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Investigation of Normal and Acne Skin Surface Lipids

RICHARD A. RUNKEL, DALE E. WURSTER, and GARRETT A. COOPER

Abstract □ Human skin surface lipid samples from both acne patients and normal individuals were examined with respect to both total acidity and the detailed composition of the free acid fraction. On the basis of this study, there is no apparent relationship between the total acidity of the surface lipid and the severity of the acne condition, nor is there a gross difference in the composition of the free acid fraction between the normal and the acne condition. *Corynebacterium acnes* has been cultured in a medium containing certain glycerides normally found in sebum and its esterase activity established.

Keyphrases □ Skin surface lipids—analysis □ Acne, normal skin—surface lipid acidity □ Microbiological analysis—artificial sebum □ GLC—analysis □ IR spectrophotometry—analysis

The composition of the mixed free fatty acid fraction of lipids from both the surface of human skin and the hair has been studied using a gas-liquid chromatography technique (1, 2). In 1959 Bougton *et al.* considered the possibility that the composition of the skin lipid might be different in the normal and acne conditions and, therefore, studied the total fatty acid mixture (3). They concluded that there was no important difference between the mixture of acids (free and esterified) found on normal skin and those of acne skin. The concept persisted, however, that the free acids might play a role in the inflammation associated with the acne lesion when the follicular contents are discharged into the dermis. Indeed, Strauss *et al.* indicated this to be the case (4, 5). It then became important to know whether the composition of just the free acid fraction is different in the acne state, and if so, the manner in which it differs. In this investigation the detailed composition of the free acid fraction has been studied and the results obtained with both normal and acne lipid samples compared. The relationship of the acid to ester fractions was followed with the aid of IR spectroscopy. An attempt was also made to determine if there was a relationship between the total acidity (acid number) of the lipid and the severity of the acne condition.

The presence of acne bacillus in and on the skin was established in 1911 (6). Subsequently this anaerobic bacteria was designated as *Corynebacterium acnes* (7). In 1946 Douglas and Gunter (8) suggested that it should more appropriately be called *Propionbacterium acnes.* General esterase activity on the glycerides of sebum present in and on the skin has been shown (9, 10); however, the current work shows specifically that *C. acnes* is capable of causing hydrolysis when cultured in the presence of glyceride esters.

EXPERIMENTAL

Materials and Apparatus—All of the solvents and chemicals used were analytical reagent grade. The ethyl ether was purged before using by bubbling nitrogen gas through it. The standard methyl esters were prepared from standard free acids (Eastman Organic Chemicals). The diazomethane was prepared with *N*-methyl-*N*-nitroso-para-toluenesulfonamide[1] (11). The IR spectra were run on a spectrophotometer (Beckman model I.R. 5A). The GLC work was performed on an Aerograph (model 204-B) equipped with dual columns, a dual-flame ionization detector, and a linear temperature programmer. A recorder (Leeds and Northrup Speedomax H) was also used.

The Lipid Samples—The lipid samples were obtained from human volunteers[2] having skin conditions classified as normal, mild, moderate, and severe acne. The patients were considered untreated if they had not received topical applications of any kind, or the systemic administration of antibiotics, sulfas, or steroids. The "deep" lipid was obtained 30 min. after the first collection and is assumed to be essentially the material in the follicles (12, 13). A series of collections was made and pooled for each of the four categories. Another series was also collected but here the sample from each individual was labeled and maintained separate. The

[1] Diazald, Aldrich Chemical Co., Milwaukee, Wis.

[2] The authors are indebted to Dr. Eli Packman of the Philadelphia College of Pharmacy and Sciences, Philadelphia, Pa., for providing a large portion of the lipid samples.

collection procedure was as follows: the forehead and nose were washed with copious amounts of distilled water and dried. The washed area was then swabbed with ether-soaked cotton which had previously been extracted with ether in a continuous extraction apparatus. The cotton containing the lipid was then extracted with ether in a continuous extraction apparatus for 4 to 6 hr. The ether solution was concentrated to 150 ml. by distilling off most of the ether at 35°. Final traces of the solvent were removed at room temperature under a stream of nitrogen gas. The lipid samples were then stored under nitrogen in a refrigerator (12).

