Lipid compositions of cells isolated from pig, human, and rat epidermis

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ABSTRACT Epidermal slices from pig, human, and rat skin were treated with dilute buffered trypsin solution (0.005%, w/v), and suspensions of mixed basal and spinous cells were obtained in good yield. Total lipids accounted for approximately 8% of the pig, 10% of the human, and 20% of the rat epidermal cell (dry weight). Phospholipids in pig, human, and rat cells accounted for, respectively, 62%, 53%, and 35% of the total lipids. Phosphatidylcholine (34–38%), phosphatidylethanolamine (18–23%), and sphingomyelin (17–21%) were major compounds in all species. The major neutral lipids were sterols (mostly cholesterol) and triglycerides. Free fatty acids were a major lipid class in pig and human cells, whereas wax esters were a major component in rat epidermal cells. Nearly half (45%) of the sterols in rat cells but less than 10% of those in pig and human cells were esterified. Cholest-7-ene-3β-ol accounted for 20% of the total sterols in rat cells. Cholesteryl sulfate and ceramide were minor lipids in the three species. The predominant glycosphingolipid (>99%) was glucosylceramide, which accounted for 7% and 9%, respectively, of the total lipids in pig and human cells. A significant proportion (pig, 17%; human, 11%) of the fatty acids in the glucosylceramides were $C_{26:0}$ and $C_{28:0}$.

Supplementary key words basal and spinous cells · phosphatidylserine · glucosylceramide · sterol esters

In the course of our studies on the lipid composition of subcellular membrane systems of the epidermal cell, we required, for comparative purposes, quantitative data on the lipid composition of the whole cell. Published data on lipids of the epidermal cell as distinct from those of the whole epidermis (1–3) or the skin surface (4, 5) are meager and mainly semiquantitative (6, 7). In this paper we present new quantitative data on the lipid compositions of cells from pig, human, and rat epidermis.

METHODS

Analytical methods

Phosphorus was determined by the method of Long and Yardley (8), plasmalogens by the method of Gray (9), and ceramide (N-acyl sphingosine) by estimation of long-chain base as described by Kisic and Rapport (10). Cholesterol, cholest-7-ene-3β-ol (lathosterol), and dihydrocholesterol were identified and estimated by GLC on 3% OV-1 and 3% OV-17 (support, Gas-Chrom Q, 80–100 mesh; Applied Science Laboratories, State College, Pa.) at 240°C with cholesteryl acetate as an internal standard. Detector (flame ionization) response was checked for linearity with various ratios of sterols and cholesteryl acetate. Total sterols (free sterols plus sterol esters) were determined in the same way after hydrolysis of the esters in chloroform–methanol–water 9:36:5 (by vol) containing 0.1 M sodium hydroxide for 18 hr at 37°C. Cholesteryl sulfate was determined by gas–liquid chromatographic estimation of cholesterol obtained after hydrolysis in 1.0 M HCl at 100°C for 1 hr. The release of cholesterol was quantitative under the conditions of hydrolysis.

The quantitative estimation of the carbohydrate portions of glycosphingolipids was made by GLC with mannitol as an internal standard as described previously (11). The compositions of the fatty acid components of phospholipids and glycosphingolipids were determined by GLC (11).

Preparation of epidermal cells

From pig epidermis. The skin from pig tails was obtained within 1 hr after death. The tails were washed with cetrimide (cetyltrimethylammonium bromide, 5% w/v aqueous solution) and water, and the skin was removed and spread flat over a damp cellulose sponge sheet (about 0.5 cm thick). The hairs were removed with clippers, and the skin was wiped thoroughly with cotton wool soaked in acetone to remove surface lipid. Epidermal cells were obtained essentially by the method of Briggaman et al. (12); slices were taken from the skin with a Castroviejo keratotome (0.2-mm cut) (13) and were floated, dermis side down, in a solution of trypsin (5 mg/100 ml; Sigma Chemical Co., type III) in PBS (137 mM NaCl, 2.68 mM KCl,

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Abbreviations: GLC, gas–liquid chromatography; PBS, phosphate-buffered saline; TLC, thin-layer chromatography.

