

Synthesis of lipids from acetate by human preputial and abdominal skin in vitro

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ABSTRACT Lipogenesis in vitro from acetate-1-¹⁴C was studied in human preputial skin and abdominal skin. Radioactive lipids were separated by column chromatography on Florisil and by thin-layer chromatography on silica gel. Radioactivity was incorporated chiefly into the triglyceride, sterol, and polar lipid fractions, while lesser amounts of ¹⁴C were found in the hydrocarbon, wax, diglyceride, monoglyceride, and fatty acid fractions; labeling of steryl esters was minimal. On thin-layer chromatography, the radioactive polar lipids had mobilities similar to lysolecithin, phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidic acid.

The radioactive fatty acids of the different lipid fractions were separated by gas-liquid chromatography. The major ¹⁴C-labeled acids were 16:0 and 18:0. Radioactivity was also detected in acids 14:0, 15:0, 16:1, 18:1, 18:2, 20:0, 20:1, 22:0, 24:0, 24:1, and 26:0. No radioactivity could be detected in arachidonic acid, although this fatty acid comprises 9% of the chromatographed fatty acids. The pattern of incorporated ¹⁴C was different from the percentage mass composition of the fatty acids.

Skin is therefore active in the biosynthesis of a wider variety of lipids than previously demonstrated.

SUPPLEMENTARY KEY WORDS lipogenesis · triglyceride · free sterols · phosphoglycerides · squalene · diglycerides · monoglycerides · fatty acid composition · waxes · steryl esters

THE SURFACE of human skin is normally covered with a lipid mixture, which has aroused increasing interest. Many of the lipids found on the surface of the skin have been characterized (1-5) and lipid components in the sebaceous gland have been analyzed (6). Changes in skin lipids in several skin diseases have also been investigated (7-9). However, the presence of various lipids in the skin gives no clue as to whether they are synthesized by the skin, are transported there from other parts of the body,

or are of dietary origin. That certain lipids are synthesized in human skin was first demonstrated in vitro by Nicolaides, Reiss, and Langdon (10), who found incorporation of ¹⁴C into squalene, cholesterol, and fatty acids after incubation of human skin specimens with ¹⁴C-acetate.

In previous communications (11,12), we described methods which allow evaluation of lipogenic activity in small biopsy specimens of human skin and reported impaired lipogenesis in skin of diabetic patients and fasting individuals. In these and other studies (13-15), the lipids synthesized by human skin in vitro were examined after hydrolysis and separation into saponifiable and nonsaponifiable fractions. In an attempt to obtain more information on the nature of the lipids synthesized in the skin, we have now chromatographically separated unhydrolyzed lipids, synthesized in vitro from acetate-1-¹⁴C by human skin from two body sites, into several major lipid classes. We have further examined the ¹⁴C-labeled fatty acids in these lipid classes.

MATERIALS AND METHODS

Skin

Specimens of preputial skin were obtained at circumcision of the newborn 4 hr after feeding. These specimens were used in our exploratory studies because they were routinely available at our nurseries. Abdominal skin specimens were excised from volunteer male students,

Abbreviations: GLC, gas-liquid chromatography; TLC, thin-layer chromatography; FAME, fatty acid methyl esters; TL, total lipid; NL, neutral lipid; PL, polar lipid; TG, triglyceride; DG, diglyceride; MG, monoglyceride; FA, fatty acid; DEGS, diethylene glycol succinate polyester. Fatty acids are designated by number of carbon atoms:number of double bonds.

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22-25 yr of age, by a 4 mm punch under local anesthesia after an overnight fast. The specimens were wrapped in gauze moistened with physiological saline solution and used within 1 hr.

Radioactive Materials

Cholesterol-4-¹⁴C was obtained from New England Nuclear Corp., Boston, Mass. Sodium acetate-1-¹⁴C, cholesteryl palmitate-1-¹⁴C, glycerol tripalmitate-1-¹⁴C, and stearic-1-¹⁴C acid were obtained from Nuclear-Chicago Corp., Chicago, Ill.

The sodium acetate-1-¹⁴C was examined for impurities by TLC on silica gel in two solvent mixtures, chloroform-ethanol-30% ammonium hydroxide (2:2:1) and pyridine-hexane (2:1) in tanks lined with filter paper. These developing solvents have effectively demonstrated impurities in acetate samples that had been stored in solution over a prolonged period, and showed that the acetate used in the present experiments had a radiopurity greater than 99%. The other radioactive compounds were examined by TLC, and the impurities, if any, were removed by column chromatography on silicic acid (16). The radiopurity of the compounds used was greater than 99% on the final test.

