Lipid Synthesis from Acetate-1-^{14}C by Suction Blister Epidermis and Other Skin Components[1] (35037)

S. L. HSIA,[2] JAMES E. FULTON, JR., DAVID FULGHUM[3] AND MARIA M. BUCH

Departments of Dermatology and Biochemistry, University of Miami School of Medicine, Miami, Florida 33136

Several previous studies have established that human skin is active in synthesizing lipids (1–4). In a recent study from our laboratory (4), the lipids synthesized from acetate-1-^{14}C by human skin *in vitro* were examined by chromatographic separation into various classes, and ^{14}C was found incorporated into all lipid classes examined, *i.e.*, hydrocarbons, sterol esters and waxes, triglycerides, sterols, diglycerides, monoglycerides, free fatty acids, and polar lipids. Thus from both quantitative and qualitative considerations, the lipids synthesized in human skin appear to merit further investigation. The present study is an extension of our previous observations, in an attempt to assess the relative contributions to lipid synthesis from the components of skin. We have taken advantage of the fact that the epidermis excised from the top of suction blisters (5, 6) is free of epidermal appendages while the epidermis separated after soaking skin in salt solutions contains attaching pilosebaceous apparatus (7, 8). Synthetic activities of the different tissue preparations were compared, and our results demonstrated that the epidermis is a particularly active site of lipid synthesis. In further experiments, an increase in the synthetic activity was observed in the blister epidermis obtained after the donor ingested 100 g of glucose. The results suggest that lipid synthesis in the epidermis is under metabolic regulation.

Materials and Methods. Sodium acetate-1-^{14}C (sp act 40 mCi/mmole) was purchased from Nuclear Chicago. Sephadex (G-25 coarse) was purchased from Pharmacia Fine Chemicals; Unisil, from Clarkson Chemical Co.; Florisil, from Floridin Co. Reagents were of analytical grade and solvents were redistilled before use. Reference lipids were products of Pierce Biochemicals, and reference ^{14}C-labeled lipids were purchased from New England Nuclear.

Tissue specimens. Five male volunteers, ages 23 to 28, participated in the initial experiments. Skin specimens were taken by two methods after an overnight fast:

Whole skin. Specimens were obtained from the lower abdomen under local anesthesia (Xylocaine) with an 8-mm punch. They were immediately blotted free of blood and trimmed of subcutaneous fat, weighed, and incubated with acetate-1-^{14}C as described under incubation.

Suction blister epidermis and dermis. A suction device for the production of blisters was fashioned after Kiistala and Mustakallio (5) with a suction cup of 15 mm in diameter, and was applied to the lower abdomen of the volunteers. Blisters were produced between 1 and 1.5 hr under suction (pressure, 250–300 mm Hg). The tops of the blisters were carefully excised, blotted gently between filter papers, weighed and immediately incubated as described under incubation. The denuded dermis was biopsied with an 8-mm punch and incubated in the same manner.

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[3] Present address: St. John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London, W. C. 2., England.

of glucose loading, specimens of blister epidermis were obtained from male volunteers after an overnight fast. Each volunteer was then given 100 g of glucose orally, and 1 hr later the blistering device was again applied to an area 2–10 cm from the first blister, and the epidermis was removed for incubation.

Incubation. Each specimen of whole skin, epidermis, or dermis was incubated in 2 ml of Krebs–Ringer phosphate buffer (pH 7.4) containing 2 μCi of sodium acetate-1-^{14}C, 200 μg of streptomycin, 200 units of penicillin, and 200 μg of gentamicin sulfate, for 6 hr at 37° in a shaking incubator. Details of this procedure were described previously (3, 4).

Separation of epidermis from dermis after soaking in 2 M NaI. In experiments with whole skin, the specimen was picked out from the incubation medium at the end of the incubation, and rinsed with distilled water. It was then soaked in 2 *M* NaI solution at 37° for 30 min following the method of Hambrick and Blank (7). The epidermal layer, with sebaceous glands attached (7, 8) was then readily peeled off under a dissecting microscope.