8.10 mM NaHPO$_4$, 1.47 mM KH$_2$PO$_4$) at pH 7.4 for 1.5–2 hr at 37°C. The epidermis was peeled from the dermis, shaken in PBS (containing either bovine serum albumin, 10% w/v, or a trypsin inhibitor, Sigma type II-0) to disperse the cells, and filtered through nylon bolting cloth with a hole size of 0.02 × 0.02 mm (Nybolt 25TII, Schweiz, Seidengazefabrik, A.G., Zurich, Switzerland). The filtrate was centrifuged at 2000 rpm (about 800 g) for 10 min, and the supernate was discarded. The cells were resuspended in PBS and centrifuged again at 2000 rpm for 10 min. This washing procedure was repeated until the cells were free from debris as judged by phase-contrast microscopy.

From human epidermis. Skin was obtained from leg and occasionally from breast. Surface lipids were removed with cotton wool soaked in acetone, and epidermal cells were obtained as for pig epidermis. It was possible to obtain cells in good yield up to 24 hr after removal of skin from the patient provided it was kept at 4°C.

From rat epidermis. Whole skins were removed from adult rats (hooded strain) immediately after death, and hair was removed with clippers. Surface lipids were removed and cells were obtained as for pig epidermis.

Extraction of lipids

Total lipids were extracted from suspensions of epidermal cells in PBS (0.5 ml of packed cells/ml) by adding 10 vol of chloroform–methanol 1:1 (v/v). The cells were shaken vigorously, allowed to stand for 0.5–1 hr, and then centrifuged. The cell residue was reextracted twice with 5 vol of chloroform–methanol 2:1 (v/v). The extracts were combined and washed once with 0.2 vol of 0.1 M KCl and twice with 0.2 vol of upper phase solvent (14) containing KCl. The upper phases were combined, and gangliosides were recovered from the total upper phase as described by Siddiqui and Hakomori (15). The lower chloroform phase was evaporated in a rotary film evaporator to dryness below 40°C, under vacuum; the lipids were weighed and then redissolved in dry chloroform and stored at −20°C.

Lipids were also recovered, by extraction with chloroform–methanol solutions, from the PBS from which the cells were isolated, including the cell washings, and from material recovered from the nylon filter, after removing the epidermal residue, by backwashing with PBS.

To allow for the possibility that the lipid composition of the cells might be altered, for example, by released lipolytic enzymes in the course of the isolation procedure and extraction of cell lipids, the following experiments were carried out. Lipids were extracted (*a*) from cells immediately after their isolation following trypsin treatment of epidermis; (*b*) from cells suspended in PBS and kept overnight at 4°C; (*c*) from cells that were freeze-dried immediately after they were isolated; (*d*) from freeze-dried cells that were reconstituted for 1 hr in PBS prior to lipid extraction;

and (*e*) from fresh whole epidermal slices (0.2 mm thick) taken with a keratotome.

Chromatography

The epidermal cell lipids were separated as described by Gray (16). The lipid extract was loaded onto a column of silica gel H (Merck, Darmstadt) in chloroform–methanol 49:1 (by vol). Lipid loading per gram of silica gel H was equivalent to a phosphorus content of not more than 0.5 mg. Neutral lipids (group *a*) were eluted from the column with chloroform–methanol 49:1; glycosphingolipids, acidic phospholipids, and phosphatidylethanolamine (group *b*) were eluted with tetrahydrofuran–methylal–methanol–water 10:6:4:1; and phosphatidylcholine and sphingomyelin (group *c*) were eluted with chloroform–methanol 1:4.

Glycosphingolipids and phospholipids in groups *b* and *c* were separated and identified by two-dimensional chromatography on thin-layer plates of silica gel H (17) developed in chloroform–methanol–water 65:25:4 in the first direction and tetrahydrofuran–methylal–methanol–4 M aqueous ammonia 10:6:4:1 in the second direction. Lipids were made visible by spraying the plate with 50% H$_2$SO$_4$ (v/v) and heating at 160–180°C for 20 min. Individual compounds were identified by cochromatography with known lipids. For further confirmation of identities, the phospholipids were separated by TLC, and each compound, made visible with iodine vapor or, if fatty acid analyses were to be done, rhodamine 6G (18), was recovered from the silica gel H by extracting with chloroform–methanol–water 10:10:1. The phospholipid was deacylated with methanolic NaOH (19), and the water-soluble phosphate ester was identified by paper chromatography (20). The fatty acids from each phospholipid were recovered after the deacylation and they were esterified by reaction with 0.8 M methanolic HCl at 80°C for 18 hr in a sealed tube. The methyl esters were analyzed by GLC. Individual phospholipids were separated by two-dimensional TLC, and the spots were visualized by charring with H$_2$SO$_4$ (see above). The areas of silica gel H containing the charred compounds were transferred to Pyrex tubes and phospholipids were quantitatively estimated by phosphorus analysis (8).

