Composition and Morphology of Epidermal Cyst Lipids

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The contents of epidermal cysts were used as a source of desquamated human keratinocytes uncontaminated by sebaceous, subcutaneous, or bacterial lipids. Lipids extracted with chloroform:methanol mixtures included six series of ceramides (41% of the total extractable lipid), cholesterol (27%), cholesteryl esters (10%), fatty acids (9%), cholesteryl sulfate (1.9%), a novel class of ceramide esters (3.8%), and a sterol diester (0.9%). Electron microscopy revealed that the lipids in the cyst contents existed as multiple intercellular lamellae, as in stratum corneum. One lamella, adjacent to the horny cell protein envelope, was resistant to lipid extraction and is thought to represent covalently bound lipid on the outer surface of the keratinocyte. The results indicate that the degradation of intercellular lipid lamellae is not required for desquamation. *J Invest Dermatol 89:419–425, 1987*

During the course of epidermal differentiation, much lipid is synthesized and packaged into lamellar granules [1,2] and in the terminal stages of differentiation, this lipid is extruded into the extracellular space, where it forms multiple extended bilayer sheets [2,3]. These extracellular lipid bilayers fill the spaces between the cells of the stratum corneum and are thought to provide the barrier to water diffusion through the skin [4,5]. Under normal conditions, keratinized cells and barrier lipids are constantly being added to the stratum granulosum/stratum corneum interface at a rate that balances the loss of these materials by desquamation at the skin surface. In this manner, the stratum corneum is maintained at a relatively constant thickness.

Attempts to explain the normally orderly desquamation of corneocytes have often invoked the idea that exfoliation results from disruption of the intercellular lipid bilayers [6]. This view was supported by several observations. First, electron microscopic examination of the stratum corneum by previous investigators [7] revealed intact intercellular lamellae primarily in the lower portion of the horny layer. Second, in the genetic disease recessive X-linked ichthyosis, rafts of cells remain firmly adherent [8]. The metabolic defect underlying this condition is the absence of sterol sulfatase activity [9], and accordingly, an elevated proportion of cholesteryl sulfate is present in this ichthyotic scale [8]. The persistence of this highly polar lipid could preclude disruption of the lamellar phase. Finally, it has been demonstrated that hydrolysis of cholesteryl sulfate does accompany normal desquamation, whereas the other epidermal lipids appear to survive exfoliation intact [10,11].

From this evidence, it appeared that the intercellular lipids, possibly due to the loss of cholesteryl sulfate, are unable to maintain a lamellar phase in the outer stratum corneum. Recent studies in vitro, however, have shown that isolated stratum corneum lipids can form bilayer structures in the absence of cholesteryl sulfate [12]. Furthermore, improved methods of sample preparation have lead to the recent demonstration that the intercellular lamellae are, in fact, present in the outer layers of the stratum corneum [13], and not only in the lower regions. This evidence would suggest that degradation of the intercellular bilayers of the stratum corneum may not be a prerequisite for desquamation. To investigate this question, desquamated material from epidermal cysts was examined to determine whether intercellular bilayers persist under these circumstances. The epithelium forming the wall of an epidermal cyst appears to be identical to normal surface epidermis as judged by histology, histochemistry, and electron microscopy [14,15]. Horny material is shed from the surface of the stratum corneum of the cyst wall and accumulates to form the cyst contents, which are arranged in laminated layers and consist of markedly flattened and elongated horny cells and intercellular material [14,15]. Desmosomes have been said to be absent from the cyst contents [15]. In addition to providing a source of desquamated human stratum corneum material, epidermal cysts provide a unique source of stratum corneum lipids uncontaminated by sebum, subcutaneous fat, bacterial products, or lipids from the environment. We have, therefore, examined both the chemical composition and physical state of the lipids from the contents of epidermal cysts.