Extraction and chromatography of lipids. The dermal and epidermal layers, after separation with NaI, were blotted gently between filter papers and weighed. Each was homoggenized in 50 ml of chloroform–methanol [2:1] with a Virtis 45 homogenizer. The tissue debris was removed by filtration on a sintered glass funnel and the residue was washed 3 times with 10 ml of chloroform–methanol [2:1]. After the incubation of dermis or epidermis obtained from suction blisters, the tissue was picked out from the incubation medium, washed, homogenized, and extracted in the same manner. The filtrate and washings were combined and evaporated to dryness in a rotary evaporator under vacuum.

The methods for separation of lipids were described previously (4). In brief, the residue obtained after evaporation of chloroform and methanol was dissolved in chloroform–methanol–water 19:1:0.1, and passed through a Sephadex (G-25 coarse) column as described by Siakotos and Rouser (9), to remove unincorporated acetate-1-^{14}C. After evaporation of the solvents, the recovered lipid mixture was dissolved in chloroform and percolated onto a column containing 5 g of Unisil (100–200 mesh) suspended in chloroform. Neutral lipids were eluted with 100 ml of chloroform and polar lipids with 100 ml of methyl alcohol.

The neutral lipids were further chromatographed on 5 g of Florisil hydrated with 7% water according to the procedure described by Carroll (10). Lipids were successively eluted from the column by the following solvents: (A) 15 ml of hexane; (B) 20 ml of 5% ether in hexane; (C) 30 ml of 15% ether in hexane; (D) 30 ml of 25% ether in hexane; (E) 20 ml of 50% ether in hexane; (F) 20 ml of 2% methanol in ether; and (G) 25 ml of 4% acetic acid in ether. Reference compounds, unlabeled and ^{14}C-labeled, were chromatographed in the same system, and showed effective separation. Squalene was eluted by (A), sterol esters and waxes by (B), tripalmitin by (C), cholesterol by (D), dipalmitin and diolein by (E), monopalmitin and monolein by (F), and palmitic acid and stearic acid by (G). The effectiveness of separation of lipids was cross-examined by thin-layer chromatography in several solvent systems. Details of the standardization of the chromatographic methods and their effectiveness have been reported previously (4).

Aliquots from the lipid fractions were assayed for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer Model 2002, with 85% efficiency.

Results. Previous studies by Hambrick and Blank (7) and by Kellum (8) have shown the attachment of pilosebaceous structures to the epidermis after epidermal–dermal separation affected by soaking skin in salt solutions. After some practice in our experiments with NaI solution, we were able to obtain epidermis specimens with most of the sebaceous glands attached. This was verified by observations with a dissecting microscope and histologic examinations. Similar examinations of the epidermis and dermis of suction blisters revealed that the sebaceous glands

TABLE I. Weights of Epidermis[a] and Dermis in Specimens of Abdominal Skin Separated After Soaking in 2 *M* NaI.

Subject	Skin specimen (mg)	Dermis (mg)	Epidermis (mg)	Wt ratio (dermis/epidermis)
1	98.9	91.6	8.0	11.5
2	69.7	59.4	7.7	7.7
3	75.2	70.0	6.4	10.9
4	104.7	91.7	8.5	10.8
5	100.3	88.0	8.6	10.2
	Mean ± SEM			10.2 ± 0.5

[a] With attached sebaceous glands.

were retained in the dermis.

The data in Table I show that in skin of the lower abdomen, the radio of the weights of dermis to epidermis was approximately 10:1. This ratio varied within a narrow range in the specimens from the 5 volunteers studied. The relative amounts of ^{14}C incorporated into lipids in these two compartments were compared (dpm of ^{14}C incorporated/mg of tissue) (Table II). The difference in the synthetic activity on the basis of tissue weight was striking, *i.e.*, from 3 up to 11 (av 5.7) times as much ^{14}C was incorporated into lipids per milligram of epidermis as per milligram of dermis.

