Skin Lipids. II. Lipid Class Composition of Samples from Various Species and Anatomical Sites

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Abstract

The literature is reviewed and new data are presented for the lipid class analysis of samples of lipid from the following skin tissues and appendages: Total epidermis, stratum corneum, and living epidermis from the human leg, total epidermis (human sole), total epidermis (rat body), sebaceous glands (human scalp), Meibomian glands (human eyelid), preputial glands (rat), preen gland (domestic goose), scalp skin surface (human), *vernix caseosa* (human fetal body), and rat skin surface from the back. Lipid yields are also given for most of these samples.

The results show that the composition of the sebaceous type excretion varies not only from species to species but for different anatomical sites within a given species. Noteworthy is the striking number of unidentified components. Evidence is presented for the existence of a wax diester in the sebaceous gland excretion of the rat.

Introduction

THIS PAPER DISCUSSES lipid class composition of three types of skin samples: epidermis, sebaceous glands, and skin surface. Literature data are reviewed for each type of sample and new data are presented for a selected number of samples from each category. The types of fatty chains found in skin lipids are discussed in the next paper (1).

The samples studied include:

1. Epidermis: a) total epidermis from human leg; b) stratum corneum from human leg; c) living epidermis from human leg; d) total epidermis from human sole; e) total epidermis from rat body.
2. Sebaceous glands: a) glands from human scalp; b) Meibomian glands from human eyelid; c) preputial glands from rat; d) preen gland from the domestic goose.
3. Skin surface: a) scalp skin from human adult; b) *vernix caseosa* from human fetal body; c) rat back.

Experimental

The preparation of the lipid samples has already been described (2). Lipid class compositions were surveyed by means of thin-layer chromatography (TLC). The relative amounts of the lipid classes present were approximated by a comparison of spot intensities with a standard preparation of human surface lipid. The composition of the standard is well established and it contains components derived from sebaceous glands and keratinizing epidermis. Additional analyses (column chromatography, infrared spectroscopy, saponification, etc.) were performed on some samples.

Most of the TLC data were obtained by techniques described by Rouser et al. (3) using an adsorbent composed of silicic acid and magnesium silicate. Plates were heated 20 min at 120–130C immediately before use, cooled 30 min, then usually 100 μg of the lipid (dissolved in $CHCl_3$ at a concentration of 5 mg/ml) applied to the plate with a microsyringe and the plate placed immediately in a developing chamber (internal dimensions 10¾ in. long × 2¾ in. wide × 10½ in. deep) lined with Whatman 3MM paper soaked with the developing solvent. Solvents used are indicated in the legends of the figures. After development (usually about 1 hr) the plates were air dried, sprayed with Rhodamine 6G (0.002% in 1 N NaOH) and photographed under ultraviolet light. This procedure was used to prepare chromatograms shown in Figures 1 through 12. Figure 13 was an alumina plate prepared as follows: Baker's alumina "Suitable for Chromatography" was pulverized in a ball mill, heat activated at 250C for 2 hr, cooled for 30 min, and spread on a plate as a slurry (30 g alumina plus 55 ml water) one minute after the slurry was prepared. The plate was heat activated at 120C for 1 hr, spotted, and developed in a chamber lined with solvent soaked Whatman 3MM paper.

The total amount of "nonpolar" lipid was determined from the amount of lipid recoverable from a 10 cm × 2.5 cm (I.D.) column of silicic acid (Mallinckrodt) eluted with chloroform containing 1% methanol. The remaining "polar" lipids were eluted with methanol. The loading factor for these columns was such that no more than 200 mg polar lipid was applied.

Acid silicic acid column chromatography was also carried out on columns the same size as used for the separation of nonpolar from polar lipids. Mallinckrodt silicic acid was washed with 6 *N* HCl on a sintered glass filter (coarse) until all pigment was removed (250 ml 6 *N* HCl/50 g silicic acid) then with distilled water (1 liter) until pH did not change (5.15). It was transferred to a 3-necked flask and heated at 120–130C under the vaccuum of a water aspirator while a stream of nitrogen was bled in. The dried deoxygenated adsorbent in the stoppered 3-necked flask was stored under nitrogen in a glove bag until used. Before use the adsorbent was equilibrated 1 hr with 2.50 ml water in a glass-stoppered Erlenmeyer flask. The loading factor for these columns was 250 to 300 mg.

Yields of Crude Lipid from Skin and Its Appendages

For general background material of the discussions to follow the reader is referred to several excellent texts and reviews (4–10).

Human Epidermis

Reinertson and Wheatley (11) have made extensive studies of lipid yield of human epidermis from various anatomical sites. In their studies epidermis was separated from dermis by the stretch method of van Scott (12) and the lipid obtained by acetone extraction. Lipid content for abdomen, back, and thigh for

TABLE I

Lipid Yield from Various Adult Human Skin Tissues [a]

	Site	Disease	Wet wt. g	Non lipid res. g	Crude lipid g	Area cm^2	% Lipid wet wt.	% Lipid dry wt.	mg lipid/ cm^2	% H_2O (whole skin)
Total epidermis										
E.W. (female)	Leg	Diabetes	3.8	0.92	0.275	486	7.2	23	0.57	68
T.H. (female)	Leg	Arteriosclerosis	3.7	0.82	0.295	311	8.0	27	0.95	70
F.S. (male)	Leg	Arterial aneurism	7.2	1.01	0.556	830	7.7	35	0.67	78
U.K. (male)	Leg	Arteriosclerosis	6.5	1.67	0.451	—	6.9	21	—	67
J.H. (male)	Sole	Arteriosclerosis	8.7	3.88	0.965	156	11.1	20	6.2	44
C.C. (male)	Sole	Osteomyelitis	11.8	5.38	0.976	161	8.3	15	6.1	46
Portions of epidermis										
M.F. (female)	Sole	Diabetes	7.1							
Stratum corneum				1.43	0.332	164	4.7[b]	19	2.02	—
Living layer				—	0.152	164	2.2[b]	—	0.93	—
E.W. (female)	Leg	Diabetes	2.3							
Stratum corneum				0.212	0.154	281	6.6[c]	42	0.55	—
Living layer				—	0.078	281	3.3[c]	—	0.28	—
Dermis										
E.W. (female)	Leg	Diabetes	54.0	13.5	2.24	486	4.1	14	4.61	71

[a] Data in this table obtained as described in (2). All lipid extracts are total extracts and contain an unknown amount of nonlipid material.
[b] Based on 7.1 g wet weight of skin.
[c] Based on 2.3 g wet weight of skin.

adult males and females ranged from 8.5 to 23.3% of dried skin. Average values for abdominal skin were 14.0% for males and 16.2% for females. Children 2 to 8 years old gave 16.2%. Soles from a pooled sample from three adults gave 4.4%.

In general, the data from the present study (Table I) are comparable to those of Reinertson and Wheatley but values for soles are considerably higher. It is not known yet whether the discrepancy reflects the manner in which the extracts were prepared or whether these are real differences. Our earlier low yield (4.75%) of lipids from a sample of palmar epidermis plus upper dermis obtained by alcohol followed by ether extraction and subsequent wash of the lipid mixture (13) corresponds more to the data of Reinertson and Wheatley for soles.

Stratum Corneum

Yield of crude total lipid for stratum corneum does not appear to have been reported previously. Stratum corneum for soles (Table I) shows a value approximately equal to that of total epidermis, but stratum corneum for one sample from the leg was 42% lipid. This high value may reflect residual sebum present in leg epidermis.

Living Epidermis

Since the sample was obtained by extraction of cells stuck to filter paper, is was not possible to obtain the weight of the nonlipid residue, but from the weight of lipid per cm^2 it appears that the living layer contributes about one third of the total lipid of the epidermis both for soles and for leg.

Dermis

Although dermis was not studied extensively in this work, its lipid content is listed for the sake of completeness. The yield of lipid from dermis is strongly influenced by the residual subcutaneous fat which is very difficult to remove completely.

Human Surface Lipid

Studies on the amount of human surface lipid present at different anatomical sites and under different conditions have been reviewed by Rothman (4). Herrmann and Prose (14) introduced the concept of the "casual level" which is defined as that amount of lipid recoverable from unprotected sites. This level was determined for many areas. Jones et al. (15) accurately determined the casual level of lipid from the forearm as 0.045 mg/cm^2, a low sebum area. Kligman and Shelly (16) found it to be 0.20 mg/cm^2 for the forehead, a high sebum area. The latter authors present evidence for the view that the sebaceous gland functions continuously without regard to what is on the surface, in contrast to an earlier view that the output of sebum is controlled by the amount of surface lipid already present.

