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Lipids of Plasma Membrane and Outer Acrosomal Membrane from Bovine Spermatozoa

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ABSTRACT

Plasma membrane (PM), primarily from the anterior sperm head, and outer acrosomal membrane (OAM), were isolated from ejaculated bovine spermatozoa, and the major lipid classes were characterized. Whole sperm (WS) lipids were analyzed for comparison. PM was removed by nitrogen cavitation and purified by sucrose density-gradient centrifugation. The OAM was removed by centrifugation through hyperosmotic sucrose and recovered by sucrose density-gradient centrifugation. The PM contained primarily spherical vesicles from the region overlying the OAM and was enriched 9- and 13-fold in 5'-nucleotidase and alkaline phosphatase activity, respectively, compared to the original cavitate. The OAM was recovered as caplike structures with associated ground substance. Protein, phospholipid, and cholesterol (PR, PL, and CH as μg/5 × 10^9 sperm) were 300, 467, and 93 for PM and 276, 111, and 25 for OAM, respectively. Corresponding values for WS (mg/5 × 10^9 sperm) were 31.4, 6.63, and 0.72. The PR/PL (w/w) and CH/PL (mol/mol) ratios were 0.66 and 0.38 for PM; 2.48 and 0.26 for OAM; and 4.39 and 0.22 for WS. Cholesterol was the only free sterol detected by gas/liquid chromatography in WS, PM, and OAM, with traces of CH sulfate present in all three preparations. Glycolipid tentatively identified as sulfogalactolipid was detected by thin-layer chromatography (TLC) in PM but not OAM. Phospholipid composition of WS and membranes was determined by TLC. Cardiolipin (3% of total PL) was present in WS only. Choline, ethanolamine, and inositol phosphoglycerides (CP, EP, PI, PIP, PIPP); sphingomyelin (SP); phosphatidylserine (PS); and lysophosphatidylcholine (LPC) were present in WS, PM, and OAM. Approximately 50% of total PL was CP in all preparations; SP was 13% of PL in PM and 17% in OAM ($p<0.05$); EP was 7% of PL in PM and 10% in OAM ($p<0.05$). The differences in composition between PM and OAM is discussed with respect to capacitation and ability of sperm to undergo the acrosome reaction.

INTRODUCTION

While numerous reports have appeared describing various prefertilization changes in spermatozoa (called capacitation) and methods for achieving capacitation in vitro have been developed, the molecular mechanisms responsible for this critical event in mammalian fertilization remain unknown. Modifications to sperm during capacitation are important for induction of the acrosome reaction, a membrane fusion event prerequisite to fertilization in mammals and characterized by vesiculation of the plasma and outer acrosomal membranes (Piko and Tyler, 1964; Barros et al., 1967; Russell et al., 1979). Reports that capacitation includes modifications in sperm lipid composition (see Yanagimachi, 1981; Clegg, 1983; Go and Wolf, 1983; Meizel, 1984; Langlais and Roberts, 1985) are consistent with the integral role of lipids in membrane fusion (Papahadjopoulos et al., 1979; Lucy, 1982; Cullis et al., 1985). Changes in sperm lipids associated with capacitation include hydrolysis of sterol sulfates to free sterols (Langlais et al., 1981; Langlais and Roberts, 1985), cholesterol efflux (Davis et al., 1979; Davis, 1982; Go and Wolf, 1985; Langlais et al., 1985), and formation of lysophospholipid by endogenous phospholipases (Meizel, 1984; Thakker et al., 1984; Langlais and Roberts, 1985; Guerette et al., 1986). These changes are generally considered to have a destabilizing effect on the membrane bilayer and thus promote fusion. However, bilayer stability depends upon the interaction of different lipid classes as well as proteins within the membrane (Cullis et al.,

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1985), so that the significance of changes in specific lipids must be evaluated on the basis of the overall membrane composition.

Much has been reported concerning the total lipids of sperm from a variety of species (see Watson and Plummer, 1985, for review). However, capacitation must affect the plasma membrane overlying the acrosome and the outer acrosomal membrane specifically, because these are the membranes involved in the acrosome reaction. Nevertheless, information on the lipid composition of these membranes is limited. Parks and Hammerstedt (1985) described developmental changes in lipid composition of ram sperm plasma membrane during epididymal maturation, and Nikolopoulou et al. (1985) conducted similar studies using the boar as a model. These studies have been extended to plasma membrane of ejaculated ram spermatozoa (Holt and North, 1985), but the lipid composition of outer acrosomal membrane has not been reported for any species. Descriptions of the lipids present in these membranes should provide insight into the relevance of lipid changes observed during capacitation. The purpose of the present study was to isolate and characterize plasma membrane from the periacrosomal region and outer acrosomal membrane from ejaculated bovine spermatozoa, with emphasis on determining the major lipid classes represented in the two membranes.

