Lipogenesis by Isolated Human Apocrine Sweat Glands: Testosterone Has no Effect During Long-Term Organ Maintenance

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Lipid synthesis by freshly isolated human apocrine glands has been measured by the incorporation of [U-^{14}C] acetate. Incorporation is linear over 6 h at 1010 ± 282 pmol/mg wet weight/h (n = 11; mean ± sem). The lipid classes, as percentages of the total lipid synthesized, were found by TLC to be cholesterol 12.3 ± 2.0, mono-glycerides 7.5 ± 1.5, 1,2 di-glycerides 3.0 ± 0.9, 1,3 di-glycerides 3.5 ± 0.5, tri-glycerides 28.4 ± 1.8, free fatty acids 2.0 ± 0.4, lysolecithin 15.4 ± 3.9, sphingomyelin 9.9 ± 4.3, phosphatidyl-choline 8.4 ± 0.4, phosphatidyl-ethanolamine -inositol and -serine 1.8 ± 0.1, phosphatidic acid and cardiolipin 3.3 ± 0.5, and unidentified 3.3 ± 0.5 (mean ± sem, n = 5). Glands were maintained on permeable supports. After 10 d maintenance, electron microscopy showed that the cellular architecture had been preserved, that the ATP contents were the same as in freshly isolated glands, and that [U-^{14}C] acetate incorporation was not significantly altered at 851 ± 237 pmol/mg/h (n = 18). The addition of 3 μM testosterone had no effect on acetate incorporation at 844 ± 231 pmol/mg/h (n = 18). The lipid classes and their proportions were similar to the values for fresh glands after 10 d maintenance both with and without testosterone. *J Invest Dermatol 92:333–336, 1989*

The biochemistry and physiology of the human apocrine sweat gland are largely unknown. It would appear that the main function of the apocrine gland is to produce a lipid rich secretion whose composition is as yet uncharacterized. The secretion is odorless and sterile [1] and is broken down by surface bacteria to produce "body odor" [2,3]. We have developed a technique for the rapid isolation of large numbers of viable glands by shearing [4]. We have used such isolated glands to study apocrine lipogenesis and have characterized the synthesized lipids by thin layer chromatography (TLC).

The development, function, and pathology of the apocrine sweat gland appear to be androgen dependent. The growth of the gland and the onset of axillary secretion occur at puberty [5], and hidradenitis suppurativa is associated with hyperandrogenism [6]. In order to study the effect of testosterone on the apocrine gland in vitro, we have developed an organ maintenance system.

MATERIALS AND METHODS

Materials Petri dishes (60 × 15mm) were supplied by Becton Dickenson (Oxford); nitrocellulose membrane filters were from Whatman (Maidstone); Williams's E, Earle's balanced salt solution (EBSS), phosphate buffered saline (PBS), L-glutamine, penicillin, streptomycin, and fungizone were from GIBCO (Paisley); all other tissue culture supplements and lipid standards were from Sigma (Poole). Solvents from BDH (Poole) were of the highest grade available; ATP monitoring reagent was from LKB Instruments (Croyden); and all radio-isotopes were from Amersham International (Amersham, UK).

Isolation of Apocrine Glands Apocrine sweat glands were isolated by "shearing" [4] female axillary skin samples taken during staging for breast cancer. The patients were 45–65 years old and had no features of androgen excess. Shearing is performed by repeatedly mincing the tissue in EBSS with scissors until a porridge-like consistency is formed; the glands are then picked out with watch makers forceps under a dissecting microscope. This technique routinely yields 30–50 glands within 45 min of skin sampling. Approval for this study was granted by the Central Oxford Research Ethics Committee.

ATP Assay ATP was measured by the luciferin/luciferase assay [7].

Electron Microscopy The glands were fixed primarily in 4% glutaraldehyde and secondarily in osmium tetroxide, processed to absolute alcohol, and embedded in epoxy resin. Ultrathin sections were counterstained with uranyl acetate and lead citrate and viewed in a Philips electron microscope EM 301 at 60 kV.

