THE JOURNAL OF INVESTIGATIVE DERMATOLOGY

Vol. 54, No. 6
Printed in U.S.A.

LIPID COMPOSITION OF COMEDONES COMPARED WITH THAT OF HUMAN SKIN SURFACE IN ACNE PATIENTS*

N. NICOLAIDES, PH.D., M. N. A. ANSARI, PH.D., HWEI C. FU, PH.D. AND DONALD G. LINDSAY, M.D.

ABSTRACT

TLC† analysis of comedo lipids from the face, neck, chest and back of acne patients of both sexes, ages 12 to 26 years, (some 65 specimens) gave the same gross composition except for hydrocarbons more saturated than squalene and lipids more polar than free cholesterol. Quantitative data (chromatography plus GLC of isolated fractions) on both comedo and sufrace lipids from each of 3 acne patients revealed the following. Free fatty acids plus triglycerides comprised ~63% of both comedo and skin surface lipids. However, for the comedo, 90% of this sum was free fatty acids compared with only 25% for surface lipid. This implies that triglycerides in comedo lipids are nearly completely hydrolyzed but only 25% hydrolyzed in surface lipids. GLC patterns of the free fatty acids were almost identical for both surface and comedo lipids in all 3 subjects except for slightly more unsaturated acids in surface lipids. For comedo and surface lipids respectively, wax esters were 14% and 24%, sterol esters 4% and 2%, free cholesterol 12% and 2%, and squalene 8% and 9%. Absence of free alcohols and constancy of GLC composition of the entire wax ester fraction indicated it was not hydrolyzed in either surface or comedo lipids. GLC composition of the entire sterol ester fraction from comedones indicated that the fatty acids were derived from epidermis and sebum.

Human skin surface lipid is unusual among natural lipid samples in that it contains a large fraction of free fatty acids. These acids originate from the hydrolysis of triglycerides, which takes place in the sebaceous gland duct and on the skin surface (1).

Several studies have been made to relate this hydrolysis to acne. Strauss and Mescon (2), for example, showed that comedones were capable of hydrolyzing *in vitro* the triglycerides of olive oil. After injecting sebum and comedones (with and without their component free fatty acids) into human dermis, Strauss and Pochi (3) inferred that the inflammatory response produced was largely due to the free fatty acids. Freinkel, Strauss, Yip and Pochi (4) found that after oral administration of tetracycline to individuals with and without acne, the free fatty acids in their surface lipids decreased while the esterified acids increased. Both trends reversed after treatment was stopped. In his docotral thesis, however, Runkel (5) could not show any difference between the acid number of "deep" surface lipids or superficial surface lipids from either normal individuals or those with mild or severe acne. Although the issue is not yet settled, the implication of these and other studies is that the free fatty acid fraction of sebum, if not the causative agent of acne, is involved in some manner.

We undertook this study to define in detail, differences between acne comedo lipids and surface lipids of the same subject. We hoped that comparison of the components present at the lesion site with those of the neighboring skin surface lipids might provide insight into the acne process.

EXPERIMENTAL

Using a comedo extractor, we obtained comedones from some 65 acne patients of both sexes, ages 12 to 26 years. We stored the pooled samples from each individual at 4° C in hexane until we could extract the lipids. Samples sites

This work was supported by Research Grant No. AM-10010 from the National Institute of Arthritis and Metabolic Diseases, U.S.DHEW and the Birely Foundation, Los Angeles, California.

Received October 10, 1969; accepted for publication January 22, 1970.

* From the Department of Medicine, Section of Dermatology of the University of Southern California School of Medicine, 2025 Zonal Avenue, Los Angeles, California 90033.

† Sat. HC, saturated hydrocarbons; TG, triglycerides; FFA, free fatty acids; Me, methyl; TLC, thin layer chromatography; GLC, gas liquid chromatography; J.H., R.C., and E.T. are initials of the 3 subjects from whom specimens were taken.