MATERIALS AND METHODS

Preparation of Sperm

Freshly ejaculated bovine semen was obtained within 30 min after collection with an artificial vagina and diluted 1:4 (v/v) with Tyrode's solution (TS, pH 7.4). Diluted semen was layered on 35% Percoll (Pharmacia, Piscataway, NJ) in TS and centrifuged at 500 × *g* for 10 min. Intact sperm pellets were resuspended in TS and washed a second time in TS alone. This procedure separated sperm from seminal plasma and cytoplasmic droplets, which remained on top of the layer of Percoll. As observed by microscope, washed sperm were virtually free of cytoplasmic droplets. Protein and lipid compositions of washed sperm were determined as described for isolated membranes.

Plasma Membrane Isolation

Washed sperm were finally resuspended to 5.0×10^8 sperm/ml in 0.15 M NaCl, 10 mM 4-(2-hydroxyethyl) - 1 - piperazineethanesulfonic acid (HEPES) (HBS, pH 7.0). Plasma membrane was removed by nitrogen cavitation following equilibration at 650 psi for 10 min at 0°C (Parks and Hammerstedt, 1985). Membranes were isolated after removal according to Noland et al. (1983), with the following modifications. Cavitated suspensions were made 1 mM ethylenediamine tetraacetic acid (EDTA) and centrifuged at 800 × *g* for 10 min (5°C). Sperm pellets were resuspended in 4 ml cold HBS and the centrifugation was repeated. Combined supernatant fluids were then centrifuged at 6000 × *g* for 20 min (5°C). Unfractionated membranes in the 6000 × *g* supernatant fluid were layered on discontinuous sucrose density gradients of 1.05, 1.16, and 1.21 g/ml. After centrifugation at 100,000 × *g* for 2.5 h, membrane at the 1.05/1.16 g/ml interface was recovered, diluted in 0.15 M NaCl, 1.0 mM HEPES, and concentrated by centrifugation at 100,000 × *g* for 1 h.

Outer Acrosomal Membrane Isolation

Outer acrosomal membrane was removed from washed sperm and isolated according to Zahler and Doak (1975), with the following modifications. Sperm washed through 35% Percoll were resuspended in hypotonic HBS (250 mOsm) and centrifuged twice through 1.3 M sucrose first at 2000 × *g* for 15 min, then at 12,000 × *g* for 10 min. Pellets were resuspended in hypotonic sucrose buffer (0.2 M sucrose, 10 mM HEPES, and 5 mM EDTA; 220 mOsm, pH 7.0) and incubated at 37°C for 30 min. This procedure loosened or detached the outer acrosomal membrane, which could be distinguished by phase-contrast microscopy. Suspensions were then placed on ice and homogenized gently by repeated aspiration into a Pasteur pipette to completely remove loosened acrosomal membranes. The homogenate was layered on a discontinuous sucrose gradient and centrifuged at 100,000 × *g* for 3.5 h. Membrane recovered from the 1.16/1.24 g/ml interface was diluted and washed as previously described.

Electron Microscopy

Sperm before and after membrane removal and purified membrane preparations were fixed with 2.5% glutaraldehyde in 175 mM cacodylate buffer (pH 7.2), washed with cacodylate, and embedded in 2% agar. Material in agar blocks was then post-fixed in 1% OsO_4, dehydrated in graded ethanol, and embedded in Spurr (Electron Microscopy Services, Fort

Washington, PA), according to Jones (1975). Thin sections were stained with 1% uranyl acetate and 1% lead citrate prior to examination with a Phillips 301 transmission electron microscope (TEM).

Lipid Extraction and Analysis

Washed sperm and membrane fractions were resuspended in 0.15 M NaCl, 1 mM HEPES (pH 7.0) and aliquot portions used for protein determination (Markwell et al., 1978) or lipid extraction (Cohen et al., 1971). Membrane for lipid extraction was supplemented with 0.25 nmol (3×10^4 dpm) [^{14}C]dipalmitoylphosphatidylcholine (New England Nuclear, Boston, MA) and 0.02 nmol (2×10^5 dpm) [^{3}H]-cholesterol (Amersham, Arlington Heights, IL), extracted, filtered, and washed to determine recoveries of sterols and phospholipids. Separate preparations for further fractionation and analysis were extracted by using the same procedure, purged with N_2 and stored at −20°C.

Total phospholipid was quantified by ashing aliquot portions of lipid extracts (Ames and Dubin, 1960) and determining phosphate content of the ashed samples (Chen et al., 1956). An average molecular weight of 750 was used for calculating phospholipid mass. Phospholipid classes were separated by two-dimensional thin-layer chromatography on silica gel H (0.25-mm thick, 1% potassium oxalate) according to Graff et al. (1984). Phospholipids were identified on the basis of their retention relative to that of purified standards. Retention of polyphosphoinositides was also determined by autoradiography of ^{32}P-labeled compounds after TLC. Regions on TLC plates corresponding to individual phospholipids were scraped and eluted. Solvent was removed from eluted samples under a stream of N_2, and phosphate was determined after ashing.

