Human epidermal lipids: characterization and modulations during differentiation

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Abstract Using thin-layer chromatography and glass capillary gas–liquid chromatography, we have quantitated the lipids in the germinative, differentiating, and fully cornified layers in human epidermis. As previously noted in nonhuman species, we found progressive depletion of phospholipids coupled with repletion of sterols and sphingolipids during differentiation. The sphingolipids, present only in small quantities in the lower epidermis, accounted for about 20% of the lipid in the stratum corneum, and were the major repository for the long-chain fatty acids that predominate in the outer epidermis. Although the absolute quantities of sphingolipids increased in the outer epidermis, the glycolipid:ceramide ratio diminished in the stratum corneum, and glycolipids virtually disappeared in the outer stratum corneum. Squalene and n-alkanes were distributed evenly in all epidermal layers, suggesting that these hydrocarbons are not simply of environmental or pilosebaceous origin. Cholesterol sulfate, previously considered only a trace metabolite in epidermis, was found in significant quantities, with peak levels immediately beneath the stratum corneum in the stratum granulosum. These studies: *1*) provide new quantitative data about human epidermal lipids; *2*) implicate certain classes of lipids for specific functions of the stratum corneum; and, *3*) shed light on possible product-precursor relationships of these lipids.—**Lampe, M. A., M. L. Williams, and P. M. Elias.** Human epidermal lipids: characterization and modulations during differentiation. *J. Lipid Res.* 1983. **24:** 131–140.

Supplementary key words epidermis • lipids • sphingolipids • n-alkanes • cholesterol sulfate

The mammalian epidermis consists of an undifferentiated germinative layer surmounted by progressively flattened cells that eventually lose recognizable organelles as they transform into corneocytes. Although there have been few quantitative studies of epidermal lipids, both biochemical analyses of lipid extracts and studies of epidermal lipogenesis suggest that the lipid composition of the viable epidermis is similar in composition to those found in other epithelial tissues (reviewed in reference 1). Phospholipids, required for the maintenance of cellular membrane bilayers, and triglycerides, utilized for energy, predominate in the lower epidermis, as in other epithelia (reviewed in reference 2). However, in addition to the usual spectrum of polar and neutral lipids, mammalian epidermis contains certain unique phospholipids (3) and glycosphingolipids (4). More importantly, in the epidermis of all mammalian species examined to date, transformation of the stratum granulosum into the stratum corneum is accompanied by: *1*) depletion of phospholipids with relative retention and enrichment of neutral lipids (5–7); *2*) generation and retention of large amounts of sphingolipids (8–11); and, *3*) enrichment of sphingolipids composed of longer chain, more saturated fatty acids than are present in lipids in the subjacent viable epidermis (9–12). Although the implications of these unique lipid modulations are not fully understood, possible functions include both the maintenance of the normal barrier to water loss and the control of stratum corneum desquamation (reviewed in reference 13).

Because of prior difficulties in obtaining viable human epidermis, more information is available about the lipids in epidermal strata in nonhuman species. This study is designed to provide detailed biochemical data about modulations in lipid composition during human epidermal differentiation, and to pinpoint possible product-precursor relationships of the lipids that are generated during differentiation. Further detailed information about the content of stratum corneum lipids can be found in the companion paper (14).

METHODS

Sources of tissue

Fresh full-thickness samples of human abdominal skin were obtained from the morgue. After removing the

Abbreviations: PBS, phosphate-buffered saline; TLC, thin-layer chromatography; FAME, fatty acid methyl esters; GLC, gas–liquid chromatography; R_t, retention time; FD, field desorption; FAB, fast atom bombardment; SB, stratum basale; SS, stratum spinosum; SG, stratum granulosum; SC, stratum corneum; NL, neutral lipid; PL, polar lipid; SL, sphingolipid; MS, mass spectrometry.