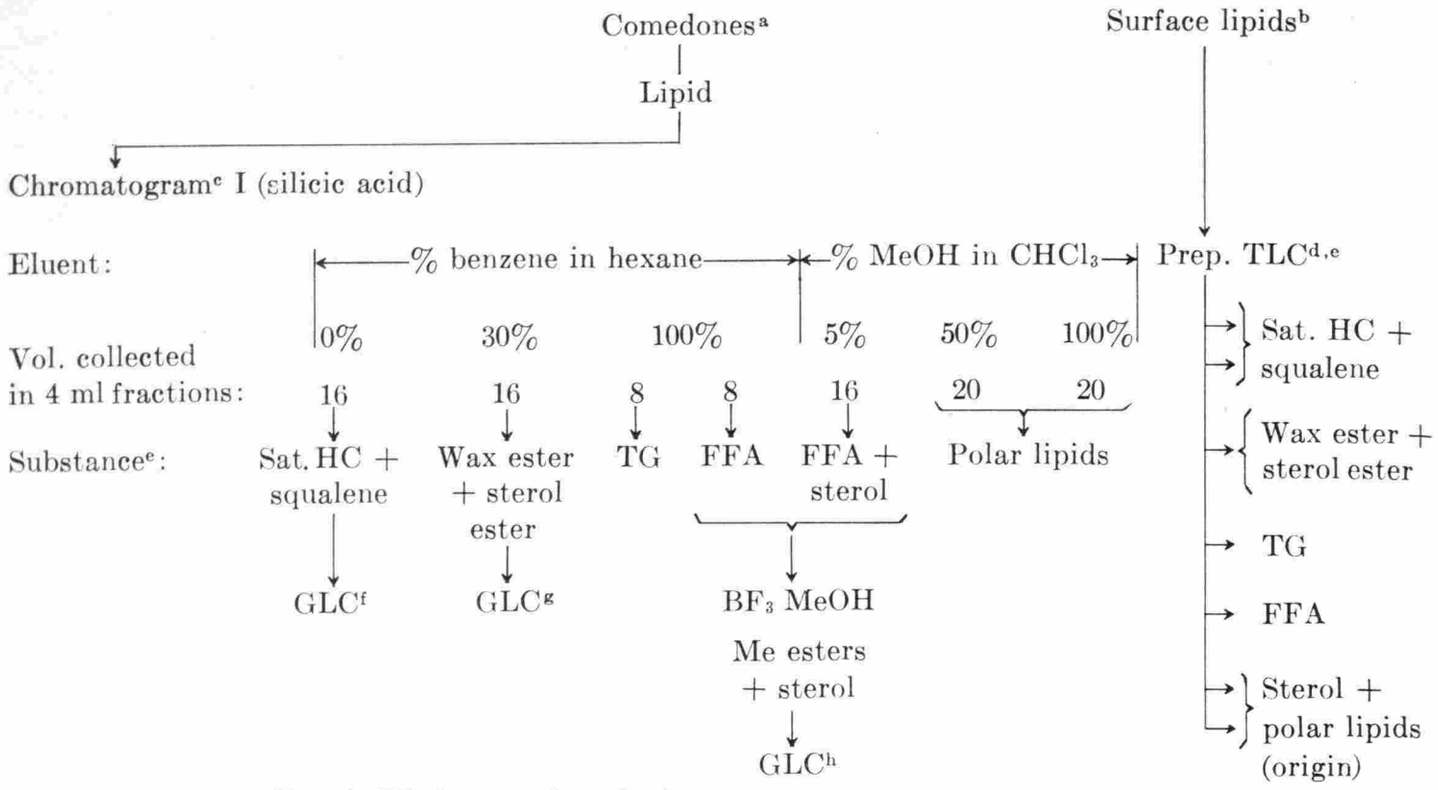

FIG. 1. Work-up and analysis of comedo and skin surface lipids

Footnotes: a. Comedones from each individual were pooled, extracted with chloroform/-methanol 2/1 by volume. Sample weights were computed from the weight (Cahn microbalance) of residue obtained from a volume aliquot of the total sample. Lipids for samples J.H. and R.C., run by column chromatography, were 3.15 mg and 5.80 mg respectively, while those from E.T., run by preparative TLC, were 5.0 mg. All solvents in this study were redistilled.

b. Surface lipids were collected from the area after comedones were removed by wiping the skin surface with fat free cotton balls "moistened" with hexane, then extracting the lipid from the cotton with hexane. Lipids from J.H., R.C., and E.T. were respectively 41.0, 67.1, and 61.0 mg.

c. The adsorbent, 2.05 g. silicic acid (Unisil 100-200 mesh, Clarkson Chemical Co. Inc., Williamsport, Pa.) was packed in a column 0.6 cm i.d. to a height of 14.1 cm. Solvents were changed when material ceased to be eluted.

d. The lipid (~2 mg) was streaked on plates 20 cm x 20 cm (0.25 mm thick) and the plates developed linearly with 3 successive solvents (ref. 6). Streaks, made visible with Rhodamine 6G, were scraped and the scrapings extracted with freshly redistilled ether on a medium sintered glass funnel and the lipid weighed. Separated components were further treated as in chromatogram I.

e. All eluates were weighed (Cahn microbalance) and identified by TLC. Where several components were present, distribution was estimated by TLC densitometry and GLC. For example, sat. HC's and squalene were isolated and weighed as a mixture to an estimated accuracy of at least ±5%. Then several standards containing known amounts of eicosane and squalene in different proportions covering the range of the unknown were spotted on a TLC plate along with the unknown mixture. The proportion of sat. HC to squalene was estimated by visual comparison. We used the same technique to estimate the amounts of FFA and free sterol although the bulk of FFA was weighed as such in an earlier eluate. The relative amounts of wax and sterol esters were determined by GLC as in footnote g.

