutral lipids, but only one prominent LCB, whereas Cer 1 is relatively free of NL, but contains a family of LCB.

rated among all of the neutral and phospholipid species, whereas ceramides and glycosphingolipids contained primarily saturated classes.

Neutral lipids. The free fatty acids were the only fraction in which the ratio of saturated to unsaturated fatty acids was greater than 1, with C16:0 representing the major component in both leg and abdominal stratum corneum. In contrast, the triglyceride fractions from both leg and abdominal stratum corneum contained more than 40% C18:1 further suggesting that some of the triglycerides may have derived from subcutaneous fat (data not shown). Again, C16:0, C18:0, C18:1, and C18:2 formed the majority of the fatty acids in the sterol/wax ester, fraction, with C18:1 comprising about 30–45% of all of the esterified fatty acids.

Phospholipids. Although the stratum corneum contained less than 4% phospholipids, they possessed abundant C18:1. However, the phospholipids displayed substantially more C18:2 and long-chain fatty acids than did the neutral lipids (Table 3).

Chain-length analysis of n-alkanes. Glass capillary GLC of the hydrocarbon fraction separated in solvent system IIIb, displayed both n-alkanes (**Fig. 6**), and a variable C28 peak identified as squalene by mass spectrometry (**Fig. 7**), depending on the age and sex of the donor. In all four sites, the n-alkanes comprised about 2–4% of the total lipid, and consisted of both odd- and even-chained compounds in a bell-shaped distribution from C19 to C34 (Fig. 6). Although the n-alkanes theoretically could have derived from certain topical medications that display a similar GLC profile (Fig. 2), their ubiquitous presence in all four skin sites, in the absence of a history of prior topical treatment, makes this source unlikely. It is also highly unlikely that they resulted from laboratory or aerosol contamination since the GLC profile of these potential contaminants differed from that demonstrated in stratum corneum samples (43). Finally, contamination by surface bacteria, or as a result of degradation of other lipid constituents, e.g., free fatty acids, is unlikely since no differences were seen in the profiles of n-alkanes or the relative proportions of other lipid constituents from skin samples allowed to incubate at 37°C for 1–2 weeks vs. samples extracted immediately after storage at −20°C (43).

DISCUSSION

Human stratum corneum lipid composition

The literature is replete with descriptions of human stratum corneum lipid composition, but most have selected only a limited spectrum of neutral (6, 7, 23–26) or polar lipids (13, 27) for analysis, or they utilized skin surface lipids (5, 28) or callous (24, 25) as starting materials. Since skin surface lipids contain a high percentage of sebaceous lipid (5, 28), such studies offer few insights into the role of lipids in cutaneous barrier function or stratum corneum cohesion (see below). Likewise, studies on callous can be criticized because the starting material derives from a nonrepresentative skin site that manifests atypical barrier function (29). In contrast, comprehension studies of both pig (9) and neonatal mouse (11, 12) stratum corneum lipids have been published. This study provides the first quantitative information about the full spectrum of human stratum corneum lipids, and, further, compares the distribution of these lipids at four sites that demonstrate distinctive variations in skin permeability (14).

As in the pig and mouse (9, 11, 12), we found almost no phospholipids in the stratum corneum, although a small amount, ranging from 3 to 5%, of the total lipid

TABLE 3. Identification of fatty acid composition of human stratum corneum lipids by comparison with straight chain fatty acid methyl esters[a]

	Free Fatty Acids				Sterol/Wax Esters				Ceramides II			Phosphatidyl-ethanolamine	
C#	Abdomen	Leg	Face	Plantar	Abdomen	Leg	Face	Plantar	Abdomen	Leg	Face	Abdomen	Leg
14:0	3.8	10.9	1.4	0.3		4.21	0.9	2.5			0.1	0.8	3.0
16:0	36.8	36.2	27.9	10.5	20.0	21.0	14.6	21.4	7.7	10.2	4.3	15.8	10.3
16:1	3.6	16.6	6.5	1.2	15.9	27.8	36.9	5.7				4.9	4.0
18:0	9.9	10.0	16.3	20.1	5.8	6.2	4.6	8.6	4.8	11.4	9.8	13.5	13.6
18:1	33.1	17.7	23.5	18.8	49.4	32.9	32.9	44.2	6.3	3.6	4.3	38.1	34.0
18:2	12.5	1.4	11.9	6.5	6.6	5.1	10.0	15.2	14.0	1.9	6.1	20.7	21.6
20:0	0.3	2.6	2.4	6.1		0.9	tr		5.9		3.8	1.3	tr
20:1	tr	1.1	0.1	1.5		0.7	tr	tr				1.0	tr
20:2		tr	0.1			tr						0.3	1.2
20:3		tr		3.1								tr	tr
20:4		tr	3.5				tr	tr			0.3	1.6	12.2
22:0	tr	3.5	4.4	9.6							7.0	0.7	
22:1			2.0	5.8			tr	tr			2.0		
22:0				16.5	0.9	1.4		2.4	50.8	43.3	43.9		
24:1					1.6						10.8	1.3	
26:0									10.5	29.6	7.7		
Total	100.0	100.0	100.0	100.0	100.2	100.2	99.9	100.0	100.0	100.0	100.1	100.01	100.01