The human surface lipid used in this study was collected by the scalp-soaking technique (17,18). The yield is variable among individuals but constant for each individual. On the average about 200 mg/scalp/24 hr is obtained from a young adult male.

Sebaceous Glands

As mentioned in (2) the yield of lipid per sebaceous gland of the human scalp is about 10 μg.

Meibomian Glands

From 539 men, women, and children, Linton et al. (19) obtained 200 mg excretion. We obtained 50 mg lipid from 60 adults (52).

TABLE II

Lipid Yield from Various Rat Skin Tissues

Rats (male)	No. 1	No. 2	No. 3	No. 4
Total weight (g)	395	388	383	359
Preputial glands				
Wet wt (mg)	82 and 66	68 and 52	91 and 138	62 and 56
Lipid-free residue (mg)	154.3			
Lipid (mg)	140.4 (22.8% wet wt, 47.5% dry wt)			
Hair				
Weight (g)	5.91	6.68	5.71	5.27
Lipid	1.105 g (4.7% hair wt)			
Body skin				
Wet wt. (g)	36.8	39.0	41.7	34.7
Area (cm^2)	184	217	190	147
Epidermis				
Wet wt. (g)	6.14		2.88	2.37
Lipid-free residue (g)	1.635		1.604	
Lipid (g)	1.001 (16.3% wet wt, 38% dry wt)		0.658 (12.5% wet wt, 29% dry wt)	
Mg lipid/cm^2	2.49		1.95	

TABLE III[a]

Composition of Human Surface Lipid[b]

	Haahti (28) Acetone Back				Nicolaides & Foster (18) Diethyl ether Scalp	
Sex	Male individuals		Female Individuals		11 males	4 females
	No. 1	No. 2	No. 3	No. 4	Lipid pooled	Lipid pooled
Age	24 to 30 years		24 to 30 years		22 to 35 years	22 to 35 years
Sample weight (lipid)	Less than 1 g		Less than 1 g		11.71 g	9.19 g
Composition	%	%	%	%	%	%
Free acids	20.1	11.7	22.5	2.3	30.7	23.2
Paraffins	1.9	1.6	1.2	1.2	0.55	0.55
Squalene	17.3	13.5	7.7	7.5	10.9	7.6
Wax esters and sterol esters	22.9	24.6	24.8	23.1	22.6[c]	22.9[c]
Sterol esters	—	—	—	—	2.37	2.50
Triglycerides	28.1	37.6	14.8	43.9	19.5	26.6
Sterols	1.5	1.2	9.5	4.6	1.40	1.40
Diglycerides	5.9	8.0	12.4	13.9		
Mixed di- and monoglycerides	—	—	—	—	7.7	10.0[d]
Monoglycerides and phospholipids[e]	2.4	1.8	7.1	3.5		

[a] Reproduced by permission of Pergamon Press, Montagna, W., "Advances in Biology of Skin," Vol. 4, 1963.
[b] For identification of components see text.
[c] Urea adduct formation of these fractoins, saponification of the resulting straight and branched chain fractions, and chromatography of both unsaponifiable fractions on alumina show scalp surface lipid to contain 16.6% urea adductable wax esters, 2.7% nonurea adductable wax esters plus oxidized squalene, and the amount of sterol esters, indicated in the Table, computed from recovered sterol.
[d] The 1-monoglyceride content of the mixture of mono- and diglycerides of this sample is 1.5% of the total lipid sample.
[e] Phospholipids have been assumed to be present in this fraction, but as yet this has not been definitely established.

Preen Gland (Domestic Goose)

After the gland was excised an the surrounding tissue removed, Weitzel et al. (21) extracted lipid from the entire preen gland of the goose with acetone followed by ether. From 96 glands they found the average weight of a gland to be 6.52 g of which 58% was water, 22% ether-soluble and 20% fat-free residue. Murray (22) found that by rubbing and squeezing the gland with the fingers he could obtain an extrudate which yielded approximately 0.4 g lipid per goose. Odham (23) obtained 40 mg per day of a secretion which could be scraped from the opening of the gland with a spatula. This secretion contained less than 1% nonlipid solids.

In the present study, the excised preen gland from one domestic goose weighed 6.1 g and an extrudate of 4.4 g was recovered after the gland was thoroughly rubbed and squeezed with the fingers. This extrudate yielded 1.417 g lipid (chloroform/methanol 2/1 extraction as with other tissues) and 0.6 g dry nonlipid residue. The extrudate was thus 70% lipid on a dry weight basis and 32% lipid on a wet weight basis. The lipid of this extrudate was 23.2% of the weight of the entire gland in agreement with the results of Weitzel et al.

Vernix caseosa. Downing (24) found that 2.5 g yielded 0.30 g lipid, i.e. 12% lipid on a wet weight basis.

In the present study, from 8.86 g *vernix caseosa* 1.036 g lipid and 0.4412 g dry nonlipid residue were obtained. Thus the sample was 11.7% lipid on a wet weight basis and 70% lipid on a dry weight basis.

Rat Skin Tissue. There is a paucity of data in the literature on the lipid yield of rat skin tissue. Nikkari and Haahti (25) found the yield of surface lipid obtainable from a rat by immersion in acetone to be 130–170 mg/day/kg body weight. Haldi *et al.* (26) showed that the fat content of total skin of female rats was significantly higher than that of males when they were fed on either a stock diet, a high carbohydrate diet or a high fat diet. For male rats, the fat content of the total skin was 6.6, 9.3 and 17.6% of the wet weight for these respective diets and for females they were 12.0, 20.1, and 31.6%. These data indicate that more subcutaneous fat is deposited in the female than in the male. Carruthers (27) gives data for the amount of total lipid in mouse epidermis and dermis.

Yields of lipid for the various rat skin tissues studied here are given in Table II.

Relative Amounts of Polar and Nonpolar Lipid [1]

Few data are available in the literature on the yields of polar and nonpolar skin lipids [see Rothman (4) for earlier and Carruthers (6) for more recent literature]. Reinertson and Wheatley (11) found the phospholipid content (computed from an analysis of lipid phosphorus) for total epidermis, "Malpighian layer" and stratum corneum, from the abdominal skin of human cadavers to be 7.9%, 9.5% and 1.1%, respectively.

By our sampling technique the nonpolar lipids constitute nearly all of the lipids of human skin surface, rat skin surface, human sebaceous glands, human Meibomian glands and goose preen glands. Wherever whole tissues are extracted, however, some polar lipid is found. The relative amounts of these two groups is tabulated for some samples:

	Nonpolar lipids	Polar lipids
Total human skin (breast)	89.5	10.5
Human epidermis (leg) (sample 1)	78	22
Human epidermis (leg) (sample 2)	78.5	21.5
Human epidermis (soles)	34	66
Human dermis (leg)	86.5	13.5
Rat epidermis	85	15

The above data can be used to calculate the relative amount of sebum and of nonpolar epidermal lipids proper found in leg epidermis. The assumption is made that the nonpolar vs. polar lipid composition of sole epidermis is the same as that of leg epidermis proper. The ratio of nonpolar to polar lipid in the soles is 1:1.94 but in leg it is 3.6:1 (or 7.0:1.94). Thus an amount of sebum (all nonpolar) 6 times the amount of nonpolar lipid found in the sole would have to be added to bring this ratio to 3.6:1. It follows, therefore, that about 14% of the nonpolar components of leg epidermis are derived from epidermis itself, the rest (86%) from sebum. Sebaceous glands and hence the relative contribution of sebum to the nonpolar lipid fraction could be much higher from other areas of the body where sebaceous glands are more numerous.

Nonpolar Lipids of Skin

Human Skin Surface Lipid

Because it is so readily available, this has been

[1] In this paper "nonpolar" lipids includes hydrocarbons, wax esters (fatty acids esterified to fatty alcohols), sterol ester, triglycerides, free fatty acids, diglycerides and monoglycerides and compounds of approximately these polarities as judged by whether they will be eluted from silicic acid columns by solvents that will elute the enumerated compounds above. "Polar" lipids are those more polar than the monoglycerides, including ceramide and other sphingolipids as well as the phosphatides.