f. GLC conditions: Beckman GC-4 gas chromatograph, 18″ x ⅛″ o.d. stainless steel column packed with 1.5% OV-101 on Chromosorb G, 100–200 mesh acid washed, DMCS treated (Johns Manville, Manville, N.J.); temp. program from 130 to 270° C in 16 min.; helium flow 35 ml/min. Saturated hydrocarbon standards were obtained from Applied Sciences, Inc., State College, Pa.).

g. GLC as in f except that we used a 12″ x ⅛″ o.d. stainless steel column packed with 1.5% OV-101 on Chromosorb G, and temp. programmed from 240–380° C in 16 min. Sterol ester standards were obtained from Applied Sciences, Inc. and wax esters synthesized by standard techniques. Wax esters greater than C_{42} or sterol esters less than C_{41} do not occur in skin surface lipids or epidermal lipids in significant amounts. Retention time of a C_{42} wax ester is sufficiently less than that of cholesterol myristate (C_{41}) as to allow complete separation of the two ester classes under our operating conditions. Furthermore GLC peak area response per unit weight of both types of ester are approximately equal. Therefore the total area of the peaks of each type of ester gave the relative amount of that ester.

h. GLC as in f except 6′ x ¼″ o.d. stainless steel packed with 3% OV-101 on Gas Chrom Q (Applied Sciences, Inc.) was used, temp. programmed from 180–280° C in 16 min., (helium flow 75 ml/min). Methyl ester standards were obtained from Applied Sciences, Inc. Presence of small amounts of sterol did not interfere with the GLC of fatty acid methyl esters.

