QUANTITATIVE DIFFERENCES IN PHOSPHOLIPIDS OF EJACULATED SPERMATOOZOA AND SPERMATOOZOA FROM THREE LEVELS OF THE EPIDIDYMIS OF THE BOAR*

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Summary. Five major phospholipids of ejaculated and epididymal porcine spermatozoa were separated and quantitatively determined by thin-layer chromatography. These were in the order of decreasing concentration, phosphatidyl choline, phosphatidyl ethanolamine, ethanolamine plasmalogen, sphingomyelin and choline plasmalogen. Phosphatidyl serine, lysolecithin and possibly phosphatidyl inositol were also present, but in low concentrations. The concentration of phosphatidyl choline and phosphatidyl ethanolamine in spermatozoa progressively decreased from the head to the tail of the epididymis, and was lowest in ejaculated semen. The plasmalogen content of spermatozoa from all levels of the reproductive tract of the boar was low and varied very slightly.

INTRODUCTION

The important role of phospholipids in spermatozoan physiology was first recognized by Lardy & Phillips (1941). Mann (1964) has cited pertinent literature which implicates the phospholipids as substrates in the metabolic activity of the spermatozoa of certain species. Phospholipids and phospholipid-containing lipoproteins have been shown to act as protective agents against harmful environmental factors encountered during storage and temperature shock (Lasley, Easley & Bogart, 1942; Kampschmidt, Mayer & Herman, 1953; Mayer, 1955; Miller & Mayer, 1960).

In contrast to ejaculated spermatozoa, the epididymal spermatozoa of the boar and the bull are highly resistant to temperature shock, as demonstrated by Lasley & Mayer (1944) and Lasley & Bogart (1944a, b). It was shown by these investigators that the most resistant spermatozoa are those isolated from the

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head-region of the epididymis and that resistance to temperature shock progressively decreases from the head-region towards the tail-region. Even so, spermatozoa from the tail of the epididymis are strikingly more resistant to cold shock than those in ejaculated semen.

Numerous reports emphasize the peculiar differences between the metabolism of epididymal and ejaculated spermatozoa and the authors have suggested possible explanations for the differences. These reports are cited and discussed by Mann (1964).

The lipid composition of the spermatozoa, seminal plasma and gel in ejaculates from boars was reported by Komarek, Pickett, Gibson & Jensen (1965). Results of a previous investigation in our laboratory by Matsumoto (1963) showed that both total lipid and a lipoprotein complex were present in greater quantities in epididymal spermatozoa of the bull than in ejaculated spermatozoa. The results obtained by Scott, Dawson & Rowlands (1963) in the rat, and by Dawson & Scott (1964) in the ram, suggest the existence of both qualitative and quantitative differences in the phospholipid composition of spermatozoa obtained from different levels of the male reproductive tract and from ejaculated semen. The object of the present study was to determine the extent to which phospholipids vary in spermatozoa from various segments of the epididymis of the boar and in ejaculated boar semen.

MATERIALS AND METHODS

Collection of samples

Ejaculated semen was collected by means of an artificial vagina from mature Poland boars. The semen was immediately strained through several layers of cheese-cloth to remove the gelatinous material. Epididymal spermatozoa were obtained from the epididymides of mature Poland boars; the epididymides were dissected into head, mid and tail portions. To obtain epididymal spermatozoa, the epididymal sections were stripped of the tunica albuginea and a slight pressure applied to the tubules with the fingers. The tubules were then punctured with a sharp needle. The milky fluid that oozed through the puncture was collected with a spatula and transferred to a beaker containing cold physiological saline.

Centrifugation and drying

Both the ejaculated and the epididymal spermatozoa were centrifuged at 2000 rev/min, the supernatant fluids were discarded and the spermatozoa were washed with physiological saline followed by a double-distilled water wash. This brief washing procedure did not remove lipids from the spermatozoa. They were frozen and lyophilized for 15 hr, then stored in the dry state under vacuum.

Lipid extraction

Lipids were extracted from pooled 100 mg samples of the lyophilized spermatozoa using a 2 : 1 (v/v) chloroform-methanol mixture with stirring at 55° C for 3 hr, and then purified according to Folch, Ascoli, Lees, Meath & LeBaron

(1951). The lipid solution was concentrated to 1 ml under vacuum. Standards for identification purposes were obtained from Sigma Chemical Co., St Louis.

Chromatography

Thin-layer chromatography plates were prepared by slurring 30 g of silica gel H, without calcium sulphate binder, with 80 ml of double-distilled water. The slurry was spread onto ten 10×20 cm plates with a Desaga applicator, previously adjusted to 0.25 mm. Just before use the plates were activated for 1 hr at 110° C. Fifty microlitres of a concentrated lipid sample were applied to the thin-layer plates with a 50 μ l micropipette. Standards were applied to the same plates for identification purposes. The chromatograms were developed in a solvent system which contained chloroform-methanol-acetic acid-water (65:25:8:4), prepared according to Skipski, Peterson & Barclay (1962). This system does not separate phosphatidyl choline from choline plasmalogen, nor does it separate phosphatidyl ethanolamine from ethanolamine plasmalogen. The average time required for the development of the plates was 60 to 80 min.

After the plates had been developed they were removed from the developing tank and allowed to dry in air for about 3 min. The plates were then placed in an iodine tank as described by Sims & Larose (1962) and the resulting dark spots due to phospholipid were outlined.