Solvents and Other Chemicals

All solvents were redistilled. Hexane, benzene, and diethyl ether were stored over sodium. Ethanol was added to chloroform (7.5 ml/liter) as a preservative. All liquid mixtures described are v/v. Standards for GLC and TLC were from Applied Science Laboratories, Inc., State College, Pa.; Florisil was obtained from Fisher Scientific Co.; silicic acid (Unisil) from Clarkson Chemical Co.; Silica Gel H from Brinkmann Instruments, Inc., and Sephadex G-25 from Pharmacia, Inc.

Incubation

The skin specimens were incubated with acetate-1-¹⁴C as previously described (11). Briefly, 30-150 mg of skin, blotted free of blood and trimmed of fat, was incubated with 2 μ c of sodium acetate-1-¹⁴C (specific activity 44 mc/mmole) for 6 hr at 37°C in 2 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing streptomycin (200 μ g), penicillin (200 units), and gentamicine sulfate (200 μ g). The incubation was terminated by the addition of 20 ml of chloroform-methanol 2:1, and the samples were kept at -10°C until analyzed.

Extraction and Chromatography

The tissue specimen together with the incubation medium was homogenized in a Virtis "45" homogenizer in 50 ml of the chloroform-methanol solvent. Tissue debris was removed by filtration through sintered glass funnels and was washed with the solvent until no more

radioactivity was detected in the washings. The residue was hydrolyzed by alkali, and after acidification the amount of ¹⁴C extractable by dichloromethane was negligible. The filtrate was evaporated in a rotary evaporator, and the lipid residue was dissolved in chloroform-methanol-water 19:1:0.1. Radioactive acetate and other small water-soluble molecules were removed by gel filtration through Sephadex G-25 by the method of Siakotos and Rouser (17). The recovered lipids were dissolved in a small amount of chloroform and separated on a silicic acid column into neutral lipid (eluted with chloroform) and polar lipid (eluted with methanol). The neutral lipid fraction was further separated into hydrocarbon, steryl ester and wax, triglyceride, sterol, diglyceride, monoglyceride and fatty acid fractions by chromatography on Florisil by the method of Carroll (18). In initial experiments, 1- or 2-ml fractions were collected and the separation of radioactive lipids was compared with reference lipid mixtures. After the effectiveness of the method was established, in routine analysis each lipid class was collected in one fraction, and the final 2 ml of each elution mixture was radioassayed to assure effective separation.

The separation by Florisil column was monitored by TLC. The plates, 5 \times 20 cm, were coated with a 0.25 mm layer of Silica Gel H with Rhodamine 6G incorporated. After exposure to iodine the lipid spots were detected under UV radiation (19). The radioactive lipids were located by a Vanguard Auto-scanner and their mobilities compared with those of reference lipids chromatographed simultaneously.

Because it has been reported (20) that phospholipids bind small water-soluble molecules, the polar lipid fractions from a number of preputial skin incubations were examined. They were hydrolyzed by 4% methanolic KOH at 60°C overnight, and after acidification the lipids were extracted with dichloromethane and subjected once more to gel filtration. The decrease in radioactivity in this fraction, possibly due to removal of radioactive acetate trapped by the phospholipids, was 0-20% (average 7%).

In other experiments the polar lipid fraction was further fractionated by TLC in the procedure of Skipsky, Peterson, and Barclay (21). Plates, 5 \times 50 cm, coated with a 0.25 mm layer of Silica Gel H (with no dye) were used. After the plates had been developed in chloroform-methanol-acetic acid-water 65:25:8:4, they were scanned for radioactivity, sprayed with 20 N sulfuric acid, and charred at 100°C to detect the lipid spots.

For GLC, the chromatographic fractions from the preputial skin were pooled according to lipid class. The mono-, di-, and triglycerides were transmethylated according to the procedure of Oette and Doss (22). The fatty acids from the Florisil column and those obtained

after hydrolysis of polar lipids or the total lipid mixture were methylated with diazomethane (23). To remove contaminating components, we chromatographed the mixture by a modification of the method of Kaplanis, Robbins, and Tabor (24) on aluminum oxide (Woelm acid, grade 1, deactivated with 1.5% water). The methyl esters were eluted with benzene-hexane 1:1. The FAME were analyzed by GLC on a 6% diethylene glycol succinate polyester column (2m × 0.96 mm OD) in an F and M model 402 gas chromatograph fitted with an effluent stream splitter, one limb of which carried less than one-tenth of the effluent to a flame ionization detector. The remainder of the effluent carried by the other limb was trapped at ice-bath temperature with a Packard gas chromatography fraction collector. The column was operated isothermally at 170–180°C, or programmed at 2°/min from 170 to 250°C. The recovery of radioactive FAME was over 90%. Quantitative results with NIH fatty acid standard mixtures A, B, C, and D agreed with the stated composition data with a relative error less than 8% for major components (>10% of total mixture) and less than 9% for minor components (<10% of total mixture).