MATERIALS AND METHODS

Collection of Cyst Contents Cysts were removed using standard surgical techniques. After excision, each intact cyst was wiped free of blood and subcutaneous fat, the capsule was slit, and the contents were squeezed into a glass vial. The capsules were submitted for routine histologic examination, which provided evidence as to whether these were typical epidermal cysts without inflammation. Two samples of the fresh cyst contents were randomly selected from each specimen for examination by electron microscopy. Cyst contents were not included in the present study

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Abbreviations:

GLC: gas–liquid chromatography

TLC: thin-layer chromatography

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if the presence of bacteria was indicated either by histology or by electron microscopy. Those found to be acceptable included one cyst from each of 5 subjects and two cysts from one subject, obtained from the face, back, neck, scrotum, and postauricular region.

Extraction of Lipids The remaining contents of each cyst were dried in a lyophilizer, weighed, and then subjected to successive extractions with mixtures of chloroform and methanol (2:1, 1:1, and 1:2, v:v). The contents of each cyst were stirred with each solvent mixture for 2 h at room temperature, and the three extracts were combined, filtered, and evaporated to dryness under a stream of nitrogen to recover the lipid. The last traces of solvent were removed from the lipid samples under vacuum, after which the samples, which ranged from 2–13 mg, were weighed and redissolved in chloroform-methanol, 2:1, at a concentration of 10 mg/ml.

Lipid Analysis The lipids from each cyst were analyzed by quantitative thin-layer chromatography (TLC) as described previously for polar [16] and nonpolar cutaneous lipids [16,17]. The developed chromatograms were charred by spraying with 50% sulfuric acid and heating slowly to 220°C.

After cooling, the chromatograms were scanned with a photodensitometer (Shimadzu CS-930) for quantitation. Preliminary examination of the lipid profiles of the two cysts removed from one patient revealed no differences, so these two extracts were combined.

For examination of the identity and composition of the individual lipid classes, the remainder of the cyst extracts were combined and fractionated by preparative thin-layer chromatography as described previously [16,18].

The cholesteryl esters and sterol diesters were saponified by treatment with 1 M sodium hydroxide in 90% methanol at 60°C for 1 h. After adjusting the pH to approximately 4 with 2 M HCl, the products of the saponification were extracted into chloroform.

Each of the five isolated ceramide fractions was chromatographed on a thin layer of silicic acid impregnated with sodium arsenite to detect phytosphingosine-containing structures [19,20]. Preparative TLC on this adsorbent also permitted the separation of two components from the fifth ceramide fraction. Each of the resulting six ceramides was then subjected to a mild saponification to liberate any ester-linked fatty acids. Those ceramides that contained phytosphingosines were oxidized with periodate to produce aldehydes from the long chain base moiety [20]. These aldehydes were further oxidized to fatty acids as previously described [20], and the fatty acids were converted to methyl esters and analyzed by gas–liquid chromatography (GLC). The residual amide linkages were cleaved by treatment with 1 M HCl in methanol containing 20 M water at 65°C for 18 h [20,21]. The liberated sphingosines were isolated by preparative TLC, oxidized to aldehydes with sodium periodate, and the aldehydes were analyzed by GLC [22]. The fatty acids and hydroxyacids were converted to methyl esters by treatment with 10% boron trichloride in methanol (Sigma Chemical Co., St. Louis, Missouri) at 60°C for 1 h. The hydroxyacid methyl esters were acetylated by treatment with a 1:1 mixture of acetic anhydride:pyridine for 2 h at room temperature. This procedure was also used to convert sterols into sterol acetates prior to analysis by GLC. Excess reagent was evaporated under a gentle stream of nitrogen.