Lipogenesis Lipid formation was measured by the incorporation of [U-^{14}C] acetate. Acetate was chosen in preference to other substrates as it enters the lipogenesis pathway most immediately and we have shown in the sebaceous gland that acetate is preferentially incorporated over glucose by 2.5:1 (unpublished data). Batches of 3–10 apocrine glands were incubated in 300 μl bicarbonate buffered medium [8] (37°C: 95% air, 5% carbon dioxide) with 2 mM [U-^{14}C] acetate (sp act 57 mCi/mmol). Control experiments demonstrated that incorporation was linear over 6 h. After incubation the glands were washed four times in phosphate buffered saline and

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the lipid was then extracted with chloroform: methanol: water: 0.88% aqueous potassium chloride 2:2:0.8:1 [9]. Lipid classes were analyzed by TLC on 250 μm silica gel plates. Two developments were employed: first with light petroleum, diethyl ether, and acetic acid (50:50:1) and then the origin was removed and further developed with chloroform, methanol, acetic acid, and water 25:15:4:2 [10]. The lipids were visualized by autoradiography and identified by standards run simultaneously. We have previously shown that sebaceous lipogenesis is enhanced by overnight maintenance (16 h) following isolation by shearing [11], and so we routinely assayed for apocrine lipogenesis after 16 h maintenance. This is defined as "fresh" lipogenesis.

Apocrine glands lie in the dermis surrounded by fat. In order to determine whether adipose tissue would survive maintenance and therefore contribute to lipogenesis, control experiments (n = 3) were performed on pure fat. Lobules of fat of similar size to apocrine glands maintained in vitro (see below for method). However, after 16 h (the time at which "fresh" lipogenesis was measured), adipose tissue was no longer viable as determined by undetectable levels of ATP.

Maintenance The glands were maintained on 11 mm nitrocellulose permeable supports with a pore diameter of 0.45 μm floating on Williams E supplemented with insulin 10 mg/ml, transferrin 10 μg/ml, hydrocortisone 10 ng/ml, triiodothyronine 3 mmol/ml, epidermal growth factor 10 ng/ml, selenium 10 ng/ml, trace element mix (GIBCO), prostaglandin E1 10 ng/ml, bovine pituitary extract 10 mg/ml, penicillin 100 u/ml, streptomycin 100 u/ml, and fungizone 2.5 μg/ml. An atmosphere of humidified air and carbon dioxide 95:5 at 37°C was used. In the testosterone treated glands 3 μM testosterone was included in the medium.

RESULTS

ATP Content Freshly isolated apocrine glands contained 345 ± 53 pmol ATP/mg wet weight (n = 6). After 10 d maintenance the ATP content was 346 ± 60 pmol/mg wet weight (n = 10) (mean ± sem).

Electron Microscopy Fresh apocrine glands showed no evidence of mechanical damage and they retained the in situ cellular structure (Fig 1) [12,13]. After 10 d maintenance, the glands were well preserved with similar appearances to the fresh glands (Fig 2). The cell membranes and their tight junctions at the intercellular border with the lumen were intact; the apices of the secretory cells consisted of numerous micro-villous projections; mitochondria were numerous and had well preserved cristae; the Golgi apparatus was plentiful. The secretory cells were filled with lipid-filled pale staining vacuoles and darker dense granules.

Lipogenesis Incorporation of [U-^{14}C] acetate in fresh glands was 1010 ± 282 pmol/mg wet weight/h (mean ± sem; n = 11). Maintained glands incorporated 851 ± 237 pmol/mg/h (mean ± sem; n = 18); this was not significantly different (p = 0.91). Glands maintained with testosterone incorporated 844 ± 231 pmol/mg/h

