FATTY ACID COMPOSITION OF LIPIDS OF BULL, BOAR, RABBIT AND HUMAN SEMEN

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Summary. Bull, boar, rabbit and human semen was fractionated into seminal plasma, sperm tails and sperm heads. The fatty acid composition of total lipid, phospholipid, phosphatidyl choline, triglycerides and diglycerides was determined in each fraction. Qualitative and quantitative differences in the fatty acid contents were found between the species and between the semen fractions. Bull and rabbit seminal plasma contained relatively higher proportions of polyunsaturated acids (PUFA) ($\omega 6$ and $\omega 3$) than that of man and boar. The sperm tails contained in all four species more PUFA than did the sperm heads. The higher metabolites of oleic acid were not present in measurable amounts. The 20- and 22-carbon acids were present in semen in much larger quantities than thus far reported, in any other mammalian tissue.

INTRODUCTION

Spermatozoa contain a considerable amount of intracellular lipid (Miescher, 1897; Zittle & O'Dell, 1941; Lovern, Olley, Hartree & Mann, 1957; Miller, 1960) which is thought to act as a source of energy (Lardy & Phillips, 1941; Hartree & Mann, 1961; Miller, Bertek & Mayer, 1960). Some data are available on the composition of lipid and fatty acids in testicular and ejaculated ram spermatozoa (Scott, Voglmayr & Setchell, 1967), and in the spermatozoa and seminal plasma of bull, boar and stallion (Komarek, Pickett, Lanz & Jensen, 1964; Komarek, Pickett, Gibson & Jensen, 1965a; Komarek, Pickett, Gibson & Lanz, 1965b). Flipse & Porter (1955) identified short chain fatty acids in bull semen. Volatile fatty acids were found in semen of dog, bull, ram, fowl, stallion and man (Scott, White & Annison, 1960). Dietz, Pickett, Komarek & Jensen (1963) and Miller (1960) reported on the fatty acid composition of bull semen, but fatty acids with 20- and 22-carbon atoms were not reported in their analyses, although studies from this laboratory showed reproductive tissues to be particularly rich in such acids (Holman & Hofstetter, 1965). Because lipids are abundant in parts of cellular structure (Locke, 1964), and because some fatty

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acids, being oxidizable, may serve as an important source of energy for spermatozoa (Mann, 1964; Scott & Dawson, 1968), the fatty acid composition of semen was re-investigated in detail, using the technique of gas-liquid chromatography (GLC). Samples of semen from four species including man were fractionated, and the composition of total lipid and of major lipid components was determined in each fraction.

MATERIALS AND METHODS

Human semen was obtained from a group of healthy donors; four pooled samples, 25 ml each, were collected over a period of about 1 month, and shipped to the laboratory in the frozen state. Four pooled samples of 15 ml of semen were collected from four groups of thirty New Zealand rabbits; the semen was collected artificially twice or thrice a week from each group. Six composite samples of the sperm-rich semen portion were collected from six Yorkshire boars of good fertility. Six pooled samples of 15 ml of semen were obtained from

TEXT-FIG. 1. Procedure for the separation of sperm heads and sperm tails.

six Holstein bulls of good fertility record. Before analysis, the semen was thawed and centrifuged at 4000 *g* for 15 min at 4° C. The seminal plasma was decanted and the spermatozoa were washed twice with 10 ml of physiological saline and recentrifuged. A detailed procedure for the separation of sperm heads and tails is given in Text-fig. 1. The purity of the fractions was estimated by microscopic examinations to be more than 90%. The fractions representing the seminal plasma (Fraction I), sperm tails (Fraction II) and heads (Fraction III) were lyophilized for 12 hr before lipid extraction. This procedure is a modification of the method described by Zittle & O'Dell (1941) and Nelson (1954). No attempt was made to separate the midpieces from the sperm tails.

The lipids were extracted from each fraction with chloroform:methanol (2:1). An aliquot of the extracted lipid was separated into neutral lipids and phospholipids by column chromatography (Hanahan, Dittmer & Warashina, 1957). The purity of each fraction was monitored by thin-layer chromatography (TLC). The phospholipid fraction was separated into various subcomponents

by TLC in a solvent system of chloroform:methanol:water (65:35:4). Only the major phospholipid component, corresponding to phosphatidyl choline ('lecithin'), was scraped off and eluted with the same solvent system that was used for phospholipid on TLC. Hartree & Mann (1961) reported that in ram semen the phosphatidyl choline component contained also the choline-based plasmalogen. It is probable, therefore, that the lecithin component which we isolated by the TLC procedure also contained the plasmalogen.