Glycosphingolipids were separated and identified by reference to standard compounds in the same TLC system used for identifying the phospholipids. Total glycosphingolipids were isolated from the group *b* lipids, after deacylation of the phospholipids, by column chromatography (21). Neutral glycosphingolipids were separated from acidic glycosphingolipids and cholesteryl sulfate by chromatography on DEAE-cellulose (22). Individual compounds were isolated (11) and quantitatively estimated by analysis of the carbohydrate moiety (11). Gangliosides were separated by TLC and identified with the resorcinol reagent described by Svennerholm (23).

TABLE 1. Distribution of polar and nonpolar lipids in
pig, human, and rat epidermal cells

	Pig	Human	Rat
	% (by weight) of total lipids		
Nonpolar (neutral) lipids[a]	30.2 ± 7.8 (5)[b]	35.5 ± 4.0 (4)	64.2 ± 8.5 (6)
Polar lipids			
Phospholipids[c]	62.3 ± 9.7 (5)	53.0 ± 4.0 (4)	34.7 ± 8.5 (6)
Glycosphingolipids (glucosylceramide)[d]	7.3 ± 1.5 (3)	9.5 ± 0.5 (2)	ND[e]
Cholesteryl sulfate	0.3 ± 0.06(2)	1.0 ± 0.14(2)	1.12 ± 0.04(3)

[a] Calculated from weights of isolated total neutral lipids and from total lipid minus polar lipid.

[b] Figures in parentheses indicate number of separate cell preparations. All values are given with standard deviation.

[c] Phospholipid weight (average mol wt 775) calculated from phosphorus content × 25.

[d] Glucosylceramide (average mol wt 785) calculated from glucose content × 4.35.

[e] ND, not determined.

Neutral lipids were separated by TLC on silica gel H. Plates (20 × 20 cm) were developed in petroleum ether–diethyl ether–acetic acid 70:30:1 to 15 cm and then in hexane to 19 cm. Lipid classes were identified by cochromatography with the following standards: cholesterol, cholesteryl oleate, mono-, di-, and triglycerides (palmitate), oleic acid, squalene, n-eicosane, ceramide, and wax esters (mainly C_{18}–C_{18} from the Tasmanian muttonbird). With the exception of ceramide, sterols, and sterol esters (see Analytical Methods) the estimation of the other lipids in the neutral lipid fraction was semiquantitative, as follows. A known sample of the total neutral lipids was separated into components by TLC with the two solvents described above. After charring the compounds by spraying with 50% H_2SO_4 (v/v) and heating for 30 min at 180°C, the intensities of the compounds were compared with standards in a range of concentrations that were also carried through the procedure with the sample. Amounts of cholesterol estimated in this way were in reasonable agreement (±10%) with those determined by GLC.

RESULTS

The cell populations obtained by treatment of the epidermis with dilute trypsin were mainly cells[1] from the basal and spinous layers of the epidermis. The cells appeared undamaged when viewed by phase-contrast microscopy and were birefringent. An arbitrary assessment of viability by the exclusion of the dyes nigrosine and eosin indicated that in most preparations 60–80% of the cells were viable. Contamination by cells from the upper, granular layer of the epidermis, easily recognized by their granulat-

[1] Throughout this paper "epidermal cells" refers to mixed populations of basal and spinous cells only.

ed appearance, large size, and flat, angular shape, was 2–5% for pig cells, 5–10% for human cells, and 10–15% for rat cells. Yields of cells varied with each preparation, but about $1 × 10^8$ cells/g wet weight were obtained from pig epidermis, $7 × 10^7$/g from human epidermis, and $4 × 10^7$/g from rat epidermis.

Lipids recovered from material discarded during the preparation of the cells were equivalent to approximately 20% (by weight) of the total lipids isolated from the cells. An equivalent of about 15% (by weight) of total cell lipid (mainly neutral lipids) was extracted from the saline washings of the cells. These contained fragments of cells, stratum corneum, and some lipid droplets, probably from the dermis. The material washed from the nylon filter was mostly clumps and sheets of epidermal cells. The amount of lipid extracted from this was equivalent to about 5% (by weight) of the lipid extract from the cells and was of similar composition.