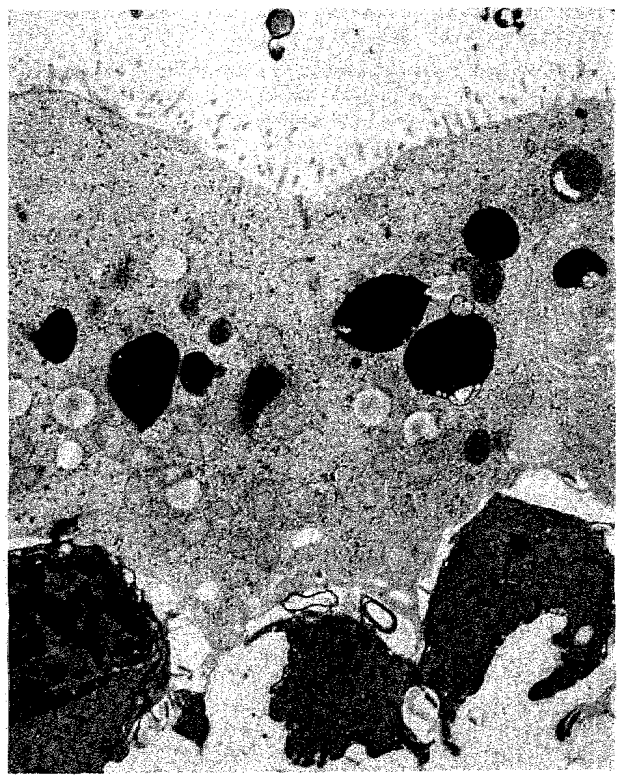

Figure 1. Ultrastructure of a freshly isolated apocrine gland. The secretory cell has numerous microvillous processes reaching into the lumen. There are pale lipid filled and dark granular vacuoles within the secretory cell. The mitochondria show healthy cristae and the Golgi apparatus is plentiful. Magnification: ×7,800.

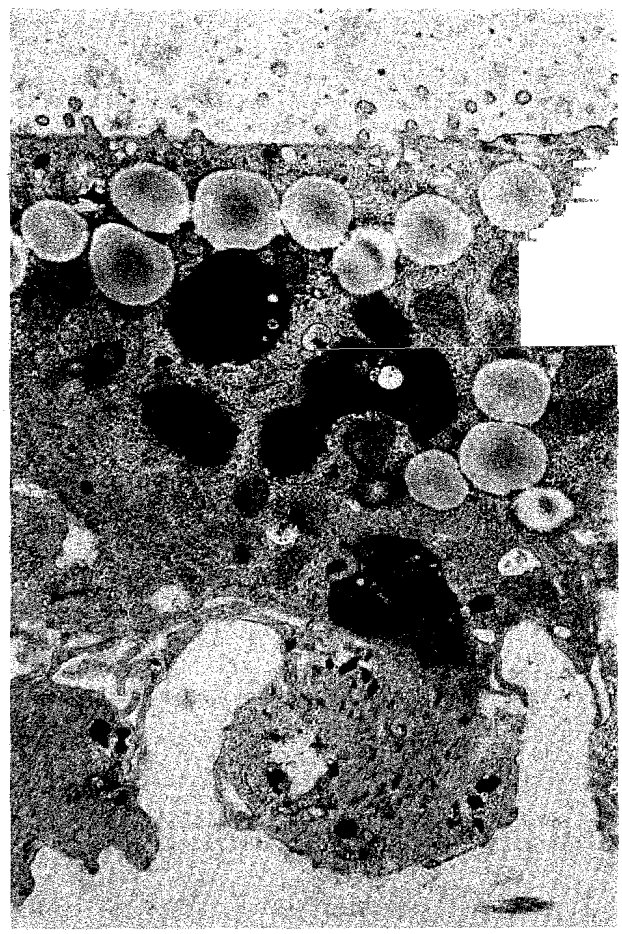

Figure 2. Ultrastructure of a maintained apocrine gland. The appearance is similar to the fresh gland with microvilli, lipid-filled and dark granular vacuoles, and mitochondria with preserved cristae. Magnification: ×15,600.

Table I. Total Incorporation of [U-^{14}C] Acetate by Isolated Apocrine Glands[a]

Fresh	Maintained	
	3μM Testosterone	No Testosterone
(n = 11)	(n = 18)	(n = 18)
1010 ± 282	844 ± 231	851 ± 237 (pmol/mg wet weight/hr)

[a] In-vitro lipogenesis from [U-^{14}C] acetate was performed as described in the *Methods* section. All incubations were for 3 h. There was no significant difference between either the lipogenesis of fresh or maintained glands, or between glands maintained with or without testosterone.

(mean ± sem; n = 18). Table II illustrates the different lipid classes synthesized; no difference can be seen between fresh glands or from those maintained either with or without the addition of testosterone with the exception of lysolecithin whose synthesis is reduced sevenfold on maintenance. There was no relationship between ATP levels and acetate incorporation (12 paired samples, Spearman correlation coefficient 0.280; p = 0.379).