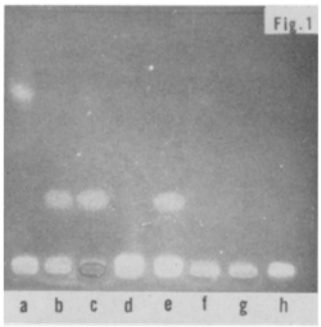

FIG. 1. The amount of 100 μg each of the lipids described below were spotted at the origin and the plate developed in hexane. The samples were a) rat skin surface (back), b) human scalp skin surface, c) sebaceous glands, d) Meibomian gland, e) *vernix caseosa*, f) soles epidermis (osteomyelitis, the initial extract of the epidermis with chloroform/methanol 2/1 where the more superficial lipids are concentrated, g) soles epidermis (osteomyelitis, the final extract of the lipids after f was removed), h) 20 μg each of cholesteryl palmitate, triglycerides, and free cholesterol. In chromatogram (a) the spot that migrates far toward the solvent front is probably saturated hydrocarbon. Traces can also be seen in chromatograms b), c) and f). The spots with Rf of about 0.3 are squalene. Note that human surface, human sebaceous gland, and *vernix caseosa* lipids show significant amounts of squalene. In these chromatograms most of the material remains at or very near the origin.

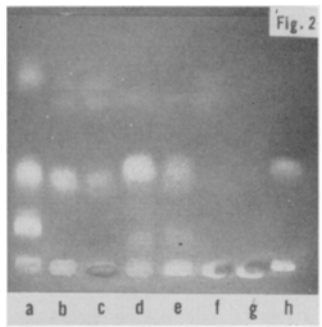

FIG. 2. Thin-layer chromatogram spotted with the same amounts of the same lipids as in Fig. 1 and developed with hexane-diethyl ether 95/5 (v/v). Reading from the solvent front toward the origin spots are a) rat surface lipid—faint and intense spots of hydrocarbon, a rather large and intense spot consisting of wax esters and sterol esters followed by faint and an intense spot (close together) of more polar wax esters, then one unidentified spot overlapping with material at the origin; b) human surface lipid—a trace of saturated hydrocarbon followed closely by squalene, then an intense spot of a mixture of wax esters plus sterol esters; c) sebaceous gland lipid—essentially as b); d) Meibomian gland lipid—a trace of squalene and other hydrocarbon, an intense spot of wax esters plus sterol esters followed by a very faint and a moderately intense spot of unidentified lipids; e) *vernix caseosa*—these lipids match up remarkably closely to those of d); f) sole epidermal lipid first extract (see Fig. 1)—two hydrocarbon spots close together (although the second spot migrates as squalene on this chromatogram it did not on others) and a faint trace of sterol esters; g) sole epidermal lipid second extract (see Fig. 1)—no hydrocarbon but a trace of sterol esters; h) 20 μg cholesteryl palmitate, 20 μg triglyceride and 20 μg cholesterol. Cholesteryl palmitate migrates into the body of the chromatogram, triglycerides just off the origin and cholesterol at the origin. It is evident that much material in these chromatograms remains at the origin.

the most widely studied of all skin lipid samples. It is an exceedingly complex lipid mixture. Its analysis is considered here in some detail.

Table III summarizes literature data on the lipid class composition of this material and Table IV data on saponifiable and nonsaponifiable matter. Skin surface lipids of scalp are compared with those of back and forearm.

Free (nonesterified) Fatty Acids. Surface lipid usually contains a very large fraction of free fatty acids in contrast to lipids of internal tissues. Considerable evidence is available indicating the presence of lipases in the sebaceous gland duct and on the skin surface that degrade triglycerides to di- and monoglycerides and free acids (31,30). Thus, the tri-, di-, and monoglycerides plus free fatty acids form a group of compounds of related origin. Quantitatively, when the free acids are high, the triglycerides are low and *vice versa.*

Triglycerides. The presence of triglycerides in human surface lipid was first inferred by Zehender (32) from the identification of glycerol in the aqueous phase after saponification of the lipid. MacKenna, Wheatley and Wormall (33) also identified glycerol in the aqueous phase after the latter was evaporated to dryness *in vacuo.* Triglycerides were isolated by silicic acid chromatography of surface lipid after the free fatty acids were removed (18). Identity was established by infrared spectroscopy and saponification. Fatty acids of the triglycerides were characterized by neutralization equivalent and iodine value determinations. No 1,2-diols other than glycerol were found in the aqueous phase after saponification. Triglycerides were also found in human skin surface lipid by Haahti (28), identified from its elution behavior on silicic acid chromatograms.

Diglycerides. From the low acetyl value for the entire lipid (~2), MacKenna, Wheatley and Wormall concluded (33) that only triglycerides are present. Silicic acid chromatography, however, revealed up to 10% of the total lipid as a mixture of di- and monoglyceride (18). The presence of diglyceride was confirmed by comparison of the infrared spectra of chromatographic eluates corresponding to diglycerides. Spectra were identical with 1,3-distearin. This may indicate that the fatty acid in the β position is the first to be lipolyzed from the triglyceride. To test the possibility that the diglyceride fraction was an artifact arising from chemical hydrolysis of triglycerides during isolation of the nonesterified acids (0.06 *N* NaOH in 50% ethanol was used) or from the chromatography on silicic acid, a portion of the isolated triglycerides of human surface lipid was treated in the same manner as the original total surface lipid was treated. At most it was found that 2.7% of the triglycerides (0.72% of the total lipid) could be decomposed by these procedures. Since 10% of the total lipid was found to be mono- and diglycerides, it was concluded that the bulk of the diglycerides found must have been present in the original sample. Diglycerides were also reported by Haahti (28) to be present in human surface lipid.

TABLE IV

Analysis of Human Surface Lipid [a,b]

	Wheatley, 1956 (29)	Nicolaides & Wells, 1957 (30)	
Extraction solvent	Acetone	Diethyl ether	
Anatomical source	Forearm	Scalp[c]	
Composition			
Free fatty acids	28.3	27.1	28.1
Acids as esters	34.6	41.8	38.5
Unsaponifiables	30.1	30.3	30.6
Total recovery	93.0	99.2	97.2
Paraffins	8.1	1.1	0.7
Squalene	5.5	12.7	12.4
Wax alcohols	6.2	11.2	12.2
Sterols	4.4	2.5	2.6
Alkane diols	2.0	....	....
Unidentified	....	0.7	1.5
Total recovery of unsaponifiables	26.2	28.2	29.4

[a] Reproduced by permission of Pergamon Press, Montagna, W.: "Advances in Biology of Skin," Vol. 4, 1963.
[b] Determined by removal of free fatty acids by washing with dilute base, saponification of the neutral lipid, then chromatography of the unsaponifiable fraction on alumina.
[c] Replicate 24-hr samples from the same young adult male.

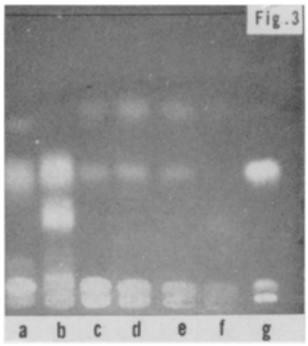

FIG. 3. Thin-layer chromatogram prepared as in Fig. 1 and developed with solvent as in Fig. 2; 100 μg of each sample were spotted. Reading from the solvent front to the origin for a) rat preputial gland lipids, the first spot is hydrocarbon; the second spot a mixture of wax esters and sterol esters; the third spot (faint), unidentified; and the fourth intense spot just off the origin from triglycerides; finally a spot at the origin; b) rat epidermal lipid—an intense spot of a mixture of wax esters and sterol esters followed by a double spot of more polar wax esters, then a faint unidentified spot followed by an intense spot of triglycerides, and much material at the origin; c) human leg epidermal lipids (diabetes)—hydrocarbon, a mixture of wax esters and sterol esters, triglycerides, considerable material at the origin; d) human leg epidermal lipids (arteriosclerosis)—similar to c); e) human leg stratum corneum lipids (diabetes)—similar to c); f) human leg living epidermal lipids (diabetes)—trace of material in the sterol ester region, a faint unidentified spot of ~0.3 rf, triglycerides and material at the origin; g) standards and their migrations are as in Fig. 2.