To test the reliability of our methods, we mixed radiolabeled reference lipids with nonradioactive lipids extracted from skin and subjected the mixture to extraction and column chromatography as described above. The recovery of radioactivity was over 90%. Addition of radioactive acetate to the nonradioactive lipids and washing by the usual Folch procedure (25) showed that a considerable amount of radioactivity remained with the lipids. Gel filtration through Sephadex, on the other hand, removed all but a small fraction of the ¹⁴C (less than 10⁴ dpm from 4 × 10⁶ dpm added). This radioactivity remained with the polar lipid after silicic acid chromatography; the neutral lipid was free of contaminating radioactivity. The persistent radioactivity in the polar fraction could be removed by hydrolysis and a second gel filtration through Sephadex. The incorporation of label into the polar lipid fraction reported in the Results section was the amount of ¹⁴C found after the second passage through Sephadex.

Detection of Unsaturation

Unsaturated FAME were established by GLC before and after hydrogenation and bromination. Hydrogenation was carried out in cyclohexane in the presence of palladium-charcoal, and bromination was effected by addition of bromine to a hexane solution of the FAME.

The unsaturated FA were also examined by argentation TLC. A slurry of Silica Gel H in 12.5% (w/v) aqueous silver nitrate was spread on plates (5 × 40 cm) to a thickness of 0.25 mm. The silica gel was activated at 110°C, FAME from TL of preputial skin were applied,

and the plate was developed with hexane-ether 7:1 in an unlined chamber. The solvent was allowed to migrate 30 cm from the origin. This procedure satisfactorily separated the saturated from the mono-, di-, tri-, and tetraunsaturated reference FAME. After development the plates were scanned for radioactivity, the areas corresponding to the saturated, mono-, di-, tri-, and tetraunsaturated FAME were scraped from the plate, and the FAME were eluted with dichloromethane for analysis by GLC.

Radioassays

Radioactivity was determined by assaying a portion of a benzene solution of the lipid fraction in a Packard Tri-Carb liquid scintillation counter. No significant quenching was detected after the addition of an internal standard. The distribution of ¹⁴C on TLC plates was traced with a Vanguard Autoscanner 880.

RESULTS

The results of Florisil chromatography of the neutral lipid fraction from an incubation with preputial skin are shown in Fig. 1. Similar chromatograms were obtained from the neutral lipid fraction after incubations of abdominal skin. The distribution of ¹⁴C in the various lipid fractions is shown in Table 1. The synthetic activities of preputial skin and abdominal skin are compared in Fig. 2. These data indicate that preputial skin incorporated more ¹⁴C, but the patterns of distribution of ¹⁴C in lipids of the two sites were similar. The most highly labeled were the TG, sterol, and PL fractions, and the least highly labeled was the steryl ester plus wax fraction. This fraction was further analyzed by TLC in hexane-benzene 3:1, most of the labeled material resembled waxes rather than steryl esters in mobility. The hydrocarbon fraction had mobility similar to that of squalene on TLC in

TABLE 1 INCORPORATION OF ACETATE-¹⁴C INTO SKIN LIPIDS

Fraction	Reference Lipid	Preputial Skin		Abdominal Skin	
		%		%	
I	Squalene	6 ± 1	14 ± 4		
II	Cholesteryl palmitate	2 ± 0.02	3 ± 1		
III	Glycerol tripalmitate	16 ± 1	13 ± 4		
IV	Cholesterol	26 ± 2	22 ± 8		
V	Glycerol dipalmitate	1 ± 0.1	4 ± 1		
VI	Glycerol monopalmitate	5 ± 0.4	5 ± 0.5		
VII	Stearic acid	5 ± 0.4	12 ± 2		
PL	Lecithin	36 ± 1	28 ± 2		

Fractions I–VII were obtained from chromatography of NL on Florisil, and PL from Unisil. The results were obtained from 14 incubations of preputial skin specimens and 4 incubations of abdominal skin specimens with acetate-¹⁴C for 6 hr at 37°C and pH 7.4. The figures are the average percentages of total ¹⁴C in lipids ± SEM.

