Specific sites of fatty acid and sterol synthesis in isolated skin components

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ABSTRACT Metabolic studies on isolated mouse skin components were undertaken to determine the specific sites of fatty acid and sterol synthesis. The concentrations of long-chain fatty acids and sterols and the incorporation of radioactivity from acetate-1-^{14}C into these lipids are reported for various skin components and intact whole skin.

Only fatty acids having chain lengths of 18 carbons or less were produced by the connective tissue cells of the dermis, while fatty acids containing 20 carbons or more, as well as the acids of 18 carbons or less, were synthesized in the upper dermis (papillary reticulum). The upper dermis also produced significant quantities of eicosenoic acid and of an octadecadienoic acid (not linoleic acid), and incorporated labeled acetate into fatty acids containing an odd number of carbons.

Removal of the epidermis and adnexa diminished sterol synthesis. However, the upper region of the dermis was capable of synthesizing, from acetate, large quantities of unidentified nonsaponifiable lipids which were neither sterols nor squalene.

KEY WORDS metabolism · skin components · epidermis · dermis · mouse · biosynthesis · fatty acid · cholesterol · Δ^7-cholestenol · squalene · acetate-1-^{14}C · gas–liquid chromatography · trimethylsilyl ethers

Skin has been shown to be one of the most active lipid-synthesizing tissues in animals (1). Rodent skin is unique in its synthesis of fatty acids containing 20 carbon atoms or more (2) and in its production of large quantities of the Δ^7-sterols (3, 4). The cellular heterogeneity of skin makes it impossible to demonstrate accurately the sites of synthesis of the various lipids using techniques with whole skin either in vitro or in vivo. Previous investigations with mechanically separated epidermis and dermis have shown important differences in lipogenesis by these two parts of skin (4–6). In this laboratory rodent skin has been partitioned successfully by employing a combination of enzymatic and mechanical procedures (5). The present paper describes metabolic studies undertaken on these isolated mouse skin components to determine the specific sites of fatty acid and sterol synthesis.

METHODS

Four- to nine-week old, A_f/Sp strain mice were kept in stainless steel cages and fed Purina chow and water ad libitum. Hair in the dorsal region was plucked 15 hr before sacrifice. Only those mice were selected for which this procedure was easy and painless; this ensured that the skin of all experimental animals was in the telogen or resting phase of the hair cycle (5). After the dorsal skin was excised with scissors, it was scraped to remove the panniculus adiposus and panniculus carnosus, then cut into small pieces.

The prepared skin was partitioned into its structural components by the following procedures. Whole skin pieces were subjected to exhaustive treatment with trypsin (eight times with a 0.1% solution), and then poured through a nylon screen to isolate the free *epidermal and adnexal cells* (filtrate) from the *dermal residue* containing the papillary reticulum, mast cells, fibroblasts, etc. Additional whole skin pieces were digested with collagenase (0.1% solution), then filtered through nylon to separate the free *dermal cells*, i.e. mast cells, fibroblasts, etc. (filtrate) from the *epidermis with intact adnexa and reticulum.* Filtrates were spun at 600 × *g*

This paper is Part II of a series entitled Metabolic Studies on Skin. Part I is reference 5.

Abbreviations: TMS, trimethylsilyl; DEGS, diethylene glycol succinate polyester; GLC, gas–liquid chromatography; P.E., petroleum ether; BZ, benzene.

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to collect the cells. These isolation procedures have been described in greater detail in a previous publication (5).

Components derived from 0.5 g of whole skin, as well as intact whole skin pieces (0.5 g), were incubated in Warburg flasks containing 1 μc of acetate-1-^{14}C, 10 mg of glucose, and Krebs-Ringer phosphate buffer added to make a total volume of 2 ml. Incubations were carried out under an oxygen atmosphere for 3 hr at 37°; incubation mixtures were then refluxed for 8 hr in 50% aqueous ethanol with 15% KOH.

Nonsaponifiable lipids were obtained by four petroleum ether extractions of the alkaline mixture. In some experiments these lipids were fractionated by chromatography on acetic acid-deactivated alumina into hydrocarbon, triterpene, and sterol groups (7). After preparing the trimethylsilyl (TMS) ether derivatives (8), the total nonsaponifiable lipids, as well as the different fractions, were analyzed by GLC. Loss of significant quantities of 7-dehydrocholesterol would be expected in the procedures used to isolate the sterols.

Saponifiable lipids were obtained from the acidified hydrolysate by three extractions with diethyl ether. The extract was washed with water to remove any remaining acetate-1-^{14}C. Long-chain fatty acids were routinely esterified with boron trifluoride in methanol (9), and the methyl esters analyzed by GLC. However, in some experiments the free fatty acids, with appropriate carriers added, were partitioned by the reversed-phase liquid–liquid chromatographic system of Howard and Martin (10).