Sterols were separated by TLC on silica gel H (0.25 mm) according to Nikolopoulou et al. (1985). Regions on TLC plates corresponding to free sterol, sterol ester, and sterol sulfate (based on relative retention of purified standards) were scraped, supplemented with cholestane as an internal standard, and eluted. Sterol sulfate was solvolyzed (Iwamori et al., 1976), and sterol esters were transesterified to provide free sterols for analysis by gas/liquid chromatography (GLC). Sterols were separated on a Hewlett Packard 5790 chromatograph with a hydrogen flame ionization detector. The column was packed with 3% SP2401 on 100/120 Supelcoport (Supelco, Bellefonte, PA) and maintained at 250°C. Peaks with retention times corresponding to purified standards were integrated, and sterol was quantified relative to added cholestane.

Glycolipids were eluted with acetone from the origin of TLC plates developed for sterol separation. Material eluting with acetone was separated by TLC according to Nikolopoulou et al. (1985), and glycolipid was identified on the basis of its retention relative to purified beef brain sulfatide (Sigma Chemical Co.).

Electrophoresis

One-dimensional polyacrylamide gel electrophoresis (PAGE) was carried out on slab gels of 15% acrylamide, as described by Laemmli and Favre (1973). Sample loads of 15–20 μg protein were separated, and individual protein bands, along with molecular weight standards, were detected by Coomassie Brilliant Blue and silver staining.

Biochemical Assay

Alkaline phosphatase (EC 3.1.3.1.) was determined by the modified procedure of Bowers and McComb (1966), using p-nitrophenyl phosphate as a substrate (see Sigma Diagnostics #245). 5'-Nucleotidase (EC 3.1.3.5.) was assayed on the basis of the liberation of phosphate according to Bickerstaff and Burchell (1980). Acrosin (EC 3.4.21.10) was assayed according to Zaneveld et al. (1973), with benzoylarginine ethylester as a substrate.

Statistical Procedures

Enzymatic and biochemical analyses of washed sperm and isolated membranes were completed on four or more separate preparations of 2–4 pooled ejaculates. Each analysis was completed on a separate aliquot portion of the same preparation. Data were analyzed by analysis of variance (Barr et al., 1979), and means tested using Duncan's new multiple-range test (Duncan, 1955).

RESULTS

Effects of procedures to remove membranes from washed, ejaculated bovine spermatozoa and the resulting membrane preparations were examined by using light and transmission electron microscopy. Plasma membrane of sperm centrifuged through Percoll followed by a second wash remained intact