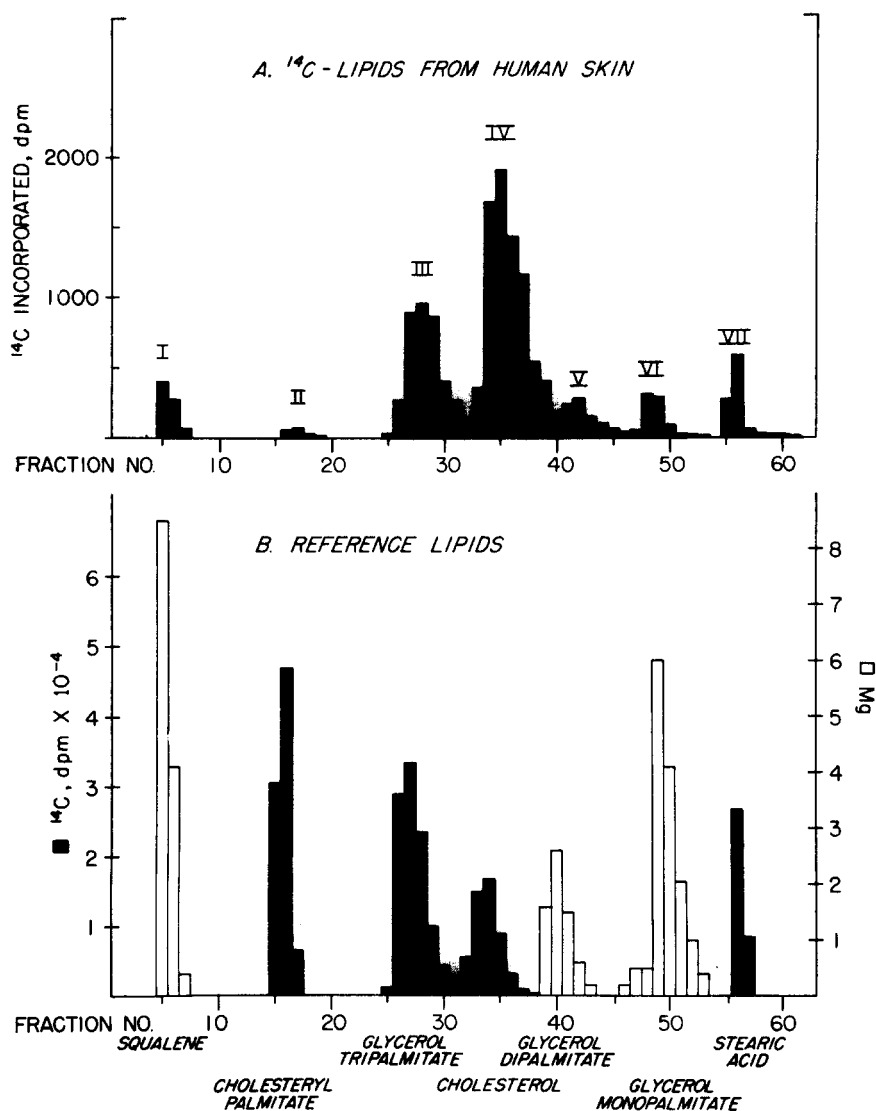


FIG. 1. Chromatographic separation (18) of neutral lipids on 5 g of Florisil after incubation of 30–150 mg of skin with acetate-¹⁴C for 6 hr at 37°C and pH 7.4. 2-ml fractions were collected. A. Neutral lipids from prepubertal skin. B. Reference lipids: 10 mg each of radiolabeled or unlabeled compounds was applied to the column. For identification of fractions I–VII see Fig. 2.

hexane. TLC of the sterol fraction in benzene-ethyl acetate 9:1, and of the DG, MG, and fatty acid fractions in ether-benzene-ethanol-acetic acid 40:50:2:0.2, afforded no further separation, and the radioactivity moved in single peaks with mobilities of the appropriate reference lipids.

The polar lipid fraction was subjected to TLC in hexane-ether-acetic acid 85:15:1, and no contamination with neutral lipids was detected. Fractionation in the system of Skipsky et al. (21) resulted in a separation shown in Fig. 3. Four peaks of radioactivity (peaks 1–4, Fig. 3) corresponded in mobility to lysolecithin, phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidic acid. The mobility of a nonradioactive spot between peaks 2 and 3, was similar to that of phos-

phatidyl serine. Further studies are needed for more detailed identification of these labeled lipids.