Extraction of the Free Fatty Acids—The whole lipid was extracted with 1.0 *N* KOH in methanol at room temperature. The KOH solution was in contact with the whole lipid for a period not exceeding 1 min. Samples of 20 to 50 mg. were commonly extracted. The methanol was then removed in a rotating vacuum evaporator at 30°. The potassium salts of the mixed acids were taken up in distilled water and the solution acidified. The mixed free acids were extracted into carbon tetrachloride, and the solvent removed at room temperature under a stream of nitrogen gas. The free acids represented from 20 to 40% of the weight of the whole lipid sample (14). A typical sample of 40.5 mg. whole lipid yielded 10.3 mg. of free acids. The samples of free acids were esterified using diazomethane and analyzed as the methyl esters. All of the samples of mixed methyl esters were analyzed by GLC. Two different columns were used: one column was 2.13 m. (7 ft.) long, 0.31 cm. (0.125 in.) inner diameter, packed with acid-base washed, dimethyl chlorosilane (Chromosorb W), treated and coated with 20% butanediol succinate polyester.[3] The injector block and the detector-oven temperatures were held at 275° and the column oven was temperature programmed from 160 to 220° at a rate of 4°/min. The carrier gas flow rate was 30 ml./min.; a second column of identical dimensions, but with the solid support coated with 3% grease (Apiezon L) was subsequently employed. This column was temperature programmed from 200 to 275° at a rate of 4°/min. The injector block and detector-oven temperatures were held at 325° and the carrier gas flow rate was 30 ml./min. Samples of 3 to 6 mcg. were normally injected. The linear range of the instrument was verified by using a methyl palmitate standard.

Identification of Peaks—The major peaks were tentatively identified by using log retention time *versus* carbon-chain length plots as shown in Fig. 1, analysis on polar and nonpolar stationary phases (15), hydrogenation of the methyl ester sample, and the comparison of unknown peaks with the retention times of standards. The peak areas were determined and the major acids reported as the percent

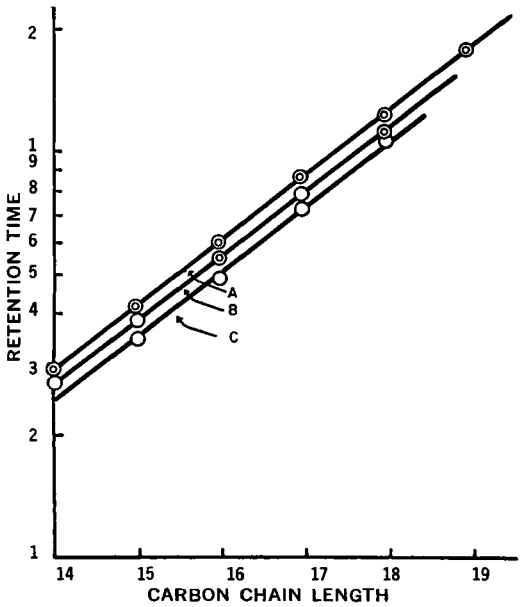

Figure 1—*Log retention time* versus *carbon number plot for normal skin lipid sample. Small circles indicate standard acids. Key: A, normal saturated series; B, normal unsaturated series; C, branched saturated series. Sample 140-D, normal.*

[3] Wilkins Instrument and Research Inc., Walnut Creek, Calif.

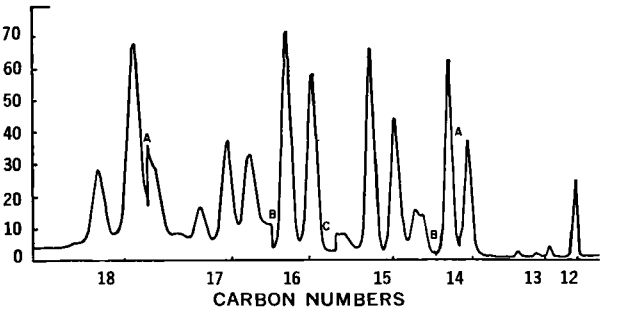

Figure 2—*Gas chromatogram of free fatty acids from mild acne. (sample W.K., mild acne, Apiezon L column, temperature program, 200 to 255° at 2°/min.)*

of the total peak area. A sample chromatogram, Fig. 2, illustrates the good separation achieved. Each sample was analyzed at least three times on both columns. Duplicate free acid extractions, methylation, and analysis were performed on most of the samples to establish the reproducibility of the extraction procedure.