The normal and α-O-acetyl fatty acid methyl esters were analyzed by GLC on a 50-m quartz capillary column with a bonded stationary phase of CP SIL 88 (Chrompak, Inc., Bridgewater, New Jersey). For the normal fatty acid methyl esters, a temperature program was used with an initial temperature of 160°C for 5 min followed by a 5°C per min increase, until a final temperature of 220°C was attained. Standards for the fatty acid analyses included a series of saturated fatty acid methyl esters ranging from 14–24 carbons (kit KF, Applied Science, State College, Pennsylvania) and a mixture including methyl palmitoleate, methyl oleate, and methyl linoleate (kit CE1-62, NuCheck Prep, Elysian, Minnesota). The α-O-acetyl fatty acid methyl esters were chromatographed isothermally at 220°C, and standards from wool wax [23] and from previously characterized pig epidermal ceramides [20] were used. The aldehydes produced from the sphingosines were also chromatographed isothermally at 210°C on the CP SIL 88 column. Sterol acetates and ω-O-acetyl fatty acid methyl esters were chromatographed isothermally at 300°C on a 50-m quartz capillary column with a bonded phase of BP-1 (Scientific Glass Engineering, Austin, Texas). Previously characterized ω-O-acetyl fatty acid methyl esters from pig epidermal lipids [20] and from carnauba wax [24] served as standards. The O-acetyl fatty acid methyl esters were examined by argentation TLC to determine if unsaturated species were present prior to analysis by GLC.

On saponification, one minor lipid fraction yielded free fatty acids and a series of components with TLC mobilities like those of the epidermal ceramides 2–6. The freed fatty acids were isolated from the saponification products, converted to methyl esters and analyzed by GLC as described above. The combined ceramides resulting from saponification were subjected to a more vigorous acid hydrolysis, and the products were examined by TLC, which indicated the presence of both hydroxy and nonhydroxy acids and sphingosine bases.

Electron Microscopy Samples of the fresh cyst contents were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 and postfixed in 0.2% RuO_4 in cacodylate buffer [25] for efficient visibility of the highly saturated stratum corneum lipid bilayers. The tissues were then dried in a graded acetone series and embedded in Spurr's low viscosity epoxy resin. Ultrathin sections were cut and stained with uranyl acetate and lead citrate before examination in a Hitachi Model H-600 electron microscope.

RESULTS

Electron microscopy of the sections of the cyst contents showed multiple lipid bilayers that were fully preserved in the intercellular spaces between the exfoliated corneocytes. A representative electron micrograph is shown in Fig 1. Similar examination of the tissue recovered after solvent extraction of the lipids showed that the intercellular lamellae had been completely removed, but a lucent band on the outside of the electron-dense horny cell protein envelope remained intact (Fig 2).

The composition of the extractable lipid from each cyst is presented in Table I. More than 40% of the total lipid consists of ceramides of several chromatographically distinct types. Other major cyst lipids include cholesterol, cholesteryl esters and fatty acids. Minor cyst lipids include cholesteryl sulfate and two previously unidentified lipids—sterol diesters and ceramide esters.

The ceramides contain sphingosine or phytosphingosine with amide-linked normal, α-hydroxy or ω-hydroxy acids (Table II). Ceramide 1 has an ester-linked fatty acid, 21% of which is linoleic acid, and ceramide 6I bears an ester-linked α-hydroxy acid (Table III). The major sphingosine bases are the 18- and 20-carbon species, whereas the major phytosphingosines contain 18 through 22 carbons (Table IV). For each of the amide-linked normal or α-hydroxy acid-containing ceramides, the major fatty acid species are 24:0 and 26:0 (Table V). The principal amide-linked ω-hydroxy acids from ceramide 1 are 30:0 and 32:0.

A minor lipid class present in the cyst was identified as a series of ceramide esters. As judged by comparison on TLC, these sphingolipids are similar to ceramides 2–6 except that they have an additional fatty acid present in ester linkage. The ester-linked fatty acids were isolated, and the composition is presented in Table III. The principal ester-linked fatty acids in this lipid group are 16:0, 18:0, 18:1, and 24:0. In contrast, the major ester-linked fatty acid in ceramide 1 is linoleic acid, which comprises 20.7% of the total (Table III).