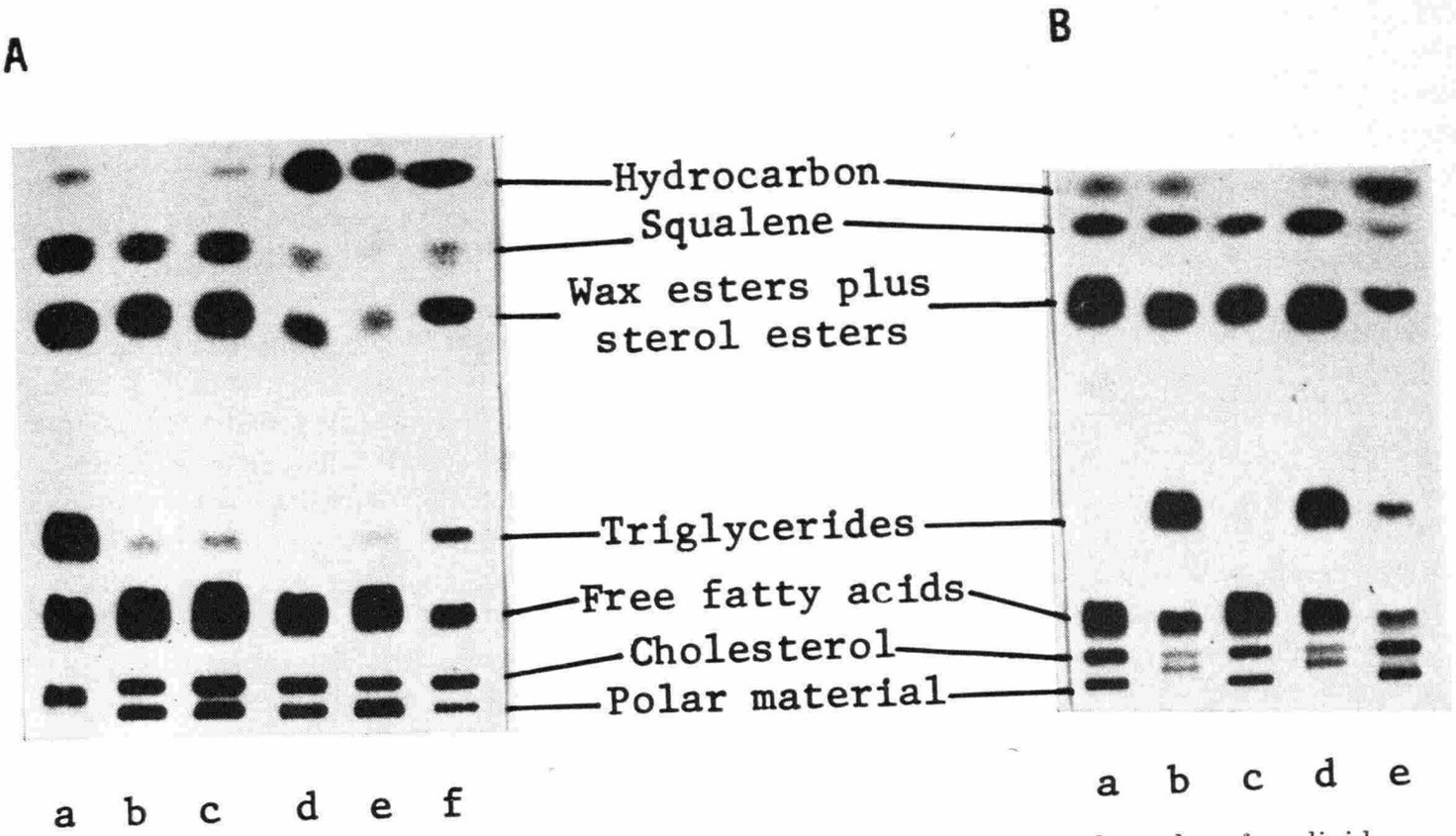

FIG. 2. Photographs of thin layer chromatograms of various comedo and surface lipid samples. Except for standards, the lane farthest right in each plate, 200 μg lipid was applied to each lane.

A. Lanes *a*, *b* and *c* are respectively surface lipids obtained from the back and chest, comedo lipids from the back, and comedo lipids from the chest of a 16 year old boy. Lanes *d* and *e* are respectively lipids from the upper one-third ("tops") and lower two thirds ("bottoms") of the extruded comedo strand from the back of a 19 year old boy. Lane *f* reading from top downward: 20 μg each of eicosane, squalene, cholesteryl oleate, triolein, palmitic acid and cholesterol. Material at the origin is unidentified.

B. Lanes *a* and *b* are comedo and surface lipids respectively from the back of a 20 year old boy. Lanes *c* and *d* are comedo and surface lipids respectively from the back of an 18 year old boy. Lane *e*: standards as in Fig. 2A lane *f*.

TABLE I

Lipid composition of comedones vs skin surface in the same acne patient

Subject, sex, age	J. H., male, 19		R.C., male, 17		E.T., male,16		Averages[a]	
Where taken	Back	Back and face	Face, back and chest	Back	Back	Back		
	Comedo	Surface	Comedo	Surface	Comedo	Surface	Comedo	Surface
	%	%	%	%	%	%	%	%
Saturated hydrocarbons	12	2	2	2	2	2	—	—
Squalene	5	6	7	8	6	11	8	9
Wax esters	6	19	15	22	11	25	14	24
Sterol esters	2	1	3	1	4	2	4	2
Triglycerides	5	51	5	39	5	38	7	47
Free fatty acids	32	10	39	17	51	17	55	16
Free cholesterol	10	1	8	2	9	2	12	2
Polar lipids[b]	28	10	22	9	11	4	—	—

[a] Averages are for the 3 subjects. Saturated hydrocarbons and polar lipids are omitted for reasons described in text.

[b] Material eluting after cholesterol in silicic acid column chromatography or remaining at the origin in TLC; this includes mono- and diglycerides, glyco- and phospholipids, and oxidized squalene products.

TABLE II

Saturated free fatty acids in comedo vs surface lipids in acne patients expressed as percent of total FFA

Carbon number	J.H.		R.C		E.T.		Averages	
	Comedo	Surface	Comedo	Surface	Comedo	Surface	Comedo	Surface
	%	%	%	%	%	%	%	%
12	1.8	1.1	.5	.7	.6	.6	1.0	.8
12.5	.1	.2	.2	.2	.2	.4	.2	.3
12.7	.1	.1	.3	.2	.1	.2	.2	.2
13	.4	.5	.4	.4	.3	.5	.4	.5
13.5	.2	tr	.4	.6	tr	tr	.2	.2
13.6	.3	.3	.7	1.3	.3	.2	.4	.6
14	8.6	11.5	8.5	10.7	6.5	9.0	7.9	10.4
14.5	.5	.7	.8	1.1	1.2	1.2	.8	1.0
14.7	.9	1.1	1.2	2.1	1.2	1.4	1.1	1.5
15	7.3	7.0	8.6	6.6	5.0	6.4	7.1	6.7
15.5	.3	.3	.2	.5	.5	.5	.3	.4
15.6	1.0	1.0	2.7	3.2	1.0	1.0	1.6	1.7
16	31.8	27.9	33.0	23.5	28.6	27.3	31.1	26.3
16.5	1.1	1.1	1.1	1.8	1.5	2.0	1.2	1.6
16.7	.6	.5	.6	.9	.5	.8	.6	.7
17.0	2.6	1.5	2.1	1.6	1.8	1.8	2.1	1.6
17.6	.2	.3	.6	.7	.1	.2	.3	.4
18	6.9	3.8	4.6	3.4	4.9	3.8	5.5	3.6
18.7	.2	.1	tr	tr	.1	.1	.1	.1
19	.6	.3	.2	.2	.6	.3	.5	.3
19.6	.1	.2	.1	.1	.1	.1	.1	.1
20	.7	.6	.7	.5	1.2	1.0	.9	.7
20.7	tr	tr	.1	tr	.1	tr	.1	tr
21	.1	tr	.1	.1	.3	.1	.2	.1
21.6	tr	.1	.1	.2	tr	tr	.1	.1
22	.7	.8	.6	.5	1.2	1.0	.8	.8
22.7	tr	.1	tr	.1	.3	tr	.1	.1
23	.2	.3	.2	.1	1.1	.3	.5	.2
23.6	.2	.1	.3	.4	.5	.3	.3	.3
24	1.3	1.4	1.0	1.6	3.9	2.2	2.1	1.7
24.7	.1	.1	.1	.3	1.1	.5	.4	.3
25	.4	.4	.3	.5	1.3	.5	.7	.5
25.6	tr	tr	.1	.3	.3	.2	.1	.2
26	.4	.5	.3	.6	1.5	1.0	.7	.7
27	.1	tr	tr	tr	tr	tr	tr	tr
27.6	—	tr	—	tr	—	tr	—	tr
28	.1	.2	.1	.3	.2	.2	.1	.2
30	.1	.2	.1	.3	.2	.2	.1	.2
Totals	70.0	64.3	70.9	65.6	68.3	65.3	69.9	65.1

