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Received October 23, 1963. P.S.E.B.M., 1964, v115.

Fatty Acid Composition of the Phosphatide and Triglyceride Fractions of Human Epidermis.* (28873)

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Although considerable work has been done on the determination of the lipid composition of sebum(1-5), and on the lipid fractions obtained from sebum, particularly by Haahti and co-workers(5), the lipid composition of epidermis has interested few investigators recently. This may be due in part to the relative ease in obtaining sebum as compared with adequate amounts of epidermis. Reinertson and Wheatley(6) have carried out lipid analysis not only on total human epidermis, but also on the "Malpighian" layer and stratum corneum. In this paper the fatty acid composition of the triglycerides and phosphatides of human epidermis is reported.

Methods. Human skin† was obtained from extremities and radical breast operations as soon as feasible or within a few hours after surgery. Aside from the pooled breast skin samples, 3 were obtained from females of ages 18, 61, and 83, and 6 from males of ages 41, 56, 58, 59(2) and 71. The extremities had been removed from patients having such afflictions as arteriosclerosis, diabetes, frostbite, and tumors. After removal of the skin from the extremities, it was cut into about 4 × 6 inch square pieces, washed free of blood, etc., and frozen immediately at –25°C if not used at once. Prior to removal of the epidermis the adipose layer was scraped off thoroughly, and the skin surface was cleaned in one of two ways: (A) The epidermal surface was washed with cotton soaked in distilled water, dried with clean cotton, then the epidermis was removed by the procedure of Baumberger *et al*(7). The epidermis was dipped into anhydrous ether quickly several times, set at room temperature to remove the excess ether, then weighed for immediate extraction or for storage at –25°C. (B) The epidermal surface was washed with cotton wet with distilled water, dried with cotton, and rubbed gently with cotton soaked with anhydrous ether. The surface was then washed again with distilled water, dried with cotton, and the epidermis was removed as described under (A). Both procedures gave the same results, but the second was preferred. Care is needed in cleaning the epidermis for the following reasons: (A) Ex-

* Aided in part by grant from Nat. Cancer Inst., U.S.P.H.S.

† Author indebted to Dr. Brennan, Buffalo General Hosp., and to Dr. Pickren of this Institute for skin samples.

TABLE I. Lipid Composition of Human Epidermis.*

Source of epidermis	Sterol and wax esters	Triglycerides	Cholesterol	Ether eluate†	Phosphatides
	%				
Male extremities (6) and breast (1)	3.8 ± .9	45.8 ± 5.4	10 ± 1.1	11.6 ± 1.9	26.5 ± 2.8
Female extremities (3)	2.4 ± 1.4	78.7 ± 3.1	4.2 ± .8	3.8 ± .2	10.9 ± 2.0
Sebum‡	23.8	31.1	4.2	13.7§	

* No. of different samples analyzed indicated in parentheses (mean ± standard deviation).
† Mono- and di-glycerides and other lipid constituents.
‡ From Haahti *et al.*(1). Rest of sebum composed of free fatty acids (14.1%), paraffins (1.2%), squalene (11.5%).
§ Contains phosphatides also.

tremities as obtained usually have fresh blood on portions of the skin along with various disinfectants applied prior to surgery. (B) Upon removal of the excessive fatty layer found in human skin, it is difficult to prevent some of the fat from getting onto the epidermis. (C) The sebum present on the skin surface should be removed since its lipid composition is vastly different from that present in the epidermal cells(1). The lipids were extracted from the epidermis (1.6 to 9.6 g samples, average 5.5 g) by homogenization in a Waring blendor in a chloroform-methanol mixture by the procedure of Schwarz *et al* (8). The solvents were removed *in vacuo* under N_2 at 40°-50°C, and the total lipids were finally dried under N_2 in small flasks and stored at –25°C until ready for fractionation. Silicic acid chromatography(9) was used to separate the sterol and wax esters, triglycerides, cholesterol, mono- and di-glycerides and phosphatides. The solvents from these fractions were removed as above, samples dried under N_2 in small flasks and stored at –25°C until ready for use. The methyl esters of the triglycerides and phosphatides were made by refluxing the samples with absolute methanol containing 5% H_2SO_4. Some of the mono- and di-glycerides samples were pooled for transesterification although these fractions, as indicated by paper chromatography(10), contained substances other than glycerides. The esters thus obtained were purified on silicic acid by the method of Luddy *et al*(11) to remove unsaponifiables such as sterols, higher alcohols etc. and were then stored under N_2 at –25°C until ready for gas-liquid chromatography.

