Origin of Amniotic Fluid Lipids
III. Fatty Acids (37233)

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Our early observations led us to suggest that amniotic fluid lipids have a multicentric origin (1, 2). Possible sources include maternal and fetal plasma, placenta, and fetal organs. Placenta exerts qualitative and quantitative control of lipids transferred to the fetus: limited amounts of specific unesterified fatty acids and free cholesterol are transferred directly from maternal to fetal plasma and thence possibly to amniotic fluid. Esterified compounds are not transferred (3). Recent work indicates that some phospholipids found in amniotic fluid originated in extruded lung surfactant (4), but the origin of other lipids is unknown. The present communication is the first detailed report of fatty acid composition of all human amniotic fluid lipid classes.

Methods and Materials. Amniotic fluids from eight normal pregnancies at term were collected during elective cesarean sections. The fluids were spun at 900*g* for 20 min and kept frozen at −20° until lipid extraction. Thirty to fifty milliliters were extracted with 20 vol of chloroform–ethanol 2:1 (v/v) at 37° for 2 hr. After purification, lipid classes were obtained by preparatory thin-layer chromatography (tlc) (5). The lipids were eluted twice from silica gel as described for phospholipids (6). The extracts were then evaporated to dryness in teflon-lined screw-cap centrifuge tubes and subjected to methanolysis (7). Fatty acid methyl esters obtained from cholesterol esters were purified by rechromatography on tlc plates developed in heptane–benzene 1:1 (v/v) and eluted with hexane–diethyl ether 9:1 (v/v). Total lipid extract was methylated as for phospholipids and then purified as for cholesterol esters. Gas liquid chromatography was done at 175° in a Barber-Coleman Series 5000 equipped with a flame ionization detector. The column was packed with 15% C6 diethylene glycol succinate on Anakrom absorbent (100–110 mesh) obtained from Analabs, Inc., Hamden, CT. The machine was calibrated each day with a standard fatty-acid–methyl-ester mixture obtained from Applied Science Labs., Inc., State Park, PA. Peak areas were obtained by triangulation. Several unidentified peaks were sometimes seen, but their sum did not exceed 6% of any lipid class except triglycerides (10%). They were usually situated between palmitoleic and stearic acids. In no case did any one unidentified peak exceed 5%. The unidentified peaks were not included in the calculations.

Results. Palmitic acid was the largest component of all classes (Table I). In phospholipids it amounted to about 53% and in cholesterol esters to about 30%. Phospholipids characteristically contained arachidonic acid, which was absent in nonpolar esters. The fatty acid composition of total lipid extract was similar to that of phospholipids, probably reflecting the high phospholipid content (1). Triglycerides differed mainly in low palmitic acid and high oleic acid, but their composition was essentially similar to that of unesterified fatty acids and partial glycerides. The composition of cholesterol esters was unlike that of other lipid classes in that it had a low percentage of palmitic acid and high percentages of palmitoleic and oleic acid. As a result they contained equal amounts of saturated and unsaturated acids, whereas in other lipid classes saturated acids predominated.

Discussion. The finding of high phospholipid palmitic acid content is in agreement with the hypothesis that lung surfactant is extruded into amniotic fluid close to term

TABLE I. Fatty Acid Distribution of Lipid Classes of Normal Human Term Amniotic Fluid.