The other components, such as phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl inositol, constituting together only about 35% of total phospholipid, were not separated for fatty acid analysis. The identification of the phospholipid components was carried out by comparison with known standards on the TLC plate (available from Applied Science Laboratories, College Park, Pa.). Similarly, the neutral lipid fraction obtained by means of column chromatography was separated into various subclasses on TLC plates using 70:25:2 petroleum ether:diethyl ether:acetic acid as solvent system. The major components, triglycerides and diglycerides, were scraped from the TLC plates and eluted with the solvent that was used for separation of neutral lipids on TLC. The minor fractions of cholesterol esters, free fatty acids and methyl esters were not isolated for the fatty acid analysis. The lipid components were *trans*-esterified using 5% HCl in methanol, and the methyl esters were analysed by GLC using a Beckman GC2A gas chromatograph with flame ionization detector, and a column packed with 20% ethylene glycol succinate and 2% phosphoric acid (Ahluwalia & Holman, 1966). Methyl esters in excess of 10% of total fatty acids were isolated on preparative GLC and hydrogenated to determine the chain length.

RESULTS AND DISCUSSION

In human semen, the fatty acid composition of total lipid, phospholipid, phosphatidyl choline (lecithin), triglyceride and diglyceride was determined using the three main fractions; that is, seminal plasma, sperm heads and sperm tails. Only data for lecithin and triglycerides are presented in Table 1. They indicate that both seminal plasma and spermatozoa are relatively rich in polyunsaturated fatty acids (PUFA). Almost all the known metabolites of linoleic acid and linolenic acid were present in measurable amounts in human semen. The higher metabolites of oleic acid (18:1 ω 9) were, however, not detectable in human semen, although in animal reproductive organs, e.g., bovine and swine testes and ovaries (Holman & Hofstetter, 1965) the 20:1 ω 9, 20:2 ω 9, 22:3 ω 9 and 22:4 ω 9 acids were reported to be present in measurable amounts.

Similar analyses were made on the main three fractions from semen of bulls, boars and rabbits. The fatty acids were qualitatively the same, but differed in quantity. Data relating to polyunsaturated derivatives of linoleic (ω 6) and linolenic (ω 3) acids in lecithin and triglycerides are given in Table 1. These data show that long chain PUFA are abundant in seminal lipids. In fact, the sum of ω 6 and ω 3 acids accounts for up to 63% of the lecithin fatty acids in bull seminal plasma (see also Text-fig. 2). PUFA ranged from 12 to 65% of the total fatty acid content in the semen fractions from all four species. The sperm

TABLE 1
FATTY ACID COMPOSITION OF LECITHIN AND TRIGLYCERIDES OF SEMINAL PLASMA,
SPERM TAILS AND SPERM HEADS OF HUMAN SEMEN

Fatty acids	Seminal plasma		Sperm tails		Sperm heads	
	Lecithin	Triglyceride	Lecithin	Triglyceride	Lecithin	Triglyceride
12:0	2.6±1.0	3.0±0.8	2.7±0.8	2.6±0.4	1.6±0.4	4.8±0.5
14:0	3.0±0.4	7.2±1.4	3.8±1.2	4.8±1.6	2.9±1.1	5.8±0.7
14:0 unknown	3.0±0.1	7.0±0.9	3.6±0.9	2.0±0.5	1.8±0.8	7.9±0.8
16:0	16.0±1.6	21.6±1.7	20.0±1.7	20.0±1.6	20.0±1.9	15.4±1.2
16:1 ω 7	2.8±0.3	1.4±0.3	9.6±0.8	9.2±1.2	7.1±0.5	6.0±1.3
17:0	3.2±0.3	2.9±0.4	2.8±0.8	1.5±0.8	2.8±0.7	2.8±0.3
18:0	5.9±0.8	3.0±0.7	5.4±1.3	6.8±2.1	7.9±1.0	6.2±0.6
18:1 ω 9	14.4±1.1	2.0±0.3	19.8±2.5	27.7±2.2	13.8±1.9	6.4±0.6
18:2 ω 6	2.0±0.4	0.7±0.2	1.9±0.4	1.8±0.6	1.9±0.6	1.8±0.5
18:3 ω 3	3.6±0.6	3.4±0.6	2.8±0.8	2.8±1.0	3.9±0.4	3.0±0.2
19:0	0	1.3±0.2	1.7±0.5	0	3.0±1.8	2.9±0.4
20:3 ω 6	2.9±0.4	2.6±0.3	1.2±0.3	1.5±0.1	1.9±0.6	2.8±0.2
20:4 ω 6	8.7±1.8	9.6±1.4	4.0±0.5	3.2±0.4	5.7±1.5	6.0±0.8
20:5 ω 3	8.1±1.3	18.5±2.0	9.4±1.2	12.3±2.7	10.4±1.2	12.0±0.5
22:3 ω 6	3.0±0.3	0	0	tr	0	0
22:4 ω 6	9.3±1.1	6.0±0.5	1.4±0.2	tr	2.4±0.3	5.2±0.6
22:5 ω 6	2.7±0.6	4.8±0.5	1.0±0.1	4.5±2.0	4.6±1.0	3.0±0.3
22:5 ω 3	7.4±1.2	3.6±0.6	10.6±1.5	0	10.6±2.1	2.8±0.3
22:6 ω 3	0.8±0.2	2.5±0.6	tr	0	tr	1.9±0.4
24:4 ω 6	4.8±0.5	0	tr	0	tr	0
Total ω 6	33.4	23.7	9.5	11.0	16.5	18.8
Total ω 3	20.0	28.0	22.8	15.1	24.9	19.7

TEXT-FIG. 2. Fatty acids derived from linoleic and linolenic acid expressed as a percentage of the total fatty acids present in lipids of fractions obtained from bull, boar, rabbit and human semen.

tails were especially rich in PUFA, particularly so in the rabbit. In all cases, the percentage of PUFA in the total fatty acids of sperm heads was less than in the sperm tails.