The GLC column packing was prepared by a filtration method (11). The fatty acid methyl esters were chromatographed on a column containing 18% (w/w on 60–80 mesh Chromosorb W) diethylene glycol succinate polyester (DEGS); the TMS ethers of the nonsaponifiable lipids were analyzed on a column coated with 6% (w/w) DEGS. The effluent gas stream was split so that 8% passed to the detector and 92% to the collector. Collections were made each minute (or occasionally each half-minute) in 0.6 mm i.d. stainless steel capillaries (12), which were then eluted with a toluene solution containing 2,5-diphenyloxazole and 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene. Radioactivity was monitored in a liquid scintillation spectrometer unless stated otherwise. Plotting the radioactivity recovered each minute facilitated the determination of column background and location of labeled compounds which were present in concentrations too low for detection by the mass detector.

The ^{90}Sr ionization detector (volume 5 ml) used in these experiments has been shown to be linear, at constant argon pressure, in its response to sterols and fatty acids over the range of concentrations studied (12). Similar linearity in the detection of TMS ethers of sterols and methyl esters of fatty acids has also been observed.[1]

TABLE 1 Recoveries of Microgram Quantities of Methyl Palmitate-1-^{14}C and Cholesterol-4-^{14}C TMS Ether from GLC

Methyl Palmitate-1-^{14}C		Cholesterol-4-^{14}C	
Injected*	Recovered†	TMS Ether Injected‡	Recovered§
μg	%	μg	%
1.5	64.0	1.8	57.0
3.1	68.0	3.6	61.8
7.6	72.9	9.2	61.4
15.3	72.5	18.1	58.5
38.3	77.5	45.9	54.6
76.6	73.7	90.5	53.0

Methyl palmitate-1-^{14}C was chromatographed on a 6 ft × 0.25 inch glass column packed with 18% DEGS on 60–80 mesh Chromosorb W. Operating conditions: column temperature 185°, flash heater 225°, detector 300°, and collector 250° (minimum temperature at tip); outlet argon flow 130 ml/min. Cholesterol-4-^{14}C TMS ether was chromatographed on a 6 ft × 0.25 inch glass column of 6% DEGS on 60–80 mesh Chromosorb W. Operating conditions: column temperature 208°, flash heater 238°, detector 300°, and collector 250° (minimum temperature at tip); outlet argon flow 151 ml/min. All collections were made in 0.6 mm i.d. stainless steel capillaries (12).

* Specific radioactivity of methyl palmitate-1-^{14}C was 418 cpm/μg.

† The mean of 14 collections of methyl palmitate-1-^{14}C between 1.5 and 76.6 μg was 71.8 ± 4.5% SD.

‡ Specific radioactivity of cholesterol-4-^{14}C TMS ether was 337 cpm/μg.

§ The mean of 10 collections of cholesterol-4-^{14}C TMS ether between 1.8 and 45.9 μg was 58.7 ± 3.2% SD.

Standard fatty acid methyl esters or free fatty acids were obtained from Applied Science Laboratories, Inc. (State College, Pa.), Lachat Chemicals Inc. (Chicago, Ill.), and the Lipid Distribution Program of the National Institutes of Health (Bethesda, Md.). Sterol and squalene standards were prepared and purified as described previously (12). Solvents were redistilled before use.

RESULTS AND DISCUSSION

Collection of Fatty Acids and Sterols from GLC

A method was presented in a previous report for the quantitative collection of free sterols and squalene from GLC (12). For the present study it was necessary to determine the recovery of methyl esters of fatty acids (methyl palmitate-1-^{14}C) and TMS ethers of sterols (cholesterol-4-^{14}C trimethylsilyl ether) from the DEGS columns (Table 1). From earlier work (12) and the data in Table 1, it may be concluded that the collection from each column was characteristic and reproducible within a ±5% standard deviation for quantities between ap-

[1] Brooks, S. C., and V. C. Godefroi, unpublished data.