[a] Straight chain analysis such as this may overlook hydroxy- or branched-chain fatty acids (see Methods).

was phosphorus-containing by TLC and by a qualitative colorimetric assay (22). This residual phospholipid cannot represent nonhomogeneity of the starting material because we have shown repeatedly by electron microscopy that our preparations contain no nucleated cells (12; Fig. 1, reference 30). Furthermore, the outer stratum corneum (OSC) also shows the same spectrum of phospholipids, although in lesser quantities (30).

As in earlier studies of human stratum corneum lipids (6, 7, 24, 27), we found large quantities of free sterols, and lesser quantities of esterified sterols. About 3- to 5-times more free than esterified sterols were present in all four skin sites, a finding that diminishes the likelihood that sterol esterification is an important concomitant of cornification (31). These large concentrations imply an important, but as yet unknown, role for free sterols in stratum corneum function. The association of abnormal stratum corneum retention (i.e., ichthyosis) with administration of hypocholesterolemic drugs in man and animals (reviewed in 1, 32), suggests an important role for sterols in one stratum corneum function, namely, intercellular cohesion. However, since these ichthyotic abnormalities are not accompanied by abnormal barrier function (33), it is less likely that free sterols alone play a central role in cutaneous barrier function.

As in pig (9) and neonatal mouse stratum corneum (11, 12), we found a substantial amount of sphingolipid in all four skin sites (15–35%), although the quantities encountered are somewhat less than those recently reported (34). Such an extensive accumulation of sphingolipids appears to be a unique feature of keratinizing epithelia and even central nervous system and intestine, which are major repositories of these lipids, contain only about 10% sphingolipid by lipid weight, of which only a minor fraction is ceramide (35, 36). Of the sphingolipids present in human stratum corneum, only a small proportion is glycosylated (Table 2), the remainder fractionating identically to a series of closely-related ceramides, as described in pig (9) and neonatal mouse (12) stratum corneum. In the accompanying paper, we demonstrate that a further loss of glycosphingolipids, with

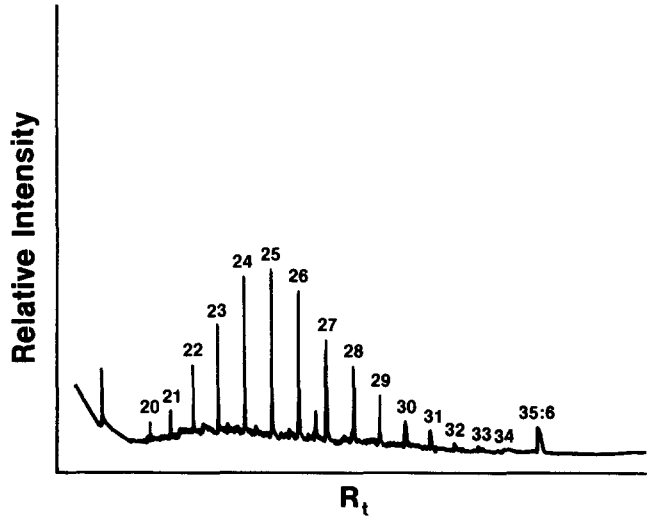

Fig. 6. Glass capillary GLC profile of n-alkane fraction. Note the bell-shaped distribution of n-alkanes from C19 to C34, with equal proportions of odd- and even-chained compounds.