Upon TLC of the TG fraction in hexane-ether-acetic acid 85:15:1, approximately one-third of the radioactivity was found in a more polar fraction with mobility resembling that of a long-chain alcohol (octadecanol). After transmethylation of the TG fraction and chromatography on alumina, the methyl esters were eluted by hexane-benzene 1:1, while another radioactive component was eluted by ether. The identity of this component has not been established.

The radioactive FAME obtained from the TL after incubation of prepubertal skin were analyzed by GLC. A typical chromatogram is shown in the upper panel of Fig. 4. In this preliminary examination, 25 distinct peaks

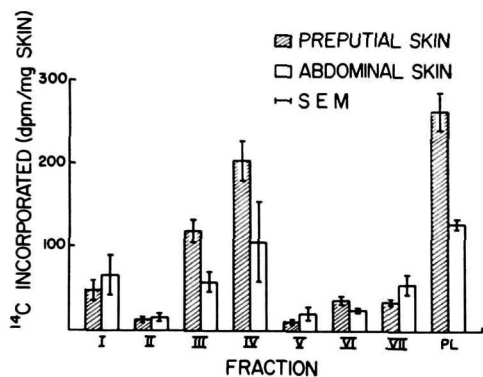


FIG. 2. Distribution of radioactivity in lipid fractions from preputial and abdominal skin after incubation with acetate. The heights of the bars indicate the averages of results of 14 incubations of preputial skin and 4 incubations of abdominal skin. The designations for lipid fractions are: I, hydrocarbons; II, steryl esters plus waxes; III, triglycerides; IV, sterols; V, diglycerides; VI, monoglycerides; VII, fatty acids; and PL, polar lipids.

of mass and 12 peaks of ¹⁴C were recorded. The peaks are designated by numbers corresponding to chain lengths of reference FAME. Designation of unsaturated fatty acids was further guided by results of hydrogenation and bromination. These designations were used for tentative identification of the peaks; definitive identification of the FA requires further characterization. The results of GLC after bromination of a portion of the mixture are shown in the lower panel of Fig. 4, in which the peaks corresponding to the FAME designated as unsaturated are missing. It is interesting that the radioactivity in peaks designated 22, 24, and 26 remained while the mass was reduced, sug-

gesting that these saturated fatty acids were synthesized from acetate while some unsaturated fatty acids with similar retention time were not.

The above results were supported by further evidence from argentation TLC. We found 68% of the radioactivity in the saturated, 31% in the mono-unsaturated, and 1% in the diunsaturated FAME. No detectable amount of ¹⁴C was found in the tri- and tetraunsaturated fractions. GLC of these fractions and trapping of the effluent FAME gave the results shown in Table 2. In the saturated fraction, the major amounts of ¹⁴C were found in acids 14:0, 16:0, and 18:0; and in the monounsaturated fraction, in acids 16:1 and 18:1. It is interesting that ¹⁴C was also found in acids 15:0, 17:1, 20:1, 21:1, and 18:2, but not in the tri- and tetraunsaturated FA.

We cochromatographed reference branched-chain C₁₅-C₂₄ FAME with skin FAME. *Iso*-18:0 coincided with the small peak immediately preceding peak 18 in Fig. 4, but this acid did not contain ¹⁴C. Although *anteiso*-20:0 had a retention time similar to that of 18:1, results of hydrogenation and bromination of skin FAME established that this branched-chain acid was not present in the skin lipids. In another experiment, the methyl ester of linolenic acid appeared in our chromatographic system between 18:2 and 20; since we could detect neither mass nor radioactivity in this area, it appears that if linolenic acid is a constituent of newborn preputial skin, the amount is extremely small.