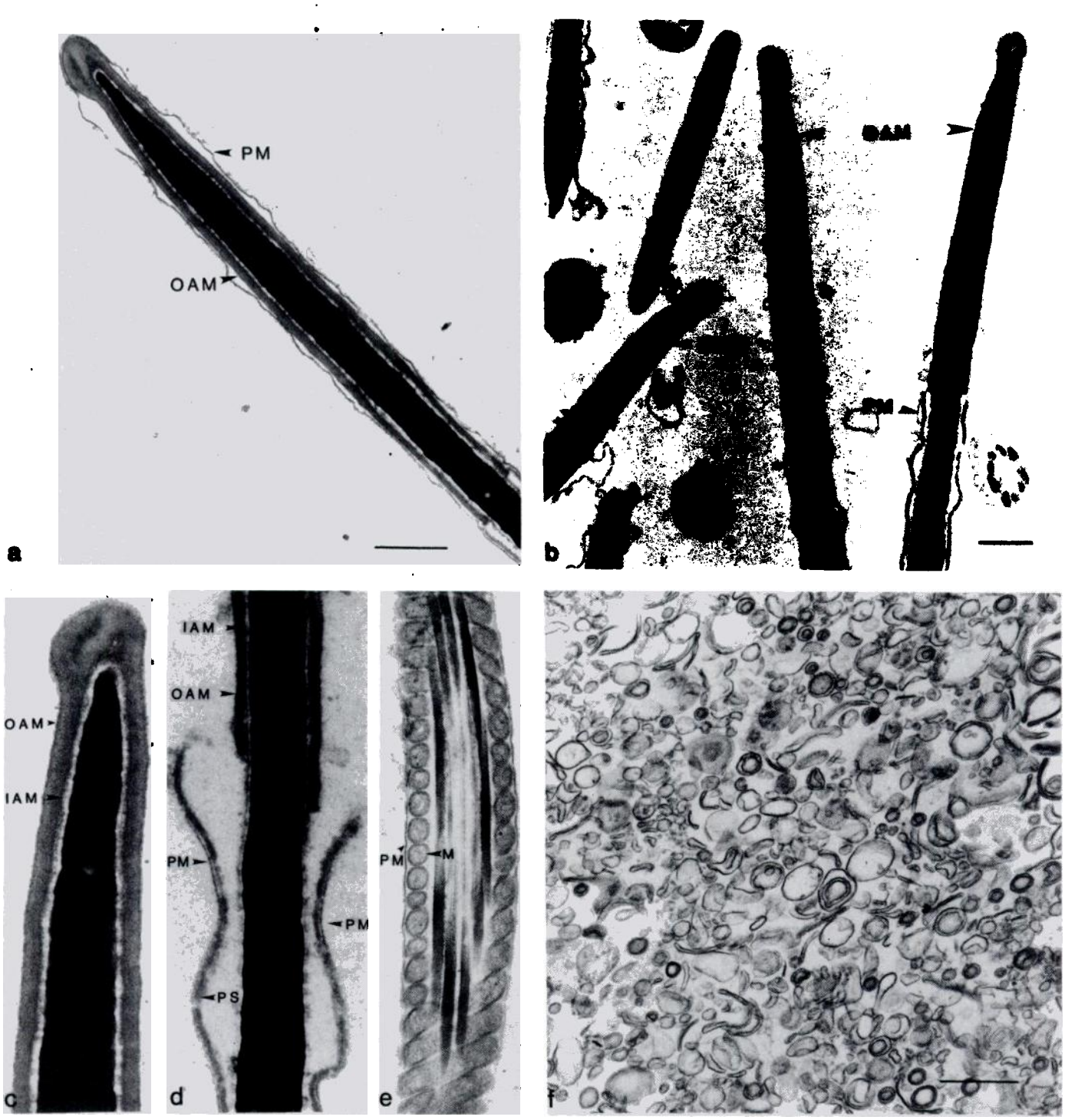

FIG. 1. Transmission electron microscopy of plasma membrane isolation and purification. *a*) Washed sperm with plasma membrane (*PM*) and outer acrosomal membrane (*OAM*) intact. *b–e*) Post-cavitation sperm with *b*) PM over the acrosome removed and OAM intact. PM was occasionally disrupted in the post-acrosomal and midpiece regions (•). *c*) Periacrosomal PM removed but OAM and inner acrosomal membrane (*IAM*) intact. *d*) PM removed from the equatorial segment of the acrosome but intact over the post-acrosomal sheath (*PS*). OAM and IAM are intact. *e*) PM and mitochondria (*M*) of the midpiece after cavitation. *f*) PM vesicles removed by nitrogen cavitation and recovered from the 1.05/1.16 g/ml interface of a sucrose density gradient. *Bars* = 0.5 μm.

until sperm were treated further (Fig. 1a). Nitrogen cavitation of sperm resulted in preferential removal of plasma membrane from the region overlying the acrosome. Plasma membrane surrounding the remaining regions of the sperm generally remained intact with occasional disruption in the post-acrosomal and midpiece regions (Fig. 1b). Outer acrosomal membrane was not removed by the cavitation procedure (Fig. 1c).

Electrophoresis patterns of membrane proteins were not qualitatively different for plasma membrane removed by cavitation and recovered from sucrose gradients at densities of 1.05 to 1.16 g/ml (Fig. 2). Therefore, preparative isolations of plasma membrane were performed by using discontinuous sucrose gradients from which membrane was recovered at the 1.05/1.16 g/ml interface. This material consisted primarily of spherical membrane vesicles with some flattened vesicles and vesicles within vesicles (Fig. 1d). When compared to the unfractionated cavitate, plasma membrane preparations were enriched approximately 13-fold in alkaline phosphatase activity and 9-fold in 5'-nucleotidase activity (Table 1).

Outer acrosomal membranes were removed as morphologically distinct caplike structures (Fig. 3a) and were isolated intact by sucrose density-gradient centrifugation. Some acrosomal ground substance remained associated with the membrane even after preparations were collected from the 1.16/1.24 g/ml interface of density gradients and concentrated by centrifugation (Fig. 3b). Final membrane preparations were characterized by large sheets of membrane and associated ground substance, with discernible remnants of the original caplike structure still present (Fig. 3c). Acrosomal membranes were not enriched in acrosin or alkaline phosphatase activity.

Major biochemical features of whole sperm and isolated membranes are presented in Table 2. Lipid content of preparations was based on extraction efficiencies of 95% for phospholipids ([^{14}C]dipalmitoylphosphatidylcholine recovery) and 98% for sterols ([^{3}H]cholesterol recovery). Measured quantities of protein, phospholipid, and cholesterol on a per cell basis were a function of the washing and isolation procedures, and differences in these values across preparations reflect the efficiency of these procedures rather than the absolute quantity of the component within the cell or membrane. However, the relative amounts of membrane components can be compared and large differences were observed. The

TABLE 1. Specific activities[a] of enzymes in plasma membrane fraction (mean ± SEM).