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TABLE 2 SYNTHESIS OF LONG-CHAIN FATTY ACIDS BY WHOLE MOUSE SKIN

Acid	Carbon Number*	Concentration	Incorporation of Labeled Acetate†	Specific Radioactivity
		μg/g skin	%	*cpm/μg*
14:0	14.00	76.3 (55.6–97.0)‡	6.5 (5.9–7.1)	6.3 (5.5–7.0)
14:1 + 15:0	14.90	19.0 (13.4–24.6)	1.4 (1.2–1.5)	5.3 (4.5–6.0)
16:0	16.00	804 (676–932)	15.5 (14.7–16.2)	1.4 (1.3–1.4)
16:1	16.62	220 (206–234)	2.2 (1.8–2.5)	0.67 (0.56–0.78)
17:0	17.00	tr.	2.5 (2.2–2.7)	—
18:0	18.00	262 (238–286)	10.1 (9.6–10.5)	2.7 (2.5–2.9)
18:1	18.55	1200 (1020–1380)	18.3 (16.9–19.7)	1.1 (0.90–1.3)
18:2	19.40	692 (594–790)	7.9 (7.8–8.0)	0.81 (0.73–0.89)
20:0	20.00	6.8 (5.7–7.9)	3.6 (3.4–3.7)	37.3 (32.7–41.8)
20:1 (18:3)	20.40	107 (89–124)	10.9 (10.2–11.6)	7.2 (6.9–7.5)
21:0	21.00	tr.	4.0 (3.5–4.5)	—
22:0	22.00	tr.	2.9 (2.0–3.7)	—
20:4 + 22:1	22.60	216 (212–220)	5.0 (4.5–5.4)	1.6 (1.4–1.8)
24:0	24.00	tr.	1.2 (1.0–1.3)	—
24:1	24.60	41.0 (26.0–55.0)	2.8 (2.7–2.9)	5.5 (4.0–6.9)

The fatty acids were obtained from incubations of 0.5 g of skin with 1 μc of acetate-1-^{14}C and analyzed by GLC. The total saponifiable lipid mass and radioactivity per gram of skin in these experiments were: 13.7 ± 1.7 mg (average deviation) with 16,700 ± 900 cpm.

* Carbon numbers were calculated according to the method of Woodford and Van Gent (13). Retention times used to calculate the carbon numbers 14.00 through 20.40 were obtained with an outlet argon flow of 130 ml/min; the carbon numbers 21.00 through 24.60 with an outlet argon flow of 353 ml/min.

† Counts incorporated from labeled acetate into each fatty acid expressed as a percentage of the total fatty acid counts recovered from GLC.

‡ Numbers in parentheses indicate range of values derived from two experiments.

proximately 1.5 and 50 μg. Inasmuch as the percentage of the sample recovered was dependent on the condition of the column, as well as on a gas-tight chromatographic system, the efficiency of collection was determined routinely for each experiment by chromatographing standard solutions of labeled methyl palmitate or cholesterol TMS ether. These collection values were used to correct for column loss in calculating the radioactivity present in experimental fatty acid and sterol samples.

Sites of Fatty Acid Synthesis

As reported previously, incubation of whole mouse skin with 1 μc of acetate-1-^{14}C resulted in the conversion of 0.74% of the available radioactivity to saponifiable lipids (5). In the present experiments data for the synthesis of fatty acids in whole skin are given in Table 2; carbon numbers, quantities, and specific radioactivities of the acids determined, and the incorporation of labeled acetate are listed. Both saturated and unsaturated fatty acids ranging in length from 14 to 24 carbons were present and contained radioactivity. Oleic (18:1), palmitic (16:0), and octadecadienoic (18:2) acids occurred in the greatest quantities, while most of the radioactivity was found in oleic, palmitic, stearic (18:0), and eicosenoic (20:1) acids. Incorporation of a significant amount (7.9%) of labeled acetate into an octadecadienoic acid by whole skin was noteworthy. Since as much as 30% of the radioactivity in long-chain fatty acids was found in acids having 20 or more carbons, the unique ability of rodent skin to synthesize these very long-chain acids was apparent in these experiments. The following series of decreasing specific radioactivity were noted: (*a*) myristic > palmitic > palmitoleic acid; (*b*) stearic > oleic > octadecadienoic acid; and (*c*) arachidic > eicosenoic acid. Of special interest was the extremely high specific activity of arachidic acid, which greatly exceeded the values of the shorter-chain acids investigated.

Further quantitative analysis of fatty acids synthesized in whole mouse skin is presented in Table 3. Since characterization by more than one chromatographic system is essential to identification, the fatty acids were also analyzed by reversed-phase liquid–liquid chromatography. This method permitted the determination of radioactivity in each acid. Losses, however, would be expected in acids which were chromtographed twice and subjected to hydrogenation to separate unsaturated acids from shorter-chain saturated acids. Data obtained by partition chromatography substantiated the results from GLC (Table 2) with respect to the relative amounts of labeled acetate converted by whole skin to fatty acids of 14 to 22 carbon atoms in length. Synthesis of an octadecadienoic acid was also confirmed.