Table 3 shows the distribution of the fatty acids in the different lipid fractions. (The fatty acids of the steryl esters plus wax fraction were not examined because the

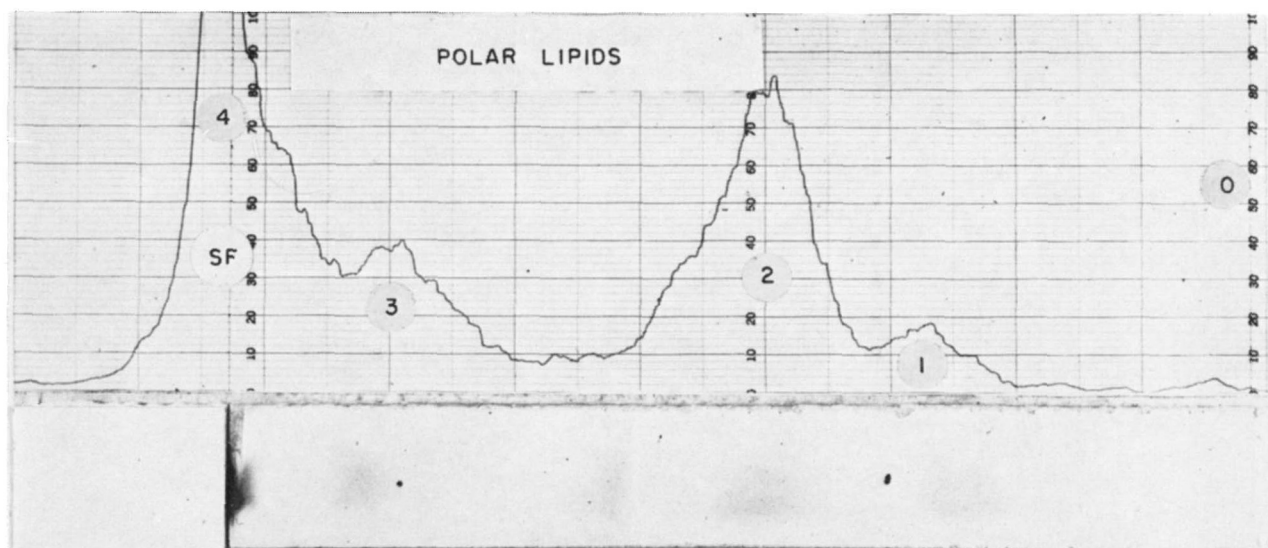


FIG. 3. TLC of polar lipid fraction from preputial skin in chloroform-methanol-acetic acid-water 65:25:8:4 (21). Radioactivity was located by scanning with a Vanguard Autoscanner (tracing on top). Lipids were made visible by spraying with 20 N sulfuric acid, and charring at 100°C (photograph at bottom). Peak 1 moved like lysolecithin, peak 2 like phosphatidyl choline, peak 3 like phosphatidyl ethanolamine, and peak 4 like phosphatidic acid. The spot that contained no detectable amount of ¹⁴C (between peaks 2 and 3) moved like phosphatidyl serine.

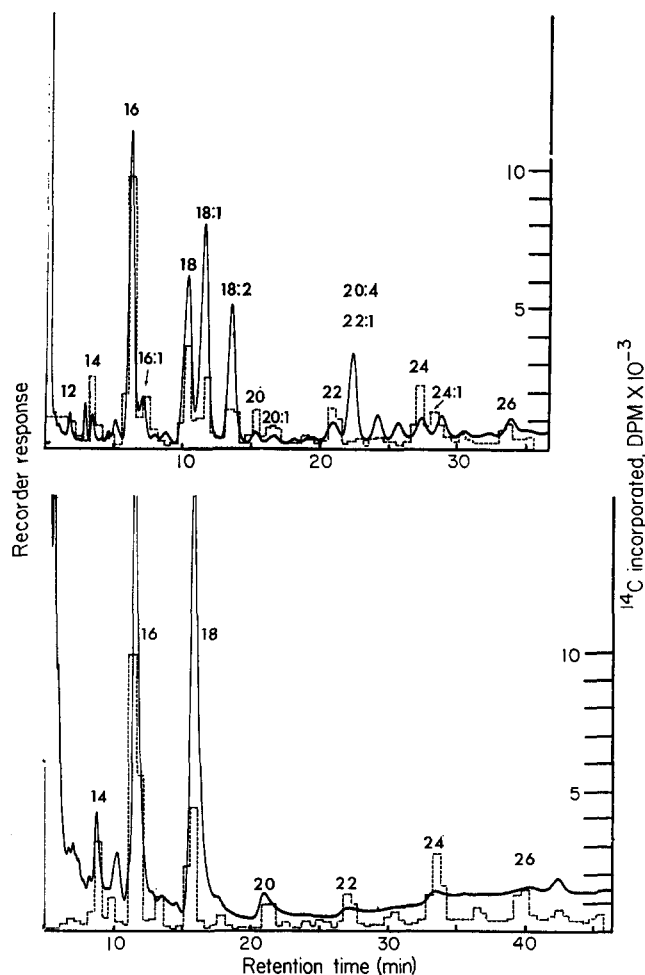


FIG. 4. Gas chromatography of FAME from total lipid after incubation of preputial skin. The column used was 6% diethylene glycol succinate polyester programmed at 2°C/min from 170° to 250°C. Half-minute fractions were collected in a Packard sub-ambient fraction collector and radioassayed. Solid line is response of mass detector and the height of bars enclosed by broken lines indicates radioactivity (dpm of ^{14}C). Lower panel, after bromination. Saturated fatty acids are designated solely by chain-length.