Phosphorus determinations

The outlined phospholipid spots were carefully removed from the plate and quantitatively transferred to 15 ml conical centrifuge tubes. A control sample of silica gel was taken from a phospholipid-free section of the plate. One-half millilitre of 10 N-H₂SO₄ was added to the phospholipid-silica gel mixture in each of the 15-ml conical tubes. After the samples were digested, they were diluted with 1 ml of water, centrifuged, and the supernatant solutions transferred to 5-ml volumetric flasks. To insure complete recovery, the samples were washed twice more and the wash fluids transferred to the respective flasks. The general procedure described by Bartlett (1959) was used for digestion and colour development.

Plasmalogen determinations

The plasmalogens were quantitatively separated and determined by applying to the thin-layer plates 250 μ l samples of the concentrated lipid solution. The gel areas on the plates which encompassed the phospholipids containing choline and ethanolamine were quantitatively removed and digested with acetic acid for 17 hr at 37° C; this procedure, according to Gray (1957), should convert the plasmalogens to lysophosphatidyl ethanolamine and lysophosphatidyl choline. The acid digests were evaporated to dryness under vacuum, taken up in 1:1 chloroform-ethanol, and spotted on a thin-layer plate. The spots representing phosphatidyl choline and lysolecithin on the one hand, and phosphatidyl ethanolamine and lysophosphatidyl ethanolamine on the other, were again carefully scraped from the plate and their phosphorus content determined. The amount of lyso compound formed was taken as an index of the plasmalogen content.

RESULTS

Phosphatidyl choline, phosphatidyl ethanolamine, choline plasmalogen, ethanolamine plasmalogen and sphingomyelin were the five major phospholipid components of both epididymal and ejaculated spermatozoa of the boar. Lysolecithin was present in ejaculated and in epididymal sperm cells, the latter having a higher concentration. The amounts of lysolecithin present in ejaculated spermatozoa or in spermatozoa from the tail of the epididymis were too small for a quantitative analysis. In the middle and head sections of the epididymis the lysolecithin content of the spermatozoa ranged from 0.3 μ moles to 0.35 μ moles/100 mg of dried porcine spermatozoa.

It is evident from the results presented in Table 1 that epididymal spermatozoa from all three sections of the boar epididymis contained more of each of the five major phospholipids than the ejaculated spermatozoa.

TABLE 1
MAJOR PHOSPHOLIPIDS OF BOAR SPERMATOZOA

Phospholipid	Spermatozoa			
	Ejaculated	Epididymal		
		Tail	Mid	Head
Phosphatidyl choline	1.95 (16)	3.91 (16)	6.05 (16)	8.79 (16)
Phosphatidyl ethanolamine	1.14 (16)	1.56 (12)	2.04 (16)	3.63 (16)
Choline plasmalogen	0.515 (3)	0.535 (4)	0.615 (4)	0.89 (4)
Ethanolamine plasmalogen	0.49 (4)	1.08 (7)	1.74 (6)	2.39 (4)
Sphingomyelin	1.02 (12)	1.19 (12)	1.27 (14)	1.84 (14)

The number in parentheses represents the number of samples analysed.
The results are expressed in micromoles of phospholipid/100 mg of dried spermatozoa.

Secondly, in the case of each of the five phospholipids cited in Table 1, a marked decrease in concentration was observed as the sperm cells progressed from the head to the tail of the epididymis. The most pronounced changes were in the concentration of phosphatidyl choline and phosphatidyl ethanolamine.

Phosphatidyl serine and possibly phosphatidyl inositol were present in ejaculated and in epididymal spermatozoa. The phosphatidyl inositol, if present, occurred in very low concentration. In some cases a spot occurred on the thin layer chromatoplates in a position suggesting phosphatidyl inositol as lipid component. However, the spots for the serine and inositol phospholipid often formed an intermixture upon the chromatoplate, making it difficult to determine their concentration separately. The spermatozoa from the head, mid-section and tail of the epididymis contained 0.899, 0.639 and 0.507 μ moles of the mixture of phosphatidyl serine and phosphatidyl inositol per 100 mg of dried sperm cells, respectively. Again the progressive decrease in concentration of lipid components of epididymal spermatozoa from head to tail of the epididymis was evident.

DISCUSSION

The thin-layer chromatographic procedure utilized in this study enabled us to separate and characterize five major phospholipid fractions in ejaculated and epididymal spermatozoa of the boar and to suggest the presence of at least two, and perhaps three minor phospholipid fractions.

There was approximately 3.7 times the quantity of total phospholipids in the spermatozoa from the head-section of the epididymis than in the ejaculated spermatozoa. The choline-containing phospholipids (phosphatidyl choline and its plasmalogen analogue) represented approximately 50% of the total phospholipids both of ejaculated and of epididymal spermatozoa. Ethanolamine-containing phospholipids were also present in appreciable amounts. Both phosphatidyl choline and phosphatidyl ethanolamine decreased in quantity in an almost parallel manner as the sperm cells traversed the epididymis and became a part of the ejaculate. This progressive decrease suggests that the phospholipids may be implicated in the maturation process of spermatozoa.

In contrast to ram spermatozoa (Hartree & Mann, 1959, 1960, 1961; Lovern, Olley, Hartree & Mann, 1957) the plasmalogen content of boar spermatozoa is low. This is particularly true for choline plasmalogen. Unlike phosphatidyl choline and phosphatidyl ethanolamine, the plasmalogen analogues remained relatively unchanged in the sperm cells from the different levels of the boar reproductive tract. This was also true of the sphingomyelin fraction.

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