Enzyme	Homogenate	Plasma membrane	Enrichment
Alkaline phosphatase (n=9)	33.0 ± 2.7	430.0 ± 75.0	13.1
5-Nucleotidase (n=4)	4.80 ± 0.02	40.8 ± 4.4	8.5

[a]Nanomoles p-nitrophenol/min/mg protein for alkaline phosphatase, nmol P_i/min/mg for 5'-nucleotidase.

protein-to-phospholipid ratio of whole sperm (4.39) was approximately 2-fold higher than for outer acrosomal membrane (2.48), and the ratio for outer acrosomal membrane was approximately 4-fold greater than for plasma membrane (0.66). The cholesterol-to-phospholipid ratio for whole sperm (0.22) did not differ significantly from that for outer acrosomal membrane (0.26), but this ratio was approximately 50% greater for plasma membrane (0.38) than for outer acrosomal membrane ($p<0.01$).

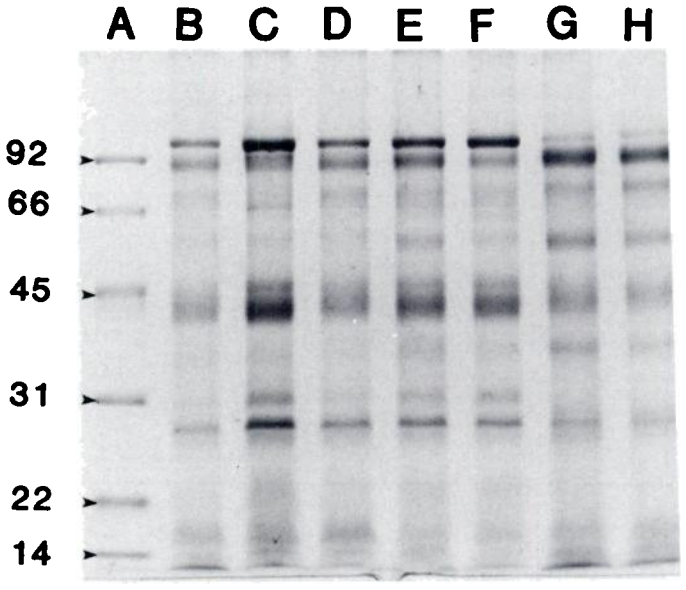

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins in plasma membrane fractions recovered from sucrose density gradients. *A*) Molecular weight standards were phosphorylase B, 92,500; bovine serum albumin, 66,200; ovalbumin, 45,000; carbonic anhydrase, 31,000; soybean trypsin inhibitor, 21,500; lysozyme, 14,400. *B*) Unfractionated membranes. Other adjacent lanes represent fractionations of two separate membrane preparations. Membrane collected from the *C* and *D*) 1.05/1.13 g/ml; *E* and *F*) 1.13/1.16 g/ml; and *G* and *H*) 1.16/1.21 g/ml interaces of discontinuous sucrose gradients.