The fatty acid composition of the methyl esters was determined by gas-liquid chromatography in an Aerograph Hi-Fi model 600 Chromatograph with a hydrogen flame ionization detector. Identification of the fatty acids was based upon comparison of the retention values (16:0 = 1) of pure and known fatty acid methyl esters on ethylene glycol adipate and Apiezon M columns previously employed for a complex mixture of fatty acid methyl esters(12). Plots of the log of the retention time *vs* the number of carbon atoms in an homologous series were occasionally used for tentative identification. Areas under the curves were measured with a Disc Integrator. Reduction of the unsaturated fatty acid methyl esters was done by the micromethod of Farquhar *et al*(13), as an aid in identification of some of the esters.

Results and discussion. The results of the fractionation of 10 samples of human epidermis (Table I) indicate that in male epidermis of extremities the triglycerides and phosphatides represent the largest proportion of the total lipid, whereas in female epidermis of extremities, the triglycerides account for 79% of the total lipid. Also in male epidermis the cholesterol and ether eluate, which contains mono- and di-glycerides as well as other components, were present in greater amounts than in female epidermis. In contrast to these values human sebum has a high content of sterol and wax esters, free fatty acids and squalene, but relatively small levels of cholesterol, mono- and di-glycerides and

TABLE II. Fatty Acid Composition (% of Total) of the Triglyceride and Phosphatide Fractions of Human Epidermis.*

Acid	Triglycerides (9)	Phosphatides (8)
14:0	2.5 ± .03	.6 ± .07
14:1	.8 ± .02	
15:0	.7 ± .02	.6 ± .2
anteiso 15:0		3.0 ± .3
16:0	20.1 ±1.5	15.0 ± .8
16:1	9.4 ±2.0	2.8 ± .6
17:0		1.6 ± .2
iso 18:0		1.8 ± .38
18:0	1.8 ± .05	14.1 ±1.1
18:1	52.5 ±1.7	23.0 ±1.2
18:2	11.0 ±2.0	24.1 ±1.2
20:0	.6 ± .2	.9 ± .2
20:1	.5 ± .1	.5 ± .1
20:un		1.5 ± .6
20:4?		7.3 ± .8
22:0		1.0 ± .3
24:0		2.2 ± .5

* No. of different samples analyzed indicated in parentheses. (Mean ± standard deviation.)

phosphatides(1). Reinertson *et al*(6) reported that the total lipids of human epidermis contained 7.9% phosphatides and 9.3% sterols which values agree quite well for male and female epidermis respectively (Table I). Glycerides were not determined by Reinertson *et al*(6).

The fatty acid composition of the 9 triglyceride and 8 phosphatide fractions is shown in Table II. The unsaturated fatty acids palmitoleic (9.4%), oleic (52.5%) and linoleic (11.0%) account for 73% of the total fatty acid composition of the triglycerides whereas the saturated fatty acids myristic (2.5%), palmitic (20.1%) and stearic (1.8%) make up about 25% of the total. In this connection it is interesting that Reinertson *et al*(6) reported that total human epidermal lipid contained 54.8% of unsaturated and branched C_{18} acids and 22.7% palmitic acid. Likewise Gellhorn *et al*(14) found that human adipose tissue and lipomas contained about the same amount of some fatty acids as epidermis, namely, palmitoleic (5.6%), linoleic (9.5%), oleic (50%) and palmitic acid (24%). Again Dabney(15) reported that human breast adipose tissue contained oleic acid (45%), linoleic acid (14%), palmitoleic acid (5.9%) and palmitic acid (21.4%).

