Lipogenesis in isolated human sebaceous glands

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Lipogenesis in isolated human sebaceous glands from [U-^{14}C]glucose, [U-^{14}C]leucine, [U-^{14}C]isoleucine, and [U-^{14}C]valine has been determined by thin-layer chromatography. Total lipogenesis from 2 mmol/l [U-^{14}C]glucose was 114.8 ± 22.3 pmol/gland per h (mean ± SE), with 53.8% being incorporated into triglycerides, 20.2% into squalene, 12.8% into phospholipids, 2.1% into cholesterol and 7.1% into wax monoester and cholesterol ester and 5% into di- and monoglycerides and free fatty acids. Total lipogenesis from 2 mmol/l [U-^{14}C]leucine, 2 mmol/l [U-^{14}C]isoleucine, and 2 mmol/l [U-^{14}C]valine in the presence of 2 mmol/l glucose was 26, 29 and 9%, respectively, of that seen with 2 mmol/l glucose alone. The pattern of ^{14}C distribution in the various lipid classes from the three U-^{14}C-labelled branched-chain amino acids was not significantly different from that seen with [U-^{14}C]glucose.

Lipogenesis *Sebaceous gland* *TLC*

1. INTRODUCTION

The study of the biochemistry and physiology of the human sebaceous gland has been hindered by the difficulty in isolating viable glands [1,2]. We have now described a technique of shearing which yields up to 30 glands within 3 h of biopsy and the glands have been shown to be viable as determined by light and electron microscopy, energy charge, and rates of ^{14}C incorporation into total lipid following incubation with [U-^{14}C]glucose [3]. However, no characterisation has yet been made of the different classes of lipid that isolated glands synthesise. This study reports, therefore, on the thin-layer chromatographic characterisation of the lipids synthesised in vitro.

Sebum contains an unusual class of mammalian lipid, the branched-chain fatty acid components of triglyceride and wax esters, which are predominantly the iso and anteiso-isomers (having a methyl substituent on the penultimate or ante-penultimate carbon atoms of the chains, respectively) but also smaller amounts in which a methyl branch occurs at other positions in the chains, and fatty acids having two or three methyl branches [4]. It has been suggested that these might derive from valine, leucine and isoleucine [4,5], but no study has yet been made on lipogenesis from branched chain amino acid and so it was decided to investigate this.

2. MATERIALS AND METHODS

L-Phosphatidylcholine, L-phosphatidic acid, L-phosphatidylethanolamine, L-phosphatidylinositol, L-phosphatidylserine, cardiolipin, L-lysolecithin, sphingomyelin, squalene, cholesterol *n*-decylate, *n*-hexadecane, cholesterol, monopalmitin, 1,2-dipalmitin, 1,3-dipalmitin, stearic acid, tricaprin and stearic acid stearyl ester were from Sigma. [U-^{14}C]Glucose, [U-^{14}C]leucine, [U-^{14}C]-isoleucine, [U-^{14}C]valine, [4-^{14}C]cholesterol, 1,2-di-[1-^{14}C]palmitoyl-3-phosphatidylcholine, glycerol tri[1-^{14}C]palmitate, were from Amersham International. The solvents were from BDH and of the highest purity available.

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2.1. *Isolation and maintenance of sebaceous glands*

Sebaceous glands were isolated by shearing [3]. Ethical Committee permission has been obtained. Glands were incubated overnight at 37°C in 10 ml tissue-culture medium RPMI-1640 (Gibco) supplemented with 5% (v/v) foetal calf serum (Gibco), penicillin (Glaxo) (100 units/ml), streptomycin (Evans) (100 μg/ml) and buffered with 5% CO_2.

2.2. *Sebaceous gland lipogenesis*

Batches of 4–8 sebaceous glands were incubated for 3–5.25 h in 150 μl bicarbonate-buffered medium [6], equilibrated with $O_2:CO_2$ (95:5) at 37°C with addition of radioactive glucose and amino acids as detailed in table 1. Following incubation, the glands were removed, blotted and total lipids extracted [7]. Control experiments showed that the rate of total lipogenesis was linear over 6 h for glucose and all 3 amino acids, and that the recovery of exogenous [4-^{14}C]cholesterol and glycerol tri[1-^{14}C]palmitate was over 90% when added to sebaceous glands and subjected to the lipid extraction procedure.

2.3. *Identification of lipid classes by thin-layer chromatography (TLC)*

The dried sebaceous gland lipid extract was redissolved in 100 μl chloroform-methanol-water (5:5:1, by vol.) and spotted onto a 20×20 cm 0.2 mm silica gel chromatography plate. This was developed in solvent 1 (light petroleum 40–60°C, diethyl ether, acetic acid [50:50:1, by vol.]), solvent 2 (light petroleum 40–60°C, benzene [70:30, v/v]), and solvent 3 (light petroleum 40–60°C), successively in the directions indicated in fig.1. Polar lipid separation was effected by cutting the plate 3.5 cm from the base (interrupted line in fig.1) and developing in solvent 4 (chloroform, methanol, acetic acid, water [25:15:4:2, by vol.]) [8]. Each sebaceous gland extract test plate was developed concurrently with a lipid standard plate. Radioactivy spots on the test plate were located using a Spark Chamber, and cut out, transferred into vials and counted by the external standard channels ratio method until 2000 counts were collected.