Total lipids accounted for approximately 8%, 10%, and 20%, respectively, of the pig, human, and rat epidermal cells (dry weight). The relative proportions of nonpolar and polar lipids differed (Table 1). The phospholipid compositions were generally similar (Table 2) except for phosphatidylserine, which was proportionally much lower in human than in either pig or rat epidermal cells. In all three species the major components were phosphatidylcholine, sphingomyelin, and phosphatidylethanolamine. Only minor amounts of plasmalogens were present. Minor amounts of two unidentified phospholipids (PL-X and PL-Y) were present in the phospholipids isolated from the cells of all three species. Palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), and linoleic ($C_{18:2}$) were the major fatty acids in the diacylphospholipids of pig epidermal cells (Table 3). The major sterol component of epidermal cells was cholesterol. Rat epidermal cells were exceptionally rich in sterols

TABLE 2. Composition of total phospholipids in epidermal cells of pig, human, and rat

	Pig (5)[a]	Human (4)	Rat (6)
	% of total phospholipids		
Diphosphatidylglycerol (cardiolipin)	3.4 ± 0.6	3.6 ± 0.7	4.7 ± 1.7
Phosphatidic acid	1.3 ± 0.5	2.4 ± 0.8	4.2 ± 1.6
Phosphatidylethanolamine	17.7 ± 1.5	{19.1 ± 1.7[b]	{23.5 ± 3.2[b]
Ethanolamine plasmalogen	2.4 ± 1.1		{[2.1][c]
Phosphatidylserine	{10.0 ± 4.7[b]	{ 3.8 ± 0.4[b]	{ 7.9 ± 2.9[b]
Serine plasmalogen	{[0.45][c]		{[0.4][c]
Phosphatidylinositol	9.0 ± 2.3	9.5 ± 2.3	6.2 ± 2.9
Phosphatidylcholine	36.0 ± 1.8	{38.5 ± 2.7	{33.4 ± 7.4[b]
Choline plasmalogen	1.9 ± 0.6		{[1.5][c]
Sphingomyelin	17.2 ± 2.0	20.8 ± 2.7	19.6 ± 4.0
Phospholipid PL-X	1.3 ± 0.5	2.4 ± 0.8	4.2 ± 1.6
Phospholipid PL-Y	tr[d]	1.9 ± 0.7	tr

[a] Figures in parentheses indicate number of separate cell preparations.
[b] Values include plasmalogens.
[c] Values are from a single determination of plasmalogens.
[d] tr, trace (<0.3).

(Table 4) and sterol esters. Also, about 20% of the total sterols was cholest-7-ene-3β-ol and its esters, whereas pig and human epidermal cells contained only small amounts of esterified sterols and very little cholest-7-ene-3β-ol. Human epidermal cells also contained traces of dihydrocholesterol.

A minor polar lipid in epidermal cells was identified as cholesteryl sulfate (Table 1). It was isolated from the acidic phospholipid fraction (group *b*), after removal of the phospholipids by deacylation with mild alkali, by DEAE-cellulose chromatography (24). Cholesterol was identified by GLC and TLC as the sole lipid component obtained

TABLE 3. Fatty acid composition of phospholipids and glycosphingolipids in pig and human epidermal cells