Bacterial Cultures—Bacterial cultures containing broth and artificial sebum were employed to study the possible esterase activity of both *C. acnes* and *S. albus*.[4] The artificial sebum was a solid mixture containing 28.5% free fatty acids ranging from C_7 to C_{22}, 26% of nonsaponifiable matter consisting of squalene, cholesterol, paraffin, and long-chain alcohols, and 45.5% of glycerides (mono-, di-, and tristearins). This artificial sebum had an acid number of 56.6, a saponification number of 153.5, and an iodine number of 64.3. These values were determined using the methods described in USP XVII. The test samples employed were as follows (10 ml. per tube):

I Uninoculated controls (triplicate tubes)
- (*a*) Eugon broth
- (*b*) Eugon broth, 0.25% sebum
- (*c*) Eugon broth, 0.25% sebum, 1% keratin (on weight of sebum)
- (*d*) *Staphylococcus albus* (*S. albus*) in sterile distilled water

II Inoculated samples (triplicate tubes)
- (*a*) Eugon broth, *S. albus*
- (*b*) Eugon broth, *S. albus*, 0.25% sebum
- (*c*) Eugon broth, *S. albus*, 0.25% sebum, 1% keratin (on weight of sebum)

III Uninoculated controls (triplicate tubes)
- (*a*) Evans broth
- (*b*) Evans broth, 0.25% sebum
- (*c*) Evans broth, 0.25% sebum, 1% keratin (on weight of sebum)
- (*d*) *C. acnes* in sterile distilled water

IV Inoculated samples (triplicate tubes)
- (*a*) Evans broth, *C. acnes*
- (*b*) Evans broth, *C. acnes*, 0.25% sebum
- (*c*) Evans broth, *C. acnes*, 0.25% sebum, 1% keratin (on weight of sebum)

The *S. albus* cultures were incubated aerobically for 48 hr. at 37° then refrigerated. The *C. acnes* cultures were incubated under an atmosphere of 95% nitrogen and 5% carbon dioxide for 96 hr. at 36° and then refrigerated. The aqueous broth was extracted with carbon tetrachloride at the end of the incubation period. The solvent was then removed in the same manner as shown previously and the IR spectra obtained.

RESULTS AND DISCUSSION

Free Fatty Acids—The data on the composition of the free fatty acids in the samples studied are divided into four main categories: normal, mild, moderate, and severe acne. The data from pooled samples are reported in Table I. In Table II the data from individual samples are shown. The major acids reported are in agreement with

[4] The authors are indebted to the Vick Chemical Co., Mount Vernon, N. Y., for providing the bacterial cultures.