were face, neck, chest, and back, and in some cases we separated the strand of extruded comedo contents into "tops" (upper one third) and "bottoms" (lower two thirds). Our subjects understood the general nature of this study and agreed not to use cosmetics or topical preparations.

By methods already described (6), we analyzed all samples routinely by TLC. In order to compare in detail the lipid components from comedones with those of skin surface from the same area, we took these samples from three caucasian males, ages 16, 17 and 19 years, and analyzed them by the scheme outlined in Figure 1.

RESULTS

The comedo lipids of all the specimens gave, by TLC, the same gross composition regardless

of sex, age or anatomical site, except for variations in the amount of hydrocarbons more saturated then squalene (Fig. 2). Comparison of comedo lipids with surface lipids from the same general area showed a striking difference in the relative amount of triglycerides and of free fatty acids. The usual human skin surface lipid sample has a small, variable amount of hydrocarbon more saturated than squalene. Most evidence indicates that these hydrocarbons are not endogenous (7–10). Despite all precautions taken to avoid such substances, including sampling from the back, we always found some of these hydrocarbons for both comedo and surface lipid (Fig. 2). Table I gives the total composition of comedo and surface lipids for 3 individuals. Note that for comedo lipids the polar lipids are variable in amount and appreciably higher than those of surface lipids. These polar lipids were unidentified except for small amounts of mono and diglycerides. We estimate the sum of the mono and diglycerides to be no greater than 1% for comedo lipids and 5% or less for surface lipids of these individuals. To compare only those components that were identified, we computed an average percentage composition for the 3 subjects and omitted all polar lipids and the variable amounts of saturated hydrocarbons (see column marked "averages" Table I).

Tables II and III present detailed analyses by GLC of the free fatty acids of both comedo and surface lipids from the 3 subjects of Table I. Table II gives the chain distribution of the saturated acids and Table III the unsaturated acids, and all are summarized in Table IV.

Table V gives GLC data for sterol and wax

TABLE III

Unsaturated free fatty acids in comedo vs surface lipids in acne patients expressed as percent of total FFA

Carbon Number	J.H.		R.C.		E.T.		Average	
	Comedo	Surface	Comedo	Surface	Comedo	Surface	Comedo	Surface
	%	%	%	%	%	%	%	%
12	—	—	tr	—	—	—	—	—
12.7	tr	tr	tr	tr	tr	tr	tr	tr
13	tr	.1	tr	tr	tr	tr	tr	tr
13.6	.1	tr	.1	.3	tr	tr	.3	.1
14	1.0	1.5	.8	.6	1.4	1.0	1.1	1.0
14.7	.2	.5	tr	.3	tr	.4	.1	.4
15	.5	1.1	.1	1.2	.9	1.2	.5	1.2
15.6	.4	.5	1.7	1.4	.1	.6	.7	.8
16	13.2	16.1	12.5	15.3	14.7	16.4	13.1	16.0
16.7	.4	1.1	.9	.3	.7	.8	.7	.7
17	1.7	2.0	1.7	1.9	1.9	1.8	1.8	1.9
17.6	.9	1.0	1.1	.6	.7	.8	.9	.8
18	10.0	10.1	9.7	10.3	9.4	9.9	9.7	10.1
18.7	tr	tr	tr	tr	tr	tr	tr	tr
19	.2	.2	.1	.2	.3	.1	.2	.2
19.6	.3	tr	tr	.8	.5	.2	.3	.3
20	.6	.6	.1	.5	.6	1.3	.4	.8
20.7	tr	tr	tr	tr	tr	tr	tr	tr
21	tr	tr	tr	.1	tr	tr	tr	tr
21.6	tr	tr	tr	tr	tr	tr	tr	tr
22	.1	.1	.1	tr	.2	.1	.1	.1
22.7	tr	tr	tr	tr	tr	tr	tr	tr
23	.1	.1	tr	tr	tr	tr	tr	tr
24	.1	.3	.3	.1	.3	.1	.2	.2
25	.1	.4	tr	.4	tr	tr	tr	.3
26	.1	tr	tr	.1	tr	tr	tr	tr
Totals	30.0	35.7	29.1	34.4	31.7	34.7	30.1	34.9