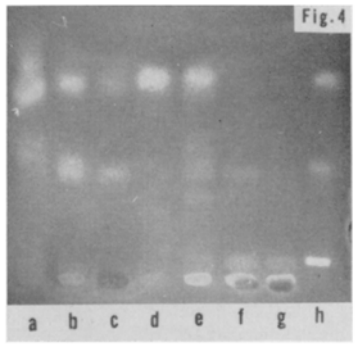

FIG. 4 is to be compared with Fig. 7. Chromatogram prepared and spotted as in Fig. 2, but developed with hexane/ether 4/1. Reading from the solvent front to the origin the spots are for a) rat surface lipid—saturated hydrocarbon (2 spots close together), wax esters plus sterol esters (most intense spot), more polar wax, small amount of triglycerides followed by a small amount of material near and at the origin; b) human scalp surface lipid—small amount of material in the hydrocarbon region followed by an intense spot in the region where wax esters plus sterol esters migrate, an intense spot in the triglyceride region followed by material at and near the origin. The purple halo in the lower part of this chromatogram is due to free fatty acid; c) human sebaceous gland lipids—very similar to human surface fat; d) Meibomian gland lipids—most of the material is wax ester and sterol ester, although a few spots are seen above and below the triglyceride region; e) *vernix caseosa* shows several unidentified components ahead and behind the triglyceride region and much material at the origin; f) and g) soles lipid extracts (see Fig. 1)—show a trace of sterol ester, some triglycerides, a faint unidentified spot behind triglycerides, cholesterol and material at the origin; h) standards 20 μg cholesteryl palmitate (upper spot), 20 μg triglyceride (middle spot) and 20 μg cholesterol (lower spot).

Monoglycerides. Monoglycerides have not been conclusively demonstrated to be present in human surface lipid. The presence of 1-monoglycerides has been inferred by periodate oxidation of a relatively polar chromatographic eluate (18) and Haahti (28) isolated a monoglyceride fraction by silicic acid column chromatography.

Paraffins. Paraffins are usually found in surface fat in an amount roughly inversely proportional to the care exercised to avoid contamination, i.e. distilling solvents, thoroughly washing adsorbents, keeping hands and clothing away from skin surface from which lipid is to be obtained, etc. Mass spectrometry of these materials showed them to resemble petroleum products (34) in keeping with the observations of Haahti (28) who used gas-liquid chromatography. Since petroleum products are widely distributed, small and variable amounts easily find their way to the skin surface, from cosmetics, floor wax, furniture wax, wax paper, waxed cartons, automobile grease, air pollutants, etc.

Squalene. Although squalene was reported to occur in human surface lipid by colorimetric measurements (35), its presence was first clearly demonstrated by MacKenna *et al.* (33). Two derivatives were prepared, both having the correct elemental analysis, namely, the hexahydrochloride and the dodecabromide. Squalene occurs in rather considerable quantities in surface fat, the amount corresponding to the density of sebaceous glands.

Wax Esters. These are a group of monoesters in which a fatty acid is esterified with a primary fatty alcohol. Both the acidic portion and the alcoholic portion of the esters are complex so that many individual molecular species are present.

The wax esters of human skin surface lipid were isolated originally (18) from a fraction of wax esters containing sterol esters and oxidized squalene obtained by silicic acid chromatography. Urea adduct formation enabled it to be separated into a "straight chain" (73.5%) and a "branched chain" (23%) fraction (18). Urea adduct separations are not clean-cut; i.e. some branched chain compounds can be incorporated into the adduct (36), and a small amount of straight chain material is not incorporated. A small amount of sterol ester (0.6%) was carried along into the adduct.[2] By far the greatest proportion of the "straight chain" material was wax ester, as was proved by infrared spectra, and by saponification and characterization of resulting acid and alcoholic material. Molecular weight determinations showed the average chain length (alcohol plus acid moieties) to be 37 carbon atoms $\pm$ ~2 methylene groups (18).

Two independent gas chromatographic studies of the wax esters (37,38) showed them to be esters ranging from C_{28} to C_{40} in total carbon content (i.e. alcohol and acid) with odd as well as even total chain lengths represented. In a more detailed study of the wax esters, Haahti (28) showed the following:

1. The wax esters range in total chain length from C_{26} to C_{42}.
2. The major fractions are: a) C_{36} with unsaturation on both the acid and the alcohol moieties; b) C_{36} with a single methyl branch on either the acid or the alcohol moieties; c) C_{36} with a double bond on either the acid or the alcohol moieties.
3. Chain lengths of an odd carbon number are present confirming earlier work.
4. Doubly branched wax esters are present.
5. Very little straight chain saturated wax ester of any chain length is present. Almost always, double bonds or methyl branches are present either in the acid or the alcohol moiety.
6. The distribution of acid-alcohol combinations is not random, even though many, if not all, of the combinations are represented.

[2] The puzzling fact that a faint but definite Liebermann-Burchard test on the straight chain portion was found even after the latter was subjected to repeated adduct formation might be understood if the fatty acid part of the ester got incorporated into the channel and the sterol moiety then terminated that channel.

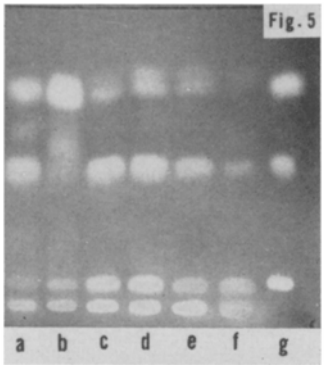

FIG. 5. is to be compared with Fig. 6. The plate was prepared and developed as in Fig. 4 and spotted as in Fig. 3. a) Rat preputial gland lipid—an intense spot in the region where wax esters and sterol esters migrate followed by a faint spot of an unidentified substance, then an intense spot in the triglyceride region, an appreciable cholesterol spot and an intense spot at the origin; b) rat epidermal lipid—a very intense spot in the region where wax esters and sterol esters migrate followed by unidentified more polar waxes perhaps of the diester type (see text), a small amount of triglycerides, then traces of other unidentified material, cholesterol, then an intense spot at the origin; c) human epidermal lipid (arteriosclerosis)—the upper of the two spots in the sterol ester region is undoubtedly hydrocarbon, this is followed by a mixture of wax esters and sterol esters. Next comes an intense spot of triglycerides, then sterols and finally an intense spot at the origin; d) human leg epidermal lipids (diabetes)—very similar to c); e) human leg stratum corneum lipids—very similar to c) and d); f) human leg living epidermal lipids—very similar to stratum corneum lipids except in amount. There is also present a faint spot between the sterol esters and the triglycerides, unidentified; g) standards are as in Fig. 4.

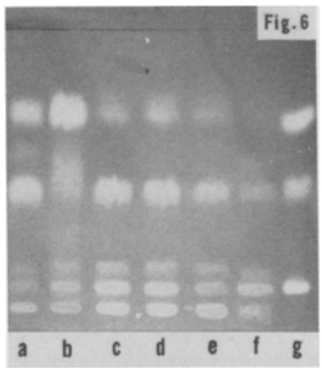

FIG. 6. Thin-layer plate prepared and spotted as in Fig. 5; the developing solvent was hexane/ether 4/1 to which 1% glacial acetic acid was added. In this solvent system the free fatty acids migrate just ahead of cholesterol. Note the nearly exact duplication of Fig. 5, except for the free fatty acids in all samples. They are minimally present in rat preputial gland lipids a).

Haahti et al. (39) showed that on $AgNO_3$ impregnated silica gel the wax esters of skin surface lipid could be separated into four fractions: 1) saturated, 2) monounsaturated, 3) diunsaturated, and 4) some unknown polyunsaturated wax esters.

The mixed wax esters melt from the heat of the palm of the hand and are thus oils at body temperature. Straight chain wax esters of a comparable carbon content would melt some 20C higher. Thus, the extra structural features on the acidic and alcoholic moieties of these wax esters enable them to be oils at body temperature (38,41).

Sterol Esters. The sterol esters of human surface lipid are a mixture of sterols, chiefly cholesterol, esterified to a complex group of fatty acids.

A crude concentration of sterol esters was achieved in the "branched chain" fraction of the urea adduct separation described under wax esters, above. This material yielded 42.2% acid and 57.4% unsaponifiable matter. The latter after chromatography on alumina consisted of 36% wax alcohol and 46% sterol; the remaining material, distributed in different portions of the chromatogram, behaved in elution and odor as oxidized squalene. The fatty alcohol and sterol content of this chromatogram showed that the original fraction was about half wax ester, half sterol ester. The neutralization equivalent and iodine value of the mixture of acids obtained by saponification of this entire "branched chain" fraction were 268 and 78.8, respectively (18).

Haahti et al. (40) showed that the wax esters could be separated from sterol esters by TLC on alumina plates. They obtained 9 mg wax ester (14%) and 1.5 mg (2.3%) sterol ester from 65 mg surface lipid from the back. The sterol esters were then subjected to TLC on $AgNO_3$ impregnated silica gel (39). The fatty acids esterified to the sterols were found to give a gas chromatographic pattern similar to that found for other skin surface lipids, especially pronounced branched chain peaks at C_{14} to C_{16}.