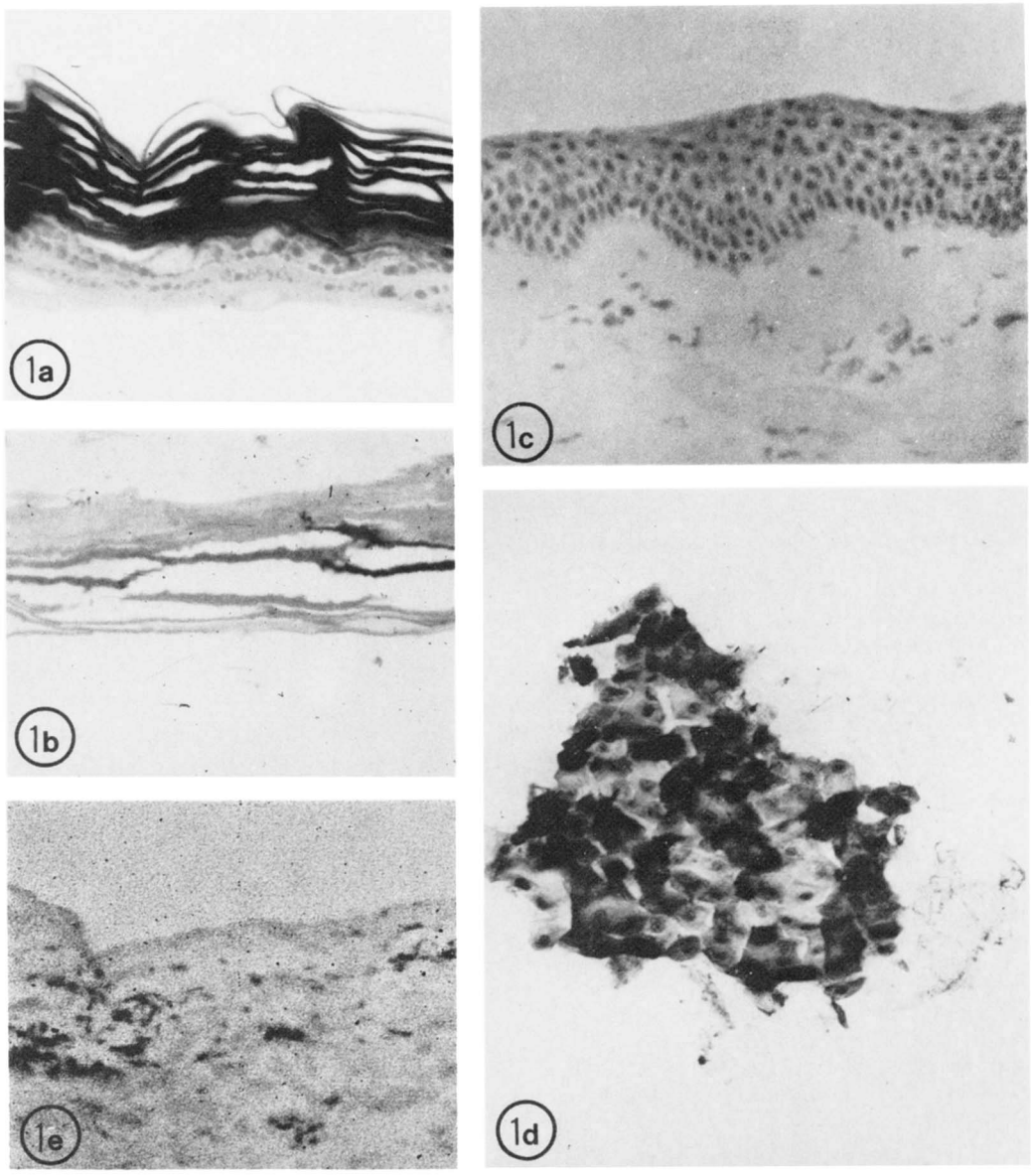

Fig. 1. Demonstration of the homogeneity of the various epidermal preparations. Trypsinization causes a clean cleft between the stratum corneum and stratum granulosum (Fig. 1a) that leaves intact both the overlying stratum corneum and stratum granulosum (Fig. 1d), and the underlying layer of loosened stratum spinosum and stratum basale cells (Fig. 1c). After further trypsinization and vortexing, the residual granular cells are removed, leaving a homogeneous preparation of stratum corneum (Fig. 1b). Removal of the basal and spinous cells by scraping is also complete, and does not enter the dermis or dislodge dermal elements (Fig. 1e).

subcutaneous fat with a #15 surgical blade, each sample was spread dermis-side-downward on filter paper soaked with 0.5% trypsin (Sigma, St. Louis, MO) in phosphate-buffered saline (PBS). After incubating for 12–16 hr at 4°C, sheets of stratum corneum and stratum granulosum were peeled off. These sheets were re-trypsinized as described previously (11) to obtain separate populations of granular and cornified cells. After 3 hr at 37°C, intact stratum corneum sheets were separated from granular cells by washing and vortexing three times in PBS. The granular and cornified cell preparations have been shown to be over 99% homogenous by light and electron microscopy (**Fig. 1,** and reference 11). A mixed basal and spinous cell population was obtained by scraping epidermal cells off the dermis with a clean spatula, a procedure which does not remove any dermal elements (Fig. 1, and reference 11). Outer stratum corneum preparations ("scale") were obtained either following orthopedic cast removal (n = 5) or from desquamating skin after sunburns (n = 3).

Extraction and thin-layer chromatography

Total lipid extraction of all samples was accomplished by the method of Bligh and Dyer (15). Addition of 1 M KCl to the extraction solvents forced cholesterol sulfate into the organic phase, permitting total recovery of this amphipathic lipid from lipid extracts. When KCl was not added to the extraction media, the aqueous phases were saved for subsequent quantitation of cholesterol sulfate, as described previously (16).

The lipid extract was fractionated by quantitative, sequential thin-layer chromatography (TLC) utilizing the following solvent systems: #1, tetrahydrofuran–methylal–methanol–4 M ammonium hydroxide 60:30:10:4 (v/v); #2a, petroleum ether–diethyl ether–glacial acetic acid 80:20:1 (v/v); followed by: #2b, petroleum ether (100%); #3a, chloroform–methanol–distilled water 90:10:1 (v/v) followed by: #3b, petroleum ether–diethyl ether–glacial acetic acid 70:50:1 (v/v); #4, chloroform–methanol–distilled water–glacial acetic acid 60:35:4.5:0.5 (v/v). Solvent system #1 separates cholesterol sulfate from both polar and neutral lipids; #4 fractionates the phospholipids; #2 fractionates the major neutral lipid species; and #3 separates glycolipids from ceramides. Further details of our extraction and chromatographic methods may be found in the companion paper (14).

Following TLC, all lipid bands were visualized by spraying with 8-anilino-1 napthalene sulfonic acid (ANS), excised, extracted from the gel, and weighed as described previously (11). Cholesterol sulfate was measured by a modification of the spectrophotometric assay of Franey and Amador (17), and was definitively identified by mass spectrometry after solvolysis (see below).