Figure 1 is a plot of collected radioactivity with a superimposed gas–liquid chromatogram of saponifiable lipids (as methyl esters) from whole skin. Unfortunately, the nearly identical carbon numbers for myristoleic (14:1) and pentadecanoic (15:0) acids precluded positive identification of either of these acids. However, the presence of radioactivity in heptadecanoic (17:0) and

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TABLE 3 Analysis by Reversed-Phase Liquid–Liquid Chromatography* of Long-Chain Fatty Acids Synthesized by Whole Mouse Skin and Its Components†

Acid	Radioactivity‡		
	Whole Skin	Epidermis with Adnexa and Reticulum	Dermal Cells
	%	%	%
12:0	<1	2.5	8.4
14:0	5.5	6.1	7.0
16:0	16.5	21.1	46.5
16:1	5.5	6.4	10.8
18:0	13.6	11.3	3.6
18:1	24.9	27.9	23.7
18:2	1.4	2.5	0.0
20 total	15.6	11.7	0.0
22 total	7.9	4.1	0.0
Above 22	9.1	6.4	0.0

* After the addition of 0.01 meq each of the even numbered fatty acids from lauric (12:0) through docosanoic (22:0), the acids were partitioned by the reversed-phase chromatographic system of Howard and Martin (10). The eluate from the column was collected in 3 ml aliquots; each fraction was titrated under nitrogen with 0.01 N aqueous NaOH using bromocresol purple as indicator. Since the unsaturated fatty acids were inseparable from shorter-chain saturated acids, these acids were determined by hydrogenating isolated mixed peaks (with added carrier of the appropriate unsaturated acid), an by subsequent rechromatography (14). Hydrogenations were carried out in Warburg flasks using palladium black on charcoal in 2 ml of ethyl acetate (15). All fractions were counted on stainless-steel planchets with a gas-flow window Geiger tube.

† The fatty acids were obtained from incubations of 0.5 g of whole skin or of components derived from 0.5 g of whole skin with 1 μc of acetate-1-^{14}C.

‡ Counts incorporated from labeled acetate into each fatty acid expressed as a percentage of the total fatty acid counts recovered from the column.

heneicosanoic (21:0) acids demonstrated the incorporation of acetate-1-^{14}C into long-chain acids containing an odd number of carbons. Nonadecanoic (19:0) acid was not detectable in these experiments. Although eicosenoic and linolenic (18:3) acids have similar carbon numbers, the detected peak with its corresponding radioactivity was assigned to eicosenoic acid. This conclusion is supported by the data of Nikkari and Haahti (2), which showed eicosenoic acid to be a significant constituent of skin fatty acids, and by the fact that incorporation of labeled acetate into linolenic acid is classically unacceptable. Since arachidonic (20:4) and erucic (22:1) acids have nearly identical carbon numbers on the GLC column, the detected and radioactive peaks might be a mixture of these two acids.

It has been demonstrated in an earlier report that the trypsinized dermal residue (i.e. papillary reticulum, mast cells, fibroblasts, etc.) was as proficient as whole skin in the synthesis of saponifiable lipids. However, trypsin-released epidermal and adnexal cells, as well as intact hair follicles and dissected epidermis, apparently were incapable of synthesizing significant amounts of saponifiable lipids (5). Additional data from the present study substantiated the conclusions of the earlier work. It may be noted that the incorporation of labeled acetate into long-chain fatty acids (Tables 2 and 4) and the composition of the fatty acids (Table 4) were quite similar for the dermal residue and whole skin. These tables also show that acids synthesized by the dermal residue had significantly higher specific radioactivity than did acids from whole skin. This increase in activity may well be attributed to greater availability of the labeled acetate to the sites of synthesis in the dermal residue and to loss of inactive fatty acid pools resulting from removal of the sebaceous glands. Incomplete recoveries of radioactivity from GLC for the dermal residue (74%) and whole skin (93%) might indicate the presence of labeled long-chain fatty acids not eluted from the GLC column under the conditions used (Table 4).

By digesting whole skin with collagenase, we have isolated the dermal cells (mast cells, fibroblasts, etc.) from the epidermis with intact adnexa and reticulum and have shown these two components of skin to be important sites for the synthesis of saponifiable lipids (5). Dermal cells converted 0.58% of the available acetate into saponifiable lipids, and the epidermis plus intact adnexa and reticulum converted 0.61% (5). Data from the present experiments demonstrated that the same pattern of long-chain fatty acids found in whole skin and in the dermal residue also existed in the epidermis with intact adnexa and reticulum (Table 4). However, this pattern was changed in the dermal cells, which did not synthesize significant amounts of fatty acids containing more than 18 carbons and did not appear to incorporate acetate into long-chain acids containing an odd number of carbons. As compared to acids in whole skin, the high specific radioactivity of acids synthesized by the epidermis plus adnexa and reticulum may be explained by removal of the dermal fat deposits and accessibility of labeled acetate to the site of synthesis. The dramatic increase in the specific radioactivity of arachidonic and octadecadienoic acids was indicative of their synthesis in the upper area of the dermis apart from the fat deposits of the lower dermis which were supplied with systemic arachidonic and linoleic acids. Addition of 9,10,12,13-tetrabromostearic acid to the brominated labeled saponifiable lipids, obtained from the incubation of acetate-1-^{14}C with epidermis plus intact adnexa and reticulum, showed that the specific radioactivity of brominated linoleic acid decreased with each of five recrystallizations (Table 5). It may be concluded that the synthesized octadecadienoic acid was not linoleic acid; the work of Privett, Blank, and Romanus (16) would suggest this acid to be octadeca-8,11-dienoic acid. Although an octadecadienoic acid was