Free (nonesterified) Sterols. By far the main sterol of human surface lipid appears to be cholesterol and its presence in human surface lipid has long been known (4). Although no positive identifications of other sterols have yet been made, 7-dehydrocholesterol was implicated (42) and some ketosteroids may be present (42,43). From a comparison of the changing spectrum of the Liebermann-Burchard color reaction, we have noted the presence of four distinct patterns which are presumably due to sterols. One of these patterns matched that of lanosterol.

Free sterols also occur in human skin surface lipid. The molar ratio of free sterol to sterol ester is about 1.0 for the lipids from scalp (18). This ratio will vary with anatomical site since sterol esters are chiefly a product of the sebaceous gland, whereas free sterols are mainly a product of the keratinizing epidermis (see epidermal lipids below).

Several minor components of sebum have also been reported. A substance that reduces ferric chloride led MacKenna et al. to conclude that vitamin E is present in significant amounts (33). On further investigation of this fraction by means of ultraviolet absorption spectra little, if any, vitamin E was found (44,45). The ferric chloride reducing substance has not been identified. Vitamins A, D, or K could not be detected (44,45). A complicating factor in these analyses is the presence of oxidation products of squalene.

Alkane, 1,2-Diols. A material which reacted with periodic acid was obtained (42) from the methanol and acetic acid eluates of the unsaponifiable matter from human surface lipid chromatographed on alumina. It was believed to be an alkane, 1,2-diol although the possibility that it could be an oxidation product of squalene was also recognized. 1,2-Diols have been identified in wool wax (46,47).

An Unidentified Hydrocarbon. We have found that a nonparaffinic hydrocarbon always occurs in freshly obtained human surface lipid. This hydrocarbon appears just after the paraffins and before squalene during silicic acid column chromatography of the total surface lipid. It gives a Liebermann-Burchard test very similar to that for cholesterol. The sum of its carbon and hydrogen content was 100.0% which shows it to be a hydrocarbon. It occurs to the extent of about 0.2% of surface lipid from the scalp.

Farnesol. When the unsaponifiable matter of human surface lipid was chromatographed on alumina,

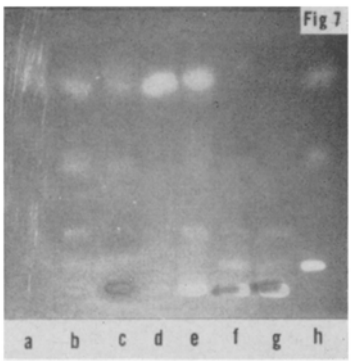

FIG. 7. The thin-layer plate was prepared and spotted as in Fig. 4. The scratches are artifacts. Note the close duplication of Fig. 4. Also note the fact that free fatty acids are present in practically all the samples except perhaps a) and d) in which it may be present only in trace amounts. In chromatograms b) and c) of this figure, namely, human surface fat and human sebaceous gland lipids the free fatty acid fraction is surrounded by a large purple and white halo. This shows the large amount of free fatty acids present.

a substance was found (in a fraction preceeding cholesterol) whose infrared spectrum corresponded to that of farnesol. The odor and behavoir on alumina columns of this substance were also identical to those of farnesol. Further identification of this material is in progress.

Thin-layer chromatograms of human skin surface lipid provide further insight into its lipid class composition (Figs. 1b, 2b, 4b, 7b, 9bc, 11bc, 12bcde, 13de). The sample used was from a young adult male and had a free fatty acid composition of 22% (by methods used in 18). Its triglyceride content is estimated to be about 30% and the remaining components about as they are for the scalp lipid of males (Table III). We thus have a crude standard whereby we can make semiquantitative comparisons of other skin lipid samples. It is emphasized that comparable migrations do not constitute an identification.

Human Sebaceous Gland Lipids

Literature on the chemical analyses of lipids of sebaceous glands is sparse. Some histochemical data are available (5). Suskind found histochemically that cholesteryl esters are present in normal human sebaceous glands but not free cholesterol (48).

TLC data on a sample of human scalp sebaceous gland lipid (2) shows a pattern similar to that of human surface lipid (Figs. 1c, 2c, 4c, 7c, 12a). Squalene, wax esters, triglycerides and free fatty acids are components of normal sebaceous glands. The presence of free fatty acids suggests that some sebaceous gland ducts were also included. Insufficient material was on hand to conclude whether or not sterol esters are also present, but the above-mentioned histochemical study and other evidence suggests that they are (41).

Lipids of Human Epidermis

Literature. The early work of Kooyman (49) shed some important light on the fate of epidermal lipids during keratinization. He analyzed total epidermis, stratum corneum, and a sample rich in basal layer cells, all taken from the sole in order to exclude sebaceous gland lipids. Phospholipids, free and esterified cholesterol and fatty acid were determined for all three samples. He found that the phospholipid content of stratum corneum was only about 5% that of the basal layer sample. This decrease was interpreted as destruction of membranes of cell organelles (50). In the basal layer nearly all the cholesterol

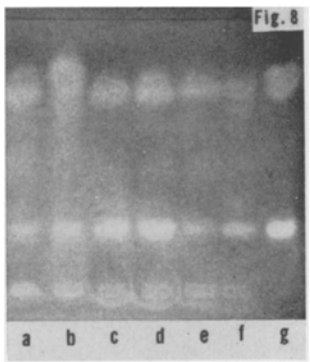

FIG. 8. The plate was prepared and spotted as in Fig. 5 and developed with hexane/ether 1/1. In this system we can see material migrating ahead of and behind cholesterol. Note that cholesteryl palmitate and triglyceride of the standard are close together while cholesterol has an Rf of about 0.3. Note that small amounts of unidentified substances are present in all chromatograms and that all have sterol present.

was as the nonesterified sterol but in the stratum corneum about 20% was esterified.

A study of the total content of fatty acids, sterols and squalene was made of forearm epidermis, forearm dermis, palmar epidermis plus upper dermis, and palmar lower dermis (13). The uptake of C^{14}-acetate in these tissues was also determined. Both the analytical and the radioactive uptake data indicated that each of the tissues synthesized all three types of lipids. In those tissues with sebaceous glands, however, squalene accumulated as such. In epidermal tissue squalene was rapidly converted to sterol.

Reinertson and Wheatley studied the lipids of epidermal tissue from a number of sites (11). On the basis of ultraviolet absorption spectra they report the presence of 7-dehydrocholesterol (pro vitamin D_3). Also present were other "fast acting" sterols [i.e. sterols that develop a color with the Liebermann-Burchard reagents much more rapidly than cholesterol (20)]. 7-Dehydrocholesterol appeared to be present only as nonesterified sterol in all specimens examined. It was higher in a "Malpighian layer" of abdominal skin (0.35% of total lipid) than it was in stratum corneum of the same skin (0.06% of total lipid). The amounts of other "fast acting" sterols for the same specimens were 0.36% for "Malpighian layer" and 0.19% for stratum corneum. Significant differences were not found between male and female adults, but a 2-week-old infant showed nearly twice as much sterol per dry weight of epidermis and the 7-dehydrocholesterol content of the sample was nearly three times that of adults. The fatty acids of the Malpighian layer were more like those from internal tissues rather than skin surface lipid. The unsaponifiable lipid, which constituted 37% of total abdominal epidermal lipid, contained 10% squalene, 39% other hydrocarbons, 5% alcohols, 26% sterols and 6% polyhydroxy substances.

Glasenapp and Leonhardi (51) found 40% free sterol in epidermis of the trunk. This is in contrast to the results of Kooyman (49) for soles (93% free sterol) and Reinertson and Wheatley for abdominal epidermis (11) (80% free sterol).

TLC Data. Four specimens of human epidermal lipids were examined by TLC in this study:

1) Total epidermis (sole) Figs. 1fg, 2fg, 4fg, 7fg, 9fg, 11fg.
2) Total epidermis (leg) Figs. 3cd, 5cd, 6cd, 8cd, 10cd.
3) Stratum corneum (leg) Figs. 3e, 5e, 6e, 8e, 10e.
4) Living epidermis (leg) Figs. 3f, 5f, 6f, 8f, 10f.

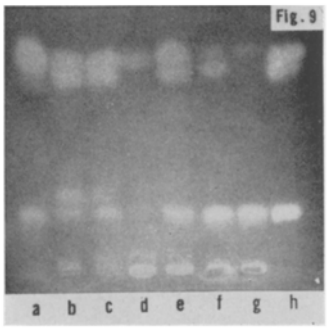

FIG. 9. Chromatogram prepared and developed as in Fig. 8 and spotted with 100 μg of the following lipids: a) rat surface lipid; b) human scalp skin surface lipid; c) human scalp skin surface lipid from which the free fatty acids were removed with dilute alkali; d) goose preen gland lipids, e) lipids from *vernix caseosa;* f) and g) first and second lipid extracts of sole epidermis respectively (see Fig. 1); h) 20 μg each of cholesteryl palmitate, triglyceride and cholesterol. Note that an unidentified substance is seen just ahead of cholesterol in the two human surface lipid samples, namely, b) and c). Note that preen gland lipids show very little if any cholesterol and that *vernix caseosa* lipids show traces of material between triglycerides and cholesterol. Note also that the amount of sterols for the two sole epidermal lipid samples, namely, f) and g) is very high. The purplish halo in the sample b) is due to free acids in the total human surface lipids, these are absent in sample c).

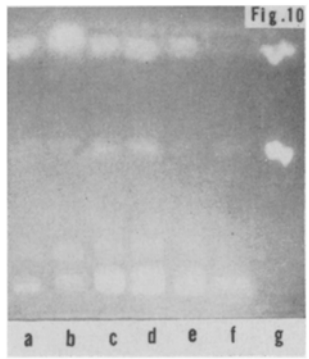

FIG. 10. The plate was prepared and spotted with lipids as in Fig. 3 and developed with chloroform/methanol 98/2. In this system substances migrating behind cholesterol (Rf ~0.6) and free fatty acids (just off the origin) are seen. Note the presence of unidentified substances in the region of Rf 0.3 and 0.2.

First note that for soles (Fig. 1fg) only traces of material are to be seen where squalene is expected to migrate (Rf ~0.3). Saturated hydrocarbons (Rf ~0.8) are also minimal. The upper part of Fig. 2, however, shows the presence of highly nonpolar material for one sample of soles (Fig. 2f) but not the other (Fig. 2g). Saturated hydrocarbon may also be present in epidermal lipids of leg (Fig. 3cd), stratum corneum and possibly the living epidermis (Fig. 3f). Whether these substances are true products of skin or contaminants remains to be determined. The difficulties of avoiding contamination of skin samples, especially from petroleum products have already been discussed.

In the wax plus sterol ester range note that sole lipid shows a small amount of material (Fig. 2fg). Some of this material was collected. From the amount of color developed with the Liebermann-Burchard reagents it is inferred that most if not all of it is sterol ester. Material that migrates as triglycerides (Fig. 4fg, 7fg), free sterols and free fatty acids is also present in lipids of soles (Figs. 4fg, 7fg).

The same components are present in total epidermis, stratum corneum and living epidermis of leg. Living epidermis shows an additional unidentified spot that migrates just behind the sterol esters (Fig. 3f). This substance, seen in a number of preparations of living epidermis, is not seen in stratum corneum lipids and apparently diminishes or disappears on keratinization. There are other unidentified substances that migrate ahead of and behind cholesterol (Figs. 8,9,10,11). Thus it appears that the nonpolar lipids of human epidermis are free sterols, glycerides, free acids, sterol esters and several unidentified substances.

Meibomian Gland Lipids (Eyelid)

The Meibomian gland lipids have been studied by Linton et al. (19). They concluded from chromatograms of the filter paper disc type and histochemical tests on the bands formed that the "neutral fats" (identified by staining with Nile blue), an unidentified lipid, and phospholipid were present. No free fatty acids or cholesterol (tested by the Liebermann-Burchard reaction) were found.

Although the Meibomian gland is of the sebaceous type, TLC shows the lipid composition to be strikingly different from that of human scalp sebaceous glands (Figs. 1d, 2d, 4d, 7d). The main component is wax ester plus sterol ester. Squalene, free fatty acids and the tri-, di- and monoglyceride group of compounds (so characteristic of scalp sebaceous gland lipids) are present in only minimal amounts. At least four unidentified nonpolar substances not seen in scalp sebaceous gland lipids are present. Some of these unidentified substances may be waxes of the diester type (see below).

Linton et al. (19) reported a melting range of 35–40C where 80% of the lipid melted. The bulk of our sample melted from 30–45C. The temperature of the material excreted on the eyelid should normally be about 30C. A more complete analysis of this lipid will be presented elsewhere (52).

Lipids of Vernix Caseosa

Table V presents data on some lipid components of *vernix caseosa.*

Haahti et al. (55) isolated four fractions by silicic acid column chromatography of 100 mg of *vernix caseosa* and studied the fatty acids from two of them. The first fraction (30 mg) was eluted with light petroleum and was presumably hydrocarbon. The second fraction (14 mg) was eluted with 1% ether in light

TABLE V

Some Lipid Components of *Vernix Caseosa*

	Free acids %	Fatty acids as esters %	Total fatty acids %	Total unsap. %	Total recovery %	Squalene %	Cholesterol %
Wheatley (53)							
Sample 1	2.3	33.3	35.6	27.0	62.6	2.6	19.7
Sample 2	9.4	42.1	51.5	48.6	100.1		20.6
Downing (24)	—	—	40	49	89	—	
Cmelic (54)	—	—		31		4.18	
This study	—	—	57	35	92	Between 2 and 12	

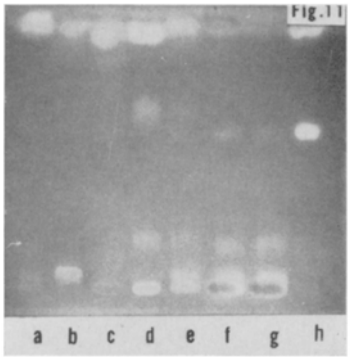

FIG. 11. Chromatogram prepared and developed as in Fig. 10, except that the sample spotted were as those in Fig. 9. Note the presence of materail just ahead of cholesterol and behind the glycerides (which are near the solvent front) in the case of the human scalp surface lipid samples b) and c). (This is better seen in Fig. 12 at higher load levels.) Note the presence of a substance migrating just ahead of cholesterol in the case of the preen gland lipids, namely, chromatogram d). There is also unidentified material at Rf of ~0.3 in this chromatogram as well as in subsequent ones. Again note that the free fatty acids migrate just above the origin in this system.

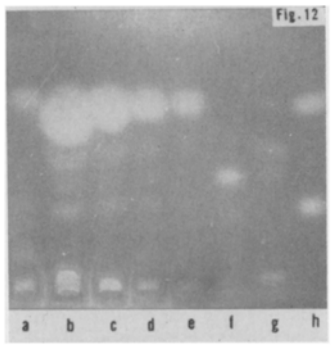

FIG. 12. The plate was prepared as in Fig. 1 and developed with chloroform/methanol 98/2. a) 100 μg of lipid expressed from a large sebaceous gland of the cheek. Note the presence of only what may be a trace of cholesterol and the presence of a large amount of fatty acids near the origin; b), c), d) and e) are 400, 200, 100, and 50 μg respectively of human scalp surface lipid. Chromatogram f) is 20 μg of lanosterol, g) is 20 μg each of a mixture of diglycerides, (a double spot appearing in the upper half of the chromatogram of which the upper spot is 1,3 diglycerides and the lower 1,2 diglycerides), and 20 μg of monoglycerides which appears just off the origin. Chromatogram h) is 20 μg each of cholesteryl palmitate and triglycerides which appear as one spot in this chromatogram, followed by 20 μg of cholesterol. Note that in the surface lipid sample, material appeared in the diglyceride region. The purple background due to the large amount of fatty acid present in these samples masks out to some extent the presence of monoglycerides, although the spot in sample a) just above the origin could well be this material pushed ahead perhaps by the large amount of fatty acid.

petroleum and was believed to be sterol ester. This fraction may have contained wax esters since the authors noted that some peaks obtained during fatty acid analysis by gas chromatography might have been from higher alcohols. Their third fraction (54 mg) was eluted with chloroform and was reported to consist of cholesterol (40 mg), glycerides (78 μmoles glyceride) and free fatty acids. There appears to be too much glyceride in this fraction. Even if it were all monoglyceride with fatty acids averaging 16 carbon atoms ~26 mg would result. Perhaps it should be 7.8 μmoles glyceride instead. Total unsaponifiable matter, obtained by calculation, also seems rather high (~80%) as compared to the other studies of Table V. Their fourth fraction (trace) was eluted with methanol and was believed to be phospholipid.

TLC data (Figs. 1e, 2e, 4e, 7e, 9e, 11e, 13f) indicate the presence of squalene (Fig. 1e), wax esters and sterol esters (Fig. 2e, 13f), triglycerides, free sterol (Fig. 4e, 7e), free fatty acids (Fig. 7e) and at least five unidentified components migrating ahead of and behind triglycerides and free sterols.

Preen Gland Lipids (Goose)

Hou (56) reviewed the very early literature on the lipids of preen glands of birds. Weitzel et al. (21) showed that an acetone extraction of the entire gland yielded about 80% octadecyl esters of optically active saturated branched chain acids. By distillation they found that the main acid was a branched C_{14} acid and the second most abundant acid a branched C_{13} acid.

Murray (22) showed the structures of these esters to be octadecyl 2,4,6,8-tetramethyldecanoate (by far the largest in amount) and octadecyl 2,4,6,8-undecanoate (the next most abundant) and very small amounts of other acids and alcohols. Murray felt that his sampling procedure, which was to squeeze out the contents of excised preen glands with the fingers, would include lipids other than preen gland excretion. (This would also apply to the procedure of Weitzel et al.) Odham (23) proved that this was the case. He collected the excretion daily with a spatula (40 mg/day/bird). He then chromatographed the lipid on silicic acid whereupon 90% of the material was found to be wax ester. Seven other more polar fractions comprising 4% of the sample were also eluted. Nothing further was done with these fractions. Only two fatty acids were found in this highly purified wax ester preparation in contrast to Murray's findings of branched chain acids below C_{14} and above C_{15}, and also palmitic, stearic and C_{18} unsaturated acids. Odham confirmed the structure found by Murray for the principal wax esters by means of mass spectrometry and infrared spectra. The sign and magnitude of the optical rotation of the natural products seemed only to be compatible with optically active centers of D-configuration at positions 2 and 8.

TLC data on preen gland lipids (Figs. 9d, 11d, 13c) are consistent with the above findings that over 90% of the lipid is wax ester. Small amounts of two unidentified components are present, one which migrates ahead of and the other somewhat behind cholesterol (Fig. 11d).

Rat Skin Lipids

Literature. Considerable work has been done with rat skin on the study of sterols and their metabolism. The sterol content of skin tissues has been competently reviewed recently by Kandutsch (57) and will not be treated here in any detail. Biosynthesis of skin sterols will be discussed subsequently (41).

Surface Lipids: Wheatley and James (58) studied the composition of the saponifiable and the unsaponifiable fractions of the surface lipid from the rat, mouse, rabbit, and guinea pig. The total surface lipid was examined for melting point, acid number, iodine value, lipid phosphorus, total nitrogen, fatty acids (nonesterified), fatty acids esterified, unsaponifiables and glycerol. Unsaponifiable matter was examined for hydrocarbon, wax alcohols, alkane, 1,2-diols, cholesterol, lathosterol and other sterols. The fatty acids were analyzed by gas chromatography.

Noteworthy findings were that in contrast to the surface lipids of man, the nonesterified fatty acids were below 10% for all animals (7.4% for the rat) and glycerol was found only in trace amounts. The presence of alkane 1,2-diols for all animals was shown by the formation of formaldehyde (proved by the

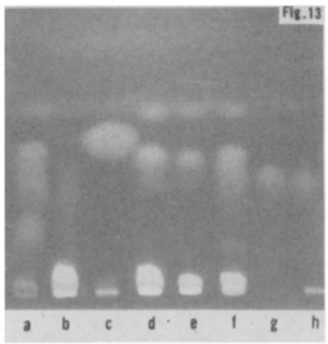

FIG. 13. Thin-layer plate of alumina prepared as described in the text. The developing solvent was hexane/benzene 9/1. a) is 50 μg rat surface lipid. Hydrocarbon is at the solvent front followed by wax esters nearly completely separated from a long beard of sterol esters. Next comes the more polar wax and material at the origin; b) is 50 μg preputial gland lipid. Note that only a minimal amount of wax esters occur. The bulk of the material in this region is sterol ester. c) is 50 μg preen gland lipid. Note that only wax esters and little else occur; d) and e) are 50 and 25 μg, respectively, of human scalp skin surface lipid; f) is 50 μg *vernix caseosa*. Note the squalene spot near the solvent front, a wax ester spot that merges somewhat with a larger sterol ester spot at Rf ~0.7 and a faint unidentified spot of Rf ~0.3; g) and h) are 20 μg each of cholesteryl palmitate and cholesteryl oleate.

dimedone complex) when a chromatographed fraction was oxidized with lead tetra-acetate (47). From the iodine value of the hydrocarbons found, squalene, if present at all, could only occur in trace amounts for all four animals. The wax alcohol content of the unsaponifiable was highly variable, constituting 69% for the rabbit, 11% for the guinea pig and mouse, and 43% for the rat.

From ultraviolet spectra, 7-dehydrocholesterol (provitamin D_3) was present to the extent of 0.01% of the total lipid for the mouse, barely detectable for the rat, and not detectable for either the rabbit or the guinea pig.

The rat had 5.3% cholesterol and 4.1% "fast acting" sterols, mainly lathosterol. The cholesterol content of the other animals was about the same except for the guinea pig which was about 20% of the lipid.

Gas chromatography of the fatty acids showed straight, branched and "highly branched" chains to be present. Mono- and di- unsaturation were also indicated.

Nikkari and Haahti (25) studied the saponifiable and unsaponifiable fraction of rat surface lipid.

TABLE VI

Probable Composition of Various Rat Skin Lipid Samples[a]

	Rat skin surface lipid[b]	Rat preputial gland lipid[b]	Rat total body epidermal lipids[c]
Fraction	%	%	%
1 Saturated hydrocarbons	5	0.1	Trace
2 Squalene	0.5	1.5	0
3 Wax esters (of low polarity) plus sterol esters	40	14	40
4 Lipid staining purple with Rhodamine 6G	~5	0	
5 Wax esters (of high polarity)	25	0	20
6 Triglycerides	7	60	5
7 Free acids	2	2	5
8 Free sterols	5	2	5
Polar lipids	0	9.2	15
Unidentified lipids	11.5	11.2	10

[a] Identification of fractions given in text.
[b] Percentages determined by combined column, paper and thin-layer chromatography. The conditions for column chromatography were as described in the experimental section. The eluting solvent for fraction 1 was n-hexane; fraction 2—10% benzene in hexane; fractions 3 and 4—30% benzene in hexane; fraction 5—60% benzene; fractions 6, 7 and 8—benzene. The polar lipids were determined as described in the experimental section. Paper chromatography was carried out as described in (65).
[c] Composition as estimated visually from TLC plates by comparison of relative intensity of spots to rat skin surface lipid which it resembles. Estimates are made to the nearest 5%.

From 67.1 mg of total lipid, 28.7 mg was fatty acid and 29.2 mg unsaponifiable matter. The free fatty acid of the original sample was only 1% (compare 7.4% found by Wheatley and James) and was not further studied. By means of TLC and gas-liquid chromatography and the Liebermann-Burchard test they found that the unsaponifiable matter consisted of 35.8% monohydric alcohols, 8.8% 1,2-diols and 52.8% sterols of which the latter possibly consisted of cholesterol 27%, lathosterol 39.1%, desmosterol derivatives 14.6%, methosterol 8.7%, agnosterol 7.5% and lanosterol 3.0%. Straight, branched and hydroxy fatty acids were also found. No unsaturates were found among the 1,2-diols. Unsaturation was found in the monohydric alcohols, the hydroxy and the normal fatty acids.

Rat Preputial Gland Lipid: No systematic studies of the lipid classes have been made of this material. Squalene, fatty acid and sterol synthesis has been studied (59–63).

Rat Epidermal Lipid: No systematic studies of the lipids of this tissue have been made. Mouse epidermal lipids have been extensively studied (see 64 and references therein).

TLC Data of Rat Skin Tissue Lipids.

Surface Lipid: Figs. 1a, 2a, 4a, 7a, 9a, 11a, 13a.
Preputial Gland: Figs. 3a, 5a, 6a, 8a, 10a, 13b.
Epidermis: Figs. 3b, 5b, 6b, 8b, 10b.

Besides the TLC data above, column chromatograms and additional analyses have been run on rat skin surface lipids and rat preputial gland lipids. Table VI summarizes available data in the form of probable compositions. The word "probable" is used here since, for the most part, identifications are based on comparisons of migrations on column, paper and thin-layer chromatograms with standard substances. The figures for rat epidermal lipids are estimates based on comparison of the relative intensities of spots on thin layer plates with corresponding spots of rat skin surface lipid. Since whole tissues were extracted in the case of rat epidermis and preputial gland, polar lipids were present.

The material labelled saturated hydrocarbon was eluted from acid silicic acid columns with hexane. It occurred in different column runs in variable amounts up to 8%. The material which was eluted with 10% benzene in hexane migrated by TLC and paper chromatography as squalene. Note that squalene occurs in these tissues in considerably smaller amounts than it does in human scalp surface lipid.