Table I—Percentage Distribution of Free Fatty Acids in Human Skin Surface Lipid

Sample, Acid	Pooled Samples 7.5[a]	8	9	10	12	14[b]	14[1c]	14	15[b]	15[1]	15	16[b]	16[1]	16	17[b]	17[1]	17	18[b]	18[1,2]	18
159-A					0.8	—[d]	2.4	8.6	1.2	3.4	4.5	0.6	20.6	26.3	3.1	3.1	1.2	1.6	16.4	6.1
173-A					0.9	—	2.0	7.1	0.9	3.2	4.9	0.6	20.0	26.2	2.4	3.1	1.3	1.5	18.8	7.1
140-D		5.7	8.1	—	2.3	—	2.4	8.0	1.4	3.4	5.1	0.8	16.0	20.5	3.1	2.9	1.3	1.4	12.3	5.2
Av. normal				—	1.3	—	2.3	7.9	1.2	3.3	4.8	0.7	18.9	24.3	2.9	3.0	1.3	1.5	15.8	6.1
140-A	2.5	—	13.0	—	0.9	—	2.0	7.2	0.8	2.8	4.2	0.5	18.1	23.2	2.5	2.7	1.1	1.1	12.7	4.8
159-B, deep			0.5	—	1.1	—	2.1	7.8	0.7	2.9	4.0	0.5	21.7	27.6	2.2	2.4	0.9	1.5	17.8	6.4
173-B					1.4	—	2.3	7.5	0.9	2.9	4.3	0.5	18.6	25.0	2.5	2.8	1.2	1.8	20.4	7.9
159-E			10.9	0.7	1.4	—	2.4	7.4	0.9	3.3	4.4	0.7	17.6	22.8	2.8	2.9	1.2	1.2	14.2	5.2
Av. mild acne		—	6.1		1.2	—	2.2	7.5	0.8	3.0	4.2	0.6	19.0	24.6	2.5	2.7	1.1	1.4	16.3	6.1
140-B	15.5	1.8	13.1	0.7	0.4	—	1.3	4.6	0.4	1.8	2.7	0.4	13.3	19.4	1.6	1.9	0.8	1.5	10.8	5.3
159-C, deep					0.3	—	2.0	5.5	0.6	2.3	3.6	0.7	17.5	28.8	3.1	4.2	2.0	2.2	18.9	8.4
173-C					1.1	—	2.6	7.8	1.0	3.0	4.3	0.7	20.2	24.5	2.6	3.1	1.2	1.6	19.5	6.6
159-F					0.6	—	2.0	7.3	0.9	3.2	5.3	0.8	21.0	27.3	2.6	3.3	1.5	2.0	15.8	6.4
Av. moderate acne					0.6	—	2.0	6.3	0.7	2.6	4.0	0.7	18.0	25.0	2.5	3.1	1.4	1.8	16.2	6.7
140-C			1.3	—	0.9	—	2.2	7.3	1.0	3.1	4.4	0.8	18.5	30.5	2.4	2.5	1.2	1.3	14.9	7.7
159-D, deep			0.5	—	1.6	—	2.7	8.2	1.3	3.8	5.4	1.1	17.6	23.0	3.1	3.5	1.6	2.5	15.5	8.8
173-D			—	—	0.6	—	1.7	6.2	0.7	3.2	5.0	0.5	20.5	27.5	2.3	3.3	1.4	1.5	17.9	7.4
Av. severe acne			0.6	—	1.0	—	2.2	7.2	1.0	3.4	4.9	0.8	18.9	27.0	2.6	3.1	1.4	1.7	16.1	8.0

[a] Fractional carbon numbers assigned according to method described in (15), these data from Apiezon L. Column. [b] Indicates methyl-branched. [c] Numerical superscript on carbon, number indicates number of double bonds. [d] Dash in above table indicates presence of a peak but less than 0.1% of total area.

Table II—Percentage Distribution of the Free Fatty Acids in Human Skin Surface Lipid