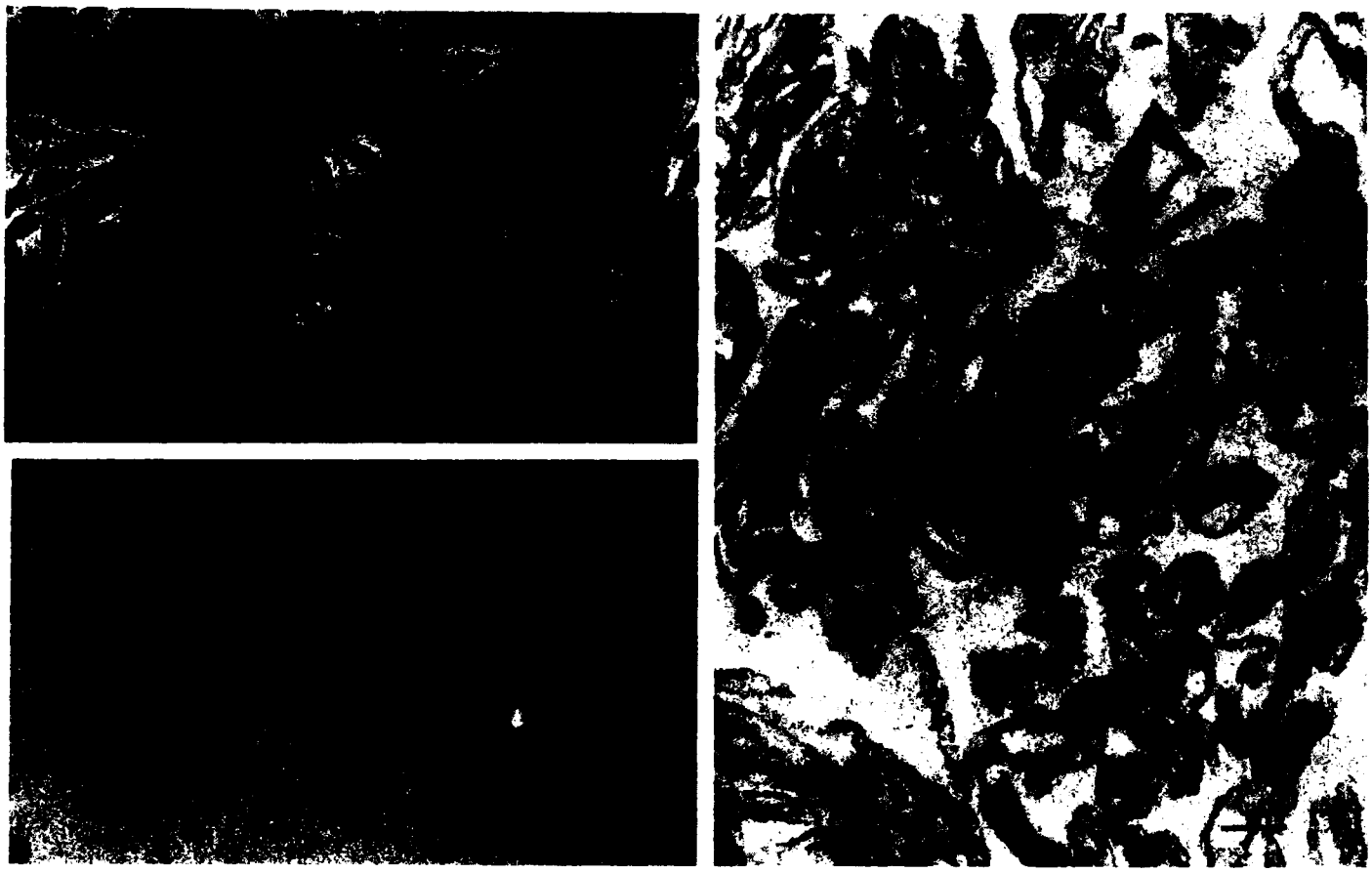

FIG. 3. Microscopy of outer acrosomal membrane isolation and purification. *a*) Phase-contrast micrograph of loosening (•) and removal of acrosomal "caps" (*arrows*) after centrifugation through hypotonic sucrose (×500). *b*) Acrosomal "caps" recovered from the 1.16/1.24 g/ml interface of a sucrose density gradient (×500). *c*) "Sheets" and "caps" of outer acrosomal membrane characterized by adherent ground substance from the acrosomal matrix. *Bar* = 0.5 μm.

Cholesterol was the only free sterol detected in lipid extracts of any of the preparations. Cholesterol sulfate was measured in whole sperm and in both plasma and outer acrosomal membranes but represented less than 1% of total sterol in each. Similarly, cholesterol ester was determined to be less than 1% of total sterol in all preparations. No further characterization of neutral lipids was attempted.

An acetone-soluble polar lipid comigrating with beef brain sulfatide during TLC was present in whole sperm and plasma membrane but not outer acrosomal membrane. This compound was tentatively identified

TABLE 2. Biochemical composition of isolated plasma membrane and outer acrosomal membrane of bovine spermatozoa (mean ± SEM, n=4).

Component	Whole sperm	Plasma membrane	Outer acrosomal membrane
Protein (μg/5 × 10^9 sperm)	31.4 ± 1.0 (mg)	300.0 ± 15	276.0 ± 26
Phospholipid (μg/5 × 10^9 sperm)	6.63 ± 0.21 (mg)	467.0 ± 28	111.0 ± 7
Cholesterol (μg/5 × 10^9 sperm)	720.0 ± 41	92.6 ± 22	24.5 ± 1.9
Protein to phospholipid (w/w)	4.39[a]	0.66[b]	2.48[c]
Cholesterol to phospholipid (mol/mol)	0.22[a]	0.38[b]	0.26[a]

[a,b,c]Values within each ratio with different superscripts are different ($p<0.01$).

TABLE 3. Phospholipid composition of plasma membrane and outer acrosomal membrane of bovine spermatozoa.[a]

Phospholipid	Whole sperm	Plasma membrane	Outer acrosomal membrane
Cardiolipin	3.2	–	–
Ethanolamine phosphoglycerides	11.8[b]	9.9[b]	6.8[c]
Choline phosphoglycerides	52.8	50.3	42.0
Lysophosphatidylcholine	1.2	1.8	1.6
Sphingomyelin	8.9[b]	12.6[c]	16.5[d]
Phosphatidylserine	1.0	1.1	1.8
Phosphoinositides[e]	1.8	2.7	2.9

[a]Values are percentage of total phospholipid plated. Approximately 8–10% of lipid phosphorus remained at the origin during thin-layer chromatography. Unidentified spots were not quantified.

[b,c,d]Values within each row with different superscripts are different ($p<0.05$).

[e]Values include phosphatidylinositol, phosphatidylinositol phosphate, and phosphatidylinositol bisphosphate.

as sulfogalactolipid and represented less than approximately 5% of total lipid in the PM fraction.