amount of ^{14}C in this fraction was small.) The data on the mass percentages, of fatty acids show that 16:0 and 18:1 were major components of all fractions examined, and 18:0 was a major component of all but the TG fraction. 18:2 comprised 8–12% of all fractions except MG, from which it was absent. There was no correlation between the relative amounts of the mass to that of the ^{14}C incorporated into the fatty acids. For example, in fraction DG, the percentages of ^{14}C in 16:0 and 18:0 were nearly the same but 16:0 was 2.5 times as much in mass. The 16:0 and 18:0 fatty acids were highly labeled in all fractions. Other highly labeled fatty acids were found in the various lipid fractions, e.g. 16:1 in PL and FA, 18:0 in DG, 20:0 in TG and MG, and 24:0 in MG. The content of labeled unsaturated fatty acids was practically the same (31–33%) in PL, TG, and DG, but was lower (18%) in MG and higher (51%) in FA.

TABLE 2 DISTRIBUTION OF ^{14}C IN FA

FA	Fraction of Total FA- ^{14}C
Saturated	
	%
14:0	8.2
15:0	3.9
16:0	22.0
18:0	7.6
20:0	2.0
22:0	1.9
24:0	4.0
26:0	3.0
Monounsaturated	
14:1	0.6
16:1	7.8
17:1	1.0
18:1	6.8
19:1	0.4
20:1	2.8
21:1	1.0
22:1	0.7
24:1	0.9
Diunsaturated	
18:2	1.3
Triunsaturated	
	Not detectable
Tetraunsaturated	
	Not detectable

The FAME were separated by argentation TLC and then by GLC. Percentages derived from peak height \times width at half height.

DISCUSSION

Skin regenerates constantly. New cells are formed in the basal cell layer and move toward the surface. After they have matured in the malpighian layer, they undergo denucleation to form the stratum corneum, which is eventually desquamated. This constant replenishment requires a constant supply of structural materials for membranes and organelles, and lipids are necessary constituents. Additionally, the sebaceous gland is a specialized structure which excretes lipids onto the skin surface. Therefore, it is not surprising that our results show active biosynthesis of a variety of lipids in human skin. Our data show that ^{14}C from acetate-1- ^{14}C is most actively incorporated into the triglyceride, free sterol, and polar lipid fractions, and in lesser amounts into the hydrocarbon, steryl ester and wax, diglyceride, monoglyceride, and fatty acid fractions. The radioactive DG and MG may be the results of either synthetic or degradative activities.

Skin from the two sites studied appears to synthesize the different lipid classes in similar proportions, but the abdominal skin appears to be less active. The quantitative difference in ^{14}C incorporation may not necessarily reflect a difference due to body sites, but it may also be due to higher activity in infant skin than in young adult skin. The similarity in distribution of label among the lipid classes in preputial skin and abdominal skin may be

TABLE 3 PERCENTAGE DISTRIBUTION OF MASS AND RADIOACTIVITY IN FATTY ACIDS OF LIPID FRACTIONS FROM PREPUTIAL SKIN AFTER INCUBATION WITH ACETATE-¹⁴C