Familiar name	Fatty acids N°–C atoms:N° unsaturated bonds	Total lipid extract	Phospho-lipids	Trigly-cerides	Cholesterol esters	Monogly-cerides	Diglycer-ides	Unesterified fatty acids
Lauric	12:0	1.2 ± 0.4[a]	1.2 ± 0.5	1.3 ± 0.2	0.8 ± 0.4	2.5 ± 0.6	2.2 ± 1.2	2.1 ± 0.1
Myristic	14:0	5.0 ± 0.2	4.1 ± 0.4	6.7 ± 1.9	11.3 ± 2.2	8.3 ± 1.4	9.3 ± 1.4	5.4 ± 0.8
Myristoleic	14:1	0.8 ± 0.2	0.6 ± 0.2	2.2 ± 0.3	5.2 ± 1.6	1.6 ± 0.4	1.5 ± 0.3	0.9 ± 0.2
Palmitic	16:0	49.7 ± 2.6	53.2 ± 2.4	40.1 ± 1.7	29.6 ± 0.9	33.5 ± 2.6	40.5 ± 2.2	37.2 ± 3.4
Palmitoleic	16:1	7.2 ± 1.2	5.1 ± 0.9	13.0 ± 2.3	21.2 ± 4.7	9.8 ± 1.6	13.9 ± 2.7	7.1 ± 0.9
Stearic	18:0	9.4 ± 0.8	8.2 ± 0.7	12.0 ± 2.0	7.8 ± 1.1	17.7 ± 0.4	8.6 ± 1.6	13.8 ± 1.4
Oleic	18:1	15.7 ± 1.0	16.4 ± 1.0	22.1 ± 1.9	19.8 ± 2.2	21.1 ± 3.0	14.6 ± 0.5	21.8 ± 2.1
Linoleic	18:2	5.7 ± 0.8	4.1 ± 0.2	2.6 ± 0.5	7.3 ± 1.0	5.5 ± 2.0	9.4 ± 3.1	6.9 ± 1.6
Linolenic	18:3	1.0 ± 0.3	0.9 ± 0.2	Tr[b]	0	0	0	0.8 ± 0.2
Arachidic	20:0	0	0	Tr	Tr	0	Tr	0.9 ± 0.3
Arachidonic	20:4	4.3 ± 0.8	6.2 ± 0.4	0	0	0	0	3.1 ± 1.8
Total Saturated		66.3	67.6	60.1	49.5	62.0	60.6	59.3
Total Unsaturated		33.7	32.4	39.9	50.5	38.0	39.4	40.7

[a] Percent average by weight of 8 fluids ± SE.

[b] Tr, trace.

(4). Frosolono *et al.* (8) demonstrated that phospholipids represented about 90% of lipids extracted from dog lung surfactant. Phosphatidyl choline was the main constituent (75%), containing 65% of palmitic acid. Body obtained similar results with pig lung surfactant (9). He showed also that surfactant phospholipids other than phosphatidyl choline contained much less palmitic acid. Gluck *et al.* (4) pointed out the high palmitic acid content on the alpha carbon and the high myristic acid content on the beta carbon of the phosphatidyl choline molecule in amniotic fluid. Arvidson *et al.* (10), however, found little myristic acid. Their results agree with ours.

The extent of the unquestionable contribution of vernix caseosa to amniotic fluid lipids is difficult to assess. Vernix is composed predominantly of hydrocarbons and free cholesterol, but other nonpolar lipids such as cholesterol esters and triglycerides are also found (11). The vernix cholesterol esters present high peaks of palmitic, palmitoleic, and oleic acids, similar to those in our analysis of cholesterol esters, but in sharp contrast to the very high linoleate content of adult serum cholesterol esters (12). Fetal serum cholesterol esters contain more oleate and linoleate than do amniotic fluid cholesterol esters.

The very low content of linoleic acid in all amniotic fluid lipids is striking. Fetal plasma linoleate is invariably lower than that of maternal plasma (12), but it is still higher in most lipids than in amniotic fluid. A low linoleic acid content was found in adipose tissue of term infants (13). Since linoleic acid is an essential fatty acid, the fetus can obtain it only by placental transfer. Its incorporation into adipose tissue and amniotic fluid lipids might be regulated by the same mechanism. The ability of skin to synthesize cholesterol esters is amply documented, but the dissimilarity between fatty acids of sterol esters of vernix and those of adult skin (14) makes vernix a doubtful source of amniotic fluid cholesterol esters. Triglyceride composition of amniotic fluid was unlike that of maternal or fetal serum in which much higher levels of oleic and linoleic acids were detected (12). The unesterified fatty acid composition was very similar to that of the fetus, perhaps because of direct influx.

Conclusion. Fatty acid analysis of amniotic fluid lipids confirms our previous hypothesis of their multicentric origin. The sources may include fetal lung, plasma, skin, and vernix caseosa, but not maternal plasma.

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