Phospholipid compositions of sperm and membrane preparations are presented in Table 3. Choline phosphoglycerides were the predominant phospholipid class in all preparations, representing approximately 50% of total phospholipid measured. Sphingomyelin and ethanolamine phosphoglycerides were relatively high also, with sphingomyelin slightly higher in outer acrosomal membrane than plasma membrane or whole sperm ($p<0.05$) and ethanolamine phosphoglycerides higher in plasma membrane and whole sperm than acrosomal membranes ($p<0.05$). Ethanolamine phosphoglycerides were higher in whole sperm than plasma membrane samples ($p<0.05$). Cardiolipin was present in whole sperm extracts but was not detected in plasma or outer acrosomal membrane. Phosphatidylserine, phosphatidylinositol, and polyphosphoinositides were found at very low levels in all preparations.

DISCUSSION

A plasma membrane fraction enriched in material from the periacrosomal region of ejaculated bovine sperm was obtained by nitrogen cavitation and sucrose density centrifugation. Preferential removal of periacrosomal plasma membrane using nitrogen cavitation has also been reported for epididymal ram (Parks and Hammerstedt, 1985) and bull spermatozoa (Noland et al., 1983) using equilibration pressures comparable to those used in the present study. Noland et al. (1983) demonstrated that post-acrosomal plasma membrane, acrosomal membranes, nuclear and mitochondrial membranes remained intact after cavitation of epididymal bull sperm. This was generally observed for ejaculated bull sperm, although some disruption of plasma membrane occurred in the post-acrosomal, midpiece, and flagellar regions. Contamination of periacrosomal plasma membrane fractions by post-acrosomal plasma membrane did not appear to be as extensive as that reported for epididymal boar sperm cavitated under similar conditions (Peterson et al., 1980).

The ultrastructure of plasma membrane vesicles isolated after cavitation was similar to that reported by Noland et al. (1983). The appearance of flattened vesicles and vesicles within vesicles in that study was partially attributed to contamination by the contents of disrupted cytoplasmic droplets. While some flattened and concentric vesicles were observed in membrane preparations in the present study, these are not attributed to cytoplasmic droplet contamination, because ejaculates containing sperm with attached droplets were not used, and detached droplets were eliminated during the sperm-washing procedure. Differences in membrane vesicle morphology possibly are related to the sperm surface origin of the membrane, although such differences have not been demonstrated.

Enrichment in the specific activity of plasma membrane marker enzymes is further indication of the purity of the plasma membrane fraction obtained. Specific activities of alkaline phosphatase and 5'-nucleotidase were equal to or greater than those for epididymal bull and boar sperm plasma membrane reported by others (Herman et al., 1976; Casali et al., 1985; Nikopoulou et al., 1985).

Preparations of outer acrosomal membrane were similar in appearance to those originally described by Zahler and Doak (1975) for ejaculated bull sperm. Membranes retained the amorphous ground substance of the acrosomal contents throughout the preparative procedures, giving the membranes their characteristic "fuzzy coat" appearance (Gillis et al., 1978; Russell et al., 1979). Outer acrosomal membrane preparations were not significantly contaminated by plasma membrane or inner acrosomal membrane because of the absence of plasma membrane vesicles in acrosomal membrane preparations, retention of the inner acrosomal membrane by treated sperm, and lack of

enrichment in the specific activities of either alkaline phosphatase or acrosin.

The high protein content of the outer acrosomal membrane is consistent with the high density of this membrane fraction relative to plasma membrane (material recovered at the 1.16/1.24 g/ml interface versus the 1.05/1.16 g/ml interface) and supports the concept that the ground substance associated with the membrane is largely protein. The identity of these proteins has not been determined, but apparently does not include an enrichment in acrosin, hyaluronidase, or other acrosomal enzymes (Zahler and Doak, 1975). Phospholipid distribution in total lipid extracts of bovine sperm is quite similar to that reported by Clegg and Foote (1973) and Poulos et al. (1973), but is characterized by less ethanolamine phosphoglyceride than reported by Pursel and Graham (1967). Furthermore, the phospholipid distribution in whole sperm lipids was similar to that for plasma membrane and outer acrosomal membrane preparations, with the exception of cardiolipin. Because cardiolipin is generally considered to be restricted to mitochondrial and lysozomal membranes (Evans, 1979; Jain and Wagner, 1980), it is perhaps not surprising that this phospholipid does not appear in plasma and acrosomal membrane preparations from bovine sperm. However, cardiolipin has been identified in plasma membrane of epididymal ram sperm (Parks and Hammerstedt, 1985), guinea pig sperm (Bearer and Friend, 1982), and ejaculated boar sperm (Clegg, 1983). Localization of this anionic phospholipid is thought to promote fusion of membranes leading to the acrosome reaction in these species (Bearer and Friend, 1982).

The slightly higher sphingomyelin content of acrosomal membranes appears to correspond to lower choline phosphoglyceride content when compared to plasma membrane. However, these differences are small, and no direct effect on the stability of these membranes can be predicted. A small difference in the ethanolamine phosphoglycerides was observed between plasma and acrosomal membranes. While a high content of phosphatidylethanolamine might be expected to destabilize membrane bilayers because of its preference for a hexagonal II configuration (Cullis et al., 1985), the relatively low level of ethanolamine phosphoglyceride compared to choline phosphoglyceride observed in this study would be expected to favor the more stable, bilayer configuration in both plasma membrane and acrosomal membranes. Perhaps during capacitation, localization of phospholipid species (Bearer and Friend, 1982) favors a non-bilayer configuration in these regions.

The phospholipid composition of bull sperm plasma membrane differs markedly from plasma membrane of boar or ram spermatozoa (Holt and North, 1985; Nikolopoulou et al., 1985; Parks and Hammerstedt, 1985). However, the similarity in phospholipid composition between bull sperm membranes evaluated in this study is surprising, since Nikolopoulou et al. (1986b), using similar methodology, found large differences in the phospholipid composition of plasma membrane and membrane released during an in vitro acrosome reaction of boar spermatozoa. The latter are presumably hybrid vesicles of plasma membrane and outer acrosomal membrane, suggesting that differences between acrosomal and plasma membranes from boar sperm were larger to begin with or that major changes occurred during the acrosome reaction.

Quantification of polyphosphoinositides after separation by TLC approached the limits of detection for the assay employed in this study. However, when sperm membranes were preincubated with [^{32}P]-adenosine triphosphate (ATP) prior to lipid extraction and TLC, both phosphatidylinositol phosphate and phosphatidylinositol bisphosphate were detected by autoradiography. Although phosphatidylinositol represents only a small percentage of total phospholipid in bovine and other sperm membranes, the importance of phosphoinositide metabolism in intracellular signaling has been demonstrated (Berridge, 1984; Nishizuka, 1984) and appears to be related to events associated with the acrosome reaction. Nikolopoulou et al. (1985) reported that hybrid vesicles resulting from the acrosome reaction of boar sperm in vitro incorporated $^{32}P_i$ into polyphosphoinositides at a rate of 5- to 15-fold that of plasma membrane alone. Furthermore, boar sperm membranes phosphorylated proteins when incubated in the presence of [^{32}P]ATP, calcium and diacylglycerol (Nikolopoulou et al., 1986a). Diacylglycerol is an important component of intracellular signaling by phosphoinositides, stimulating the activity of protein kinase C. Phorbol esters, which mimic the effect of diacylglycerol on protein kinase C, accelerate the acrosome reaction of mouse sperm once bound to the zona pellucida (Lee et al., 1986). Berruti and Franchi (1986) have presented results suggesting that the acrosome is an important site of phosphoinositide

metabolism in boar spermatozoa. Not only were phosphoinositides associated with the outer acrosomal membrane, but the acrosomal compartment was identified as a source of intracellular calcium and phospholipase C activity. In the present study, both plasma membrane and outer acrosomal membrane contained polyphosphoinositides. The significance of phosphoinositide metabolism and its effect on calcium mobilization and protein phosphorylation during the acrosome reaction is yet to be determined.

The structure of glycolipid detected in plasma membrane preparations was not identified but is in all probability the alkylacyl glyceryl monogalactoside sulfate reported in total lipids of bovine sperm (Selivonchick et al., 1980) and plasma membrane lipids of epididymal boar sperm (Nikolopoulou et al., 1985). The functional significance of this glycolipid is not known, although it may contribute to the net negative charge associated with the surface of uncapacitated sperm (Langlais and Roberts, 1985).

The high cholesterol to phospholipid ratio of sperm plasma membrane relative to outer acrosomal membrane and total sperm lipid is consistent with the disproportionate distribution of cholesterol among cellular membranes of other cell types (Yeagle, 1985). This distribution appears to be controlled, in part, by thermodynamic partitioning as a function of membrane lipid and protein composition. The similarity in phospholipid composition between the plasma and outer acrosomal membranes suggests that the higher cholesterol content of the plasma membrane may be a function of protein rather than lipid composition.

The stabilizing effect of cholesterol on cell membranes is well established (Demel and DeKruyff, 1976; Bell, 1978; Chapman, 1982; Yeagle, 1985). While cholesterol depletion may play a direct role in destabilizing the sperm plasma membrane and inducing the acrosome reaction, the decondensing effect of cholesterol removal on the membrane and the resulting increase in permeability (Chapman, 1982; Yeagle, 1985) may be more significant. Langlais and Roberts (1985) have suggested that loss of cholesterol from the sperm surface may result in increased permeability to extracellular calcium. Elevation of intracellular calcium is a prerequisite to the acrosome reaction (Yanagimachi, 1981), and it has been suggested that one effect of calcium is the activation of phospholipases, resulting in an accumulation of lysophospholipid in the membrane and destabilization of the bilayer configuration leading to vesiculation (Meizel, 1984; Langlais and Roberts, 1985).

While the description of outer acrosomal and plasma membrane presented here does not clearly establish a basis for membrane vesiculation, a foundation for examining important changes accompanying sperm capacitation has been laid. Furthermore, phospholipid-bound fatty acyl composition and other components that directly affect membrane structure and function are now being examined. These studies should provide further insight into the mechanisms involved in sperm capacitation.

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