A second minor lipid from the cyst was identified as a sterol diester. Saponification of this lipid yielded fatty acid, α-hydroxy acid and sterol, and the molar proportions of these products were

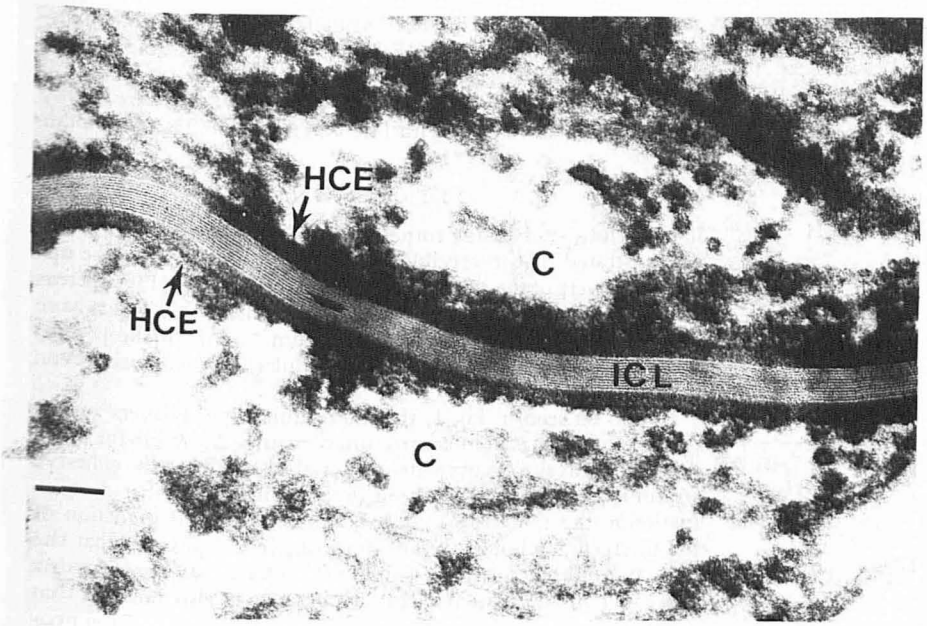

Figure 1. Transmission electron micrograph of epidermal cyst contents (×160,000). The samples were fixed with glutaraldehyde, postfixed with ruthenium tetroxide, and embedded in Spurr's resin before ultrathin sections were cut. The sections were stained with uranyl acetate and lead citrate prior to examination. C, corneocytes; HCE, horny cell envelopes (*arrows*); ICL, intercellular lamellae between a pair of corneocytes. *Bar* = 80 nm.