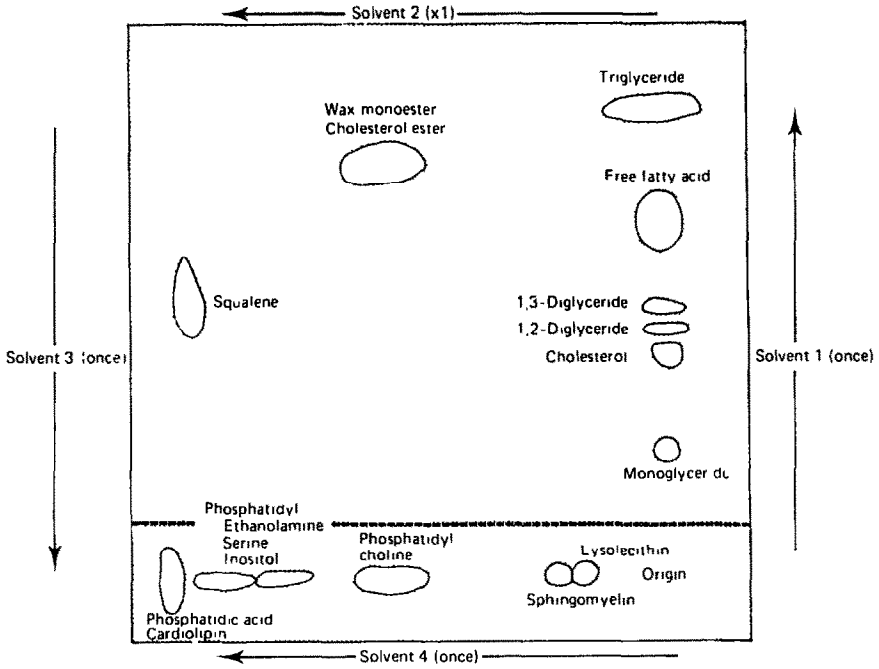

Fig.1. Thin-layer chromatogram of sebaceous gland lipid. Lipid extracts from sebaceous glands were separated by TLC as described in section 2.

Table 1

Incorporation of substrates into sebaceous gland lipids

	Conditions of incubation				
	2 mmol/l [U-^{14}C]glucose ($n=5$)	2 mmol/l [U-^{14}C]glucose, 0.66 mmol/ leucine, 0.66 mmol/l isoleucine, 0.66 mmol/l valine ($n=4$)	2 mmol/l [U-^{14}C]valine, 2 mmol/l glucose ($n=4$)	2 mmol/l [U-^{14}C]iso-leucine, 2 mmol/l glucose ($n=3$)	2 mmol/l [U-^{14}C]leucine, 2 mmol/l glucose ($n=3$)
Total lipid	114.8 ± 22.3	84.4 ± 36.8	10.2 ± 2.4[a,b]	33.3 ± 13.1[a]	30.0 ± 18.5[a]
Lysolecithin + sphingomyelin	3.1 ± 2.6 (2.7%)	1.5 ± 0.8 (1.7%)	0.2 ± 0.004 (2.1%)	0.5 ± 0.2 (1.4%)	0.5 ± 0.5 (1.6%)
Phosphatidylcholine	5.0 ± 1.6 (4.3%)	7.8 ± 4.5 (9.2%)	1.2 ± 0.2 11.9%)	1.9 ± 0.2 (5.8%)	2.3 ± 0.3 (7.6%)
Phosphatidylserine + phosphatidylethanolamine + phosphatidylinositol	3.4 ± 1.1 (2.8%)	2.2 ± 0.8 (2.6%)	0.7 ± 0.2 (7.1%)	2.6 ± 1.8 (7.7%)	1.8 ± 1.4 (6.1%)
Phosphatidic acid + cardiolipin	3.3 ± 1.1 (2.8%)				
Squalene	23.2 ± 6.7 (20.2%)	11.4 ± 6.2 (13.3%)	0.2 ± 0.05 (2.1%)	3.7 ± 1.5 (11.0%)	3.0 ± 2.6 (10.1%)
Wax monoester + cholesterol ester	8.2 ± 3.2 (7.1%)	4.8 ± 2.9 (6.5%)	0.8 ± 0.5 (7.4%)	2.7 ± 1.2 (8%)	1.7 ± 1.5 (5.5%)
Monoglyceride	0.5 ± 0.1 (0.4%)	2.8 ± 0.9 (3.3%)	0.7 ± 0.2 (6.9%)	0.8 ± 0.3 (3%)	1.8 ± 0.6 (6.0%)
Cholesterol	2.5 ± 0.8 (2.1%)	2.2 ± 0.5 (2.5%)	0.2 ± 0.04 (2.2%)	0.7 ± 0.4 (2.1%)	2.3 ± 1.2 (7.5%)
1,2-Diglyceride	1.4 ± 0.5 (1.1%)	3.7 ± 1.9 (4.4%)	0.3 ± 0.1 (2.6%)	0.6 ± 0.2 (1.7%)	0.8 ± 0.1 (3.3%)
1,3-Diglyceride	1.1 ± 0.1 (0.9%)	2.3 ± 1.1 (2.7%)	0.5 ± 0.1 (4.8%)	1.0 ± 0.5 (2.9%)	1.8 ± 0.9 (6.1%)
Free fatty acid	1.0 ± 0.1 (0.8%)	4.3 ± 1.3 (5.0%)	0.8 ± 0.3 (7.3%)	1.1 ± 0.4 (3.1%)	2.3 ± 0.8 (7.7%)
Triglyceride	61.9 ± 9.9 (53.8%)	42.9 ± 21.6 (50.8%)	4.5 ± 2.0 (43.9%)	17.7 ± 8.0 (53.1%)	13.6 ± 10 (45.3%)
Total phospholipids	14.8 ± 3.3 (12.8%)	9.6 ± 4.3 (13.6%)	2.2 ± 0.3 (21.3%)	5.0 ± 1.8 (15.1%)	36.8 ± 2.3 (15.3%)
Total glyceride	66.5 ± 9.6 (57.3%)	56.0 ± 24.9 (66.4%)	6.7 ± 1.7 (65.7%)	21.1 ± 8.9 (63.3%)	20.4 ± 12.4 (67.9%)