Gas–liquid chromatography

The fatty acid composition of the neutral (NL), polar (PL), and sphingolipid (SL) fractions was analyzed by glass capillary gas–liquid chromatography (GLC). Fatty acid methyl esters (FAME) of the neutral lipid species were prepared in 12% boron trichloride in methanol at 90°–100°C, for 1–1.5 hr (18), followed by extraction of methyl esters in hexane, and fractionation in solvent system #2a to recover purified FAME.

Fatty acid methyl esters of all these compounds were analyzed on a Hewlett-Packard 5830 Gas Chromatograph using a 15-meter SP-1000 glass capillary column (J & W Scientific, Orangevale, CA), using temperature programing: 160°–180°C at 5°C/min. Peaks were identified by comparison of retention time (R_t) values to known standards. Analysis was limited to unsubstituted fatty acids because the range of branched and hydroxy fatty acids in skin is known to be limited and the amounts of lipid available for analysis precluded application of alternate analytical methods.

Hydrocarbon analysis

The most nonpolar lipids, fractionated in solvent system #3b, and comprising squalene and n-alkanes, were analyzed further by glass capillary GLC on a 25-m FS-OV-1 column programed from 170°–340°C at 5°C/min, and assigned as squalene or n-alkanes, by comparison of R_t values with known standards, or by mass spectrometry (see below). In order to rule out exogenous sources of n-alkanes, several controls were performed, as detailed in the companion paper (14).

Mass spectrometry

Gas–liquid chromatographic–mass spectrometric (MS) analyses were carried out in the University of California Mass Spectrometry Unit, using a Kratos MS-25 double focusing mass spectrometer (Kratos Ltd., Manchester, U.K.) coupled with a direct fused silica capillary inlet to the electron impact ion source employing a Varian 3700 gas chromatograph (Varian Instrument Group, Palo Alto, CA). Grob-type splitless injection was employed using a 20-m, 0.3-mm i.d. SE-52 fused silica capillary column. On-line computer recording and analysis of the GLC–MS runs were provided by an LSI minicomputer. High resolution mass spectra (including field desorption and fast atom bombardment ion sources) were obtained when necessary, employing a Kratos MS-50S double-focusing mass spectrometer on-line to a Xerox Sigma 7/LOGOS-II computer system (19).

RESULTS

Distribution of lipid fractions

Polar lipids. The phospholipids found in the living layers of human epidermis include phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), sphingomyelin (SPH), and lysolecithin (LYS) (**Fig. 2**). This one-dimensional solvent system (#4) does not fractionate the unusual phospholipids found in two-dimensional chromatograms of pig epidermis (3, 8). If present in human epidermis, they would presumably be included in the PE fraction. Whereas these phospholipid species comprised about 45% of the total lipid in the basal and spinous layers, they consisted of only about 25% of the total lipid in the stratum granulosum, and less than 5% of the total lipid in the stratum corneum (**Table 1**). In the stratum corneum only trace quantities of phosphatidylethanolamine and phosphatidylcholine remained (Table 1, Fig. 2, c.f. 14).

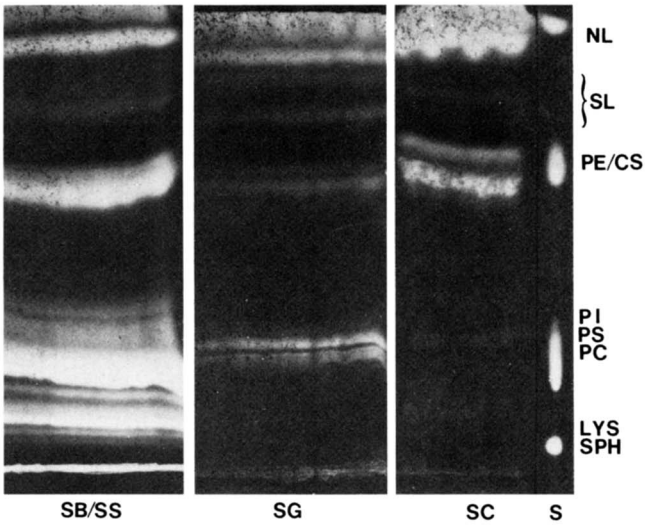

Fig. 2. TLC of phospholipids fractionated in solvent system #4 (see Methods). Whereas all of the usual phospholipid species, sphingomyelin (SPH), lysolecithin (LYS), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidylethanolamine (PE), are present in the basal and spinous layer (SB/SS), they diminish greatly in the granular layer (SG), and only two faint bands remain in the cornified layer (SC). The prominent fraction in the SC, which migrates just below PE, is cholesterol sulfate (CS), which exhibits mobility similar to PE when not previously removed by fractionation in solvent system #1.

Neutral lipids. The neutral lipids separated by solvent system #3 comprised six discrete fractions found in all epidermal layers (**Fig. 3**). Neutral lipids began to predominate over phospholipids in the stratum granulosum where they comprised over 50% of the total lipids, and they accumulated still further in the stratum corneum, where they represented about 75% of the total lipid (Table 1). Significant shifts in the distribution of the various neutral lipid species were found. During differentiation (granular to cornified layer), the amounts of free sterols (FS), free fatty acids (FFA), and highly nonpolar species, including both sterol (SE) and wax esters (WE), and n-alkanes, progressively increased (Table 1). Although the quantities of squalene (SQ) and n-alkanes (Alk) were highest in the stratum corneum, they were also identified in significant quantities in the stratum granulosum and basal/spinous layers by TLC, GLC, and mass spectrometery (Table 1, Fig. 3, and **Fig. 4**). Wax esters, which co-migrate with sterol esters in solvent system #2, were also identified in all layers of epidermis, as shown by acid hydrolysis followed by TLC of this fraction (**Fig. 5**).