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TABLE 4 SYNTHESIS OF LONG-CHAIN FATTY ACIDS BY MOUSE SKIN COMPONENTS

Acid	Whole Skin	Skin Components: Dermal Residue			Epidermis with Adnexa and Reticulum			Dermal Cells		
	Composition*	Composition	Sp. Act.	cpm†	Composition	Sp. Act.	cpm	Composition	Sp. Act.	cpm
	%	%	cpm/μg	%	%	cpm/μg	%	%	cpm/μg	%
14:0	2.1‡	1.2	18.7	6.9	1.8	28.6	4.4	0.6	581	0.3
14:1 + 15:0	0.5	tr.	—	3.8	0.6	—	3.3	tr.	—	0.2
16:0	22.0	23.8	2.1	15.3	40.7	6.3	21.6	26.3	599	16.2
16:1	6.0	7.2	1.5	3.2	10.9	2.5	2.4	4.0	1150	4.7
17:0	tr.	tr.	—	2.8	tr.	—	4.6	0.0	—	0.0
18:0	7.2	4.5	5.2	7.2	9.2	16.2	12.7	14.3	567	8.5
18:1	33.0	36.9	2.0	22.2	32.4	5.8	15.5	36.9	1580	61.8
18:2	19.0	18.8	0.85	4.9	0.7	40.4	2.4	15.3	—	0.0
20:0	0.2	0.1	61.2	2.0	0.7	76.2	4.8	0.0	—	0.0
20:1 (18:3)	2.9	1.8	18.8	10.1	2.2	38.8	7.2	2.6§	—	0.0
21:0	tr.	tr.	—	3.4	tr.	—	2.3	0.0	—	0.0
22:0	tr.	tr.	—	2.3	tr.	—	1.3	0.0	—	0.0
20:4 + 22:1	5.9	5.8	3.5	6.3	0.8	79.1	5.5	0.0	—	0.0
24:0	tr.	tr.	—	0.0	tr.	—	0.0	0.0	—	0.0
24:1	1.1	tr.	—	2.4	tr.	—	5.4	0.0	—	0.0
Recovery of radioactivity from GLC	93%		74%			74%			100%	

The fatty acids were obtained from incubations of components derived from 0.5 g of whole skin with 1 μc of acetate-1-^{14}C and analyzed by GLC. The total saponifiable lipid mass and radioactivity per gram of skin in these preparations were: dermal residue, 13.0 ± 0.9 mg (average deviation) with 34,300 ± 6,100 cpm; epidermis with adnexa and reticulum, 5.9 ± 0.2 mg with 23,100 ± 4,100 cpm; and dermal cells, less than 0.5 mg with 134,000 ± 5,200 cpm.

* Amount of each fatty acid expressed as a per cent of the total fatty acid quantity.

† Counts incorporated from labeled acetate into each fatty acid expressed as a percentage of the total fatty acid counts recovered from GLC.

‡ Each value in the table is an average derived from two experiments. Ranges were similar to those shown in Table 2.

§ The absence of radioactivity indicates this acid to be linolenic rather than eicosenoic.