[a] Incorporation of ^{14}C from a U-^{14}C-labelled branched-chain amino acid, in the presence of 2 mmol/l glucose, into total lipid was significantly different ($p<0.05$ as determined by Mann-Whitney U test) when compared with the incorporation of ^{14}C from 2 mmol/l [U-^{14}C]glucose

[b] Incorporation of ^{14}C from a U-^{14}C-labelled branched-chain amino acid, in the presence of 2 mmol/l glucose, into total lipid was significantly different ($p<0.05$ as determined by Mann-Whitney U test) when compared with the incorporation of ^{14}C from 2 mmol/l [U-^{14}C]glucose in the presence of 0.66 mmol/l leucine, 0.66 mmol/l isoleucine and 0.66 mmol/l valine

Values are expressed as pmol substrate incorporated/gland per h and are means ± SE (%)

Each area of radioactivity corresponded to a lipid standard spot as identified by 2′,7′-dichlorofluorescein spray under UV light. Control experiments showed that the recovery of radioactive tripalmitate, cholesterol and phosphatidylcholine after TLC was 90–98%.

3. RESULTS AND DISCUSSION

Fig.1 shows that TLC effects a satisfactory separation of the major sebum lipid classes except that cholesterol ester and wax monester have the same R_f value.

Table 1 shows that the incorporation of ^{14}C from [U-^{14}C]glucose into total lipid was 114.8 ± 22.3 (mean ± SE) which is approx. 2.5-times that we have previously reported [3]. The only difference in technique between the two studies is that the glands of this one were maintained overnight. This might have allowed any cell trauma associated with isolation to be corrected.

Table 1 shows the incorporation of ^{14}C from [U-^{14}C]glucose into the various lipid classes. The composition of human sebum is not known precisely because skin lipid derives from two sources, sebaceous glands and epidermal cells, and sebum cannot be collected without epidermal lipid contamination. However, the surface lipid from different skin sites with known variations in sebaceous gland density has been sampled and it has been calculated [9] that the major classes of sebum lipid are: triglycerides, diglycerides, free fatty acids, 57.5%; wax esters, 26%; squalene, 12%; cholesterol, 1.5%; cholesterol esters, 3%. It will be seen that calculation is in broad agreement with our reported pattern of [U-^{14}C]glycose incorporation.

Sebaceous glands were incubated in U-^{14}C-labelled branched-chain amino acids in the expectation that their ^{14}C incorporation would be concentrated, relative to that of glucose, in the fatty acid-containing lipids because of the previous suggestion that they were the source of the branch points of fatty acids [4,5]. In the absence of such concentration (table 1) it remains to be shown whether the amino acids are indeed responsible for the fatty acid branch points.

Table 1 shows that there appears to be substrate competition between glucose and the branched-chain amino acids, and that there is significant catabolism of these essential amino acids. However, the relative concentration of these substrates in blood are so different in vivo (serum glucose concentration is greater than 4.5 mmol/1 [10]; the branched-chain amino acids are approx. 0.1 mmol/1 [11]) that the actual loss in vivo of branched-chain amino acids in sebum may be small. The incorporation of ^{14}C from valine appeared to be much less than that for the other branched-chain amino acids and this may be a reflection of its exclusively gluconeogenic metabolism.

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