Sphingolipids. All of the epidermal layers contained sphingolipids that comprised a mixture of ceramides and glycosphingolipids (**Fig. 6**), separable into at least ten bands in solvent system 3. The four most polar bands were orcein-positive and divided into two groups. The three bands closest to the origin were quantitated as a group since there was no obvious basis for subdivision. The fourth band was taken separately, since it demonstrated the mobility described for the unique o-acyl-glycolipid, "problemin" (2, 4), previously described in human and pig epidermis. The remaining six orcein-negative bands were subdivided into two groups

TABLE 1. Variations in lipid composition during human epidermal differentiation and cornification[a]

Fraction	Strata Basale/Spinosum (n = 5)	Stratum Granulosum (n = 7)	Stratum Corneum	
			Whole (n = 4)	Outer (n = 8)
Polar lipids*	44.5 ± 3.4	25.3 ± 2.6	4.9 ± 1.6	2.3 ± 0.5
Cholesterol sulfate**	2.4 ± 0.5	5.5 ± 1.3	1.5 ± 0.2	3.4 ± 0.5
Neutral lipids	51.0 ± 4.5	56.5 ± 2.8	77.7 ± 5.6	68.4 ± 2.1
Free sterols	11.2 ± 1.7	11.5 ± 1.1	14.0 ± 1.1	18.8 ± 2.1
Free fatty acids***	7.0 ± 2.1	9.2 ± 1.5	19.3 ± 3.7	15.6 ± 3.0
Triglycerides	12.4 ± 2.9	24.7 ± 4.0	25.2 ± 4.6	11.2 ± 1.5
Sterol/wax esters[b]	5.3 ± 1.3	4.7 ± 0.7	5.4 ± 0.9	12.4 ± 1.9
Squalene	4.9 ± 1.1	4.6 ± 1.0	4.8 ± 2.0	5.6 ± 2.1
n-Alkanes	3.9 ± 0.3	3.8 ± 0.8	6.1 ± 2.6	5.4 ± 0.8
Sphingolipids****	7.3 ± 1.0	11.7 ± 2.7	18.1 ± 2.8	26.6 ± 2.3
Glucosylceramides I	2.0 ± 0.3	4.0 ± 0.3	trace	trace
Glucosylceramides II	1.5 ± 0.3	1.8 ± 0.2	trace	trace
Ceramides I	1.7 ± 0.1	5.1 ± 0.4	13.8 ± 0.4	19.4 ± 0.5
Ceramides II	2.1 ± 0.3	3.7 ± 0.1	4.3 ± 0.4	7.2 ± 0.5
Total	99.1	101.1	99.3	100.7

[a] Weight % ± SEM.
[b] Sterol/wax esters present in approximately equal quantities, as determined by acid hydrolysis (see text).
Significant differences: *, SS/SB vs. SG ($P < 0.01$); SG vs. WSC or SS/SB ($P < 0.001$). **, SG vs. WSC ($P < 0.02$); WSC vs. OSC ($P < 0.01$). ***, SG vs. WSC ($P < 0.05$). ****, SS/SB vs. SG ($P < 0.05$); WSC vs. OSC ($P < 0.05$).

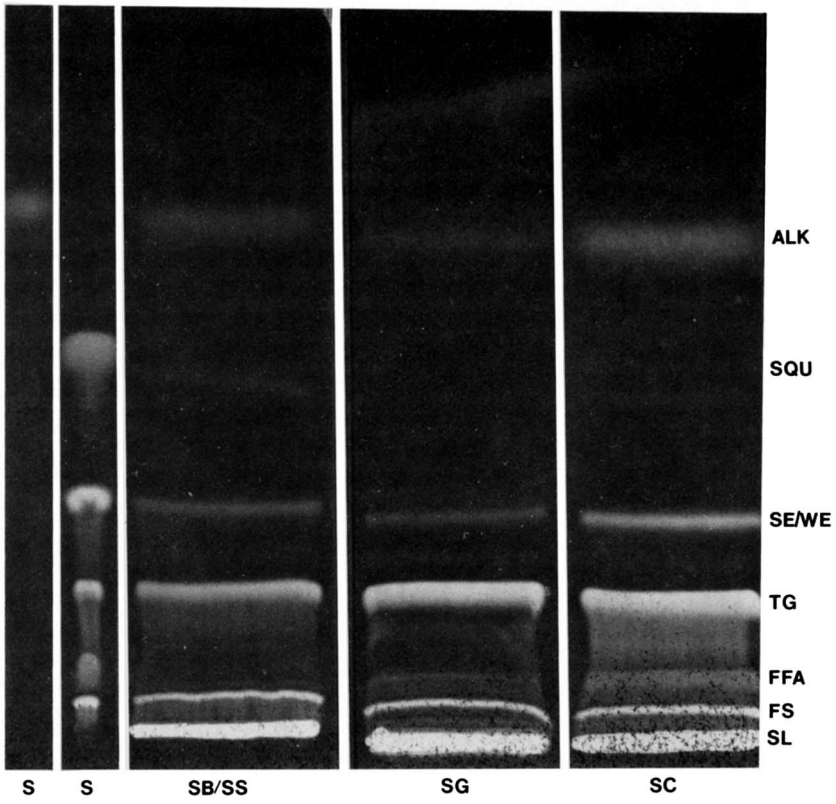

Fig. 3. TLC of neutral lipids fractionated sequentially in solvent systems 2a and 2b. An equal quantity of lipid was applied to each lane. Although all of the major neutral lipids are present in the three strata, the increased prominence of the free fatty acids (FFA), sterol/wax esters (SE/WE), and n-alkanes (n-Alk) in the cornified layer (SC) is apparent. However, major differences in the quantities of sphingolipids (SL), free sterols (FS), triglycerides (TG), and squalene (SQU) cannot be appreciated.