Sample, Acid	Individual Samples 12[a]		14[1b]	14	15[c]	15[1]	15	16[c]	16[1]	16	17[c]	17[1]	17	18[c]	18[1,2]	18
R.S.	1.1	—[d]	3.3	7.9	0.7	3.3	4.8	0.4	23.1	25.1	2.3	3.0	1.4	1.8	14.9	6.8
G.K.	1.0	—	2.5	8.0	1.0	3.6	4.7	—	21.9	29.6	2.7	3.2	1.4	1.5	12.4	6.5
J.T.	0.7	—	1.6	5.8	1.7	3.3	4.5	—	20.8	25.7	2.7	3.7	1.5	2.0	19.2	6.5
B.B.	0.8	—	2.6	6.8	1.4	3.1	3.4	1.0	26.1	25.1	2.3	2.6	1.0	1.7	14.9	7.2
Av. normal	0.9	—	2.5	7.1	1.2	3.3	4.4	0.4	23.0	26.4	2.5	3.1	1.3	1.8	15.3	6.8
W.K.	1.2	—	2.9	8.7	0.9	3.6	5.4	0.5	20.8	24.9	3.0	3.3	1.3	1.8	15.7	5.9
P.N.	0.9	—	3.4	7.8	1.7	4.9	5.2	1.3	24.0	25.2	2.7	2.6	1.0	1.7	12.2	5.5
M.F.	0.9	—	2.7	8.3	0.9	3.0	4.2	6.7	24.1	26.9	2.7	2.6	1.0	1.7	14.3	6.1
Av. mild acne	1.0	—	3.0	8.3	1.2	3.8	4.9	2.8	23.0	25.7	2.8	2.8	1.1	1.7	14.1	5.8
R.W.	0.7	1.0	2.7	7.4	0.7	3.1	4.0	2.8	24.2	26.7	2.8	2.9	1.3	1.7	13.4	4.8
B.Bl.	1.6	—	2.5	5.7	1.0	3.9	4.7	2.6	18.6	25.2	2.9	3.7	1.5	1.6	17.7	6.8
R.St.	0.5	—	2.1	6.0	1.0	3.4	3.9	—	22.4	25.7	3.4	3.2	1.3	2.0	18.2	6.2
P.D.	1.2	—	2.0	6.8	0.8	2.6	4.1	—	19.0	28.4	2.3	2.7	1.1	2.2	18.6	7.9
K.W.	1.2	—	3.4	8.4	1.1	4.1	4.8	1.2	22.1	25.6	2.0	2.8	1.0	1.5	13.6	6.9
Av. moderate acne	1.0	0.2	2.5	6.8	0.9	3.4	4.3	1.3	23.3	26.3	2.7	3.1	1.3	1.8	16.3	6.5
D.F.	0.8	—	2.3	7.0	1.2	3.9	4.9	—	21.7	24.1	3.3	3.4	1.1	1.7	17.5	6.9
G.R.	0.7	—	2.3	6.6	1.1	3.2	4.3	5.3	22.5	26.0	3.1	3.5	1.6	1.6	16.2	6.8
R.W.	1.2	—	2.5	7.1	0.6	3.1	3.1	1.7	22.4	28.9	2.7	2.5	0.9	1.2	14.8	7.2
F.A.	1.0	—	3.6	7.4	1.0	3.9	5.9	3.1	22.7	22.4	3.5	4.4	2.2	1.8	14.5	5.4
Av. severe acne	0.9	—	2.7	7.0	1.0	3.5	4.6	2.5	22.3	25.4	3.2	3.5	1.4	1.6	15.7	6.6

[a] Carbon numbers assigned according to method described in (15), these data from Apiezon L. column. [b] Numerical superscript on carbon, number indicates number of double bonds. [c] Indicates methyl-branched. [d] Dash in above table indicates presence of peak but less than 0.1% of total area.

those previously reported for human skin and hair lipid (2, 16). The percentages of the acids present are in agreement with those recently reported by Gershbein and Metcalfe (17) for the total fatty acids in human hair lipid. Acids with carbon chain length less than 12 are not present in major amounts in either the normal or the acne samples, except for pooled samples 140-A, 140-B, 140-C, 140-D, 159-E. It is possible that these samples were subjected either to excessive heat in the removal of solvents or suffered the loss of their nitrogen atmosphere in storage. The assumption has, therefore, been made that these samples were oxidized. This seems reasonable because (*a*) the acids of chain length under 12 do not appear in major amounts in any of the other samples; (*b*) these samples were much darker in color than the others; and (*c*) the percent unsaturated acids in these samples is depressed while the percent saturated acids is concomitantly elevated. The average percentages for the pooled samples are very much the same for all of the four categories. The individual samples reveal considerable variation within categories, as expected, but the same acids are present in all of them and when they are averaged, no striking differences are seen in the four categories. Tables III and IV show that the percentages contributed by normal saturated, normal unsaturated, and branched saturated are essentially the same for the four categories. The "deep" lipid samples of sebum have essentially the

Table III—Free Acid Composition of Normal and Acne Lipids

Pooled Samples	Normal Saturated, %	Normal Unsaturated, %	Branched Saturated, %
Normal, av.	50.7	43.7	6.1
Mild acne, av.	48.0	41.9	9.5
Moderate acne, av.	51.0	43.2	5.9
Severe acne, av.	50.3	43.3	6.3