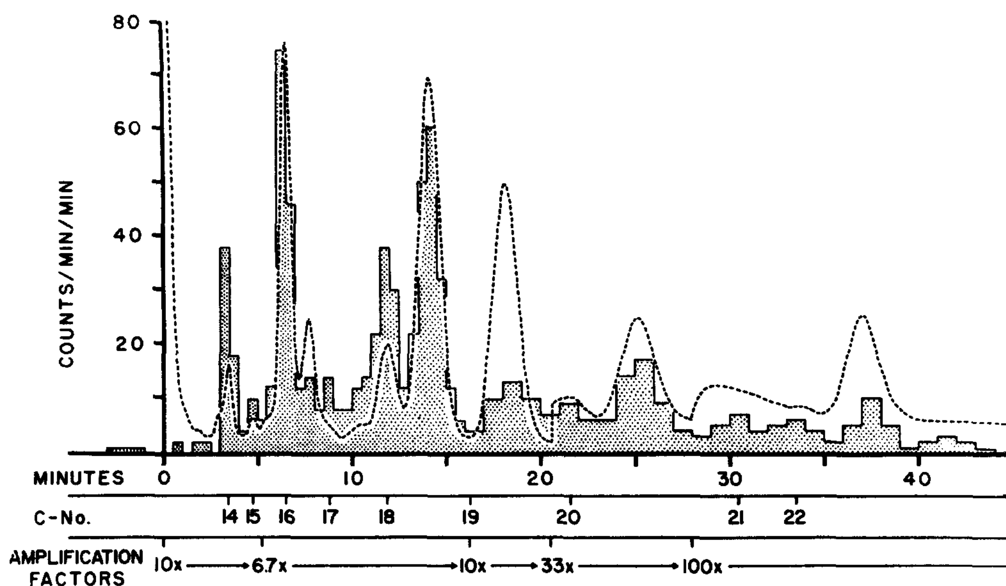

FIG. 1. Bar graph of collected radioactivity with superimposed gas–liquid chromatogram of the methyl esters of saponifiable lipids in whole mouse skin. Operating conditions: temperatures (see Table 1, first footnote); outlet argon flow 130 ml/min up to 28 min, 353 ml/min thereafter. The sensitivity of the GLC recorder, indicated by amplification factors, was changed during the run. An amplification factor of 10 × represents full scale recorder deflection at 2 × 10^{-8} μamp. Three 1-min collections were made before injection of the sample in order to determine the background radioactivity of the GLC column. The saponifiable lipid sample was obtained from incubation of 0.5 g of skin with 1 μc of acetate-1-^{14}C; a one-fifth aliquot (10 μl/50 μl in heptane) was used for the chromatogram.

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TABLE 5 Specific Radioactivity of Carrier 9,10,12,13-Tetrabromostearic Acid* Isolated from Labeled Fatty Acids of Mouse Epidermis with Intact Adnexa and Reticulum

Number of Recrystal-lizations	9,10,12,13-Tetrabromo-stearic Acid Recovered	Melting Point	Specific Radioactivity
	meq	°*C*	*cpm/meq*
1	0.145	111.0–112.0	35,600
2	0.122	113.0–114.0	14,900
3	0.105	113.5–114.0	10,300
4	0.091	115.0–115.2	6,990
5	0.043	113.5–114.5	4,060

* Tetrabromostearic acid (0.333 meq) was added to a brominated fatty acid sample having a total radioactivity of 105,000 cpm.

present in the dermal cells (Table 4), it was devoid of radioactivity and probably represented systemic linoleic acid. Most of the radioactivity from these cells was found in oleic acid, the acid occurring in greatest quantity. Complete recovery from GLC for the dermal cell samples indicates the absence of longer-chain acids that remain on the GLC column under the conditions used.

Fatty acids synthesized by the epidermis with intact adnexa and reticulum, and fatty acids produced by dermal cells were also analyzed by reversed-phase liquid–liquid chromatography (10) to confirm the distribution of the label (Table 3). The data from this method, showing the incorporation of labeled acetate into fatty acids, were similar to those obtained by GLC (Table 4).

TABLE 6 Synthesis of Nonsaponifiable Lipids in Whole Mouse Skin and Its Components

Tissue	Total Radioactivity in Nonsaponifiable Lipids	Squalene		Δ^7-Cholestenol		Cholesterol		Recovery*
		Concn.	Sp. Act.	Concn.	Sp. Act.	Concn.	Sp. Act.	
	cpm	*μg/g skin*	*cpm/μg*	*μg/g skin*	*cpm/μg*	*μg/g skin*	*cpm/μg*	*%*
Whole skin	7,350	6.4	92	262	10.9	1,500	1.38	75
	5,830	5.6	69	105	14.9	1,640	1.22	67
Epidermis with adnexa and reticulum	10,500	4.2	73	134	33.0	690	2.97	68
	6,950	3.2	89	78	43.9	490	3.20	76
Dermal residue	12,500	4.0	66	74	12.5	892	1.36	19
	5,450	5.0	79	36	9.9	680	0.89	25

Nonsaponifiable lipids were obtained from incubations of 0.5 g of whole skin or of components derived from 0.5 g of whole skin with 1 μc of acetate-1-^{14}C and analyzed by GLC. The total nonsaponifiable lipid mass per gram of skin in these preparations were: whole skin, 7.5 ± 0.3 mg (average deviation); epidermis with adnexa and reticulum, 3.8 ± 0.1 mg; and dermal residue, 5.3 ± 0.8 mg.
* Percentage of total radioactivity in nonsaponifiable lipids recovered from GLC as identified compounds.