for quantitation. The two least polar bands ran with the neutral lipids and upon further analysis were shown to contain traces of neutral lipids as well as ceramides. The relative quantities of sphingolipids increased progressively from the basal/spinous layers to the stratum granulosum, and still further during subsequent formation of the stratum corneum (Table 1). Both glycosphingolipids and ceramides increased in the granular layer, but the glycosphingolipids diminished as ceramides alone increased in the stratum corneum (Table 1). Finally, whereas glycosphingolipids were still demonstrable in whole stratum corneum preparations, the glycosphingolipid:ceramide ratio diminished still further in the outer stratum corneum (Table 1, Fig. 6).

Cholesterol sulfate. This highly amphipathic sterol metabolite is present not only in the stratum corneum, as previously described (8, 11, 14), but also in all viable epidermal layers (**Fig. 7**), where the greatest concentration occurred in the stratum granulosum (Table 1). The identification of cholesterol sulfate was proven by mass spectrometry using both desorption and fast atom bombardment (FAB) sources, and by identification of free sterols after solvolysis (14, 16).

Fatty acid and hydrocarbon analysis

In all of the layers examined, the fatty acid composition ranged from C12 to C24, with C16–18 representing the major species (**Table 2**). Whereas the major saturated fatty acids were C16:0 and C18:0, among monoenoic acids C18:1 was always found in greater quantities than C16:1. Trace quantities of both C20:3 and C20:4 were found, with C20:4 invariably present in greater quantities than C20:3 (Table 2, representative experiment).

Neutral lipids. Shorter-chain fatty acids, particularly C12:0 and C14:0, occurred in greater quantities in the neutral lipid fraction than in the phospholipid or sphingolipid fractions. Moreover, none of the C20 and longer-chain unsaturated fatty acids occurred in the neutral lipids. During differentiation few significant changes in fatty acid distribution occurred, although the content of C18:1 decreased slightly during the transition from the granular to the cornified layer (Table 2).

In all three strata, the n-alkanes comprised a spectrum of odd- and even-chained compounds ranging from C18 to C35 with a peak at C25 or C26 (Fig. 4).

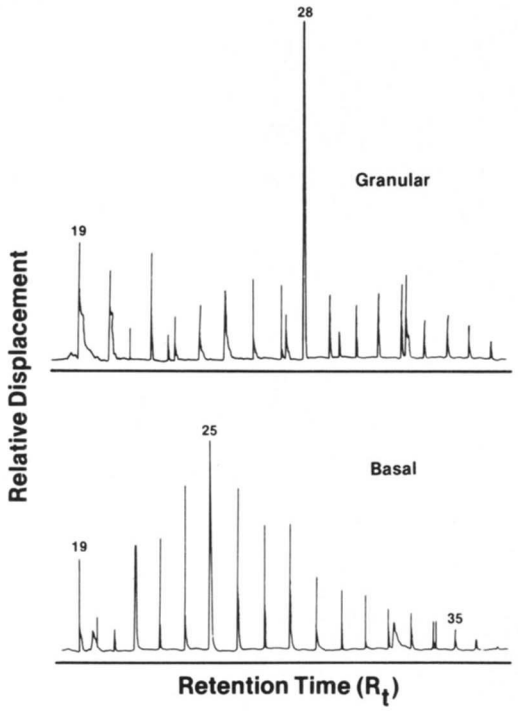

Fig. 4. Glass capillary-GLC fractionation of n-alkanes derived from basal/spinous (SS/SB) and granular (SG) layers from the same individual. Some squalene (C28) remains in the n-alkane fraction after TLC of the SG, but not SB sample. A bell-shaped distribution of n-alkanes ranging from C19 to C35, and consisting of both odd- and even-chained lipids, is present.

Although squalene was usually separated cleanly from the n-alkanes by solvent system #3b, in some samples a small amount of squalene co-migrated with the C28 peak (Fig. 4, mass spectrometry data not shown).

Sphingolipids. The quantities of sphingolipid in the spinous/basal layer were too small for fatty acid analysis. In both the stratum granulosum and the stratum corneum, the sphingolipids demonstrated a greater proportion of the longer-chain fatty acids (C22–C24), and less shorter-chain fatty acids (C12 through C16), than encountered in either the neutral or phospholipids. In the stratum corneum the sphingolipids contained still more long-chain fatty acids than the stratum granulosum, with fully 32% of the weight in C22:0 and C24:0 alone (Table 2). After hydrolysis of the sphingolipids and rechromatography to isolate the fatty acid methyl esters, several bands that migrated in the region of the sphingosine standard were present. Further analysis of these presumed long chain bases was not performed.

Phospholipids. The usual range of fatty acids was present in the phospholipids, except that no C16:1 was found in any epidermal layers (Table 2). Whereas both C16:0 and C18:1 decreased in the stratum granulosum and stratum corneum, C18:2 increased dramatically, and the small amount of phospholipid remaining in stratum corneum demonstrated a large proportion of long-chain (C24:0) fatty acids.