Fatty Acid	Pig									Human	
	PE	PI	PS	CL	PA	PL-X	PC	SP	GSL	SP	GSL
14:0	tr	2.7	2.4	4.4	3.6	0.8	14.7	1.7	tr	2.6	5.1
15:0	tr	2.0	2.7	2.3	1.8	1.0	1.2	1.0	tr	1.1	3.4
16:0	11.5	18.5	11.3	15.4	11.9	11.0	26.4	22.5	9.4	14.6	8.2
16:1	5.5	3.6	3.9	6.8	4.5	5.6	6.4	tr			
17:0	tr	tr	2.5	0.5	1.2		tr	tr	tr	2.0	2.4
18:0	16.3	25.2	16.5	7.4	13.5	12.9	7.5	4.5	2.7	6.4	4.3
18:1	24.6	13.3	17.4	21.6	19.7	31.5	19.1	3.1	24.7	2.8	17.9
18:2	34.4	14.9	20.7	34.2	22.9	21.0	22.9				
18:3	7.7	0.8	2.1	3.5	3.5	1.3	tr				
20:0	tr				1.0	1.9		24.6	15.8	11.6	7.7
20:1		3.3	5.9	1.7		0.6					
20:2	tr	1.7	2.0								
20:3	tr	1.5	4.3			3.3					
20:4		7.2	5.3			4.1					
21:0								0.8	tr	1.3	1.7
22:0								8.1	9.8	8.9	4.3
23:0								1.0	1.6	1.6	1.7
24:0						3.1		14.4	14.5	18.8	10.0
24:1								10.5	2.6	9.5	2.0
25:0								0.8	tr	2.0	5.2
26:0								2.8	9.0	5.8	5.4
28:0									7.8	0.7	5.9
24:0:OH									tr		2.6
26:0:OH									tr		5.6
NI		1.8	2.9	1.6	12.5	1.9	1.8	4.2	2.1	10.3	8.6

Values are percentages (by weight) of total fatty acids. NI, not identified; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; CL, diphosphatidylglycerol (cardiolipin); PA, phosphatidic acid; PL-X, unidentified phospholipid; PC, phosphatidylcholine; SP, sphingomyelin; GSL, glycosphingolipid; tr, trace (<0.2).

TABLE 4. Sterol content of pig, human, and rat epidermal cells

	Pig (4)[a]	Human (2)	Rat (6)
	mmoles of sterol/mole of epidermal cell phospholipid		
Cholesterol	270 ± 23	310 ± 20	550 ± 200
Cholesteryl esters	22 ± 8	22 ± 10	330 ± 190
Cholest-7-ene-3β-ol	tr[b]	38 ± 14	74 ± 37
Cholest-7-ene-3β-ol esters	tr	6 ± 2	126 ± 22

[a] Figures in parentheses indicate number of separate cell preparations.
[b] tr, trace (<1).

TABLE 5. Composition of nonpolar (neutral) lipids in epidermal cells of pig, human, and rat

	Pig	Human	Rat
	% (by weight) of total nonpolar lipids		
Hydrocarbons	8	6	4
Squalene		tr	
Cholesteryl esters	3	2[a]	11[a]
Wax esters			26
Triglycerides	25	25	19
Free fatty acids	20	25	
Cholesterol	30	25[a]	18[a]
Ceramide	3	3	ND
Unidentified	11	13	22

Except for cholesterol, cholesteryl esters, and ceramide, all values are semiquantitative only (see Methods section). ND, not determined.
[a] Values include cholest-7-ene-3β-ol.

after hydrolysis with methanolic HCl (11). The chemical and chromatographic properties of the intact lipid were indistinguishable from those of pure synthetic cholesteryl sulfate (24, 25).

In the course of isolating cholesteryl sulfate from the group *b* lipids of human epidermal cells, another alkali-stable compound was obtained whose chromatographic properties on thin-layers of silica gel H were very similar to cholesteryl sulfate. However, the compound was not retained on DEAE-cellulose columns (22) and was eluted from the column with chloroform–methanol 2:1 along with neutral glycosphingolipids. Cholesterol was the only lipid product of acid hydrolysis (0.7 M methanolic HCl for 18 hr at 80°C), and the only other product was tentatively identified as glucose by GLC (11). Thus, the compound might be cholesteryl glucoside, and its concentration in human epidermal cells was similar to that of cholesteryl sulfate. There were traces in pig cells but it was not detected in rat epidermal cells.

The glycosphingolipids in epidermal cells were of one class, the ceramide monohexosides, though rarely some preparations from human epidermis did contain traces of ceramide dihexosides. Analyses by GLC showed that the ceramide monohexosides from most preparations contained only glucose, but occasionally some galactose (<1%) was also present. Thus, glucosylceramides accounted for virtually all of the glycosphingolipids in epidermal cells (Table 1). The glucosylceramide in rat epidermal cells was not estimated quantitatively, but its concentration judged by TLC was less than that in either pig or human epidermal cells. The major portion (approximately 60%) of the fatty acids in the pig and human glucosylceramides consisted of C_{20} to C_{28} compounds (Table 3), and oleic acid ($C_{18:1}$) was the major individual acid. Small amounts of gangliosides were detected in the epidermal cells of these species. There was insufficient material to identify individual compounds unambiguously, but TLC indicated that they were mostly hematosides and higher gangliosides similar to G_{M1} (26).