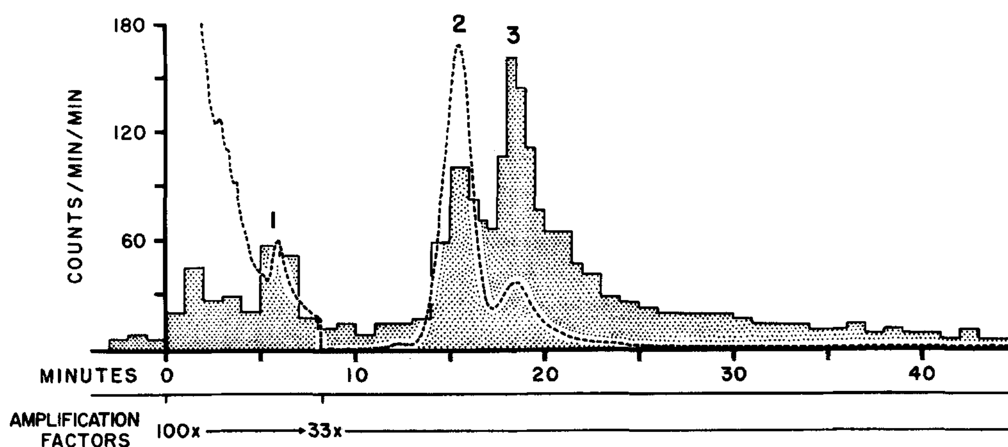

Fig. 2. Bar graph of collected radioactivity with superimposed gas–liquid chromatogram of the TMS ethers of nonsaponifiable lipids in whole mouse skin. Operating conditions as in Table 1, first footnote. Amplification factors, collections before injection, and sample preparation as in Fig. 1, with the exception that butanone was used instead of heptane. The identified peaks are: *1*, squalene; *2*, cholesterol; and *3*, Δ^7-cholestenol.

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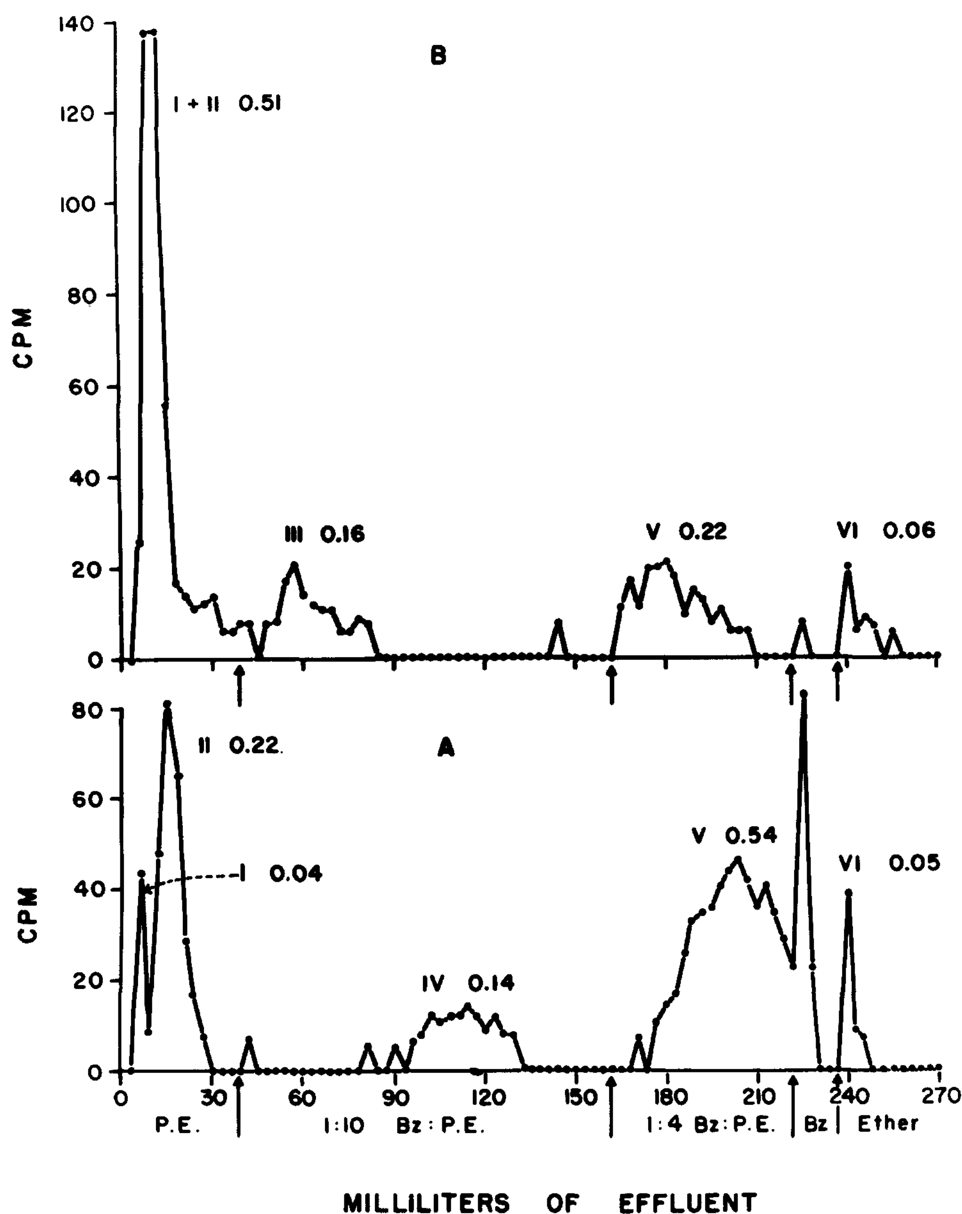