DISCUSSION

Changes in lipid composition during epidermal differentiation (Fig. 8)

This study revealed essentially the same spectrum of lipid species in the lower epidermis that are encountered in other epithelia, where an abundance of phospholipids is required to maintain organelle membrane bilayers (20). The initial observation that epidermal lipids lose phospholipids and generate free sterols during differentiation is now almost 50 years old (5), and has been described repeatedly since that time (6–11). In contrast, appreciation that sphingolipids are present in large quantities in mammalian epidermis is quite recent (8), and since then both ceramides and glycolipids have been identified in pig (8), human (10), rat (21), and neonatal mouse (9, 11) epidermis. Although sphingolipids have been reported to comprise from 15–40% of the total lipid in the stratum corneum in several mammalian systems, information about quantities in humans is more fragmentary. Our studies demonstrate that sphingolipid enrichment occurs in human epidermis, as previously shown in the pig, specifically in the stratum granulosum, and that the sphingolipids are the major repository of long-chain, saturated fatty acids (see also 8, 11) in the stratum corneum. The fact that these lipids are gen-

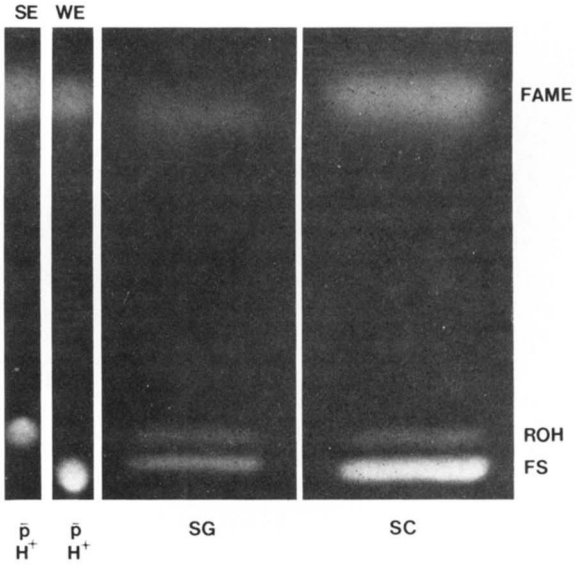

Fig. 5. After acid hydrolysis of the sterol/wax ester fraction, both alcohols and free sterols are generated in the granular (SG) and cornified (SC) layers. The ratio of wax:sterol esters appears to be less in the SC than in the SG despite the presumed greater contribution of pilosebaceous lipids to the SC than to the SG (see text).

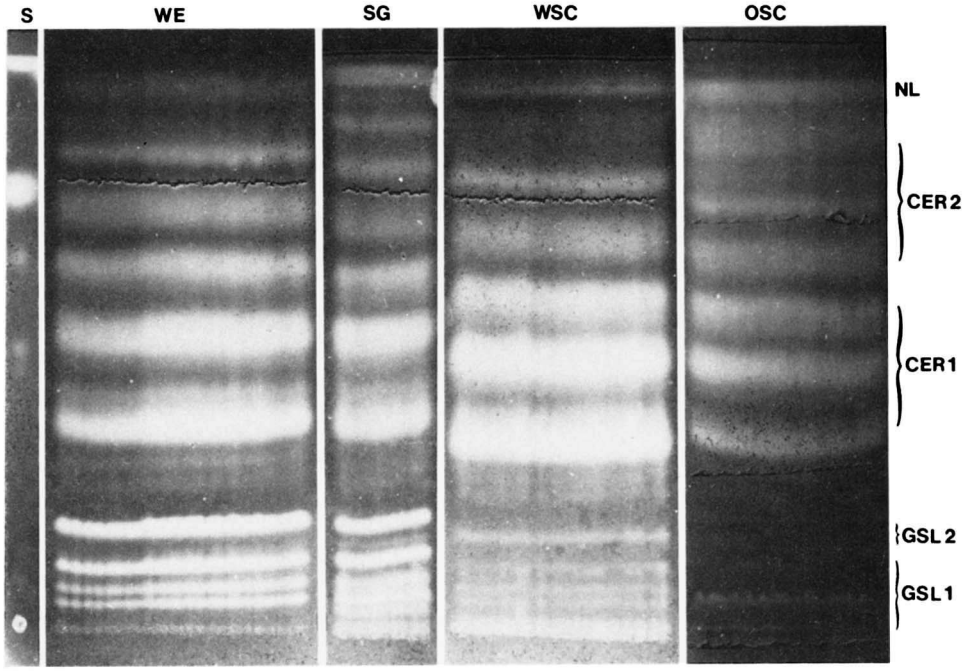

Fig. 6. The sphingolipids are fractionated into four or five orcein-positive glycosphingolipids (GSL I and II) and six orcein-negative ceramide (Cer I and II) species in solvent system #3 (see Methods). Both GSL and Cer are prominent in the SB/SS and the SG, but GSL begin to diminish in the SC, and disappear from the outer stratum corneum (OSC), while Cer persist in the SC.

erated near the skin surface suggests that they may play a central role in stratum corneum function. Stratum corneum lipids are thought to control permeability barrier phenomenon (22–25), and evidence is also accumulating that stratum corneum lipids modulate stratum corneum cohesion and desquamation (26–27).