DISCUSSION

This study describes the first in vitro analysis of apocrine lipogenesis. Using the technique of [U-^{14}C] acetate incorporation, we have shown that the apocrine gland synthesizes glycerides, cholesterol, and phospholipids. This confirms a single description of the nature of apocrine secretion in vivo [3], which reported the presence of cholesterol, glycerides, squalene, and wax and cholesterol esters (see Table II). We performed five separate analyses of apocrine incubations with [U-^{14}C] acetate using the four-dimensional TLC method described by Cooper et al [10] but did not find any of the latter three classes of lipids; subsequent experiments were performed in two dimensions as described. The presence of squalene and wax and cholesterol esters in the in vivo study may have been due to contamination by sebum [11,14] which shares a common duct to the skin surface with apocrine secretions. A further difference between the two studies is the presence of phospholipids in our in vitro study. This may be explained by the incorporation of phospholipids into membranes during cell turnover and would, therefore, not be secreted. The considerable quantities of glycerides synthesized by apocrine glands would be a suitable substrate for the release of volatile fatty acids by skin surface bacteria in the genesis of body odor [1,2,3].

Whole glands were maintained on permeable supports floating on a defined medium for 10 d. After this period, biochemical and structural measurements determined that cellular integrity was preserved at similar levels to the fresh state. This enabled us to study the effect of testosterone on apocrine function.

Glands were maintained in the presence and absence of testosterone. After 10 d there was no difference between those glands maintained with or without testosterone. In particular, the pattern of lipid synthesis was unchanged. There was a diminution in the quantity of lysolecithin produced on maintenance, but this was not affected by the presence of testosterone; the significance of this finding is unknown. A similar lack of effect of testosterone on the apocrine gland in vivo was found by Shelley and Hurley [15] who injected testosterone into the axillae of male volunteers and found no change in size or function. This would suggest that, although androgens may be responsible for the development of the apocrine gland, they are no longer required after terminal differentiation has occurred.

In summary, we have defined the classes of lipid synthesized by the apocrine gland in vitro and have developed a method for long-term maintenance which may prove to be a valuable tool in the study of apocrine physiology and pathophysiology.

We are grateful to Mrs. Ysanne Smart for the electron micrographs and to Professor Sir Philip Randle FRS for valuable discussions.

Table II. Lipid Classes Elaborated by Apocrine Glands[a]

	Isolated			in-vivo
		Maintained		
	Fresh	3μM Testosterone	No Testosterone	Leyden et al[3]
	n = 6	n = 5	n = 4	
Cholesterol	12.3 ± 2.0	15.6 ± 2.1	24.7 ± 10.1	76.2
Mono-glycerides	7.5 ± 1.5	11.9 ± 2.8	7.7 ± 3.9	
1,2 di-glycerides	3.0 ± 2.2	2.0 ± 0.5	3.0 ± 0.4	
1,3 di-glycerides	3.5 ± 0.5	6.9 ± 1.7	4.9 ± 0.5	
tri-glycerides	28.4 ± 1.8	35.8 ± 7.7	32.4 ± 13.3	19.2
Free fatty acids	2.0 ± 0.4	3.9 ± 0.2	4.3 ± 0.4	
Sphingomyelin	9.9 ± 4.3	6.7 ± 2.5	4.3 ± 2.1	
Lysolecithin	15.4 ± 3.9	2.1 ± 0.9	2.4 ± 1.0	
Phosphatidyl-choline	8.4 ± 0.4	4.4 ± 3.6	8.5 ± 2.9	
-ethanolamine -inositol -serine	1.8 ± 0.1	3.6 ± 0.5	3.0 ± 0.4	
Phosphatidic acid Cardiolipin	3.3 ± 0.5	4.6 ± 1.0	8.1 ± 2.0	
Unidentified	3.6 ± 0.9	4.1 ± 0.9	2.0 ± 0.7	
Cholesterol esters	-	-	-	0.9
Wax esters	-	-	-	3.6
Squalene	-	-	-	0.2

[a] In-vitro lipogenesis from [U-^{14}C] acetate was performed as described in the *Methods* section. All incubations were for 3 h. Lipid classes were characterized by TLC as described in the *Methods* section. There was no significant difference between either the lipogenesis of fresh or maintained glands, or between glands maintained with or without testosterone, except for lysolecithin. This was significantly lower in glands maintained with or without testosterone compared to fresh glands ($p < 0.002$). The figures for in-vivo lipogenesis were those reported by Leyden et al [3].

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