In the column chromatogram of the rat surface lipid and rat preputial gland lipid the wax ester plus sterol ester fraction was eluted as a mixture with 30% benzene in hexane. From the intensity of the sterol ester spot and the wax ester spot just ahead of it for rat surface lipid (Fig. 13a) there appears to be more sterol ester than wax ester in this fraction. Rat preputial gland lipids (Fig. 13b) appear to be mainly sterol esters. The Liebermann-Burchard test for these fractions were atypical of cholesterol (purple to blue to gray) and aliquots of successive tubes for this eluate showed several sterols to be present in both samples.

In the region where wax esters and sterol esters migrate on TLC of the total lipid, all three samples showed a distinct spot (Figs. 2a, 3a, 3b). Another distinct spot (Figs. 2a, 3b) of slightly lower Rf is seen in rat surface and rat epidermal lipid but not in rat preputial gland lipid (Fig. 3a). Material collected from the slower migrating spot gave an infrared spec-

trum indistinguishable from wax ester. Furthermore, absence of bands in the OH absorption regions indicated absence of OH groups. It did not give a Liebermann-Burchard color test which rules out most sterol esters. Saponification and paper chromatography of unsaponifiable matter showed one main spot for wax alcohol. The fatty acids showed two main spots, one corresponding to palmitic acid and one to α-hydroxy palmitic acid. A structure which will account for all these observations is a diester of an α-hydroxy fatty acid in which the carboxyl group is esterified to a wax alcohol and the α-hydroxyl group is esterified to another fatty acid. There is also some evidence for the presence of this type of ester in wool wax (66).

The presence of 1,2-diols in rat surface lipid in the unsaponifiable matter (25) makes it conceivable that other types of diesters may also exist, i.e., both hydroxyl groups of the diol esterified to a fatty acid. Indeed, the spot of the polar wax ester itself (Fig. 2a, 3b) appears to be complex.

Since rat preputial gland lipid does not contain this more polar wax ester, in the rat (as in the human) there are glands of the sebaceous type that synthesize different lipids. There are also differences in the triglyceride content (Table V); the rat preputial gland has more triglyceride than either the rat surface or the rat epidermis. The possible adherence of subcutaneous fat to the whole excised rat preputial gland might account, in part at least, for the large amount of triglyceride found. Other differences in composition are also evident. The functions that such differences can serve are discussed subsequently (41).

Sterols occur in low concentrations in lipids of rat surface and preputial gland but occur in higher concentrations in total epidermal lipids. Thus, in rat skin (as in human skin) it would appear that sterol esters arise primarily in sebaceous glands whereas unesterified sterols are mainly a product of the keratinizing epidermis (41).

The large number of components in these samples, many of which are unknown, is a striking feature. One such component stains purple with Rhodamine 6G. Tocopherols, vitamin K and Coenzyme Q stain similarly but their migrations in TLC are different from the unknown lipid in the rat skin surface. This substance was present in the unsaponifiable matter with unchanged chromatographic migration.

The Polar Lipids of Skin

The hydrolytic technique of Dawson (67) was used in two studies of the phospholipids of the human epidermis. This technique employs mild alkaline and acid hydrolysis of the lipid sample so that water soluble esters of phosphoric acid are formed. All lecithins, for example, would yield glycerophosphorylcholine; all phosphatidyl ethanolamines would yield glycerophosphorylethanolamine, etc. The yield of a particular phosphorus diester is thus a measure of a particular class of phospholipid. These water soluble diesters of phosphoric acid are identified and determined by paper chromatography and phosphorus assay. Bloomstrand (68) determined phosphorus by neutron activation and Gerstein (69) by colorimetry of the eluted spots (Table VII).

By means of paper chromatography Carruthers (70) finds the same classes of lipids (plasmalogen forms not determined) as well as phosphatidic acid. While these indirect studies are suggestive that the substances listed are present, in order to establish their presence definitely additional work needs to be done (isolation and characterization of each lipid class).

TABLE VII

Phospholipids of Human Epidermis

Phospholipid	Percent of total phosphorus	
	Bloomstrand et al. (68)	Gerstein (69)
Phosphatidylcholine[a]	33.76	31.2
Phosphatidylethanolamine[a]	10.60	18
Phosphatidylserine[a]	12.23	13.2
Phosphatidylinositol[a]	12.90	2.8
Phosphatidylcholine[a] plasmalogen	3.83	2.4
Phosphatidylethanolamine plasmalogen		10.0
Phosphatidylserine plasmalogen		1.4
Sphingomyelin	10.75	20.7
Unidentified	15.93	

[a] Includes any of the corresponding lyso compounds if present.

Flesch and Esoda (71) have isolated what they call a glycoproteolipid from human horny matter (callus, nails or scales from patients with scaling dermatoses). Long periods of extraction (3 to 4 days) with ether were used followed by a water wash for 24 hr, then 2 weeks of shaking with absolute alcohol at room temperature). No statements were made as to what precautions were taken to avoid oxidation. The authors state "... since the water soluble components and all the ether soluble fats have been extracted we assume we are dealing with one complex and not with a mixture." It is well known, however, that nonlipid material can easily be extracted with solvents such as alcohol, consequently whether protein, lipid and carbohydrate material are actually in some form of chemical combination has yet to be proved. Furthermore, artifactual material arising from oxidation, polymerization and further reaction of labile phosphatides with other substances must also be ruled out.

The phospholipids of whole rabbit skin were analyzed by Schwartz et al. (72). The main components found were lecithin, phosphatidyl ethanolamine and phosphatidyl serine. Lecithin accounted for 50% of the lipid phosphorus; polyglycerophosphatides, lysolecithin and sphingomyelin were present in appreciable quantities; and traces of two unidentified lipids (one believed to be lysocephalin) made up the remainder.

Carruthers and Heining (73) have analyzed the phosphatides of mouse skin epidermis under normal growth conditions (induced by hair plucking) and under abnormal conditions (where the epidermis was made hyperplastic by application of methyl cholanthrene). The phosphatides from both tissues as well as from tumor tissue itself (induced by the latter procedure) all showed a similar composition as determined by silicic acid column and paper chromatography. The products reported are 1) a cardiolipin-like substance, 2) phosphatidyl ethanolamine, 3) lysophosphatidyl ethanolamine or an oxidized product of phosphatidyl ethanolamine, 4) phosphatidyl inositol, 5) phosphatidyl serine, 6) lecithin, 7) sphingomyelin and unidentified substances.

Two dimensional TLC of the polar lipids of skin has revealed the presence of many components. Their identification is under active investigation at the present time and will be reported in future publications.

ACKNOWLEDGMENTS

This work was supported in part by the following grants: Research Grant No. AM-05120-04 and Training Grant No. 5-TI-AM-5300-04 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service; and U.S. Army Medical Research and Development Command, Department of the Army, Research Contract No. DA-49-139-MD-2184.

Dr. George Rouser provided laboratory assistance; The City of Hope Medical Center provided laboratory facilities.

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Skin Lipids. III. Fatty Chains in Skin Lipids. The Use of *Vernix Caseosa* to Differentiate Between Endogenous and Exogenous Components in Human Skin Surface Lipid

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Abstract

The literature on the types of fatty chains that occur in skin lipids is reviewed and new data are presented.

To ascertain whether certain unusual fatty acids found in human skin surface lipids are truly products of the human skin or are due to some type of external contamination (possibly bacterial), the fatty acids of *vernix caseosa*, (the lipoidal material covering the human fetus) were analyzed and compared to those found in human skin surface lipid. The same unusual fatty acids were found in *vernix caseosa*. This indicates that these acids are products of human skin.

These acids consist of five classes of saturated branched chain acids and of three classes of monoenes: straight chain, *iso* and *anteiso*. All the monoenes are Δ^6 or derivable from this position by addition of an integral number of 2 carbon units to the carboxyl group. On the polar phase diethylene glycol succinate polyester, the saturated branched chain methyl esters have fractional carbon numbers (by gas chromatography) of 0.15, 0.23, 0.45, 0.63 and 0.75. The series at 0.63 and 0.75 are *iso* and *anteiso*, respectively. The series at 0.15 and 0.23 appear to be two newly identified classes of branched chain fatty acids.

I. Types of Fatty Chains that Occur in Skin Lipids

The unusual nature of the fatty acids present in the skin surface lipids first shown by A. W. Weitkamp in two classic investigations (1,2). In the first (1), he determined the structures of 32 fatty acids that occur in degras, a refined wool grease. He distinguished four homologous series by means of a variety of techniques, especially two novel ones that he de-