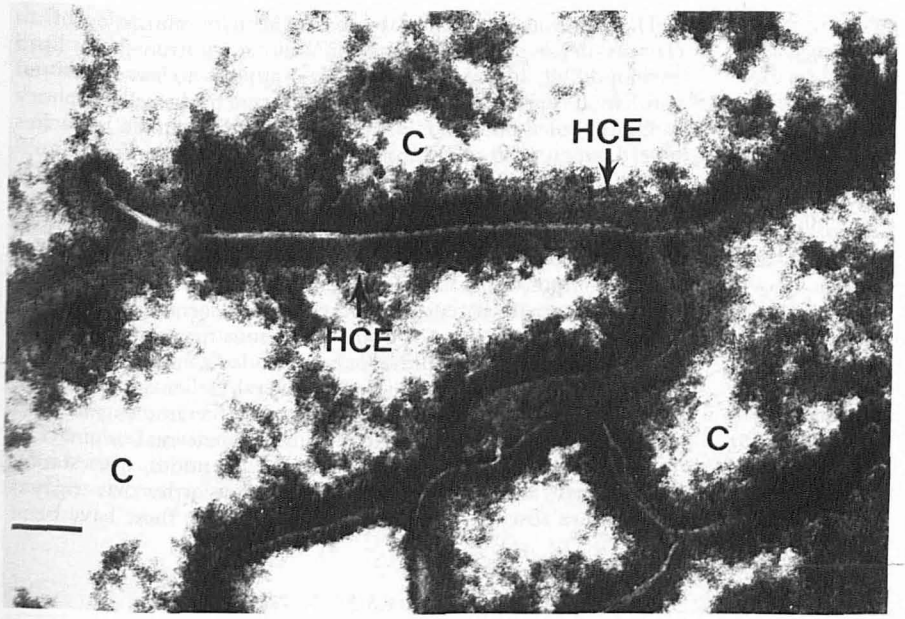

Figure 2. Transmission electron micrograph of epidermal cyst contents after lipid extraction (×160,000). Lipids were extracted from the cyst contents with chloroform:methanol mixtures, and samples were prepared for electron microscopy as indicated in *Fig 1*. As in *Fig 1*, C, corneocytes; HCE, horny cell envelopes (*arrow*). *Bar* = 80 nm.

Table I. Class Composition (wt. %) of Lipids Extracted From Epidermal Cysts[a]

	1	2	3	4	5	6	Mean	SD
Sterol ester	14.5	5.7	7.8	12.0	7.2	12.9	10.0	3.3
Sterol diester	1.1	0.1	0.9	0.9	0.4	1.9	0.9	0.6
Fatty acids	10.7	6.2	5.9	14.6	6.6	10.3	9.1	3.1
Cholesterol	23.3	31.3	34.9	22.1	28.0	22.0	26.9	4.9
Ceramide ester	3.7	4.3	3.0	5.0	3.9	3.1	3.8	0.7
Ceramide 1	4.8	2.2	1.4	1.9	4.3	4.4	3.2	1.4
Ceramide 2	12.1	10.1	7.9	4.9	11.4	7.2	8.9	2.5
Ceramide 3	4.7	6.5	5.1	4.7	3.9	4.2	4.9	0.8
Ceramide 4/5	10.3	13.9	11.8	14.1	10.1	10.4	11.8	1.7
Ceramide 6I	0.7	1.0	1.3	1.2	3.1	1.4	1.5	0.8
Ceramide 6II	6.0	9.9	11.6	9.3	14.4	13.3	10.8	2.8
Cholesteryl sulfate	2.9	2.3	2.6	2.0	0.9	0.6	1.9	0.9
Others	5.2	6.3	5.8	7.2	5.8	8.3	6.4	1.0

[a]In order of decreasing thin-layer chromatography mobility.

Table II. Summary of the Structures of the Ceramides From Epidermal Cysts

Ceramide	Amide-Linked FA	Bases	Ester-Linked FA
1	ω-OH	Sph	Nonhydroxy
2	Nonhydroxy	Sph	—
3[a]	Nonhydroxy	Phyto	—
4/5	α-OH	Sph	—
6I	α-OH	Phyto	α-OH
6II	α-OH	Phyto	—
Ceramide esters	— mixture	—	Nonhydroxy

[a]Sometimes contains small amount of ω-hydroxyacylsphingosine.
FA, fatty acid; Sph, sphingosine; Phyto, phytosphingosine.

Table III. Compositions (wt. %) of the Ester-Linked Fatty Acids From Ceramides of Epidermal Cysts

Chain Structure	Ceramide 1 (non-OH)	Ceramide 6I (α-OH)	Ceramide Ester (non-OH)
14:0	2.0	—	1.0
14:1	2.3	—	—
15:0	0.1	—	0.6
16:0	18.0	30.2	17.2
16:1	4.8	—	1.3
17:0	1.2	—	0.6
18:0	9.1	4.8	20.3
18:1	11.6	—	12.5
18:2	20.7	—	0.8
19:0	0.1	—	0.2
20:0	3.5	3.6	6.3
21:0	0.2	3.1	1.0
22:0	4.8	3.3	6.7
23:0	—	0.1	0.6
24:0	8.4	20.2	16.0
25:0	1.8	6.3	2.2
26:0	4.1	18.5	3.9
27:0	0.1	—	0.2
28:0	2.1	—	3.5
Others	5.1	9.9	5.1

estimated to be 1.0:1.2:1.1. On TLC, the sterol comigrated with authentic cholesterol and produced the same red–violet color on heating with sulfuric acid before charring. The acetylated sterol also comigrated with authentic cholesteryl acetate on GLC. The composition of the fatty acids and α-hydroxy acids from the sterol diester are presented in Table VI. In each case, the major species is 16:0.