FIG. 3. Separation by chromatography on acetic acid-deactivated alumina (7) of nonsaponifiable lipids from incubations of acetate-1-^{14}C with either whole mouse skin (*A*) or the dermal residue (*B*). Recoveries of the radioactivity were: for whole skin lipids, 99.9%; for dermal residue lipids, 96.8%. Peaks *I* (squalene), *IV* (lanosterol), and *V* (cholesterol and Δ^7-sterols) have been described previously (7). Ratios of individual peak radioactivity to the total nonsaponifiable lipid radioactivity are shown.

It may be concluded from these and earlier experiments (5) that skin possesses two distinct and differing centers of fatty acid synthesis. Cells of the dermal connective tissue comprise one of these sites and produce fatty acids of 18 carbons or less. The other site is situated in the upper dermis (papillary reticulum), which synthesizes not only acids of 18 carbons or less but also fatty acids containing 20 or more carbons. Another characteristic of this latter metabolic site is its ability to produce significant quantities of both eicosenoic acid and an octadecadienoic acid (not linoleic acid), as well as its ability to incorporate labeled acetate into acids containing an odd number of carbons.

Site of Sterol Synthesis

As previously reported, whole mouse skin incorporated 0.40% of the available labeled acetate into nonsaponifiable lipids (5). Data from the present work on concentrations and specific radioactivities of squalene, cholesterol, and Δ^7-cholestenol in whole skin are listed in

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Table 6. The order of decreasing specific activity was: squalene $>\Delta^7$-cholestenol $>$ cholesterol. As seen in Table 6, the results from GLC agreed with other methods (3, 4) in demonstrating the high specific activity of Δ^7-sterols as compared to cholesterol. Figure 2 is a plot of collected radioactivity with a superimposed gas–liquid chromatogram of nonsaponifiable lipids from whole skin. 7-Dehydrocholesterol and other sterol intermediates were detected only in trace quantities in these preparations. For whole skin, approximately 30% of the radioactivity of nonsaponifiable lipids was present in unidentified lipids and was not eluted from the GLC column (Table 6).

The epidermis with intact adnexa and reticulum obtained by collagenase digestion was very active in synthesizing nonsaponifiable lipids (Table 6). This component of skin was as efficient as whole skin in converting labeled acetate to squalene and sterols, as the recovery of approximately 70% from GLC indicates. The greater specific activity of sterols synthesized by the epidermis plus adnexa and reticulum reflected increased availability of acetate-1-^{14}C to the metabolic site and, to some extent, loss of sterols in the collagenase treatment.

An earlier study has demonstrated that trypsin-released epidermal and adnexal cells, as well as isolated hair follicles and dermal cells, produced only trace amounts of nonsaponifiable lipids. On the other hand the trypsinized dermal residue, when compared with whole skin, incorporated equal or greater quantities of labeled acetate into nonsaponifiable lipids (5). The present data, however, show that squalene and sterols contained only approximately 20% of this radioactivity. The greater part of the nonsaponifiable lipid radioactivity was present in unidentified lipids, and was not recovered from GLC.

Chromatography on acetic acid-deactivated alumina (7) of nonsaponifiable lipids, from incubations of labeled acetate with either whole skin or the dermal residue, separated these lipids into six distinct radioactive peaks (Fig. 3). Peaks I (squalene), IV (lanosterol), and V (cholesterol and the Δ^7-sterols) have been previously identified (7). Peaks II and III were the predominant radioactive fractions in the nonsaponifiable lipids of the dermal residue. Analysis of Peak II by GLC showed that it was contaminated with no more than 10% squalene; the remainder of the radioactivity was not eluted from the column. In addition, only 40% of the radioactivity in Peak III was recovered unresolved from GLC as a small increase in the column background. It may, therefore, be concluded that the upper region of the dermis was capable of producing, from acetate, large quantities of nonsaponifiable lipids which were neither sterols nor squalene.

The authors gratefully acknowledge the valuable technical assistance of Mrs. Lenore Langs and Mr. Jerome Seymour.

This investigation was supported in part by Public Health Service Research Grant CA-02903 from the National Cancer Institute and in part by an institutional grant to the Detroit Institute of Cancer Research from the United Foundation of Greater Detroit allocated through the Michigan Cancer Foundation.

Manuscript received 25 February 1965; accepted 27 August 1965.

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