TABLE IV

Summary of chain types of comedo and surface lipid free fatty acids in acne patients[a]

	Saturated		Unsaturated		Total sat. + unsat.	
	Comedo	Surface	Comedo	Surface	Comedo	Surface
Even chains[b]	50.2	45.5	24.6	28.2	74.8	73.7
Odd chains[c]	11.5	9.8	2.5	3.6	14.0	13.4
Iso chains[d, f]	2.9	3.4	2.2	2.0	5.1	5.4
Anteiso chains[e, f]	2.6	2.9	.8	1.1	3.4	4.0
Other branched[f]	2.7	3.5	—	—	2.7	3.5
	69.9	65.1	30.1	34.9	100.0	100.0

[a] Data from averages of Tables II and III.

[b] Chains with an even number of C-atoms.

[c] Chains with an odd number of C-atoms.

[d] Chains with an additional methyl group 1 C-atom from the methyl end of the fatty chain.

[e] Chain with an additional methyl group 2 C-atoms from the methyl end of the fatty chain.

[f] Structure determined from GLC retention data only.

esters, chromatographed as such, for comedo and surface lipids of the same 3 subjects. For comparative purposes, sterol ester data from epidermal cyst contents are also included. Such material provides a sample similar in lipid composition to that of normal skin epidermis but not contaminated with sebum (11). This sample also has no wax esters. The carbon numbers of the sterol esters and wax esters represent the sum of the carbon atoms of the fatty acid and the fatty alcohol (or sterol) portion of the ester. For these high molecular weight esters differences in saturation, unsaturation or branching were not detectable. Thus, for the wax esters, a carbon number of C_{36} would include all C_{16} acids esterified to all C_{20} alcohols (either portion of which could be saturated, unsaturated and/or branched), plus any other combination of acid and alcohol to give C_{36}. The same applies to the sterol esters. Since cholesterol (C_{27}) makes up by far the largest proportion of the sterols, variations in carbon number of these esters represents variations in the chain lengths of the fatty acid portion.

DISCUSSION

The strikingly large amount of free fatty acids in comedo lipids compared with surface lipids—visually apparent in TLC (Fig. 2A and B) and shown quantitatively in Table I—is in marked contrast to the relative amounts of the triglycerides in these samples. Triglycerides are in a directly opposite relationship, i.e. they are very low in comedo lipids but constitute the major component of surface lipids. Note, however, that the sum of the triglycerides plus free fatty acids for both comedo and surface lipids are nearly the same (~63%). These results imply that triglyceride hydrolysis to free fatty acids is only about 25% complete on the skin surface but nearly 90% complete in the comedo. Although we have shown earlier that the longer the surface lipids remain on skin the greater the hydrolysis of the triglycerides (1), the main message to be emphasized here is that in the comedo this hydrolysis is practically complete. This is in contrast to many other enzymatic processes where the reaction products inhibit further reaction. Furthermore this hydrolysis has gone more to completion in the "tops" of the extruded comedo strand, than in the "bottoms" (see Fig. 2A lanes *d* and *e*). This is what one might expect if the "tops" represent older formed material than the "bottoms." The free fatty acids appear to be derived *only* from the triglycerides and not from the wax esters (the other major ester class in these samples) since the wax esters are not hydrolyzed (see below).

Free fatty acids produce an inflammatory response when injected into dermis (3). Large amounts of potentially inflammatory agents within the small confines of the comedo, support the view that the free fatty acids participate in the acne process. However, large amounts of fatty acids are also present in the comedones of people without acne (12). It would thus appear that the acne prone person cannot tolerate this for some unknown reason.

It appears that only man among the animals has triglycerides in his surface lipids (6), and only man seems to have acne. Animals have mainly sterol esters and wax mono and diesters, and these are not hydrolyzed to any appreciable extent. Free fatty acids occur in very small amounts, if at all, on the skin surfaces of most animals (6).