The lipids were further separated by chromatography on Unisil into neutral and polar lipid fractions. The amounts of ^{14}C in these fractions in dermis and epidermis separated by the two methods are compared in Table III. More ^{14}C was found in neutral lipids than in polar lipids of all specimens, and the relative amounts of ^{14}C in polar lipids were higher in the epidermis than in dermis. There is in general, good agreement in the data from specimens of each individual, but the values were higher in experiments with the separated components of suction blisters, particularly in the neutral lipid fraction.

TABLE II. ^{14}C-Incorporated into Lipids in Dermis and Epidermis Separated After Soaking in 2 *M* NaI.

Subject	(dpm/mg of tissue) Dermis	Epidermis[a]
1	90	558
2	137	1175
3	172	526
4	83	935
5	395	1760

[a] With attached sebaceous glands.

The neutral lipids were further separated

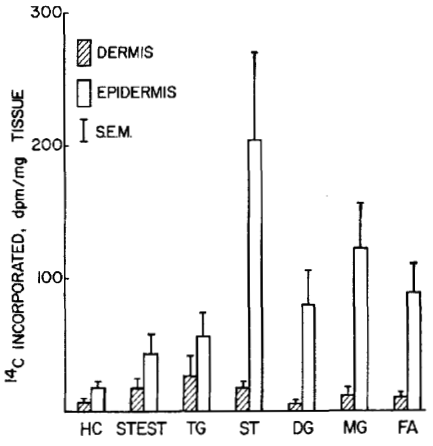

FIG. 1. Distribution of ^{14}C in neutral lipids of the dermis and epidermis separated after soaking in NaI. Specimens of human skin from the lower abdomen were incubated with acetate-1-^{14}C, and then soaked in 2 *M* NaI. The epidermis with attached sebaceous glands was peeled off from the dermis. The lipids in the two separated skin components were extracted and fractionated. The neutral lipids were separated by chromatography on Florisil. The heights of bars represent the amounts of ^{14}C incorporated into lipid fractions per milligram of dermis or epidermis. The sebaceous glands were attached to the epidermis. The results were from skin specimens of five male volunteers (ages 23–28). The abbreviations are: HC, hydrocarbons; STEST, sterol esters and waxes; TG, triglycerides; ST, sterols; DG, diglycerides; MG, monoglycerides; and FA, fatty acids.

into 7 lipid classes on Florisil columns. Figure 1 shows the results obtained from experiments in which the epidermis and dermis were separated after soaking in NaI, and Fig. 2 shows the results from suction blisters. It can be seen that ^{14}C was incorporated into all the lipid classes. A distinct difference in the distribution of the ^{14}C in these lipid classes is found in the hydrocarbon and triglyceride fractions. More ^{14}C is found in these two fractions in the dermis than the epidermis as shown in Fig. 2, while the reverse is true in Fig. 1. In Figs. 1 and 2, the sterol fraction of the epidermis contains the largest amount of ^{14}C.

Since the above experiments indicated that the epidermis is an active site of lipid synthesis, and specimens of blister epidermis can be obtained with relative ease, we tested in further experiments the response of lipid synthesis in this tissue to glucose loading, Eight male volunteers (age 25–49) participated in this study and fasted overnight. Specimens of blister epidermis were obtained