Table IV—Free Acid Composition of Normal and Acne Lipids

Individual Samples	Normal Saturated, %	Normal Unsaturated, %	Branched Saturated, %
Normal, av.	46.9	47.2	5.9
Mild acne, av.	46.8	46.7	8.5
Moderate acne, av.	46.1	48.6	6.7
Severe acne, av.	45.9	47.7	8.3

Table V—Absorbance Ratios for the Acid-Ester Fractions of Individual and "Deep" Pooled Samples

Normal	Mild Acne	Moderate Acne	Severe Acne
1.7	1.2	1.3	1.1
1.3	1.7	1.3	1.3
1.0	1.0	1.1	1.3
1.2	1.1	1.4	
		1.3	
Average Values			
1.3	1.25	1.3	1.2
"Deep" Lipids		**Pooled Samples**	
1.1	1.2	1.3	1.4

Table VI—Absorbance Ratios for the Inoculated and Uninoculated Test Samples

$A_{5.75}/A_{5.85}$, μ	$A_{5.75}/A_{5.85}$, μ
I	III
a. —	a. —
b. 1.0	b. 1.2
c. 1.1	c. 1.2
d. 1.0	d. —
II	IV
a. —	a. —
b. 1.2	b. 0.8
c. 1.1	c. 0.9

same composition as the surface lipid samples (Table I). This fact seems to validate the concept that a surface lipid sample, which is much easier to obtain, is a fairly good representation of the sebum which lies in the follicle. The free acids in lipids from normal skin are not grossly different from those present in mild, moderate, or severe acne skin lipid. Thus, when injected intradermally, one would expect to see the same degree of irritation from normal lipids as from acne lipids (5).

Composition of the Acid-Ester Fraction—IR spectral analysis should detect a change in the composition of the acid-ester fraction (12). If, as indicated by some investigators, the severity of the acne condition is related to the acid number of the surface lipid, then this relationship is not apparent from the data shown in Table V. The absorbance ratios should reflect a change in the acid number (12). The absorbance ratios for the samples of "deep" lipid might be expected to have slightly higher values than those of the surface lipid in view of the fact that it is believed by some that newly formed sebum contains no free acids, but that the free acids appear as a result of esterase activity at the sebaceous duct, in the follicle, and on the surface (18, 19). This apparent contradiction could be explained in at least two ways: (*a*) the "deep" lipid, reaching the surface 30 min. after defatting the skin, is a preformed lipid from the follicular reservoir (13), or (*b*) the hydrolysis which is said to take place is an extremely rapid process which quickly reaches a self limiting end point as does the growth of *C. acnes* in an unbuffered medium (8).

Esterase Activity of C. Acnes—The absorbance data in Table VI indicates that the absorbance ratio is relatively constant for both control sets (I, III), and for the set inoculated with *S. albus* (II), while the samples inoculated with *C. acnes* (IV) have an apparent absorbance ratio below 1.0. Any decrease in this ratio indicates an increased acid number and this must be assumed to be the result of ester hydrolysis. A literature search on this topic revealed abundant references of the esterase activity of the total bacterial flora in the sebaceous gland duct, comedones, sebaceous follicle, and the skin surface (9, 10, 18, 20). Since *C. acnes* (*Propionbacterium acnes*) is one of the major components of the bacterial flora found in these areas (7) it has been assumed that at least some of this esterase activity could be attributed to it. Nothing, however, was found in the literature which showed that esterase activity is associated specifically with the growth of *C. acnes* as has been indicated in this work.

SUMMARY

Human skin lipid samples were collected from acne patients and normal individuals and the total acidity measured. The free fatty acids from these samples were then extracted and analyzed by GLC. There is no apparent relationship between the total acidity of the lipid and the skin condition, nor is there any outstanding difference between the kinds and amounts of the acids present in the normal and acne skin lipid. Cultures of *C. acnes* were grown in the presence of an artificial sebum containing glyceride esters. An increase in the acid number of the sebum extracted from these cultures indicates that hydrolysis had taken place and this must be attributed to the esterase activity of *C. acnes*.

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