We have shown here that the proportion of orcein-positive sphingolipids vs. ceramides undergoes fluctuations during differentiation. Although pig basal and spinous layers have been reported to contain substantial sphingolipids (8), we found only small quantities below the granular layer in human epidermis. In contrast, in mouse, pig, and human epidermis (8, 10, 11) both glycolipids and ceramides are present in large quantities in the stratum granulosum. By comparing the composition of stratum granulosum to both whole stratum corneum and to outer stratum corneum (Table 1), it is apparent that the glycolipid:ceramide ratio begins to decrease in the lower stratum corneum, with glycolipids effectively disappearing from the outer stratum corneum (Fig. 5). A comparable depletion of glycolipids also has been described in pig (28) and neonatal mouse (11) epidermis, where it has been suggested that loss of glycolipids could account for several phenomena, including the formation of a more hydrophobic barrier (29) and/or the break-up of intercellular lipid membrane bilayers leading to orderly desquamation (30). However, whether sphingolipids participate specifically in either barrier function or cohesion is still not known.

Although the major modulations in phospholipids

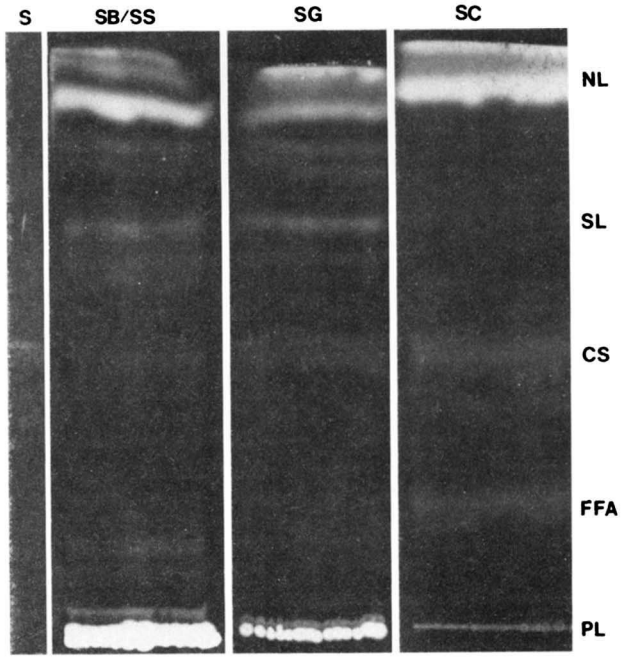

Fig. 7. Cholesterol sulfate (CS) is clearly separated from phospholipids (PL), free fatty acids (FFA), neutral lipids (NL), and glycosphingolipids (SL) in solvent system #1 (see Methods).

TABLE 2. Fluctuations in straight chain fatty acid composition of major epidermal fractions during differentiation[a]

	Neutral Lipids			Sphingolipids		Phospholipids		
	Layer			Layer[b]		Layer		
Fraction	SS/SB	SG	SC	SG	SC	SS/SB	SG	SC[b]
12:0	0.03	0.3	0.1					
14:0	1.9	3.5	2.7	0.7				
16:0	24.1	25.3	24.1	13.1	11.9	25.8	9.4	2.8
16:1	6.7	7.4	7.3	1.8	1.5			
18:0	10.7	16.7	24.7	11.4	8.3	14.1	20.6	14.9
18:1	36.8	31.1	24.1	32.3	28.2	42.1	31.0	20.0
18:2	14.5	14.3	14.7	18.8	15.9	12.3	26.5	20.1
20:0	0.5	0.3	0.3	1.2	2.0		2.1	
20:1				0.4	tr			
20:2	0.5	0.3	0.12					7.5
20:3								
20:4				1.8	tr		3.6	
22:0	0.9	0.4	0.4	2.5	4.7			
22:1								
24:0	3.8	0.7	1.6	6.8	1.6			
26:0				9.3				
Total	100.0	100.0	100.0	99.9	100.0	99.9	100.0	100.0

[a] Mole % of identified FAME. Because of methods (see text), branched and hydroxy acids are not included (14).
[b] Too little material in SS/SB fraction for GLC.
[c] Very little material present for GLC; comparisons may not be meaningful.

and neutral lipids in human epidermis appear to be remarkably similar to other mammalian species (13), several new observations, in addition to the sphingolipid accumulation, deserve comment. Cholesterol sulfate, previously thought to be present only in trace quantities in epidermis (2, 11), is actually present in substantial amounts in all epidermal layers, with the highest levels present in the stratum granulosum (Table 1). The failure of earlier workers to detect cholesterol sulfate in these quantities may reflect the ready loss of this highly polar sterol metabolite into usually discarded aqueous phases (15), or its co-migration in several TLC systems with sphingolipids (11), where it may have been overlooked. Since abnormal cholesterol sulfate degradation is associated with prolonged stratum corneum retention and clinical ichthyosis (15), the generation of cholesterol sulfate, in the quantities described here, may have important implications for stratum corneum function. This highly amphiphatic lipid, which like other stratum corneum lipids, presumably is localized to cell membranes in the stratum corneum (31), may be important for intermolecular cross-linking and cohesion of adjacent corneocytes (32). The fact that steroid sulfatase, the enzyme that catabolizes cholesterol sulfate, parallels cholesterol sulfate localization in epidermis (32) suggests that sterol desulfation may be an important factor in normal stratum corneum desquamation (26).