Fatty Acid	TL		PL		TG		DG		MG		FA	
	Mass	¹⁴ C	Mass	¹⁴ C	Mass	¹⁴ C	Mass	¹⁴ C	Mass	¹⁴ C	Mass	¹⁴ C
12:0	1.0	—	0.3	—	1.4	—	1.2	—	1.6	—	1.4	—
14:0	1.3	6.0	4.5	3.4	7.9	7.3	6.9	2.0	7.0	2.6	5.5	4.3
15:0	1.3	—	tr	—	6.3	—	4.6	—	4.3	—	3.7	—
16:0	18.3	18.9	24.4	17.3	25.5	13.6	28.2	17.1	27.5	14.2	23.0	10.1
16:1	5.1	5.5	5.7	13.7	16.0	4.9	12.2	5.8	12.1	4.3	11.4	29.2
18-iso	1.3	—	tr	—	2.4	—	tr	—	—	—	tr	—
18:0	14.9	8.6	16.1	9.2	6.4	6.0	11.3	18.7	13.0	10.0	17.1	5.1
18:1	19.4	5.4	20.3	12.3	20.3	11.5	19.2	11.4	20.2	13.2	21.1	16.7
18:2	11.5	4.0	8.2	3.3	8.6	10.2	8.3	11.9	—	—	9.3	3.5
20:0	1.3	3.0	1.8	—	0.8	17.8	3.5	2.1	14.2	22.2	0.8	8.3
20:1	1.1	4.0	1.2	1.5	tr	6.0	0.8	3.8	—	—	tr	1.8
21:0	tr	—	tr	—	—	—	tr	—	—	—	tr	—
21:1	tr	—	tr	—	—	—	tr	—	—	—	tr	—
22:0	2.7	3.8	3.8	3.6	0.8	2.4	0.9	10.1	—	—	tr	3.7
20:4	8.6	—	11.0	—	3.0	—	2.8	—	tr	—	3.2	—
24:0	2.6	6.8	2.0	7.4	0.5	3.5	tr	1.8	tr	12.7	tr	1.4
24:1	tr	2.2	—	—	—	—	—	—	—	—	—	—
26:0	2.0	3.2	tr	2.8	tr	1.0	—	—	—	—	—	—
Total unsaturated fatty acids	53.2	21.1	46.4	30.8	47.9	32.6	43.3	32.9	32.3	17.5	48.4	51.2

The numbers presented in this table are the percentages of the total mass in the chromatogram and of the recovered radioactivity. The data on TL were the average of results obtained from three incubations with preputial skin; data on other lipid fractions were from the pooled samples of 14 incubations. tr, trace. —, not detected.

a reflection of similarity in the distribution of the sebaceous glands at these sites; microscopic examination showed that sebaceous glands in both areas are sparse.

Fig. 4 and Table 3 show that not only the fatty acids of medium chain lengths but also several longer chain fatty acids (20:0, 20:1, 21:1, 22:0, 22:1, 24:0, 24:1 and 26:0) are labeled. Downing and Greene (26) in their studies of the monounsaturated fatty acids of vernix caseosa suggested that some of these acids may be synthesized in the skin. Our data show indeed that acids 14:1, 16:1, 17:1, 18:1, 19:1, 20:1, 21:1, 22:1, and 24:1 as well as 18:2 contain radioactivity. The lack of radioactivity in acid 20:4 indicates that preputial skin is incapable of synthesizing this polyunsaturated acid from acetate, although it may comprise as much as 9% of the fatty acids in skin. This inability of preputial skin to synthesize 20:4 fatty acid is in contrast to findings of Brooks, Godefroi, and Simpson (27) on the incorporation of labeled acetate into a fraction containing this acid by mouse skin, and to findings by Miyamoto, Stephanides, and Bernsohn (28) who reported a high degree of acetate incorporation into arachidonic acid by a chick brain preparation.

Our data show that the mass and labeling patterns of the FAME do not parallel each other (Fig. 4 and Table 3). There are two possible explanations: (a) the dietary fatty acids and the fatty acids synthesized elsewhere in the body contributing to skin lipids may be composed differently from those synthesized in the skin; (b) the fatty acids synthesized by chain elongation would have lower specific activities than those synthesized de novo.

The data in Table 3 show that the distribution of labeled fatty acids is not random. For example, labeled 14:0 was present in a higher percentage in TG than in MG and DG, and two labeled unsaturated acids, 18:2 and 20:1, were present in TG and DG, but were not found in MG. Furthermore, a smaller percentage (18%) of labeled unsaturated fatty acids was found in MG than in TG (33%), DG (33%), and PL (31%), and relatively large percentages of labeled 20:0 (22%) and 24:0 (13%) were found in MG. Also, labeled 20:0 was present in greater percentage in TG (18%) than in DG (2%), and was absent from PL. It would be interesting in further studies to elucidate the structural specificity of these acids in glycerides and PL, and compare with results of enzymic hydrolysis of glycerides of human adipose tissue by Brockerhoff (29), who found that unsaturated fatty acids are preferably esterified at position 2, and long-chain fatty acids at position 3.

This study has demonstrated that human skin can synthesize a variety of lipids. A wealth of more detailed and specific information can come from further studies. Since skin is more accessible than other human tissues for metabolic studies, it may be advantageous to use skin specimens in future investigations concerning lipid metabolism in the human.

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