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Effect of secretory particles in bovine seminal vesicle secretion on sperm motility and acrosome reaction

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Summary. Particles found in bovine seminal vesicle secretion were enriched by centrifugation. They varied in size and morphology and contained Mg^{2+},Ca^{2+}-activated ATPase, aminopeptidase A, alanyl aminopeptidase, γ-glutamyl transpeptidase and dipeptidyl peptidase IV activities. Hyperactivation of sperm motility and the acrosome reaction were induced by these particles in epididymal spermatozoa suspended in a modified Ringer medium. The hyperactivation, analysed with a microscopic slide test, started within minutes of exposure to membrane particles and continued for 3–4 h, during which time spermatozoa underwent the acrosome reaction. Acrosome staining, phase-contrast microscopy and transmission electron microscopy revealed that the acrosome reaction started within 60 min at 37°C and affected up to 80% of spermatozoa in 4 h. These membrane particles differed from those reported previously in other species in enzyme composition, function and organ of origin.

Introduction

Seminal fluid comprises the secretions of the testis, epididymis and sex accessory glands. The components of the seminal fluid can influence the motility and metabolism of spermatozoa (Inskeep *et al.*, 1985) with beneficial as well as deleterious effects being reported (Eliasson & Lindholmer, 1975; Dott *et al.*, 1979; Mann & Lutwak-Mann, 1981; Baas *et al.*, 1983). Metz *et al.* (1968) and Davis (1973) reported the presence of membrane particles in the seminal plasma of rabbit, bull and man. Ronquist *et al.* (1978a, b) described similar particles in the seminal fluid and prostatic secretion of man and called them prostasomes on the basis of their obvious origin from the prostate (Brody *et al.*, 1983). Membrane particles have also been reported in ram seminal plasma (Breitbart & Rubinstein, 1982). Davis (1974) and Davis & Hungund (1976) observed that the particles in rabbit seminal fluid originated from the epididymis and caused sperm decapacitation and inhibited fertility, whereas Stegmayr & Ronquist (1982) concluded that the human prostasomes were involved in stimulation of forward motility in spermatozoa. These particles have also been shown to contain high activities of ATPase and protein kinase (Ronquist & Brody, 1985), a zinc-dependent endopeptidase (Laurell *et al.*, 1982), γ-glutamyl transpeptidase (Lilja & Weiber, 1983), alanyl aminopeptidase and dipeptidyl peptidase II (Vanha-Perttula, 1984) as well as angiotensin converting enzyme (Krassnigg *et al.*, 1985). The role of these enzymes and the functional importance of the particles in seminal fluid have remained obscure or highly contradictory.

In this study we have identified a possible origin of the particles found in bull seminal plasma, analysed their enzyme composition and investigated their effects on epididymal spermatozoa with particular emphasis on sperm motility and the acrosome reaction.

Materials and Methods

Chemicals. The substrates L-alanine-β-naphthylamide HBr (AlaNA), L-α-glutamyl-β-naphthylamide (GluNA), L-γ-glutamyl-β-naphthylamide (γ-GluNA), *N*-hippurylglycylglycine (HipGlyGly), glycyl-L-proline-β-naphthylamide

(GlyProNA) and *N*-succinyl-L-analyl-L-alanyl-L-alanine-*p*-nitroanilide (Succ-$(Ala)_3$-*p*-na) as well as glycylglycine (GlyGly) were obtained from Bachem Feinchemikalien (Bubendorf, Switzerland). Theophylline, trifluoperazine, ouabain, bovine serum albumin (BSA), adenosine 5'-triphosphate (ATP) and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.). Naphthol Yellow S was from Chroma Gesellschaft (Stuttgart, West Germany), while erythrosin B, various salts and reagents were obtained from E. Merck AG (Darmstadt, West Germany) and Sephacryl S-1000 from Pharmacia Fine Chemicals (Uppsala, Sweden). Spectrapor dialysis tubing (12 000–14 000 molecular weight cut-off) was from Spectrum Medical Industries (Los Angeles, CA, U.S.A.).

Preparation of samples. Epididymides and seminal vesicles of bulls were obtained from a local slaughterhouse (Lihakunta Abattoir). The samples were transported on ice to the laboratory within 15–30 min after slaughter and immediately processed. The epididymis was divided into 5 parts: proximal and distal caput, corpus, proximal and distal cauda. The different parts were put in glass beakers containing (a) saline (9 g NaCl/l), (b) a modified Ringer solution (120 mM-NaCl, 5 mM-KCl, 10 mM-KH_2PO_4, 5 mM-$MgSO_4.H_2O$, 1 mM-Tris–HCl, pH 7·5), referred to as Medium NKM, or (c) Medium NKM containing 50 mM-theophylline and called Medium NKMT. Theophylline was dissolved in Medium NKM by constant stirring at 50°C. The epididymal parts were minced with scissors and the spermatozoa were separated from tissue pieces by filtration through a nylon net. The spermatozoa were sedimented by centrifugation (800 *g* for 10 min) at room temperature and washed 3 times with Medium NKM. After the last wash, spermatozoa were diluted to give a concentration of 2–5 × 10^7/ml.