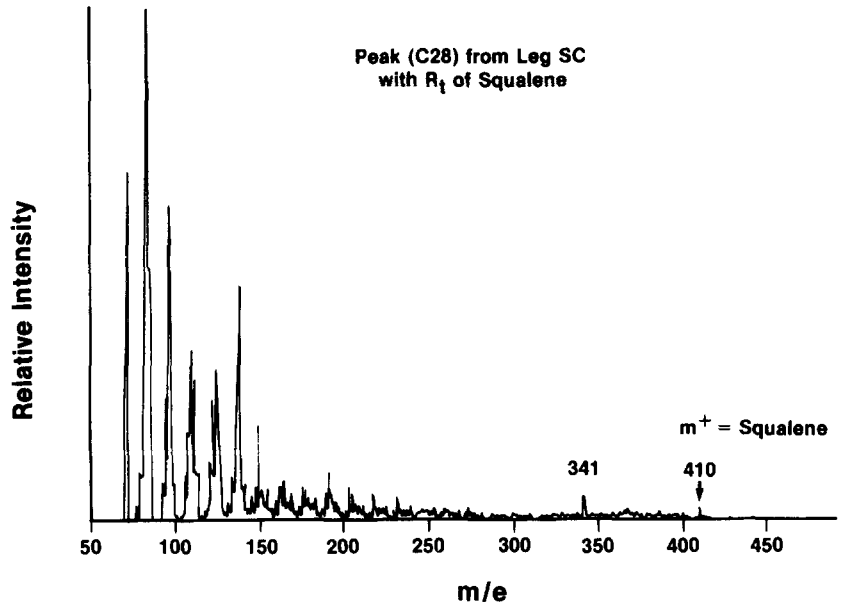

Fig. 7. C28 peak from previous sample displaying the mass spectrometric characteristics of squalene.

a proportionate increase in ceramides, occurs in the outer stratum corneum, perhaps thereby provoking stratum corneum desquamation (37). However, the opposite does not appear to be true: since ichthyosis is not a feature of the sphingolipidoses, excessive glycosphingolipid accumulation probably does not result in abnormal stratum corneum retention.

The invariable presence of cholesterol sulfate in stratum corneum lipids deserves mention in light of the recent finding of increased cholesterol sulfate in recessive x-linked ichthyosis stratum corneum (17). Although the quantities found in stratum corneum are not large (2–5%), cholesterol sulfate appears to be targeted for a specific stratum corneum function in light of: *a*) the major role of the skin in the biosynthesis of this metabolite (38); and *b*) its accumulation just beneath the stratum corneum, in the stratum granulosum (30). One possible function for cholesterol sulfate would be in the modulation of stratum corneum desquamation through its own desulfation (3), a suggestion that is supported by the preponderance of steroid sulfatase activity found in the stratum corneum (39).

Finally, the invariable occurrence of significant quantities (2–5%) of n-alkanes in the stratum corneum is noteworthy in light of the importance of these substances for barrier function in plant and insect integument (reviewed in reference 40). Other workers have found n-alkanes in skin surface lipids (9, 11, 12), but it has been suggested that they represent only environmental contaminants (41). Great pains were taken here to verify the endogenous origin of these n-alkanes. Specifically, we excluded aerosol pollutants, laboratory contaminants, surface bacterial action, and some, but not all, medications, as potential sources of these n-alkanes. The fact that the n-alkanes comprise a homologous series of both odd- and even-chained compounds also mitigates against a purely dietary source (42). Moreover, the recent description of large quantities of n-alkanes in untreated ichthyotic scale (43) further suggests an endogenous origin. Finally, the presence of n-alkanes in the living layers of the epidermis also makes an exogenous source unlikely (30). If the n-alkanes are of endogenous origin, the fact that plantar stratum corneum contains ample n-alkanes (Table 1) suggests the n-alkanes are of keratinocyte, and not of pilosebaceous, origin. Whether n-alkanes are incidental co-products of epidermal lipid metabolism, or subserve a specific function in the skin still remains to be determined.

Regional variations in stratum corneum lipids: implications for cutaneous barrier function

The role of lipids in governing skin permeability is well established (reviewed in reference 1). Despite the morphological similarity of the stratum corneum, this layer displays striking regional variations in permeability. For example, the palm and sole regulate water loss poorly (29), whereas facial and scrotal skin are highly water-impermeable, but selectively admit lipophilic probes (reviewed in reference 2). In seeking explanations for these phenomena, we previously correlated the barrier function of abdominal and leg stratum corneum with several structural and chemical parameters (14): briefly, we found no relationship between barrier function and the thickness or the number of cell layers in the stratum corneum. Instead, an inverse relationship was found between lipid weight percent and perme-

ability. In this study, the same variations were noted in lipid weight percent that were found in the earlier study (14), with face > abdomen > leg > plantar stratum corneum in the exact reverse to their known permeability (2).

Going a step further than simply comparing lipid concentrations, in this study we have attempted to pinpoint variations in the content of specific lipid fractions that might explain the above-cited permeability differences. Although statistically significant site-to-site differences in the content of several categories of lipids occurred, the observed variations in cholesterol sulfate did not correlate with known variations in permeability. In contrast, there was a reasonably good, inverse correlation between the total amount of neutral lipid and permeability according to site. In contrast, the sphingolipid content to some extent revealed a direct relationship with permeability: the skin site which demonstrates the greatest permeability to water, i.e., plantar, contains the greatest quantities of sphingolipids (Table 2). When considered together, these studies suggest that the neutral lipids and sphingolipids of the stratum corneum may subserve different functions in forming the permeability barrier. Although we suspect that neutral lipids, as the more labile component (44), are the "weak link" in the barrier, while sphingolipids comprise a more permanent sealant, further studies, utilizing appropriate in vitro model systems, will be required to clarify the precise functions of each lipid component in the barrier.

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