In the phosphatide fraction (Table II) oleic and linoleic acids were found in about equal amounts (24%), while the saturated fatty acids palmitic (15%), and stearic (14.1%), present in nearly equal levels, represent some 29% of the total. Pentadecanoic, possibly 12-methyltetradecanoic, heptadecanoic, isostearic and two unsaturated fatty acids (20:un 20:4?) were found. The unsaturated acids reduced to arachidic(13) and hence contain 20 carbon atoms. Small amounts of behenic and lignoceric acids were also found.

Several pooled samples of the methyl esters of the mono- and di-glycerides were analyzed for their fatty acid composition although paper chromatography(10) and the extent of transesterification of these fractions showed them to contain other components. Pooling of these fractions was necessary because a large part of them was not transesterified. Briefly these fractions contained aside from the common fatty acids 2 unsaturated fatty acids containing 20 carbon atoms (proved by reduction to arachidic acid), behenic, tricosanoic, lignoceric, pentadecanoic and cerotic acids. Indication of tricosanoic, pentacosanoic and cerotic acids is tentative being based upon plots of log retention times *vs* number of carbon atoms in an homologous series, and not by a comparison of retention values on ethylene glycol adipate and Apiezon M columns(12).

The part of the ether eluate fraction (Table I) which was not transesterified and hence was not eluted with 1% ether in petroleum ether could be eluted with ethyl ether. This non-transesterifiable fraction from 6 samples was combined (67 mg) and was rechromatographed on Bio Rad silicic acid. Elution was carried out fractionally (19 ml) with increasing concentrations of ether in petroleum ether which resulted in graded elution in the order 10.2%, 20.0%, 24.2% and 45.5% (91% recovery) with 100 cc 10%, 150 cc 10%, 200 cc 20% and 400 cc 30% ether in petroleum ether respectively. Increasing the concentration of ether in petroleum ether from 40 to 100% did not elute any more material from the silicic acid. These materials eluted with ether in petroleum ether

consist of long chain aliphatic alcohols(1) determinable by gas-liquid chromatography(3).

Several different samples of epidermal phosphatides were chromatographed on silicic acid impregnated paper(10). The following phosphatides were identified: phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl choline, inositol phosphatide, phosphatidic acids and sphingomyelin along with other phosphatides. These same phosphatides together with plasmalogens have been found in human epidermis by Bloomstrand *et al*(16) and by Gerstein(17).

Summary. In a limited number of analyses the lipid composition of human male and female epidermis of extremities shows differences in that female epidermis has much more triglycerides than does the male epidermis while the latter has more cholesterol, ether eluate and phosphatides than does female epidermis. The fatty acid composition of the triglyceride fraction is characterized in part by containing about 73% unsaturated fatty acids while this figure for the phosphatides is 50%. The phosphatides contain 2 unsaturated acids with 20 carbons and small amounts of pentadecanoic, heptadecanoic, behenic, lignoceric acids as well as the more common fatty acids.

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Received October 3, 1963. P.S.E.B.M., 1964, v115.

Biochemistry of the α-Glyceryl Ethers. I. Distribution in Mammalian Tissues and in Starfish.* (28874)

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A report(1) on the distribution of α-glyceryl ethers in the tissues of the dog has recently appeared. During our work on the α-glyceryl ethers we have carried out a survey of the occurrence of these ethers in various tissues of the rabbit, in a number of mammalian marrows and in the starfish.

* Supported by Massachusetts Division of Am. Cancer Soc. and by Nat. Inst. Health.

Methods. Preparation of non-saponifiable (NS) and phospholipid non-saponifiable (PNS) fractions: Tissue lipid extracts were obtained following the method of Folch(2). Usually 10-20 g of the wet tissue were used, the tissue being fresh or newly thawed from −20° storage. The total lipids were then separated into neutral lipid and phospholipid fractions by acetone precipitation as follows: