Uneven distribution of desmosterol and docosahexaenoic acid in the heads and tails of monkey sperm[1]

William E. Connor,[2,*] Don S. Lin,[*] D. P. Wolf,[†,§] and M. Alexander[§]

Division of Endocrinology, Diabetes and Clinical Nutrition Department of Medicine,[*] and Department of Obstetrics and Gynecology,[†] Oregon Health Sciences University, Portland, OR 97201, and Oregon Regional Primate Research Center,[§] Beaverton, OR 97006

Abstract **Previously we demonstrated high concentrations of desmosterol and docosahexaenoic acid (DHA, 22:6 n–3) in monkey testes and sperm. Desmosterol, a cholesterol precursor, is not present elsewhere in the body. High concentrations of DHA are found elsewhere only in the retina and brain. To examine the distribution of these compounds in the heads and tails of sperm, we separated them and determined their sterol, fatty acid, and phospholipid molecular species composition. Desmosterol predominated in tails (134.4 vs. 1.7 μg/10^9 cells in heads). The cholesterol content was also greater in the tails (66.2 vs. 30.3 μg/10^9 cells in heads).**

Sperm tails had more polyunsaturated fatty acids than the heads (34.1 vs. 12.1% of total fatty acids) which resulted mainly from the higher contents of DHA (19.6 vs. 1.1%) and arachidonic acid (20:4 n–6) (6.4 vs. 1.6%) in the tails. These differences in fatty acid composition occurred mainly in phospholipids: phosphatidyl choline and phosphatidyl ethanolamine for n–3 fatty acids and phosphatidylserine and cardiolipin for n–6 fatty acids. Fifteen phospholipid molecular species were identified. Sperm tails had more molecular species containing unsaturated fatty acids than the heads. Our results reveal the large differences in membrane lipid composition between the heads and tails of sperm. Most (99%) of the desmosterol and DHA in sperm is located in the tail. These differences may be responsible for the different functions of these two components of sperm. The large number of double bonds in DHA, six, and in desmosterol, two, may contribute to the membrane fluidity necessary for the motility of the sperm tails. —Connor, W. E., D. S. Lin, D. P. Wolf, and M. Alexander. **Uneven distribution of desmosterol and docosahexaenoic acid in the heads and tails of monkey sperm. *J. Lipid Res.* 1998.** 39: **1404–1411.**

Supplementary key words sterols • fatty acids • phospholipid • molecular species • motility • membrane fluidity • unsaturation • polyunsaturation

More than 300 years ago, Leeuwenhoek discovered spermatozoa using a microscope that had a single highly convexed lens. He noted their swimming motion brought about by the movements of the tail (1). Indeed, until the structure of spermatozoa was viewed under the electron microscope, the simple drawing of sperm in 1677 by Leeuwenhoek was remarkably accurate. As there is no fertility without sperm motility, the mechanism of how sperm are propelled remains a subject of considerable interest.

Spermatozoa are haploid highly specialized cells consisting of at least three major functional compartments: the acrosome, head, and tail (2, 3). Their development from diploid stem cells into mobile sperm is governed by several hundred genes in which the cell nucleus becomes the sperm head while portions of the Golgi, mitochondrial, and cytoplasmic material become the tail and the sheath (4). The tail and head each have distinctive functions. The tail is responsible for movement in the epididymis and, after ejaculation, in the female genital tracts. The head with its acrosome plays an important role in capacitation and in the acrosome reaction that renders the spermatozoa capable of fusing with egg plasma membrane, penetrating the egg for fertilization and transferring the genetic materials (5).

While the protein components of sperm heads and tails have been recently characterized by many investigators (6-10), there is no information available about the membrane lipid compositions of the heads and tails. Recently, we found that human and monkey sperm uniquely contain large amounts of desmosterol and have an especially high concentration of docosahexaenoic acid which is known to be important in the membranes of retina, brain, and testes (11–16). To carry out these observations further, in the present study we analyzed the sterols, fatty acids, and phospholipid molecular species of the heads and tails of the sperm of rhesus monkeys. Large differences in the concentrations of desmosterol and DHA were observed between the heads and tails.

Abbreviations: DHA, docosahexaenoic acid; GLC, gas–liquid chromatography; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography.

[1]Initially presented at the national meeting of Experimental Biology, (Federation of American Societies for Experimental Biology, 1995); Lin, D. S., W. E. Connor, D. P. Wolf, and M. Alexander. 1995. Uneven distribution of desmosterol and docosahexaenoic acid in the heads and tails of monkey sperm. *FASEB J.* **9(A):** 81.

[2] To whom correspondence should be addressed.

MATERIALS AND METHODS

Four healthy 7- to 12-year-old male rhesus monkeys (9–13 kg) were individually caged in a temperature-controlled (22°C), light-regulated (12 h: 12 h light: dark) room. Their diet consisted of Purina Monkey Chow (Ralston Purina Co., Richmond, IN) supplemented with fresh fruit three times weekly and water ad libitum. The study protocol was approved by the Animal Care and Use Committee of the Oregon Regional Primate Research Center.

Monkey semen was collected by a penile electroejaculation technique (17). Samples were twice washed in 3.0 ml Talp-HEPES medium containing 0.3% bovine serum albumin by sedimentation and resuspension. Washed sperm motility and density were determined microscopically. Sperm displayed normal motility (>90%) and sperm counts (total cells/ejaculate) for the four monkeys ranged from 291 to 529 $\times$ 10^6 cells. The cellular content was found to be 99% sperm cells. These monkeys had a record of inducing successful pregnancies in breeder females.

A small portion of the sperm sample was taken for total sterol and fatty acid analysis. Most of the sample was treated with sodium dodecyl sulfate to dissociate the intermolecular linkage of the sperm head and tail (18). The heads and tails were then separated by sucrose gradient centrifugation. To obtain enough material for analysis, semen samples were collected several times (several days apart) from each monkey. Samples were properly pooled for analysis.

Sterol analysis

The lipids of the intact sperm and sperm head and tail were extracted by the method of Folch, Lees, and Sloane Stanley (19). For determining the composition of sterols and fatty acids, aliquots of sperm lipid extract were saponified with alcoholic KOH. The recovered sterols and fatty acids were analyzed by gas–liquid chromatography (GLC). The detailed procedures will be described in subsequent sections.

For analysis of free and esterified sterols in sperm, aliquots of the lipid extracts were plated on silica gel G thin-layer chromatography (TLC) plates after [4-^{14}C]cholesterol and cholesteryl [^{14}C]oleate (New England Nuclear Corp., Boston, MA) were added as internal standards. The plates were developed in hexane–chloroform–ether–acetic acid 80:10:10:1. The free sterol band containing both cholesterol and desmosterol was removed and extracted with ether. Sterol esters were saponified with alcoholic KOH and the sterols were extracted with hexane. The sterol ester fatty acids were recovered by acidifying the aqueous phase and re-extracting with hexane for fatty acid analysis by the procedures described later. The sterol content was determined by GLC (Perkin-Elmer Model 8500, Norwalk, CT) on a 30 M SE-30 capillary column. The temperatures of column, detector, and injection post were 260°, 300°, and 300°C, respectively. Helium was used as the carrier gas. Cholestane was used as internal standard (20).

For the analysis of sterol sulfates, another aliquot of liquid extract was applied on silica gel H TLC plates. [4-^{14}C]cholesterol sulfate was added as internal standard. The TLC plates were developed in chloroform–methanol–acetic acid 80:20:2 (21). Sterol sulfates were extracted from the TLC gel by chloroform and solvolyzed to liberate free sterols (22). The quantity of sterols was determined by GLC as described in the previous paragraph.

Analysis of fatty acids

The four lipid classes, phospholipids, free fatty acids, triglycerides, and sterol esters, were separated by TLC as described above. The individual phospholipids were separated by another TLC system (23), using pre-coated silica gel K6 plate (Whatman, Clifton, NJ) and a solvent system of chloroform–methanol–petroleum ether (bp 35–60°C)–acetic acid–boric acid 40:20:30:10:1.8 (v:v:v:v:w).

The fatty acids in each lipid class or phospholipid class were transmethylated with boron trifluoride–methanol (24) and their methyl esters were analyzed by GLC (25) on an instrument equipped with a hydrogen flame ionization detector (Perkin-Elmer Model Sigma 3B, Norwalk, CT) and a 30-m SP2330 fused silica capillary column (Supelco, Bellefonte, PA). The temperatures of the column, detector, and injection port were 195°, 250°, and 250°, respectively. Helium was used as the carrier gas. The split ratio was 1:170. The retention time and area of each peak were measured by an HP-3390 integrator, and a computer (HP85, Hewlett-Packard, Palo Alto, CA) identified and quantified each individual fatty acid. A mixture of fatty acid standards was run daily.

Analysis of phospholipid molecular species

The molecular species of sperm ethanolamine glycerophospholipids were determined by established techniques (15). As noted above, sperm phospholipid classes were separated by TLC (23). The ethanolamine glycerophospholipids were extracted from gel scrapings with two washes of 5 ml of chloroform–methanol 1:1 (v/v), followed by one wash with 5 ml of chloroform–methanol–water 65:45:12 (by volume) and one more with 5 ml of chloroform–methanol 1:1 (v/v) (26). The molecular species of ethanolamine glycerophospholipids were analyzed by the method of Blank et al. (27). Briefly, ethanolamine glycerophospholipids were hydrolyzed with phospholipase C for 4 h at room temperature (28) . Diradylglycerols were extracted from the hydrolysate by the Bligh and Dyer method (29) and benzoate derivatives were prepared by reaction with benzoic anhydride and 4-dimethyl aminopyridine for 1 h at room temperature (30). The reaction was stopped by addition of concentrated ammonium hydroxide, and the resulting diradylglycerobenzoates were extracted with hexane.

The diradylglycerobenzoates were separated into the alkenylacyl, alkylacyl, and diacyl subclasses by TLC on silica gel G with benzene–hexane–ethyl ether 50:45:4. Bands were scraped into a 1:1 ethanol and water mixture and the diradylglycerobenzoates were extracted with hexane–ethyl ether 50:45:4. Bands were scraped into ethanol–water 1:1 and the diradylglycerobenzoates were extracted with hexane. The samples were then filtered (Millex-HV 0.45 *u*m filter unit, Millipore Corp., Bedford, MA 01730), dried under nitrogen, and redissolved in acetonitrile–isopropanol 70:30 (v/v) for HPLC injection.

The separation of molecular species was accomplished with a Perkin-Elmer Model 410 LC BioPump system fitted with a *u*Bondapak C18 pre-column insert and a 3.9 mm $\times$ 30 cm analytical column packed with Nova-pak C18 (Water Associates, Milfor, MA). Peaks were monitored at 230 nm with a Perkin-Elmer LCI-100 integrator. Molecular species within the diacyl-, alkenylacyl-, and alkylacyl-glycerobenzoates were separated by isocratic elution with acetonitrile–isopropanol (v/v) in the ratios of 70:30, 65:35, and 63:37, respectively. Column flow rate was 1 ml/min.

RESULTS

Eighty-six percent of the sperm sterols were located in the tails (**Table 1**). Both sperm heads and tails contained two sterols: cholesterol and desmosterol. However, there were vast differences in the sterol composition between the heads and tails. Almost all of the desmosterol was present in the tails (99%). The tails also had twice as

TABLE 1. Sterol distribution in the heads and tails of monkey sperm

Sterols	Heads (n = 4)	Tails (n = 4)	Heads + Tails (n = 4)	Intact Sperm (n = 4)
			μg/10⁹ cells	
Cholesterol	30.3 ± 5.9	66.2 ± 22.2[a]	96.5 ± 21.5	97.9 ± 22.4
Free	17.6 ± 3.7	36.7 ± 8.3[b]	54.3 ± 11.0	
Ester	6.9 ± 3.5	23.9 ± 16.5	29.6 ± 16.2	
Sulfate	5.7 ± 5.0	5.7 ± 3.8	11.4 ± 3.2	
Desmosterol	1.7 ± 1.0	134.2 ± 14.7[c]	135.9 ± 13.9	125.2 ± 9.7
Free	1.1 ± 1.3	132.3 ± 18.4[c]	133.4 ± 17.5	
Ester				
Sulfate	0.6 ± 0.5	1.9 ± 3.8	2.5 ± 3.7	
Total sterols	32.0 ± 5.5	200.4 ± 20.2[c]	232.4 ± 22.3	223.1 ± 29.0

Values are given as mean ± SD. Total sterols = cholesterol + desmosterol.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$: heads versus tails.

much cholesterol as the heads. Consequently, the desmosterol to cholesterol ratio was 0.06 in the heads and 2.03 in the tails.

Cholesterol was present in three forms (free, ester, and sulfate) (Table 1). In both heads and tails, free cholesterol was the major fraction contributing 55–58% of the total cholesteryl. Cholesteryl esters accounted for 23% and 36% in heads and tails, respectively. Cholesterol sulfate amounted to 19% of total cholesterol in the heads and 9% in the tails.

Unlike cholesterol, desmosterol was present in only two forms (free and sulfate). In the heads, the free and sulfate forms amounted to 65 and 35% of the total desmosterol, respectively. In the tails, almost all the desmosterol was in the free form (99%).

There was a very striking difference in the fatty acid composition between sperm heads and tails as well (**Table 2**). Sperm tails had a much greater docosahexaenoic (DHA) content. The DHA concentration was only 1.1% of total fatty acids (weight percent) in the heads and 19.6% in the tails (**Fig. 1**). From the DHA concentrations in the heads, tails, and intact sperm, we estimated that 99% of the sperm DHA was contained in the tails. Arachidonic acid and dihomo-γ linolenic acid (20:3 n–6) were also higher in the tails than in the heads, 6.4 versus 1.6 percent and 2.2 versus 0.2%, respectively. On the other hand, oleic acid (13.2 vs. 9.4%), palmitoleic (16:1 n–7) (7.0 vs. 0.9%) and myristic acid (14:0) (5.3 vs. 2.1%) were higher in the heads. The n–6/n–3 ratio was higher in the heads (3.32 vs. 0.67).

TABLE 2. Fatty acid composition of the intact sperm and the heads and tails of the sperm of rhesus monkeys

Fatty Acids	Heads (n = 4)	Tails (n = 4)	Intact Sperm (n = 4)
		% of total fatty acids	
14:0	5.3 ± 2.2	2.1 ± 0.3[a]	2.1 ± 0.7
16:0	29.2 ± 1.1	28.6 ± 3.5	28.9 ± 1.5
18:0	14.2 ± 2.3	12.7 ± 3.3	12.2 ± 0.06
Saturated	55.6 ± 2.9	46.7 ± 3.7[b]	46.5 ± 3.5
16:1 n–7	7.0 ± 1.8	0.9 ± 0.2[c]	0.9 ± 0.3
18:1 n–9	13.2 ± 2.8	9.4 ± 0.3[a]	12.0 ± 3.9
Monounsaturated	22.8 ± 3.7	11.3 ± 0.7[c]	13.6 ± 3.9
18:2 n–6	5.0 ± 0.9	4.1 ± 0.6	5.7 ± 0.5
20:3 n–6	0.2 ± 0.1	2.2 ± 0.1[c]	1.7 ± 0.2
20:4 n–6	1.6 ± 1.0	6.4 ± 0.3[c]	5.4 ± 0.6
Total n–6	8.3 ± 1.8	13.6 ± 0.5[c]	14.5 ± 1.8
18:3 n–3	0.6 ± 0.4	0.1 ± 0.08[a]	0.2 ± 0.08
22:6 n–3	1.1 ± 1.3	19.6 ± 1.7[c]	16.0 ± 1.5
Total n–3	2.5 ± 1.4	20.2 ± 1.7[c]	16.8 ± 1.5
Polyunsaturated	12.1 ± 1.3	34.1 ± 1.5[c]	31.5 ± 2.3
P/S	0.22	0.73	0.67
n–6/n–3	3.32	0.67	0.86

Values are given as mean ± SD.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$: heads versus tails.

The fatty acid composition of different lipid classes of heads and tails is depicted in **Table 3**. The same differences in fatty acid composition between heads and tails observed in total lipids were seen in the different phospholipids. No difference was observed in other lipid classes (free fatty acids, triglycerides, and cholesteryl esters).

The fatty acid composition of seven individual phospholipids is presented in **Table 4**. Because of the limited amount of material available, fatty acid data of phospholipid classes and phospholipid molecular species represent duplicate runs of pooled samples. The differences in fatty acid composition between heads and tails observed in total phospholipids were delineated by phosphatidylethanolamine (PE), choline (PC), and-serine (PS). In PE and PC, DHA was 34.3 and 27.6% in the tails and 6.5 and 5.9% in the heads, respectively. The arachidonic acid of PE and PS was 11.3 and 19.5% in the tails and 2.5 and 3.0% in the heads respectively. Conversely, myristic acid was 1l.1, 12.8, and 11.8% in the PE, PC, and PS of the heads and 3.3, 1.5, and 8.0% in the tails, respectively. Oleic acid was 13.0 and 9.8% in PE and PS of the heads and 4.7 and 5.5% in the tails, respectively. It was of interest that the linoleic acid content in the cardiolipins of the tails was very high (24.3 vs. 4.4% in the heads).

As DHA is the predominant polyunsaturated fatty acid in sperm, and as the ethanolamine glycerophospholipids of sperm are richest in this fatty acid, we chose this phospholipid class for molecular species analysis (**Table 5**). Molecular species were determined for each of the three subclasses of this phospholipids (diacyl, alkenylacyl, and alkylacyl). In the diacyl subclass, a total of 15 different molecular species were identified and quantified. While the *sn*-1 position always contained only 16:0, 18:0, or 18:1, the fatty acids in the *sn*-2 position differed markedly. There were three n–3 species that contained an n–3 fatty acid in the *sn*-2 position, five n–6 species, four n–9 species, and two saturated species containing saturated fatty acid in both *sn*-1 and *sn*-2 positions. For a given fatty acid at *sn*-2 position, the species with 16:0 at *sn*-1 position was the dominant species. For example, the mole % ratios of 18:1–22:6, 16:0–22:6, and 18:0–22:6 in sperm heads were 1, 5.9, and 1.1, respectively. The membranes of the tails contained more molecular species with polyunsaturated fatty acid at *sn*-2 position and fewer molecular species with saturated and monunsaturated fatty acid at *sn*-2 position. For example, in the diacyl subclass, the molecular species with polyunsaturated fatty acid at *sn*-2 position contributed

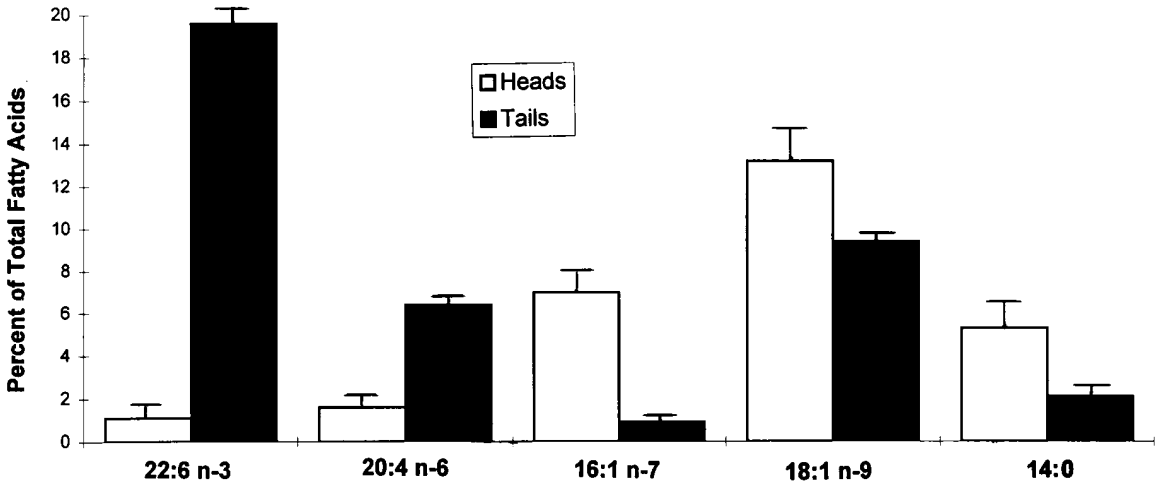

Fig. 1. The concentration of certain fatty acids in the heads and tails of monkey sperm (mean ± SE).

39.5% in the heads and 78% in the tails. In the alkenylacyl subclass, a total of 14 different molecular species were identified. There were three n–3 species, five n–6 species, five n–9 species, and one saturated species. In the alkylacyl subclass, 11 different species were identified in heads; only 7 species were detected in the tails.

As the HPLC detector response is proportional to the molar concentration of the glycerobenzoate derivatives of the different molecular species (27), the relative concentrations of the three subclasses (diacyl, alkenylacyl, and alkyacyl) could be calculated by summing the total area of all peaks (**Table 6**). The relative distributions of diacyl alkenylacyl, and alkylacyl subclasses in ethanolamine glycerophospholipids of sperm heads were 66, 26, and 8%, respectively, and for tails 67, 26, and 7%, respectively. These were all very similar values.

DISCUSSION

The present study characterizes for the first time the composition of the membrane lipids of the heads and tails of spermatozoa. Great differences in the composition of sterols, fatty acids, and the phospholipid molecular species were observed. As these lipids have an important role in membrane integrity, fluidity, stability, and permeability (31–39), these differences in membrane lipid composition between sperm heads and tails may contribute to their unique functions.

TABLE 3. Fatty acid composition of lipid classes of the heads and tails of sperm

	Phospholipids		Free Fatty Acids		Triglycerides		Cholesteryl Esters	
Fatty Acids	Heads (n = 4)	Tails (n = 4)	Heads (n = 4)	Tails (n = 4)	Heads (n = 4)	Tails (n = 4)	Heads (n = 4)	Tails (n = 4)
				% of total fatty acids				
14:0	5.5 ± 1.3	1.9 ± 0.4	4.2 ± 1.8	2.1 ± 0.6	4.3 ± 1.8	4.3 ± 2.0	6.5 ± 2.4	7.0 ± 1.0
16:0	25.1 ± 1.4	25.5 ± 0.6	24.6 ± 4.8	23.4 ± 2.1	19.5 ± 3.4	19.1 ± 3.5	14.1 ± 3.6	15.8 ± 1.7
18:0	16.7 ± 4.1	14.0 ± 7.7	13.3 ± 2.4	16.2 ± 5.1	9.1 ± 1.0	9.3 ± 1.6	7.5 ± 1.9	7.9 ± 1.4
Saturated	49.9 ± 6.0	45.6 ± 8.6	50.2 ± 6.7	50.2 ± 8.2	40.2 ± 8.2	41.0 ± 7.3	38.6 ± 1.9	41.0 ± 3.5
16:1 n–7	3.9 ± 0.1	1.9 ± 0.8[c]	2.6 ± 1.3	1.9 ± 0.1	4.7 ± 2.1	4.1 ± 2.4	4.5 ± 4.7	4.4 ± 3.4
18:1 n–9	21.4 ± 6.2	13.0 ± 2.5[a]	24.7 ± 10.0	26.8 ± 6.0	30.7 ± 7.1	31.5 ± 7.9	28.9 ± 2.6	28.4 ± 6.9
Monounsaturated	28.2 ± 8.2	17.0 ± 3.1[a]	35.6 ± 9.2	36.8 ± 6.0	42.2 ± 8.0	42.1 ± 9.2	40.7 ± 4.2	41.4 ± 6.4
18:2 n–6	2.8 ± 0.4	3.4 ± 0.7	2.7 ± 1.1	1.6 ± 0.6	3.1 ± 0.7	3.3 ± 0.9	2.5 ± 1.9	1.9 ± 0.5
20:3 n–6	0.4 ± 0.4	1.9 ± 0.3[d]	0.2 ± 0.2	tr.	0.4 ± 0.3	0.4 ± 0.2	0.2 ± 0.1	tr.
20:4 n–6	2.0 ± 1.2	5.8 ± 1.1[c]	1.0 ± 0.9	tr.	0.8 ± 0.5	0.8 ± 0.5	0.3 ± 0.2	0.4 ± 0.3
Total n–6	6.5 ± 1.6	11.9 ± 2.1[b]	4.9 ± 1.5	3.1 ± 0.6	5.9 ± 0.8	6.1 ± 1.6	5.0 ± 2.0	4.7 ± 2.0
18:3 n–3	0.3 ± 0.0	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.0	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.3	0.7 ± 0.5
22:6 n–3	4.5 ± 2.0	20.0 ± 0.5[d]	0.2 ± 0.3	0.2 ± 0.2	0.4 ± 0.2	0.8 ± 1.2	1.3 ± 2.4	1.6 ± 2.9
Total n–3	5.0 ± 2.0	20.8 ± 0.6[d]	1.8 ± 0.7	1.1 ± 0.3	1.7 ± 0.5	1.6 ± 1.3	2.5 ± 2.8	3.6 ± 2.9
Polyunsaturated	13.9 ± 4.9	33.6 ± 1.3[d]	7.3 ± 2.3	5.0 ± 0.4	8.5 ± 0.5	8.6 ± 2.2	8.3 ± 4.3	9.5 ± 4.2
P/S	0.28	0.74	0.15	0.10	0.21	0.21	0.22	0.23
n–6/n–3	1.30	0.57	2.72	2.81	3.47	3.81	2.00	1.31

Values are given as mean ± SD.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.005$; [d] $P < 0.001$: heads versus tails.

TABLE 4. Fatty acid composition of individual phospholipids of the heads and tails of monkey sperm

Fatty Acids	Phosphatidylethanolamine		Phosphatidyl Choline		Phosphatidyl Serine		Phosphatidyl Inositol		Phosphatidyl Glycerol		Cardiolipin		Sphingomyelin	
	Heads	Tails	Heads	Tails	Heads	Tails	Heads	Tails	Heads	Tails	Heads	Tails	Heads	Tails
							% of total fatty acids							
14:0	11.1 ± 0.0[a]	3.3 ± 3.0	12.8 ± 2.4	1.5 ± 0.8	11.8 ± 4.4	8.0 ± 1.0	11.0 ± 4.3	4.1 ± 0.8	8.9 ± 3.7	3.8 ± 0.8	18.4 ± 2.5	6.6 ± 1.3	7.5	7.7
16:0	20.1 ± 4.9	16.4 ± 2.7	23.1 ± 7.0	33.1 ± 1.1	23.0 ± 4.2	17.0 ± 4.4	25.2 ± 2.1	19.9 ± 4.3	24.6 ± 3.0	59.4 ± 8.2	18.9 ± 4.8	16.2 ± 2.2	24.5	24.1
18:0	9.5 ± 1.2	6.1 ± 2.1	9.2 ± 0.7	4.7 ± 0.8	10.1 ± 0.2	20.5 ± 1.5	9.5 ± 5.4	28.2 ± 8.0	6.9 ± 0.1	3.8 ± 1.1	7.1 ± 1.3	5.7 ± 3.8	8.8	6.5
Saturated	53.5 ± 8.1	36.6 ± 0.2	54.2 ± 4.5	41.9 ± 2.3	61.0 ± 7.1	51.5 ± 4.8	55.8 ± 17.1	56.7 ± 6.5	60.0 ± 19.5	74.9 ± 9.1	64.8 ± 7.1	32.8 ± 4.9	50.8	49.5
16:1 n–7	2.5 ± 1.6	1.1 ± 0.3	2.0 ± 0.4	2.1 ± 1.2	3.1 ± 2.5	2.0 ± 0.6	4.0 ± 3.3	1.9 ± 0.2	3.9 ± 1.2	1.3 ± 0.0	3.2 ± 1.6	2.0 ± 1.1	4.3	13.6
18:1 n–9	13.0 ± 3.2	4.7 ± 1.5	14.0 ± 2.8	10.9 ± 3.9	9.8 ± 0.5	5.5 ± 1.2	13.2 ± 2.7	11.2 ± 3.7	16.8 ± 9.6	6.8 ± 1.7	8.8 ± 2.3	13.7 ± 3.7	9.7	11.2
Monounsaturated	17.2 ± 0.2	6.0 ± 0.9	18.6 ± 2.1	13.0 ± 2.5	13.8 ± 3.8	8.3 ± 1.8	19.0 ± 1.9	15.0 ± 3.0	23.4 ± 7.6	11.7 ± 5.1	17.6 ± 0.2	16.7 ± 5.2	27.0	31.3
18:2 n–6	4.0 ± 0.2	2.7 ± 1.6	4.1 ± 1.5	2.3 ± 0.2	4.6 ± 2.3	2.1 ± 0.1	3.8 ± 0.5	2.6 ± 0.4	5.1 ± 3.1	2.1 ± 0.3	4.4 ± 0.6	24.3 ± 13.2	2.9	1.8
20:3 n–6	4.9 ± 2.6	1.7 ± 0.3	1.2 ± 0.4	3.3 ± 0.8	0.3 ± 0.2	0.5 ± 0.3	1.5 ± 0.2	1.7 ± 1.4	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	7.7 ± 0.6	0.3	0
20:4 n–6	2.5 ± 1.0	11.3 ± 4.2	3.6 ± 0.5	4.8 ± 0.4	3.0 ± 0.8	19.5 ± 4.5	2.5 ± 1.2	10.3 ± 1.8	0.3 ± 0.1	0.9 ± 0.1	0.6 ± 0.3	3.4 ± 0.9	1.2	2.1
Total n–6	13.8 ± 6.5	15.9 ± 3.0	14.1 ± 5.9	13.2 ± 0.8	10.1 ± 1.4	26.5 ± 4.9	10.0 ± 0.2	15.8 ± 2.1	7.1 ± 2.0	4.3 ± 0.5	8.0 ± 1.1	38.1 ± 10.1	8.3	7.3
18:3 n–3	0.8 ± 0.3	0	0.8 ± 0.4	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	1.0 ± 1.3	0.4 ± 0.3	0.4 ± 0.1	tr.	0.4 ± 0.1	0.2 ± 0.1	0.4	0.4
22:6 n–3	6.5 ± 0.6	34.3 ± 3.9	5.9 ± 4.4	27.6 ± 2.8	6.2 ± 1.5	5.2 ± 1.3	4.9 ± 0.1	4.7 ± 0.8	0.7 ± 0.6	1.3 ± 0.0	0.9 ± 0.1	3.9 ± 2.4	1.8	1.4
Total n–3	7.4 ± 0.9	35.2 ± 2.6	7.8 ± 3.4	29.0 ± 1.4	6.5 ± 1.9	7.3 ± 0.4	6.8 ± 1.9	5.2 ± 1.1	1.1 ± 0.6	1.7 ± 0.0	1.8 ± 0.8	4.5 ± 2.4	4.3	2.8
Polyunsaturated	22.3 ± 6.5	51.2 ± 5.7	22.2 ± 2.1	42.4 ± 0.4	17.1 ± 1.2	33.8 ± 4.3	17.2 ± 1.2	21.4 ± 1.7	8.6 ± 0.9	6.4 ± 1.7	10.2 ± 0.1	42.9 ± 8.7	14.2	12.2
P/S	0.42	1.40	0.41	1.01	0.28	0.66	0.31	0.38	0.14	0.09	0.16	2.31	0.28	0.25
n–6/n–3	1.86	0.45	1.81	0.46	1.55	3.63	1.47	3.04	6.45	2.53	4.44	8.47	1.93	2.61

Values are given as mean ± SD.
[a] Duplicate run of two pooled samples.

The most striking differences between heads and tails were in the DHA and desmosterol concentrations. Over 99% of the sperm DHA and desmosterol were localized to the tails. The precise reason for this selective accumulation of these compounds in the tails is not known. Studying membrane-associated particles in guinea pig sperm tails, Koehler and Graddum-Rosse (40) observed that the amplitude of the tail waves increased during incubation with capacitating media. This change in the pattern of flagellar beat was temporally correlated with the redistribution of particles within the tail membranes. These findings focus the attention upon the possibility that the internal specializations of the tail phospholipid membranes may have important functions in coordination of the beat, control of the ionic environment of the motor elements, or access of substrate to the energy generating enzyme systems of the mitochondria (40-42). Treen and colleagues (43) reported that the DHA content in retinoblastoma cells was directly correlated with membrane fluidity. Therefore, it is possible that selective concentration of polyunsaturated fatty acids and sterols in the tails is necessary to increase membrane fluidity to provide for the bending and flexing of tails required for motility. In a recent study we found that the DHA and desmosterol concentrations in monkey testis increased greatly during puberty, suggesting the importance of these two compounds for spermatogenesis (16).

DHA (22:6 n–3) is a highly polyunsaturated long-chain fatty acid and is highly concentrated in three organs of the body: the outer segments of the rods of the retina, the synaptosomal membranes of the brain, and, from these data, the tails of the sperm. Although the specific role of this highly polyunsaturated fatty acid is uncertain, the selective enrichment of the phospholipid membranes of vital organs with DHA has structural and functional significance. In our previous study, we found that monkeys fed DHA-deficient diets had impaired vision (13). A deficiency of DHA or the substitution of less polyunsaturated fatty acids in the membranes of photoreceptors may disturb membrane fluidity and function or it could alter regeneration of rhodopsin or the process of outer segment renewal (44). Studying the effect of DHA in cultured Y-79 retinoblastoma cells, Treen et al. (43) suggested that the effect of DHA content on retinal function may be mediated by changes in membrane fluidity and associated enzyme and transport activities.

Sperm and retina have attributes in common. Both sperm and the rod outer segments of retina have a high concentration of DHA and cilia (45, 46). Recently, we had an opportunity to explore the link of retina and sperm abnormality. We studied patients with retinitis pigmentosa in whom there is degeneration of the photoreceptors (12). In these patients there was low DHA content in their sperm and also poor sperm motility. Sperm motility was found to be positively correlated to the DHA concentration. In the late 1980s, Hunter et al. (45, 46) reported that these patients had low sperm motility, abnormal sperm tails, and abnormal axonemes in both sperm and retina. These findings of the association of low sperm DHA, poor

TABLE 5. Major molecular species of ethanolamine glycophospholipids in the heads and tails of monkey sperm

Molecular Species	Diacyl		Alkenylacyl		Alkylacyl	
	Heads	Tails	Heads	Tails	Heads	Tails
	mol %		*mol %*		*mol %*	
n–3						
18:1–22:6	3.2 ± 2.3[a]	5.7 ± 1.1	4.6 ± 2.9	24.8 ± 7.0	9.1 ± 0.6	10.9 ± 4.3
16:0–22:6	18.8 ± 9.3	40.3 ± 11.1	24.3 ± 12.4	38.5 ± 15.1	12.9 ± 2.1	32.8 ± 10.5
18:0–22:6	3.6 ± 2.2	6.3 ± 0.8	5.0 ± 1.3	9.7 ± 2.8	8.5 ± 1.1	18.5 ± 10.2
n–6						
18:1–20:4	0.8 ± 0.4	2.2 ± 1.7	3.1 ± 1.6	5.0 ± 2.1	6.7 ± 0.2	11.7 ± 2.6
16:0–20:4	4.6 ± 2.9	9.9 ± 0.3	3.2 ± 0.4	1.0 ± 0.8	7.6 ± 2.4	8.8 ± 4.0
18:0–20:4	3.5 ± 2.8	7.4 ± 3.3	4.9 ± 1.0	2.5 ± 1.1	6.5 ± 1.8	7.4 ± 4.9
16:0–20:3	4.0 ± 2.8	5.7 ± 1.1	2.5 ± 0.7	3.8 ± 1.2	—	—
18:0–20:3	1.0 ± 0.4	1.3 ± 0.4	0.8 ± 0.3	1.2 ± 1.0	—	—
n–9						
18:1–18:1	5.0 ± 1.3	2.0 ± 1.4	8.3 ± 0.7	2.5 ± 1.5	7.6 ± 2.9	—
16:0–18:1	12.3 ± 2.2	6.0 ± 2.5	11.7 ± 1.6	4.7 ± 2.0	13.4 ± 1.5	3.5 ± 1.1
18:0–18:1	8.3 ± 8.8	4.6 ± 3.9	9.9 ± 2.8	1.2 ± 0.9	8.6 ± 1.7	—
17:0–18:1	—	1.5 ± 1.4	6.8 ± 3.3	1.3 ± 1.1	4.3 ± 1.5	—
18:0–20:1	2.5 ± 1.4	—	8.4 ± 4.5	1.5 ± 0.6	8.4 ± 4.5	—
Saturates						
16:0–16:0	18.7 ± 10.9	3.1 ± 1.6	—	1.0 ± 0.9	—	—
16:0–18:0	8.4 ± 3.3	2.9 ± 2.0	—	—	—	—

Values given as mean ± SD.
[a]Duplicate run of two pooled samples.

motility, and abnormal axonemes in addition to the low DHA in the blood of these patients suggests the possibility that the DHA concentration in the retina of these patients might also be low.

Desmosterol is an intermediate compound in the cholesterol synthetic pathway. Normally, no desmosterol is found in the blood. Desmosterol is found in the brain during development in utero (47–49) and is present in earthworms and in snails and slugs (50, 51). In the tissues of mammals, however, desmosterol in high concentrations is found only in sperm and in the testis (11, 12, 16). As testicular cholesterol is mostly synthesized locally (52–54), high levels of desmosterol may result from low levels of the Δ^{24}-reductase enzyme which converts desmosterol to cholesterol. While desmosterol is well known as a transitory trace product in the synthesis of cholesterol, the biological significance of desmosterol has not been studied. High concentration of desmosterol in the tails of spermatozoa may relate to its greater unsaturation than cholesterol (it has two double bonds compared to the single double bond of cholesterol) which could improve the membrane fluidity. In our previous study we found that desmosterol/cholesterol ratio was positively correlated to sperm mobility (12). Sperm tails had a high desmosterol/cholesterol ratio 2.03 in contrast to a ratio of 0.06 for sperm heads.

A higher proportion of the cholesterol in sperm heads was in the sulfate form. Based on the ability of cholesterol sulfate to stabilize erythrocyte membranes and the finding of sterol sulfatase activity in female reproductive tract (55), Langlais et al. (56) hypothesized that cholesterol sulfate acts as a membrane stabilizer and enzyme inhibitor during the maturation of spermatozoa in the epididymis. According to this hypothesis, the cleavage of the sulfate moiety in sperm within the female reproductive tract triggers a cascade of events leading to sperm capacitation and fertilization (56). Radioautography at the level of the electron microscope revealed that the sterol sulfate is localized on the plasma membrane, mostly in the region of the acrosome of the head (55, 56).

In conclusion, the data of this study demonstrate large differences in the lipid composition of sperm heads and tails. These differences may have a role in the unique functional characteristics of these two components. The selective concentrations of DHA and desmosterol in sperm tails raise many interesting questions. Why is DHA concentrated in the cells of only three organs (outer segments of rod of the retina, synaptosomal membrane of the brain, and the tail of sperm cells). Is there a common role of DHA in these cells or does DHA have a different specific role in each of these cells which have different functions? The high concentration of desmosterol in sperm cells is also of great interest. Desmosterol is found in sizable amounts only in sperm and testes (11, 12, 16, 47–49) and is commonly considered as a transitory inter-

TABLE 6. Distribution of the subclasses of ethanolamine glycerophospholipids in heads and tails of monkey sperm

Subclass	Head	Tail
	% of total	
Diacyl	65.5 ± 11.2[a]	66.7 ± 5.0
Alkenylacyl	25.6 ± 13.0	25.5 ± 5.4
Alkylacyl	8.9 ± 1.8	7.6 ± 0.4

Values are given as mean ± SD.
[a]Duplicate run of two pooled samples.

mediate product in the cholesterol synthetic pathway. Both DHA and desmosterol are polyunsaturated. The greater number of double bonds in DHA and desmosterol may contribute to membrane fluidity necessary for the bending and flexing of tails required for motility.

Supported by National Institutes of Health grants DK 29930 and RR 00163 of the Oregon Health Sciences University and the Oregon Redional Primate Research Center.

Manuscript received 27 October 1997 and in revised form 3 March 1998.

REFERENCES

1. Fawcett, D. W. 1975. The mammalian spermatozoan. *Dev. Biol.* **44:** 397–436.
2. Lindsley, D. L., and K. T. Tokuyasu. 1980. Spermatogenesis. *In* The Genetics and Biology of Drosophila. M. B. Ashburner, and T. R. F. Wright, editors. Academic Press, London. Vol. 2. 225–294.
3. Guyaya, S. S. 1987. Biology of Spermatogenesis and Spermatozoa in Mammals. Springer-Verlag, Berlin.
4. Hackstein, J. H. P. 1987. Spermatogenesis in *Drosophila. In* Results and Problems in Cell Differentiation. W. Hennig, editor. Springer-Verlag, Berlin Heidelberg. Vol. 15. 63–116.
5. Yanagimachi, R. 1988. Sperm–egg fusion. *Curr. Top. Membr. Transp.* **32:** 3–43.
6. Holger, H., H. Heid, and W. W. Frank. 1993. Molecular characterization of mammalian Cylican, a basic protein of the sperm head cytoskeleton. *J. Cell. Biol.* **122:** 1043–1052.
7. Holger, H., H. Hess, H. Heid, R. Zimbelmann, and W. W. Frank. 1995. The protein complexity of the cytoskeleton of bovine and human sperm heads: the identification and characterization of cyticin II. *Exp. Cell. Res.* **218:** 174–182.
8. Neesen, J., S. Padmanabhan, and H. Bunemann. 1994. Tandemly arranged repeats of a novel highly charged 16 amino-acid motif representing the major component of sperm-tail-specific anoneme-associated protein family. Phmst 101 from extended x-helicae rods with in the extremely elongated spermatozoa of *Drosophila hydie. Eur. J. Biochem.* **225:** 1089–1095.
9. Higgy, N. A., A. Ladia, T. Partoor, C. Renz, H. A. Turnasky, and F. A. van der Hoorn. 1994. Testes-specific RT7 protein localized to sperm-tail and associates with itself. *J. Reprod.* **50:** 1357–1366.
10. Kim, J. H., J. R. McFarlane, G. Hlmahboki, P. G. Stanton, P. D. Temple-Smith, and D. M. DeKretser. 1995. Isolation and partial characterization of rat sperm tail fibrous sheath proteins, and comparison with rabbit and human spermatozoa using a polyclonal antiserum. *J. Reprod. Fertil.* **104:** 107–114.
11. Lin, D. S., W. E. Connor, D. P. Wolf, M. Neuringer, and D. L. Hachey. 1993. Unique lipids of primate spermatozoa: desmosterol and docosahexaenoic acid. *J. Lipid Res.* **34:** 491–499.
12. Connor, W. E., R. G. Weleber, C. A. DeFrancesco, D. S. Lin, and D. P. Wolf. 1997. Sperm abnormalities in retinitis pigmentosa. *Invest. Ophthalmol. Vis. Sci.* **38:** 2619–2628.
13. Neuringer, M., W. E. Connor, D. S. Lin, L. Barstad, and S. Luck. 1986. Biochemical and functional effects of prenatal and postnatal omega–3 fatty acid deficiency on retina and brain in rhesus monkeys. *Proc. Natl. Acad. Sci. USA.* **83:** 4021–4025.
14. Lin, D. S., W. E. Connor, G. J. Anderson, and M. Neuringer. 1990. Effects of dietary n–3 fatty acids on phospholipid molecular species of monkey brain. *J. Neurochem.* **55:** 1200–1207.
15. Lin, D. S., G. J. Anderson, W. E. Connor, and M. Neuringer. 1994. The effect of dietary n–3 fatty acids upon the phospholipid molecular species of the monkey retina. *Invest. Ophthalmol. Vis. Sci.* **35:** 794–803.
16. Connor, W. E., D. S. Lin, and M. Neuringer. 1997. Biochemical marker for puberty in monkey testis: desmosterol and docosahexaenoic acid. *J. Clin. Endocrinol. Metab.* **82:** 1911–1916.
17. Lanzedorf, S. E., P. M. Giessman, A. E. Archibong, M. Alexander, and D. P. Wolf. 1990. Collection and quality of rhesus monkey semen. *Mol. Reprod. Dev.* **25:** 61–66.
18. Young, R. J., and G. W. Cooper. 1983. Dissociation of intermolecular linkage of sperm head and tail by primary amines, aldehydes, sulphydryl reagents and detergents. *J. Reprod. Fertil.* **69:** 1–10.
19. Folch, J., M. B. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
20. Rapp, J. H., W. E. Connor, D. S. Lin, T. Inahara, and J. M. Porter. 1983. The lipids of human atherosclerotic plaques and xanthomas: clues to the mechanism of plaque progression. *J. Lipid. Res.* **24:** 1329–1335.
21. Nikolopoulou, M., D. A. Soucek, and J. C. Vary. 1985. Changes in the lipid content of boar sperm plasma membranes during epididymal maturation. *Biochim. Biophys. Acta.* **815:** 486–498.
22. Iwamon, M., H. W. Moster, and Y. Kishimoto. 1976. Cholesterol sulfate in rat tissues. Tissue distribution, developmental change and brain subcellular localization. *Biochim. Biophys. Acta.* **441:** 268–279.
23. Gilfillan, A. M., D. A. Smart, and S. A. Rooney. 1983. Single plate separation of lung phospholipids including disaturated phosphatidyl choline. *J. Lipid Res.* **24:** 1651–1656.
24. Morrison, W. R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride–methanol. *J. Lipid Res.* **5:** 600–608.
25. Connor, W. E., M. Neuringer, and D. S. Lin. 1990. Dietary effects upon brain fatty acid composition: the reversibility of n–3 fatty acid deficiency and turnover of docosahexaenoic acid in the brain, erythrocytes and plasma of rhesus monkeys. *J. Lipid Res.* **31:** 237–248.
26. Louie, K., R. D. Wiegand, and R. E. Anderson. 1988. Docosahexaenoate-containing molecular species of glycerophospholipids from frog retinal rod outer segments show different rates of biosynthesis and turnover. *Biochemistry.* **27:** 9014–9020.
27. Blank, M. L., M. Robinson, V. Fitzgerald, and F. Snyder. 1984. Novel quantitative method for determination of molecular species of phospholipids and diglycerides. *J. Chromatogr.* **298:** 473–482.
28. Marvis, R. D., R. M. Bill, and P. B. Vageler. 1972. Effect of phospholipase c hydrolysis of membrane phospholipids on membranous enzymes. *J. Biol. Chem.* **247:** 2835–2841.
29. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
30. Blank, M. L., E. A. Ceres, T. C. Lee, N. Stephens, C. Piantadosi, and F. Snyder. 1983. Quantitative analysis of ether-linked lipids as alkyl- and alk-l-enylglycerolbenzoates by high-performance liquid chromatography. *Ann. Biochem.* **133:** 430–436.
31. Yeagle, P. L. 1985. Cholesterol and cell membrane. *Biochim. Biophys. Acta.* **822:** 267–287.
32. Kuiper, J., A. Livne, and N. Meyerstein. 1971. Changes in lipid composition and osmotic fragility of erythrocytes of hamster induced by heat exposure. *Biochim. Biophys. Acta.* **248:** 300–305.
33. Horwitz, A. F., M. E. Hatten, and M. M. Burger. 1974. Membrane fatty acid replacements and their effect on growth and lectin-induced agglutinability. *Proc. Natl. Acad. Sci. USA.* **71:** 3115–3119.
34. Farias, R. N., B. Bloj, R. D. Morero, F. Sineriz, and R. E. Trucco. 1975. Regulation of allosteric membrane-bound enzymes through changes in membrane lipid composition. *Biochim. Biophys. Acta.* **415:** 231–250.
35. Engelhard, V. H., M. Glase, and D. R. Storm. 1978. Effect of membrane phospholipid compositional changes on adenylate cyclase in LM cells. *Biochemistry.* **17:** 3191–3200.
36. Bloj, B., R. D. Morero, R. N. Farias, and R. E. Trucco. 1973. Membrane lipid fatty acids and regulation of membrane-bound enzymes: allosteric behavior of erythrocyte Mg^{2+}-ATPase, (Na^+ + K^+)-ATPase and acetylcholinesterase from rats fed different fat-supplemented diets. *Biochim. Biophys. Acta.* **311:** 67–69.
37. Clandinin, M. T., C. J. Field, K. Hargreaves, L. Morson, and E. Zsigmond. 1985. Role of diet fat in subcellular structure and function. *Can. J. Physiol. Pharmacol.* **63:** 546–556.
38. Holub, B. J., and A. Kuksis. 1978. Metabolism of molecular species of diacylglycerophospholipids. *Adv. Lipid. Res.* **16:** 1–125.
39. Lynch, D. V., and G. A. Thompson. 1984. Retailored lipid molecular species: a tactical mechanism for modulating membrane properties. *Trans. Biochem. Sci.* **9:** 442–445.
40. Koehler, J. K., and D. Graddum-Rosse. 1975. Media-induced alterations of the membrane associated particles of the guinea pig sperm tail. *J. Ultrastruct. Res.* **51:** 106–118.
41. Friend, D. S., and D. W. Fawcett. 1974. Membrane differentiation in freeze-fractured mammalian sperm. *J. Cell. Biol.* **63:** 641–664.

