Phospholipids and phospholipid-bound fatty acids and aldehydes of spermatozoa and seminal plasma of rhesus monkeys

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Summary. The major components of the phospholipids of rhesus monkey spermatozoa are phosphatidyl choline (33%), phosphatidyl ethanolamine (25%), ethanolamine plasmalogen (16.1%), sphingomyelin (8.1%), choline plasmalogen (6.9%) and cardiolipin (4.5%). The major phospholipid-bound fatty acids are 16:0, 18:0, 18:1 and 22:6; the major fatty aldehydes are 15:0, 16:0 and 18:2. The same phospholipids are also present in the seminal plasma.

The composition of sperm phospholipids and phospholipid-bound fatty acids and aldehydes has been established in a number of species (see Darin-Bennett, Poulos & White, 1974) including man (Poulos & White, 1973; Poulos, Darin-Bennett & White, 1973). However, no data seem to be available on the nature of these membrane components in non-human primates. In view of the use of the monkey as a model for research in human reproduction and the possible evolutionary significance, the phospholipids of the spermatozoa and seminal plasma of the monkey were studied to determine the extent of similarity to human spermatozoa.

Ejaculates from 2 to 5 male rhesus monkeys (*Macaca mulatta*) were pooled, diluted with 3 volumes of 0.9% (w/v) NaCl and centrifuged at 500 g for 20 min at room temperature (20–25°C). The pellet was resuspended to 10 ml in 0.9% NaCl. The sperm suspension and seminal plasma supernatant were extracted within 90 min of collection with 3.75 volumes of chloroform : methanol (2:1, v/v) and stored under liquid nitrogen at –79°C during transport to the laboratory where it was kept at –15°C until analysis.

The treatment of the lipid extracts and the method of analysing the phospholipids have been previously described (Darin-Bennett *et al.*, 1973). The phospholipid-bound fatty acid and aldehyde composition were estimated by gas–liquid chromatography according to the methods of Poulos, Darin-Bennett & White (1973).

The major components of rhesus sperm phospholipids (Table 1) are phosphatidyl choline, phosphatidyl ethanolamine and ethanolamine plasmalogen, with lesser amounts of sphingomyelin, choline plasmalogen, and cardiolipin. These components were also present in the seminal plasma but not in the same proportions. Comparison of the phospholipid composition of monkey spermatozoa and seminal plasma with that of other species such as the ram (Quinn & White, 1967; Neill & Masters, 1973; Darin-Bennett *et al.*, 1973), bull (Pursel & Graham, 1967; Poulos, Voglmayr & White, 1973; Neill & Masters, 1972; Darin-Bennett *et al.*, 1973), boar (Johnson, Gerrits & Young, 1969; Darin-Bennett *et al.*, 1973), man (Poulos & White, 1973), dog and fowl (Darin-Bennett *et al.*, 1974), and rabbit (A. Darin-Bennett, unpublished observations) indicate clear species differences. The pattern of phospholipid composition of monkey spermatozoa would seem to resemble most closely that of the dog. Monkey spermatozoa contain higher amounts of both choline and ethanolamine plasmalogen and a lower proportion of sphingomyelin than are found in human spermatozoa.

Monkey seminal plasma phospholipids are also present in different proportions from those of human seminal plasma, the main differences being in the levels of sphingomyelin and phosphatidyl choline. Apart from the lower proportion of choline plasmalogen in monkey seminal plasma,

Table 1. Phospholipid composition of rhesus monkey spermatozoa and seminal plasma

Component	Spermatozoa	Seminal plasma
Origin	0.9 ± 0.6	0.9 ± 0.5
Sphingomyelin	8.1 ± 1.2	16.9 ± 1.3
Phosphatidyl serine	1.6 ± 0.7	1.7 ± 0.4
Phosphatidyl inositol	<1.0	2.0 ± 1.1
Choline plasmalogen	6.9 ± 1.8	3.5 ± 1.4
Phosphatidyl choline*	33.0 ± 0.6	21.8 ± 1.6
Ethanolamine plasmalogen	16.1 ± 1.1	24.0 ± 0.5
Phosphatidyl ethanolamine†	25.0 ± 1.1	15.1 ± 0.9
Cardiolipin	4.5 ± 0.2	1.3 ± 0.4
Miscellaneous lipids	4.0	11.3

Each result is expressed as a percentage of the total phospholipid extracted and is the mean of three analyses ± standard error.

* The phosphatidyl choline zone may include 1-alkyl,2-acyl glycerol-3-phosphorylcholine.

† The phosphatidyl ethanolamine zone may include 1-alkyl,2-acyl glycerol-3-phosphoryl-ethanolamine.

the phospholipid composition bears some resemblance to that of bull seminal plasma (Pursell & Graham, 1967). It would seem, therefore, that the phospholipid composition of both the spermatozoa and the seminal plasma of the monkey differ from those of other species so far examined.

Table 2. Phospholipid-bound fatty acids of rhesus monkey spermatozoa and seminal plasma

Fatty acid	Spermatozoa	Seminal plasma
14:0	N.D.	1.5 ± 0.1
16:0	29.2 ± 1.0	38.2 ± 3.8
18:0	11.2 ± 0.9	18.3 ± 1.7
18:1	14.8 ± 1.5	14.1 ± 1.6
18:2	3.8 ± 0.2	1.6 ± 0.2
18:3	N.D.	4.6 ± 0.6
20:0	3.1 ± 0.1	1.5 ± 0.8
20:4 ‡	8.9 ± 0.4	8.9 ± 0.6
22:5 *	2.8 ± 0.8	N.D.
22:6	25.2 ± 0.9	10.2 ± 5.3
Miscellaneous †	1.2	0.7
	(14:0, 16:1, 17:0)	(17:0)

Each fatty acid (chain length : number of double bonds) is expressed as a percentage of the total weight of fatty acid. Mean values of three analyses are given ± standard error. N.D., indicates that the fatty acid was either not detected or was present in amounts below 1%.

* Values for this fatty acid are expressed as a percentage of total peak area.

† Fatty acids identified in this fraction are indicated in parentheses.

‡ This fraction may contain 20:3.

This, however, is not the case for the phospholipid-bound fatty acids of monkey spermatozoa (Table 2), since the composition bears a close resemblance to those of the rabbit, dog and man (Poulos, Darin-Bennett & White, 1973; Darin-Bennett *et al.*, 1974). The similarity to human spermatozoa is particularly striking in that the major polyunsaturated acid was docosahexaenoic acid; high levels of arachidonic acid were also present. The main saturated acid was palmitic acid, and the 18-carbon fatty acids were also prominent. The ratio of polyunsaturated : saturated fatty

Table 3. Phospholipid-bound aldehydes of spermatozoa and seminal plasma of rhesus monkeys

Fatty aldehyde	Spermatozoa	Seminal plasma
14:0	3.2 ± 0.1	8.7 ± 1.4
15:0 br	9.9 ± 0.6	9.9 ± 2.6
15:0	21.5 ± 2.6	7.0 ± 2.4
16:0	43.7 ± 1.6	25.6 ± 2.5
16:1	4.3 ± 1.7	17.0 ± 6.1
17:0	N.D.	4.4 ± 0.9
18:0	1.4 ± 0.4	7.8 ± 1.0
18:1	2.6 ± 0.7	5.6 ± 1.7
18:2	12.7 ± 3.7	13.1 ± 1.6
Miscellaneous aldehydes	0.8 (17:0)	0.0

Each aldehyde (chain length : number of double bonds) is expressed as a percentage of the total peak area. Mean values for three analyses ± standard error are given. N.D., indicates that the fatty acid was either not detected or was present in amounts below 1%.

acids in monkey sperm phospholipids is 1.08, which would probably influence the membrane reaction of monkey spermatozoa to environmental stress (Poulos, Darin-Bennett & White, 1973; Darin-Bennett *et al.*, 1974) and justify grouping with human, rabbit, dog and fowl spermatozoa, anticipating that monkey, like human spermatozoa, might be comparatively resistant to cold shock. The phospholipids of monkey seminal plasma have a polyunsaturated : saturated fatty acid ratio of 0.46. Palmitic acid predominated and a high level of arachidonic acid, which may act as a precursor of prostaglandins, was present.

The aldehydes of the phospholipids of monkey spermatozoa (Table 3) are also similar to those of human, rabbit, dog and fowl and unlike those of ram, bull and boar (Poulos, Darin-Bennett & White, 1973; Darin-Bennett *et al.*, 1974) spermatozoa in that, although palmitaldehyde predominated, there were high levels of 18-carbon aldehydes. In monkey seminal plasma there was also a high proportion of palmitolealdehyde.

It would seem, therefore, that monkey spermatozoa are similar, although by no means identical, to human spermatozoa with respect to phospholipid-bound fatty acids and aldehydes, and are somewhat different in respect of phospholipid composition. This would indicate some differences in the structure of the membrane but the significance of this is not at present clear.

We thank Professor C. W. Emmens for his interest and advice, and W.H.O. and the Australian Research Grants Committee for financial support.

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Received 13 May 1976