The nonpolar lipids of pig and human epidermal cells were similar, with sterols, triglycerides, and free fatty acids (**Table 5**) as major components. Sterols and triglycerides, but not free fatty acids, were major components in rat epi-

dermal cells. Wax esters, which were another major lipid class in rat epidermal cells, were not detected in either pig or human epidermal cells. All three species contained some lipid with properties characteristic of long-chain hydrocarbons. A relatively polar compound in the neutral lipids was identified as ceramide by cochromatography (27) with authentic ceramide and by analysis of long-chain bases and fatty acids, which were the only products after acid hydrolysis (10). Other "polar" compounds with chromatographic properties (on TLC) similar to ceramide were present in the epidermal cells of the three species, but they were not identified.

The compositions of the lipids extracted from epidermal cells after procedures *a* to *d* (see Methods section) were similar, and no change was observed in the relative amounts of any individual lipid class. Furthermore, none of the lipid extracts from the cell preparations contained lipids that were not present in the lipid extract from fresh whole epidermis. Relative levels of triglycerides, cholesteryl esters, and free fatty acids, which might be considered most prone to attack by an "activated lipase," in all cell preparations were not significantly different from those in whole epidermis.

It was also noted that the lipid compositions of cells that were isolated with or without contact with bovine serum albumin (a possible source of contamination by fatty acids) were the same.

DISCUSSION

The cells in the basal and spinous layers of the epidermis probably account for the majority of the viable cells in the tissue. Cells passing through the granular layer are degenerating, and with the loss of their nuclei, subcellular membrane structures, and phospholipids their differentiation into the dead cells of the stratum corneum is complete. Be-

cause of this heterogeneity in the cell population of the whole epidermis, what little quantitative data there are (1–3, 28) on the lipids of epidermis are difficult to interpret. The data·presented in this paper provide new information on the lipids of the viable cells of the epidermis.

Our cell preparations were free from stratum corneum cells, and those from pig epidermis were only slightly contaminated (2–5%) with granular cells. The proportions of granular cells in preparations from human epidermis (5–10%) and rat epidermis (10–15%) were higher. The removal of the skin surface lipids prior to cutting the epidermal slices and the thorough washing of cell suspensions to remove tissue debris and lipid droplets ensured that the lipids isolated originated only from the epidermal cells. Supportive evidence for this were the absence of squalene in the neutral lipids from human epidermal cells (cf. human skin surface lipids [5])and the absence of glycosphingolipids containing more than one carbohydrate unit, which indicated a minimal contamination by cells of dermal origin such as fibroblasts (29).

Though similar to pig and human epidermal cells in phospholipid composition, rat epidermal cells contained, respectively, approximately three and six times the amounts, as a percentage of the cell dry weight, of nonpolar lipids. Nearly half (45%) of the sterols in rat cells were esterified (Table 4), and approximately 20% of the sterols was cholest-7-ene-3β-ol. The wide variation in the amount of sterols present in each preparation of cells was possibly related to differences in age, weight, and sex of each batch of rats. On the other hand, values for both polar and nonpolar lipids from different batches of pig and human epidermis showed little variation (Tables 2 and 3). It has also been noted that the lipid compositions of cells from human leg and breast are very similar, as are those from pig tail and ear.[2]

The phospholipids of the three species were notable for their high content of sphingomyelin. Most mammalian tissues contain proportionally far less sphingomyelin, and very few contain more (30). It is probable that the endoplasmic reticulum and/or the plasma membrane phospholipids of pig and human epidermal cells are very rich in sphingomyelin because their nuclei and mitochondria (31) contain proportionally less sphingomyelin than the phospholipids of the whole cell.

Pig and human epidermal cells were rich in glycosphingolipid in comparison with reported levels (32) in other tissues. However, the glycosphingolipid composition is unusually simple, being restricted to one class only, the glucosylceramides. They contained significant quantities of C_{26} and C_{28} fatty acids (Table 3), which are not normally found in glycosphingolipids or phospholipids from mammalian sources with the exception of brain, which does

contain C_{26} fatty acid (30). Glucosylceramide was not found in either mitochondria[3] or nuclei[3] from pig and human epidermal cells and may be localized predominantly in the plasma membrane of the cell (33).