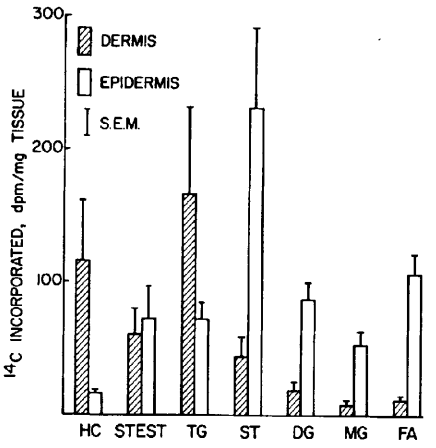

FIG. 2. Distribution of ^{14}C in neutral lipids from dermis and epidermis of suction blisters. Suction blister epidermis and the underlying dermis were separately incubated with acetate-1-^{14}C. The neutral lipids were separated by Florisil column chromatography. The heights of bars represent the amounts of ^{14}C incorporated into lipid fractions per milligram of dermis or epidermis. The sebaceous glands remained in the dermis. The blisters were obtained from the same five volunteers who participated in the study shown in Fig. 1. The abbreviations are the same as in Fig. 1.

TABLE III. ^{14}C Incorporated into Neutral Lipids (NL) and Polar Lipids (PL) by Dermis and Epidermis.

	I. Separation after soaking in NaI				II. Suction blister			
	Dermis (dpm/mg)		Epidermis[a] (dpm/mg)		Dermis[a] (dpm/mg)		Epidermis (dpm/mg)	
Subject	NL	PL	NL	PL	NL	PL	NL	PL
1	63	8	394	123	231	20	328	130
2	88	18	686	400	114	11	687	436
3	116	20	374	60	966	25	1194	362
4	67	20	737	145	391	25	616	125
5	353	35	1330	164	871	39	739	460
Mean ± SEM	137 ± 55	20 ± 4	704 ± 172	178 ± 58	514 ± 171	24 ± 5	713 ± 139	302 ± 73

[a] The sebaceous glands were attached to the epidermis after separation subsequent to soaking in NaI, but remained with the dermis of suction blisters.

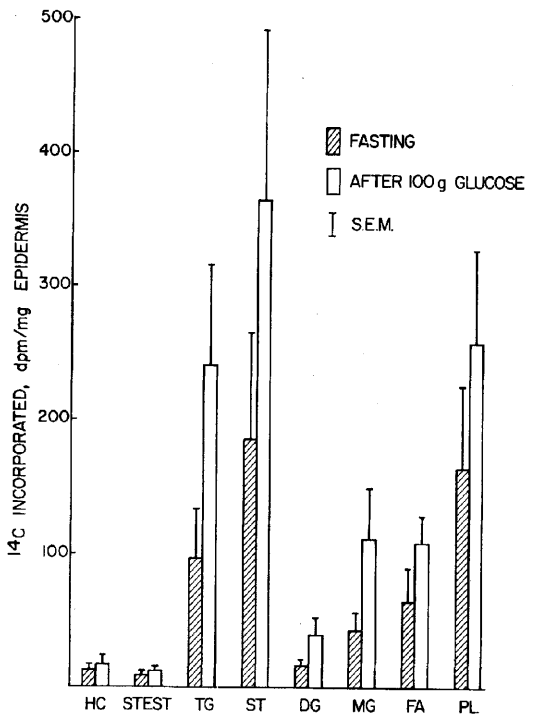

FIG. 3. The effect of glucose loading on the incorporation of ^{14}C from acetate-1-^{14}C into various lipid classes by blister epidermis. Specimens of blister epidermis were obtained from eight male volunteers (ages 25–49), before and after the ingestion of 100 g of glucose. After the incubation with acetate-1-^{14}C, the lipids recovered from the specimens were separated by Unisil into neutral lipids and polar lipids. The former were further separated into seven classes by Florisil column chromatography. PL denotes polar lipids. Other abbreviations are the same as in Fig. 1.

from the lower abdomen before and after the ingestion of 100 g of glucose. After these specimens were incubated with acetate-1-^{14}C, the amounts of ^{14}C incorporated into the lipid fractions were determined (Fig. 3). The results indicated increases in the ^{14}C incorporated into lipids after the ingestion of glucose.