The composition of the fatty acids from the free acid, cholesteryl ester, and sterol diester fractions are given in Table VI. The major free fatty acids are 22:0 and 24:0, which account for 42.1% of the total. The fatty acids from the cholesteryl esters are mainly of medium chain-length, with 18:1 making up 68.2% of the total.

DISCUSSION

In a previous study using ruthenium tetroxide postfixation, it was demonstrated that intercellular lipid bilayers persist into the uppermost layers of the stratum corneum of mouse skin [13], whereas with osmuim tetroxide fixation, only fragments of bilayers have been seen in the outer stratum corneum [7,26]. In the present study, it was observed that the intercellular bilayers persist, even after desquamation.

As can be seen in Fig 1, the intercellular lipid bilayers appear to be as uniform as in intact stratum corneum. Although the plane at which cleavage occurs when material is shed from the cohesive stratum corneum has not been defined, it is clear that desquamation in the epidermal cyst does not depend on degradation of the intercellular lipid bilayers. It is, however, possible that the reduction in the content of cholesteryl sulfate [10,11] results in a loosening of interlamellar cohesiveness. It is also possible that proteolytic activity contributes to the overall desquamation process, and this possibility is supported by the observation that desmosomes are found in the stratum corneum but are reported to be absent from the cyst contents [15].

The compound envelope of the corneocyte, consisting of an electron-dense protein envelope [27] and an electron-lucent lipid envelope [28], like the lipid lamellae, appears to have survived exfoliation (Fig 2), although limited damage to the cell periphery cannot be ruled out. The lipid envelope has been noted by earlier investigators, who called it the horny cell "plasma membrane;" [29] however, its persistence after extraction of the intercellular lipids, combined with recent chemical evidence [30], has lead to the conclusion that this lucent band is actually a covalently bound layer of lipid.

The composition of the lipids extracted from epidermal cysts can be presumed to represent pure stratum corneum lipids, uncontaminated by sebum or other extraneous materials. Contamination with sebum has previously precluded a complete analysis of stratum corneum lipids, so that several earlier investigations [10,11] were limited to examination of the ceramides and cholesteryl sulfate, which are known to be of epidermal origin. The major lipids found in the cyst included ceramides, cholesterol, sterol esters, and free fatty acids. It is noteworthy that triglycerides were absent from the cyst contents since these have been

Table IV. Compositions (wt. %) of the Long-Chain Bases From Epidermal Cyst Ceramides

Carbon Chain	Ceramide 1 Sphingosine	Ceramide 2 Sphingosine	Ceramide 3 Phytosphingosine	Ceramide 4/5 Sphingosine	Ceramide 6I Phytosphingosine	Ceramide 6II Phytosphingosine
16:1	2.4	0.7	—	7.1	—	—
16:0	0.1	0.6	3.1	1.8	5.7	2.6
17:1	11.9	5.7	—	4.7	—	—
17:0	5.4	2.1	8.0	2.4	6.1	6.5
18:1	31.5	35.6	—	31.4	—	—
18:0	4.4	11.7	12.8	11.4	20.7	15.0
19:1	4.4	4.6	—	6.3	—	—
19:0	0.1	1.6	16.0	1.8	24.4	12.7
20:1	11.5	20.7	—	15.2	—	—
20:0	5.4	6.5	14.4	5.5	13.9	16.6
21:1	9.7	2.3	—	3.4	—	—
21:0	3.3	1.5	10.1	1.7	8.9	13.1
22:1	6.7	5.0	—	5.4	—	—
22:0	3.2	1.5	21.4	2.1	10.0	20.3
23:0	—	—	2.1	—	1.6	1.8
24:0	—	—	5.1	—	1.7	5.3
25:0	—	—	2.1	—	2.1	2.1
26:0	—	—	4.9	—	4.7	3.9