Detailed GLC examination of the free fatty acids of both comedo and surface lipids gave very similar patterns, not only for the same subject but for all 3 subjects of this study (Tables II, III and IV). However, there was a small but

TABLE V

Wax and sterol esters in comedo and surface lipids of acne patients

Carbon No.[a]	J.H.		R.C.		E.T.		Averages		Epidermis[b]
	Comedo	Surface	Comedo	Surface	Comedo	Surface	Comedo	Surface	
Sterol esters									
41	10.8	12.6	12.8	14.7	9.8	18.2	11.1	15.2	1.7
42	3.6	5.0	11.8	3.5	3.1	7.5	6.2	5.3	2.5
43	40.5	67.7	53.0	62.1	54.8	61.0	49.3	63.6	15.2
44	2.5	3.5	.3	2.8	2.5	tr	1.8	2.2	4.6
45	38.7	8.8	21.0	13.1	23.9	12.0	27.6	11.3	72.8
46	2.4	tr	1.1	1.5	1.5	tr	1.7	.5	3.0
47	1.5	2.1	tr	2.3	4.4	1.3	2.3	1.9	.2
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Wax esters									
28	1.0	.8	.6	.6	2.2	1.7	1.3	1.0	
29	.8	.8	.5	.4	1.1	1.1	.8	.8	
30	4.1	3.9	3.2	2.3	4.9	6.6	4.1	4.3	
31	3.7	2.8	2.2	2.0	3.9	3.1	3.3	2.6	
32	7.7	6.9	7.8	7.6	11.2	11.2	8.9	8.6	
33	7.3	3.7	5.9	5.7	6.2	6.6	6.5	5.3	
34	12.9	14.9	13.9	15.0	13.6	13.7	13.5	14.5	
35	8.4	8.3	10.2	9.0	8.0	7.7	8.7	8.5	
36	21.0	25.5	22.9	28.1	19.1	18.2	21.0	23.9	
37	6.5	7.5	7.0	7.3	6.7	6.0	6.7	6.9	
38	11.7	12.2	13.1	11.7	9.1	10.9	11.3	11.6	
39	5.1	3.9	4.1	4.1	3.6	3.2	4.3	3.7	
40	6.2	7.5	5.5	4.8	7.1	6.8	6.3	6.4	
41	1.5	.5	1.5	.5	1.1	1.5	1.4	.8	
42	2.1	.8	1.6	.9	2.2	1.5	1.9	1.1	
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	

[a] Represent total number of C-atoms in the alcohol (or sterol) plus the fatty acid moieties. Additional structural details such as unsaturation and/or chain branching, undoubtedly present in the alcohols and fatty acids, was not determined.

[b] Sterol esters obtained by column chromatography (similar to that described in Fig. 1) of a sample of lipids from epidermal cyst wall (11). No wax esters were detected.

higher percentage of unsaturated fatty acids in surface lipids compared with comedo lipids for all 3 subjects (Table IV).

Histologic studies show large amounts of epidermal cells in comedones (13). Therefore, comedo lipids will have components from epidermis as well as from sebum. Major components of epidermal lipids are free cholesterol and polar lipids (14, 15). Thus, in comparing comedo lipids with surface lipids (Table I) the relative amounts of wax esters (products of sebum only), free cholesterol and polar lipids reflect the dilution of sebum lipids with epidermal lipids. In surface lipids, a mixture also derived from epidermal lipids, the latter make a considerably smaller contribution at the acne sites.

Comedo lipids have a small but significantly higher sterol ester content than surface lipids (Table I). Enzymatic esterification of free cholesterol could account for this, for Freinkel showed that epidermis and material on the skin surface can incorporate labeled sterol or acid into the

sterol ester fraction (16). Fatty acids for this esterification could come either from sebum or epidermis or both. Wilkinson recently showed that at least some of the unsaturated fatty acids of the sterol esters of surface lipids were of the sebum type (17), but Kooyman much earlier showed that some sterol esters are formed entirely from fatty acids of epidermis (18). Our data support a dual origin for these fatty acids. In Table V peaks with carbon numbers 43 and 45 are mainly cholesterol esterified to the C_{16} and the C_{18} fatty acids respectively. For epidermis the ratio of C_{18} to C_{16} esters is 4.8, whereas for surface lipids it is 0.18. For the comedo the ratio is between these extremes i.e. 0.56, which would result if sebum provided more of the fatty acids than epidermis. Since the iodine value of sterol ester fatty acids is nearly twice that of triglyceride fatty acids of surface lipids (8), unsaturated fatty acids are preferentially utilized in sterol ester synthesis. If these unsaturated fatty acids are taken from the free fatty acid pool of the comedo, then this could account for the relatively lower amount of unsaturated free fatty acids we found in comedo lipids compared with those of surface lipids.

The wax esters appear not to be affected by all this esterase activity, for both comedo and skin surface lipids show nearly identical wax ester compositions. Hydrolysis of comedo wax esters is extremely low, if it occurs at all, since material migrating where free fatty alcohols would migrate in TLC, if consisting entirely of free fatty alcohols, could be at the most only 0.3% of the sample.

If epidermal lipids dilute sebum lipids in the comedo, the squalene content of comedo lipids would be lower than that of surface lipids, but apparently this is not so (Table I). Perhaps the reason is that squalene on the skin surface is far more exposed to atmospheric oxidation than squalene encapsulated in the comedo. In our TLC systems oxidized squalene produces considerable amounts of polar lipid. Interestingly, only those surface lipid samples that show low squalene values have higher polar lipid values.