We also found surprisingly large quantities of squalene in the viable epidermis (Table 1). Although it is generally assumed that all squalene originates from sebaceous glands, it is possible that much of the squalene that we encountered reflects a high level of cholesterol metabolism in epidermis (33). This possibility is supported by the relatively low quantities of wax esters,

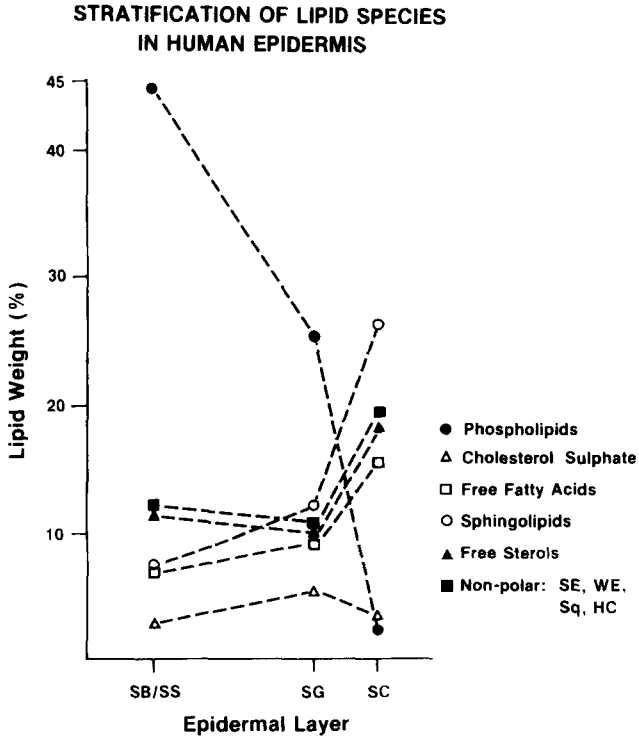

Fig. 8. This line drawing depicts the major fluxes in lipids that occur during epidermal differentiation.

which also are presumed to originate from sebaceous glands (34), that are present in the same levels. Yet, wax esters are present in measurable quantities in both the lower epidermis and stratum granulosum, and are also present in nonsebaceous gland-bearing skin sites, such as the sole, as well (14). These results suggest that squalene, in particular, and wax esters to a lesser extent, though traditionally considered markers of pilosebaceous lipid (34), cannot be assumed to be completely of pilosebaceous origin.

A new observation in this and the companion (14) studies, and confirmed by both co-chromatography and mass spectrometry, is the presence of n-alkanes in substantial quantities in both the stratum corneum and in the viable epidermis (Table 1). When n-alkanes have been previously noted in stratum corneum or skin surface lipids they have been discounted as being either environmental contaminants (35, 36), or of exogenous origin from either the diet or sebaceous glands (34, 37). The studies reported here tend to discount these possibilities. First, the fact that the quantities of n-alkanes are nearly as great in the viable epidermis as in the stratum corneum, and lower in the outer than in whole stratum corneum (Table 1), mitigates strongly against environmental contamination as the sole source of this lipid. Also, the n-alkane composition of human lipid samples does not show the same spectrum as the hydrocarbons found in laboratory air (38). Second, the observation that the n-alkanes comprise a mixed series of odd- and even-chained compounds effectively rules out accumulation of nonmetabolized hydrocarbons of dietary origin, which are predominantly odd-chained (reviewed in 39). Third, although certain topical medications display a similar spectrum of n-alkanes to those found in endogenous lipids (14), our samples possessed n-alkanes in substantial quantities, despite absence of prior topical treatment. Moreover, we have found that the total lipid weight of the initial extract from stratum corneum is a reliable indicator of prior topical treatment,[1] and here the n-alkanes were present regardless of the initial stratum corneum lipid weight present. Finally, in some scaling dermatoses, particularly lamellar ichthyosis, the n-alkane content of stratum corneum is extremely high, approaching that found in insect and plant integument (39). Although active biosynthesis of n-alkanes in epidermis has not been demonstrated, it is possible that hydrocarbons are generated for some specific function(s) in mammalian epidermis.

As reviewed above, phospholipids largely disappear from the stratum corneum. Yet, small quantities do persist into the lower stratum corneum (Table 1). In both the stratum granulosum and in the stratum corneum, the phospholipids reveal a disproportionate amount of long-chain fatty acids (Table 2), suggesting that phospholipid catabolism may also participate in the generation of lipids of importance for the epidermal permeability barrier.

Product-precursor relationships among epidermal lipids

The distinctive sequence of modulations in lipid composition that occurs during epidermal differentiation allows certain inferences to be drawn about lipid metabolic pathways in epidermis. The epidermis possesses broad lipid biosynthetic and catabolic capabilities (2). Yet, to what extent epidermal lipids in general, and stratum corneum lipids in particular, result from degradation or de novo synthesis is not known. Doubtless most of the long-chain free fatty acids derive from catabolism of sphingolipids and phospholipids, but whether the fatty acids persist simply because they are not utilized as an energy source, as one worker has suggested (12), or whether the epidermis generates these long-chain, highly saturated species specifically for its barrier function is still not known. Since sphingomyelin is present in relatively large quantities in the basal layer, might epidermal ceramide metabolism be interrelated with this phospholipid? Similarly, some of the free sterols that are generated in such abundance may derive from degradation of sterol esters, but it is extremely unlikely that catabolism alone can account for a majority of the free sterols. These, and several other unresolved questions about epidermal lipid metabolism, are likely to be resolved when de novo epidermal lipid biosynthesis is studied in the light of the analytical biochemical observations reported here.

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[1] Williams, M. L., and P. M. Elias. Unpublished observations.

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