Table V. Compositions (wt. %) of the Amide Linked Fatty Acids From Epidermal Cyst Ceramides

Chain Structure	ω-Hydroxy Acid Ceramide 1	Nonhydroxy Acid Ceramide 2	Nonhydroxy Acid Ceramide 3	α-Hydroxy Acid Ceramide 4/5	α-Hydroxy Acid Ceramide 6I	α-Hydroxy Acid Ceramide 6II
14:0		0.2	0.3			
15:0		0.3	0.5			
16:0		3.6	4.9	7.1	1.7	3.5
17:0		0.4	0.7	1.0	1.4	0.1
18:0		4.4	5.1	4.0	9.9	0.6
19:0		1.0	1.3	1.4	1.7	0.1
20:0		3.8	3.9	1.6	1.6	1.7
21:0		1.2	1.0	0.1	1.4	0.1
22:0		8.7	7.5	3.1	1.7	3.9
23:0		5.7	4.8	3.5	1.3	4.5
24:0	3.2	30.4	26.6	37.1	27.9	47.4
25:0	1.0	7.8	7.7	12.0	11.3	13.2
26:0	2.1	18.3	20.1	25.1	34.8	22.7
27:0	1.3	2.8	3.4	1.9	2.9	1.1
28:0	2.8	8.3	9.4	2.0	2.7	1.1
29:0	4.5	1.2	1.2			
30:0	58.7	1.9	1.6			
31:0	9.6					
32:0	16.7					

reported to represent as much as 25% of the total stratum corneum lipid [31]. Although we cannot entirely rule out the possibility that triglycerides may have been hydrolyzed by an epidermal lipase in the cyst, it seems highly probable that all previous attempts to analyze stratum corneum lipids were complicated by the presence of triglycerides from sources other than epidermis. Sebum is a ubiquitous source of triglycerides, as well as other lipids, and any small epidermal samples that are prepared from excised skin will be contaminated by subcutaneous fat. It is suggested that the triglycerides reported to exist in normal human stratum corneum are largely, if not entirely, contaminants.

These various sources of contamination present severe obstacles for the investigation of human epidermal or stratum corneum lipid compositions, and if ignored, may lead to erroneous or unwarranted conclusions. For instance, one recent report [31] claimed to present the first detailed quantitative analysis of human stratum corneum lipids and to demonstrate anatomic variation in this composition. The compositions reported, however, included squalene and wax esters, which are of sebaceous origin, and paraffin hydrocarbons, which are probably environmental. Triglycerides, which may arise from sebum or from subcutaneous fat as noted above, were reported to be one of the major and most variable stratum corneum lipid classes [31]. Although anatomical variation in epidermal lipid composition may actually exist, the claims made in the single report on this subject must be viewed with some reservation.

Two of the minor components of the epidermal cyst lipids were not previously known and would have been obscured in sebum-contaminated samples. Of these newly identified lipids, the sterol diesters consist of a normal fatty acid esterified to the hydroxyl group of an α-hydroxy acid, which in turn is esterified to cholesterol. This unusual lipid has a mobility on TLC similar to triglycerides, with which it may have been confused in earlier studies. Also present in the cyst lipids are ceramide esters, which are less polar than ceramide 1 and have an ester-linked fatty acid attached to one hydroxyl group of the long-chain bases. It is not anticipated that these lipids will prove to be unique to cysts. In fact, preliminary evidence has already been obtained indicating the presence of both ceramide esters and sterol diesters in normal pig stratum corneum (data not shown).