The effect of glucose loading was further observed in another 11 male volunteers (age 17–77). The amounts of ^{14}C incorporated into total lipids by blister epidermis before and after the ingestion of 100 g glucose are compared in Fig. 4. An increase in ^{14}C incorporation was observable after glucose ingestion in each case. Although there seems to be a tendency of increasing ^{14}C incorporation with age, the limited number of observations did not allow statistical evaluation.

To further investigate the effect of glucose loading, three volunteers donated blister epidermis on two occasions separated by a 2-week period. It was found that the amounts of ^{14}C incorporated into total lipids by blister epidermis from the same individual varied within a reasonable range in the 2-week period, and the rise in ^{14}C incorporation into lipids after glucose loading was apparent on both occasions. The results are shown in Table IV.

Discussion. This study has examined lipid synthesis in human epidermis, dermis, and sebaceous glands and revealed certain differences. The data in Table II show that on the average, greater amounts of ^{14}C were incorporated into lipids from acetate-1-^{14}C by the epidermis than dermis of a given skin specimen and Tables II and III show that on per milligram basis, the epidermis was several times more active than dermis.

The activities of the sebaceous glands become apparent when the results in the dermis compartment obtained by the two methods of preparation were compared. Greater amounts of ^{14}C were found in the neutral lipids of blister dermis which retained the sebaceous glands, than in that from NaI preparations (Table III). This difference

TABLE IV. The Effect of Glucose Loading on Lipid Synthesis by Blister Epidermis, and Variation in a 2-Week Period.[a]

	^{14}C incorporated into total lipids (dpm/mg of epidermis)			
	Fasting		After ingestion of 100 g of glucose	
Volunteer	Nov. 21	Dec. 5	Nov. 21	Dec. 5
JEF	1622	1193	2461	2890
SLH	2057	2025	2267	2985
DF	1315	1013	2230	1405

[a] Specimens of blister epidermis were obtained on two occasions separated by a 2-week period. The conditions for the experiment are explained in the text.

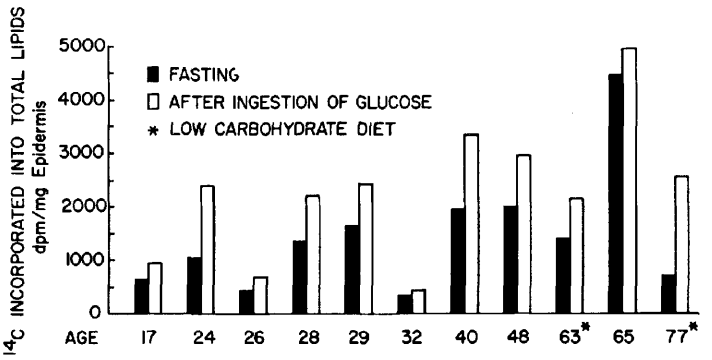

FIG. 4. The effect of glucose loading on ^{14}C incorporation from acetate-1-^{14}C into total lipids by blister epidermis. Male volunteers of various ages donated the specimens of blister epidermis before and after each volunteer ingested 100 g of glucose. Two of the volunteers (indicated by *), were on a low carbohydrate diet. Each value is the average of two incubations.

varied from specimen to specimen, possibly reflecting the uneven distribution and variations in size of the sebaceous glands in the abdominal skin. Further evidence of sebaceous activity was found by comparing Figs. 1 and 2. In Fig. 1, higher amounts of ^{14}C were found incorporated into all lipid fractions of epidermis than in the corresponding fractions of dermis, but in Fig. 2, higher amounts of ^{14}C were found in the hydrocarbon and triglyceride fractions of the dermis. These differences could be attributed to the synthesis of squalene and triglycerides by sebaceous glands which were attached to the epidermis of NaI preparations (Fig. 1), but retained in the dermis of blisters (Fig. 2). This interpretation is in accord with previous findings by Nicolaides and Wells (1), that sebaceous glands produce as major components squalene, wax esters, and triglycerides. A study by Nicolaides and Rothman (11) has given evidence for the synthesis of squalene by sebaceous glands and of sterols by epidermis.