Wheatley and Flesch (34), in a study of the lipids from normal and pathological (mainly psoriatic) human stratum corneum, reported the presence in both normal and pathological material of some unusual, highly polar, sterol compounds that yielded free sterols on acid hydrolysis. They noted that there were apparent variations in amounts of these compounds in the different disease conditions investigated. Nieminen et al. (3) reported an unknown compound in the lipids from whole epidermis (human) whose chromatographic properties in their TLC system were similar to sulfatide (O-β-D-galactosyl-(3-sulfate)-(1 $\rightarrow$ 1)-ceramide). It is probable that one of the unidentified sterol compounds described by Wheatley and Flesch (34) and the unidentified compound reported by Nieminen et al. (3) was cholesteryl sulfate. It is a minor component of the lipids of pig, human, and rat epidermis but, nevertheless, amounts in skin appear relatively large compared with some other tissues (35). Human epidermal cells contained another minor lipid that contained cholesterol. This compound may be cholesteryl glucoside and probably corresponds to another of the sterol compounds reported by Wheatley and Flesch (34). It is of some interest that, relative to other lipids, its amount is increased severalfold in psoriatic epidermis.[2]

Ceramide is another minor component of epidermal cell lipids that was not identified in earlier studies (34, 36). There is suggestive evidence (37) that it represents a catabolic product of sphingomyelin rather than a biosynthetic precursor for either this phospholipid or glucosylceramide.

Though we have found the phospholipid PL-X in all cell preparations from epidermis, we are not certain that it is a normal component of the viable, i.e., the basal and spinous, cells of the epidermis. We have some evidence (37) that its presence may relate to the small quantities of granular cells that contaminate our preparations. This may also be true for PL-Y. ∎

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[2] Gray, G. M. Unpublished results.

REFERENCES

1. Reinertson, R. P., and V. R. Wheatley. 1959. Studies on the chemical composition of human epidermal lipids. *J. Invest. Dermatol.* 32: 49–58.
2. Blomstrand, R., S. Fregert, and F. Nakayama. 1961. Phospholipid composition of human epidermis. *Acta Dermato-Venereol.* 41: 281–288.
3. Nieminen, E., E. Leikola, M. Koljonen, U. Küstala, and K.

K. Mustakallio. 1967. Quantitative analysis of epidermal lipids by thin-layer chromatography with special reference to seasonal variation. *Acta Dermato-Venereol.* **47:** 327–338.

4. Nicolaides, N., H. C. Fu, and G. R. Rice. 1968. The skin surface lipids of man compared with those of eighteen species of animals. *J. Invest. Dermatol.* **51:** 83–89.

5. Lewis, C. A., and B. J. Hayward. 1971. Human skin surface lipids. *In* Modern Trends in Dermatology. Vol. 4. P. Borrie, editor. Butterworth, London. 89–121.

6. Kooyman, D. J. 1932. Lipids of the skin. Some changes in the lipids of the epidermis during the process of keratinization. *Arch. Dermatol. Syph.* **25:** 444–450.

7. Long, V. J. W. 1970. Variations in lipid composition of different depths of the cow snout epidermis. *J. Invest. Dermatol.* **55:** 269–273.

8. Long, V. J. W., and H. J. Yardley. 1970. Phospholipids in cultured guinea pig skin. *J. Invest. Dermatol.* **54:** 174–177.

9. Gray, G. M. 1969. The preparation and assay of long-chain fatty aldehydes. *Methods Enzymol.* **14:** 678–684.

10. Kisic, A., and M. M. Rapport. 1974. Determination of long-chain base in glycosphingolipids with fluorescamine. *J. Lipid Res.* **15:** 179–180.

11. Gray, G. M. 1971. The effect of testosterone on the biosynthesis of the neutral glycosphingolipids in the C57/BL mouse kidney. *Biochim. Biophys. Acta.* **239:** 494–500.

12. Briggaman, R. A., D. C. Abele, S. R. Harris, and C. E. Wheeler. 1967. Preparation and characterization of a viable suspension of postembryonic human epidermal cells. *J. Invest. Dermatol.* **48:** 159–168.

13. Blank, H., E. W. Rosenberg, and I. Sarkany. 1961. An improved technic for obtaining uniformly thin sheets of skin. *J. Invest. Dermatol.* **36:** 303–304.

14. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.

15. Siddiqui, B., and S. Hakomori. 1970. Change of glycolipid pattern in Morris hepatomas 5123 and 7800. *Cancer Res.* **30:** 2930–2936.

16. Gray, G. M. 1967. Chromatography of lipids. I. An improved chromatographic procedure for the quantitative isolation of the neutral ceramide-containing glycolipids from mammalian tissues. *Biochim. Biophys. Acta.* **144:** 511–518.

17. Gray, G. M. 1967. Chromatography of lipids. II. The quantitative isolation of the minor (acidic) phospholipids and of phosphatidylethanolamine from the lipid extracts of mammalian tissues. *Biochim. Biophys. Acta.* **144:** 519–524.

18. Marinetti, G. V. 1962. Chromatographic separation, identification, and analysis of phosphatides. *J. Lipid Res.* **3:** 1–20.

19. Dawson, R. M. C. 1960. A hydrolytic procedure for the identification and estimation of individual phospholipids in biological samples. *Biochem. J.* **75:** 45–53.

20. Gray, G. M. 1964. The isolation of phosphatidyl glycerol from rat-liver mitochondria. *Biochim. Biophys. Acta.* **84:** 35–40.

21. Vance, D. E., and C. C. Sweeley. 1967. Quantitative determination of the neutral glycosyl ceramides in human blood. *J. Lipid Res.* **8:** 621–630.

22. Svennerholm, L., and H. Thorin. 1962. Quantitative isolation of brain sulfatides. *J. Lipid Res.* **3:** 483–485.

23. Svennerholm, L. 1957. Quantitative estimation of sialic acid. *Biochim. Biophys. Acta.* **24:** 604–611.

24. Coles, L., J. B. Hay, and G. M. Gray. 1970. Factors affecting the glycosphingolipid composition of murine tissues. *J. Lipid Res.* **11:** 158–163.

25. Mumma, R. O. 1966. Preparation of sulfate esters. *Lipids.* **1:** 221–223.

26. Svennerholm, L. 1963. Chromatographic separation of human brain gangliosides. *J. Neurochem.* **10:** 613–623.

27. Morell, P., and N. S. Radin. 1969. Synthesis of cerebroside by brain from uridine diphosphate galactose and ceramide containing hydroxy fatty acid. *Biochemistry.* **8:** 506–512.

28. Gerstein, W. 1963. The phospholipids of normal and psoriatic skin. *J. Invest. Dermatol.* **40:** 105–109.

29. Dawson, G., R. Matalon, and A. Dorfman. 1972. Glycosphingolipids in cultured skin fibroblasts. I. Characterization and metabolism in normal fibroblasts. *J. Biol. Chem.* **247:** 5944–5950.

30. White, D. A. 1973. The phospholipid composition of mammalian tissues. *In* Form and Function of Phospholipids. G. B. Ansell, R. M. C. Dawson, and J. N. Hawthorne, editors. Elsevier, Amsterdam. 441–482.

31. Gray, G. M., and H. J. Yardley. 1975. Mitochondria and nuclei of pig and human epidermis: isolation and lipid composition. *J. Invest. Dermatol.* **64:** 423–430.

32. Martensson, E. 1969. Glycosphingolipids of animal tissue. *Progr. Chem. Fats Other Lipids.* **10:** 367–407.

33. Critchley, D. R. 1973. Glycolipids and cancer. *In* Membrane Mediated Information. P. W. Kent, editor. Medical and Technical Publications, Lancaster, England. 20–47.

34. Wheatley, V. R., and P. Flesch. 1967. Horny layer lipids. II. Further studies on the overall chemical composition of lipids from normal and pathological human stratum corneum. *J. Invest. Dermatol.* **49:** 198–205.

35. Moser, H. W., A. B. Moser, and J. C. Orr. 1966. Preliminary observations on the occurrence of cholesterol sulfate in man. *Biochim. Biophys. Acta.* **116:** 146–155.

36. Nicolaides, N. 1965. Skin lipids. II. Lipid class composition of samples from various species and anatomical sites. *J. Amer. Oil Chem. Soc.* **42:** 691–702.

37. Gray, G. M., and H. J. Yardley. 1975. Different populations of pig epidermal cells: isolation and lipid composition. *J. Lipid Res.* **16:** 000–000.