Earlier reports indicated that the free fatty acids represent 25% of the stratum corneum lipids and consist mainly of 16:0, 18:0,

Table VI. Compositions (wt. %) of the Fatty Acids From Nonpolar Lipids of Epidermal Cysts

			Sterol Diesters	
Chain Structure	Free Fatty Acids	Cholesteryl Esters	Nonhydroxy acids	α-Hydroxy acids
14:0	0.8	—	3.9	2.6
15:0	0.7	—	2.6	9.8
16:0	7.4	9.9	43.6	62.5
16:1	0.7	3.0	—	—
17:0	0.8	1.1	1.9	0.9
18:0	9.1	4.6	19.3	3.0
18:1	5.7	68.2	9.5	—
18:2	1.4	—	—	—
19:0	1.1	—	2.1	2.6
20:0	5.9	6.6	2.7	2.6
21:0	1.9	1.1	—	1.8
22:0	15.3	1.3	9.0	10.4
23:0	6.2	1.0	—	1.4
24:0	26.9	1.4	1.8	2.5
25:0	5.0	1.1	—	—
26:0	8.5	1.1	1.7	—
28:0	2.7	—	2.0	—

18:1, and 20:0 [1]. In the present work, the fatty acids have been found to constitute only 10.9% of the cyst lipid and the principal components are 22:0 and 24:0, which together account for more than 40% of the total. A similar fatty acid content and composition has recently been found for pig stratum corneum (data not shown). In one recent report on human stratum corneum lipids, a fatty acid composition similar to that given in Table VI was included [32]. Presumably, the earlier results were confounded by contamination with fatty acids derived from sebaceous triglycerides or from subcutaneous fat introduced during excision of the skin.

The structural details of the ceramides reported in the present work are all in accord with the previously reported structures for ceramides of human stratum corneum [10,18]. Nevertheless, it is noteworthy that ceramide 1 contains saturated ω-hydroxy acids only. This is in contrast with the analogous lipid isolated from full thickness pig epidermis, which contains an appreciable proportion of unsaturated ω-hydroxy acids [20]. It also contrasts with the work of Bowser and associates [33], who found only monoenoic ω-hydroxy acids in the acylceramide from pig stratum corneum. More work is needed to determine differences in ω-hydroxy acid composition between species or during differentiation.

The proportion of cholesteryl sulfate relative to the ceramides in the cyst lipids is similar to that found in the lipids of the desquamated material from cast liners [10]. The ratio of cholesteryl sulfate to ceramides previously reported for intact human stratum corneum was 1:7, whereas the ratio for desquamated stratum corneum lipids was 1:27 [10]. The ratio of cholesteryl sulfate to ceramides calculated from the data in Table I is 1:22.

Although only a minor component of the cyst lipids, cholesteryl sulfate is of considerable interest. The inability to hydrolyze cholesteryl sulfate appears to underlie the aberrant desquamation in recessive X-linked ichthyosis [8]. Its hydrolysis has been the only detected change in lipid composition accompanying normal desquamation [10,11]. Furthermore, topical application of cholesteryl sulfate on the skin of hairless mice induces scaling [34]. These observations have strongly suggested a role for cholesteryl sulfate in stratum corneum cohesion. It has been suggested that this highly polar lipid may be necessary to maintain the lamellar phase of the stratum corneum lipids, and that its gradual hydrolysis may lead to loss of bilayer structure and desquamation [6,8]. The present results, however, show unambiguously that the intercellular lamellae are not lost as an antecedent to desquamation in the epidermal inclusion cyst. Perhaps cholesteryl sulfate functions in providing strong bonding between lipid bilayers, possibly by forming bridges through divalent cations such as calcium or magnesium. The hydrolytic cleavage of such bridges could reduce interlamellar cohesion without disrupting bilayer structure.

In conclusion, the desquamated material in epidermal cysts has been shown to include ceramides, cholesterol, sterol esters, free fatty acids, and cholesteryl sulfate. In addition, ceramide esters and sterol diesters have been identified as minor epidermal lipid components. The lipid in the cyst contents is arranged in multiple extracellular bilayers like those found in the cohesive stratum corneum. These observations demonstrate that breakdown of bilayer structure is not required for desquamation. We suggest that the epidermal inclusion cyst is a valid and useful model for studies of stratum corneum lipids and desquamation.

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