In the bull, the total lipids were relatively richer in total $\omega 3 + \omega 6$ fatty acids in the seminal plasma and sperm tails than in the sperm heads (see Text-fig. 2). In the boar and rabbit semen, however, the total lipids of sperm tails contained the largest proportion of the polyunsaturated acids. In the sperm tails from bull spermatozoa, the percentage of $\omega 6$ fatty acids in the total lipid was 18:2, 5.2%; 20:3, 3.6%; 20:4, 3.6%; 22:3, 0.6%; 22:4, 13.5%; and 22:5, 1.6%; making a total of 28%. The percentage of $\omega 3$ acids was 18:3, 1.6%; 20:5, 0.8%; 22:5, 20.8%; and 22:6, trace; making a total of 23%. In general,

TABLE 2
THE PERCENTAGE OF POLYUNSATURATED FATTY ACIDS IN
SEMINAL LIPID FRACTIONS

	Total $\omega 6$ acids	Total $\omega 3$ acids
<i>Bull</i>		
Seminal plasma: lecithin	35.1	28.4
triglycerides	17.6	tr
Sperm tail: lecithin	26.5	30.6
triglycerides	10.0	5.7
Sperm head: lecithin	25.8	28.3
triglycerides	14.7	1.6
<i>Boar</i>		
Seminal plasma: lecithin	25.1	7.5
triglycerides	7.7	1.3
Sperm tail: lecithin	33.4	13.3
triglycerides	10.0	2.6
Sperm head: lecithin	35.1	9.0
triglycerides	7.5	5.2
<i>Rabbit</i>		
Seminal plasma: lecithin	25.3	13.6
triglycerides	16.0	5.3
Sperm tail: lecithin	25.2	19.4
triglycerides	33.0	18.3
Sperm head: lecithin	17.6	11.7
triglycerides	24.9	16.3

linoleic acid and arachidonic acid are the major $\omega 6$ acids in mammalian tissues, including reproductive organs, and the C_{22} metabolites of $\omega 6$ acids are usually the minor components. Bull semen contained unusually large quantities of acids related to linolenic acid (18:3 $\omega 3$); together, the $\omega 3$ acids constituted about 23% of total acids in seminal plasma and sperm tails, and 14.0% in the sperm heads. In general, $\omega 3$ acids are the minor PUFA components in other tissues, including the reproductive organs. The presence of large amounts of long chain metabolites of $\omega 6$ and $\omega 3$ acids together with only relatively small amounts of their precursors (18:2 $\omega 6$ and 18:3 $\omega 3$) is striking. The metabolites of oleic acid, 18:1 $\omega 9$, were not detected in semen lipids.

Boar semen was less rich in PUFA than bull semen (Table 2). The higher metabolites of $\omega 6$ and $\omega 3$ acids were present in much smaller amounts in lipids of seminal plasma than in those of sperm heads and sperm tails. Similar amounts of total PUFA were present in lipids of the sperm heads. The pattern of total PUFA in the diglyceride fraction revealed a composition almost identical to that of the phospholipid fraction. Whether this represents a metabolic relationship or an artifact in the storage and preparation of the samples cannot be stated.

In rabbit semen the sperm tails contained larger amounts of $\omega 6$ and $\omega 3$ acids than did the other fractions. Most notable is the presence of large amounts of C20:5 $\omega 3$ which is almost 20% of total acids in total lipid, in contrast to seminal plasma and sperm head where this acid is present in small amounts, from 1 to 3% respectively. The neutral lipid fractions were relatively richer in PUFA than in other species, the triglyceride, diglyceride and phospholipid fractions containing almost identical amounts of total PUFA.

Our analyses show that each species has a distinct fatty acid pattern so far as the three semen components are concerned; that is, seminal plasma, sperm tails and sperm heads. Of particular interest are the fatty acids with 22 carbon atoms which were encountered in large quantities. For example, the 22-carbon acids ($\omega 6 + \omega 3$) in the total lipids of sperm tails were 37% for bull, 40% for boar, 20% for rabbit and 10% for man. Bieri & Prival (1965) found large quantities of C₂₂ acids in the testes of rat, mouse, hamster, rabbit, dog, chicken and man. Therefore, it would seem that these fatty acids are essential constituents of reproductive organs. The rapid loss of these fatty acids in essential fatty acid deficiency in rat testes and rabbit testes accompanied by the reproductive failure, also indicates their importance in reproduction (Ahluwalia, Shima & Pincus, 1968; Ahluwalia, Pincus & Holman, 1967). Normally, the C₂₂ fatty acids are synthesized from linoleic ($\omega 6$) and linolenic ($\omega 3$) acids by chain elongations and dehydrogenations.

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