The variable amounts of hydrocarbons more saturated than squalene found in comedo lipids need explanation. One possibility is that they are synthesized by bacteria from free fatty acids as is done by the widespread *Sarcina lutea*. Mechanisms proposed for this synthesis would require that the distribution of hydrocarbons be related in some manner to the chain distribution of the free fatty acids used (19, 20). We found no obvious relationship when we compared the hydrocarbons with the free fatty acids of the comedones from each of our 3 subjects. Each hydrocarbon fraction was exceedingly complex (GLC showing more than 75 peaks which ranged from C_{14} to C_{45}), none of the GLC patterns resembled each other, and no peak in any of the GLC patterns was especially prominent. In marked contrast (as noted above) the fatty acids of all three samples showed very definite and nearly identical distributions in which $C_{16} \gg C_{18} > C_{14}$ (Tables II and III). Thus it appears unlikely that these hydrocarbons were derived from a *S. lutea* type of synthesis. They are in all probability derived from petroleum products of the environment.

REFERENCES

1. Nicolaides, N. and Wells, G. C.: On the biogenesis of the free fatty acids in human skin surface fat. J. Invest. Derm., *29:* 423, 1957.
2. Strauss, J. S. and Mescon, H.: The chemical determination of specific lipases in comedones. J. Invest. Derm., *33:* 191, 1959.
3. Strauss, J. S. and Pochi, P. E.: Intracutaneous injection of sebum and comedones. Arch. Derm., *92:* 443, 1965.
4. Freinkel, R. K., Strauss, J. S., Yip, S. Y. and Pochi, P. E.: Effect of tetracycline on the composition of sebum in acne vulgaris. New Eng. J. Med., *273:* 850, 1965.
5. Runkel, R. A.: The chemical composition of normal and acne-skin lipids from humans. Doctoral Thesis, Dept. of Health Sciences, Pharmacy, Univ. of Wisconsin, 1967.
6. Nicolaides, N., Fu, H. C. and Rice, G. R.: The skin surface lipids of man compared with those of eighteen species of animals. J. Invest. Derm., *51:* 83, 1968.
7. Rothman, S.: Pp. 312–314, 329, *Physiology and Biochemistry of the Skin.* University of Chicago Press, Chicago, 1954.
8. Nicolaides, N. and Foster, R.: Esters in human hair fat. J. Amer. Oil Chem. Soc., *33:* 404, 1956 and references quoted there.
9. Nicolaides, N.: Chapter XI, Human skin surface lipids—Origin, composition, and possible function. *Advances in the Biology of Skin*, Vol. 4, *The Sebaceous Gland.* Eds. Montagna, W., Ellis, R. A. and Silver, A. F. Pergamon Press, Oxford, 1963.
10. Lewis, C. A. Hayward, B. J. and McKenna, R. M. B.: Saturated hydrocarbons in skin surface lipids. Brit. J. Derm., *77:* 303, 1965.
11. Nicolaides, N., Levan, N. E. and Fu, H. C.: The lipid pattern of the wen (Keratinous cyst of the skin). J. Invest. Derm., *50:* 198 1968.
12. Nicolaides, N.: Skin lipids. IV. Biochemistry and function. J. Amer. Oil Chem. Soc., *42:* 708, 1965.
13. Strauss, J. S. and Kligman, A. M.: The patho-

logic dynamics of acne vulgaris. Arch. Derm., *82:* 171, 1960.
14. Nicolaides, N.: Skin lipids. II. Lipid class composition of samples from various species and anatomical sites. J. Amer. Oil Chem. Soc., *42:* 691, 1965.
15. Nieminen, E., Leikola, E., Koljonen, M., Kiistala, U. and Mustakallio, K. K.: Quantitative analysis of epidermal lipids by TLC with special reference to seasonal and age variation. Acta Dermatovener., *47:* 327, 1967.
16. Freinkel, R. K. and Aso, K.: Esterification of cholesterol in the skin. J. Invest. Derm., *52:* 148, 1969.
17. Wilkinson, D. I.: Variability in composition of surface lipids. The problem of the epidermal contribution. J. Invest. Derm., *52:* 339, 1969.
18. Kooyman, D. J.: LXI. Lipids of the skin. Some changes in the lipids of the epidermis during the process of keratinization. Arch. Derm. Syph., *25:* 444, 1932.
19. Albro, P. W. and Dittmer, J. C.: The biochemistry of long chain, nonisoprenoid hydrocarbons. Biochemistry, *8:* 953, 1969.
20. Albro, P. W. and Dittmer, J. C.: Bacterial hydrocarbon: Occurrence, structure and metabolism. Lipids. In press. Presented at the 60th Annual Meeting of the American Oil Chemists' Society, April 20–24, 1969.