42. Stackpole, C. W., and D. Devorkin. 1974. Membrane organization in mouse spermatozoa revealed by freezing–etching. *J. Ultrastruct. Res.* **49:** 167–187.
43. Treen, M., R. D. Uauy, D. M. Jameson, V. L. Thomas, and D. R. Hoffman. 1992. Effects of docosahexaenoic on membrane fluidity and function in intact cultured Y-79 retinoblastoma cells. *Arch. Biochem. Biophys.* **294:** 564–570.
44. Weidmann, T. S., R. D. Pates, J. M. Beach, A. Salmon, and M. F. Brown. 1988. Lipid–protein interactions mediate the photochemical function of rhodopsin. *Biochemistry.* **27:** 6469–6474.
45. Hunter, D. G., G. A. Fishman, R. S. Mehta, and F. L. Kretzer. 1986. Abnormal sperm and photoreceptor axonemes in Usher's syndrome. *Arch. Ophthalmol.* **104:** 385–389.
46. Hunter, D. G., G. A. Fishman, and F. L. Kretzer. 1988. Abnormal axonemes in X-linked retinitis pigmentosa. *Arch. Ophthalmol.* **106:** 362–368.
47. Stokes, W. M., W. A. Fish, and F. C. Hickey. 1953. Metabolism of cholesterol in the chick embryo. I. The synthesis of cholesterol. *J. Biol. Chem.* **200:** 683–689.
48. Connor, W. E., R. Johnston, and D. S. Lin. 1969. Metabolism of cholesterol in the tissues and blood of the chick embryo. *J. Lipid. Res.* **10:** 388–394.
49. Edmond, J. R., R. A. Korsak, J. W. Morrow, G. Torok-Both, and D. H. Catlin. 1990. The origin of cholesterol in brain of developing rat. *FASEB. J.* **4:** (A532).
50. Zhu, N., X. Dai, D. S. Lin, and W. E. Connor. 1994. The lipids of slugs and snails: evolution, diet and biosynthesis. *Lipids.* **29:** 869–875.
51. Albro, P. N., J. L. Schroeder, and J. T. Corbett. 1992. Lipids of the earthworm *Lumbricus terrestris*. *Lipids.* **27:** 136–143.
52. Morris, D., and I. L. Chaikoff. 1959. The origin of cholesterol in liver, small intestine, adrenal gland, and testes of rat: dietary versus endogenous contribution. *J. Biol. Chem.* **234:** 1095–1097.
53. Charreau, E. H., J. C. Calvo, K. Nozu, O. Pignataro, K. J. Catt, and M. L. Dufan. 1981. Hormonal modulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in gonadotropin-stimulated and -desensitized testicular Leydig cells. *J. Biol. Chem.* **256:** 12719–12724.
54. Wilson, J. D. 1970. The measurement of the exchangeable pools of cholesterol in the baboon. *J. Clin. Invest.* **49:** 655–665.
55. Lalumier, G., G. Bleau, A. Chapdelain, and K. D. Roberts. 1976. Cholesteryl sulfate and sterol sulfatase in the human reproductive tract. *Steroids.* **27:** 247–259.
56. Langlais, J., M. Zollinger, L. Plante, A. Chapdelaine, G. Bleau, and K. D. Roberts. 1981. Localization of cholesterol sulfate in human spermatozoa in support of a hypothesis for mechanism of capacitation. *Proc. Natl. Acad. Sci. USA.* **78:** 7266–7270.