From the data in Table III, it can be calculated that the total amounts of ^{14}C in neutral plus polar lipids recovered from NaI preparations were less than those from blister components. This may indicate a leakage of ^{14}C-labeled lipids from the tissue, particularly from sebaceous glands, during soaking in the NaI solution or during the separation of the epidermis from the dermis. This leakage is reflected by data in Fig. 1, where the amounts of ^{14}C recovered in the hydrocarbon and triglyceride fractions of the epidermis from NaI preparations were too low to account for the activity of the attached sebaceous glands.

A noticeable feature of epidermal activity as indicated by Figs. 1 and 2 is the large amount of ^{14}C incorporated into sterols by epidermis. Another point of interest is found in Table III. The amount of ^{14}C in the polar lipid fraction of the epidermis was in considerably greater proportion than in dermis. Since epidermis is the site of cell proliferation, it is reasonable to expect that the formation of new cellular membranes needs active synthesis of sterols and phospholipids.

These studies indicated that epidermis is particularly active in lipid synthesis and that blister epidermis can be used as an experimental tissue. The results from experiments with blister epidermis presented in Figs. 3 and 4 and in Table IV are interesting as they indicated increases of lipid synthesis after the ingestion of glucose. Although these experiments are exploratory and the results preliminary, they are noteworthy and suggest that more elaborate experiments attempting to elucidate the regulatory mechanisms of lipid synthesis in the skin, and to evaluate the effects of age, diet and other factors, would be worthwhile.

Because tracer amounts of acetate-1-^{14}C were used in these experiments, these data do not allow the calculation of the actual rate

of lipid synthesis in terms of moles of acetate utilized. In a separate communication (12) we have evaluated the rate of lipid synthesis in rat skin, and found that the *in vitro* rate of lipid synthesis from glucose in a medium containing 5 m*M* glucose, was 624 mμ atoms of glucose carbon/hr/g of skin (equiv to 312 mμmoles of acetate incorporated into lipids/hr/g of tissue). Under similar conditions, rat epididymal fat pad is about 30 times more active (13). The rate of lipid synthesis in human skin is currently under investigation in our laboratory.

In a previous study, we observed an impairment of lipid synthesis in skin of patients in diabetic acidosis and of fasting individuals, and a return towards normal after insulin therapy and refeeding, respectively (14). Thus our present findings corroborate previous observations in supporting the concept that lipid synthesis in the skin, and in the epidermis in particular, as the present study shows, is influenced by systemic metabolism. The biochemical basis for the regulatory mechanisms of lipid synthesis has been the subject of many studies. We have recently presented evidence that the level of L-glycerol 3-phosphate may play a regulatory role in lipogenesis in rat skin (15). Whether or not the ingestion of glucose caused changes in the level of L-glycerol 3-phosphate or affected other mediators in human epidermis, *e.g.*, the level of insulin, remains to be studied.

Summary. Lipids synthesized from acetate-1-^{14}C *in vitro* in human dermis, epidermis, and sebaceous glands were examined. On the average, epidermis incorporates more ^{14}C into lipids than dermis does in a given specimen. On per milligram basis, epidermis is several times more active than dermis.

The lipids were separated into eight classes by chromatography on Unisil and Florisil columns. ^{14}C was found in all lipid classes. The major amounts of ^{14}C were found in the sterols, glycerides, and polar lipids of both dermis and epidermis, but greater proportions of ^{14}C were found in the sterol and polar lipid fractions of epidermis than in corresponding fractions of dermis. Synthesis of squalene and triglycerides appeared to characterize the activity of the sebaceous glands.

The epidermis excised from suction blisters was found active in synthesizing lipids from acetate-1-^{14}C, and the activity appeared to increase after the ingestion of 100 g of glucose, indicating metabolic regulation of lipid synthesis in the epidermis.

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