Seminal vesicle secretion was collected from the cut excretory duct by gentle manual pressure on the glands. The fluid was centrifuged at 800 *g* for 10 min in a Sorvall GLC-2 centrifuge. The pellet was discarded and the supernatant was centrifuged at 40 000 *g* for 2 h in a Sorvall RC-5 centrifuge at 4°C. The pellet was resuspended in 0·9% (w/v) saline (half of the original volume) and part of it was applied to a column (0·9 × 60 cm) of Sephacryl S-1000. The fractions eluting at V_e/V_o values 1·5 (pool A_1) and 2·2 (pool A_2) with a strong hydrolysis of AlaNA and GluNA (Agrawal & Vanha-Perttula, 1986a, b) were pooled separately and centrifuged to result in two particle preparations, A_1 and A_2. These and the original pellet were used to study their effect on sperm motility and the acrosome reaction. Parts of the samples were processed for electron microscopy. In some experiments the original pellet was further washed twice in 0·9% (w/v) saline by intervening centrifugations. The particle suspension in saline was stored at 4°C and used within 2–3 days. In addition, an aliquant of seminal vesicle secretion (after the initial centrifugation at 800 *g*) was centrifuged at 105 000 *g* for 2 h at 4°C. The pellet and supernatant were analysed for enzyme activities and were also used to evaluate the effect on sperm motility. Additional studies were carried out with pellet and supernatant preparations, which had been dialysed overnight (at 4°C) against 0·9% (w/v) saline (2000-fold in excess).

Electron microscopy. Fixation of pieces from freshly obtained seminal vesicles (secretion not expelled) as well as sperm samples and particle pellets after centrifugation was carried out with 2% glutaraldehyde in 0·1 M-sodium cacodylate buffer, pH 7·4. After fixation for 18 h, the sperm and particle specimens were pelleted in capsules by centrifugation at 2000 *g* for 30 min. Following a wash with 0·1 M-phosphate buffer, pH 7·4, the samples were post-fixed in 1% osmium tetroxide in 0·1 M-phosphate buffer, pH 7·4, for 90 min and dehydrated in a series of ethanol. After transfer to propylene oxide they were embedded in Epon. Sections of 50 nm were prepared with an Ultrotome III (LKB, Bromma, Sweden) and stained with uranyl acetate (5 min) and lead citrate (20 min) in Ultrostainer (LKB, Bromma, Sweden). After carbon coating the specimens were studied in a Jeol JEM 100 B electron microscope at 80 kV.

Hyperactivation and acrosome reaction. In studies on hyperactivation and acrosome reaction, equal volumes of sperm suspension and seminal vesicle fluid or particle suspension (~1 mg/ml protein) in different vehicles (saline, Medium NKM and Medium NKMT) were mixed in a small test-tube. The samples were then kept at room temperature (~22°C) or incubated in a water bath at 37°C. In the motility studies a 20–30-µl drop of the mixture was put on glass slides with a coverslip. After the spermatozoa had settled, they were observed by phase-contrast microscopy (Nikon Optiphot) on a temperature-regulated stage (Reichert, Vienna, Austria) at 37°C or at ambient (~22°C) room temperature. Controls consisted of spermatozoa suspended in saline, Medium NKM or Medium NKMT alone. The effects of BSA (1% final concentration) or trifluoperazine (30 µM final concentration) on the sperm motility were studied in the presence and absence of the seminal vesicle fluid or particle preparation. The sperm motility was photographically recorded with Kodak Tri-X pan film using a stroboscope with 8 Hz light frequency attached to the microscopic illumination. The sperm motility in different experimental conditions was also assessed with a computer-assisted laser Doppler spectroscopy method (Lazymot, BTG Biotechnik, GmbH, Düsseldorf, West Germany) according to Hartmann *et al.* (1983).

The acrosome reaction of spermatozoa was analysed by microscopy in bright-field illumination after staining the smear preparations with naphthol yellow S and erythrosin B according to Bryan & Akruk (1977) with a minor modification. After air-drying, the slides were dipped twice in distilled water to remove the seminal vesicle proteins obscuring the visual assessment of the acrosome reaction. With this method the acrosomal caps in intact spermatozoa stained cherry-pink, whereas sperm heads devoid of acrosomes stained pale yellow. A total of 400 spermatozoa were counted from each slide and the percentages of acrosome reaction-positive (acrosome-reacted) and -negative spermatozoa were determined. The acrosome reaction was also checked by phase-contrast microscopy and by transmission electron microscopy (TEM). In phase-contrast microscopy (× 1000, oil immersion), the spermatozoa with an intact acrosome displayed sharp margins of the head, and after the acrosome reaction the apical regions became fuzzy and the total loss of acrosomes was readily distinguished (Shams-Borhan & Harrison, 1981). The criteria for the true

acrosome reaction in TEM were those applied by Barros *et al.* (1967) and Bedford (1970), i.e. a progressive and orderly fusion of sperm plasma and outer acrosomal membranes with resultant fenestration and vesiculation.

Enzyme activities. The enzyme activities in seminal vesicle fluid and particle preparation were assayed after appropriate dilution in 0·025 M-imidazole–HCl buffer, pH 7·4. ATPase activity was assayed in the presence of 1 mM-EDTA (basal ATPase; EC 3.6.1.3), 10 mM Mg^{2+} or 10 mM Ca^{2+} (Mg^{2+},Ca^{2+}-activated ATPase) with and without ouabain (80 μM) at pH 7·5 (Breitbart & Rubinstein, 1982). After incubation the inorganic phosphate released was measured according to Chen *et al.* (1956). Aminopeptidase A (AP-A; EC 3.4.11.7) and alanyl aminopeptidase (AAP; EC 3.4.11.2) activities were assayed as described earlier (Agrawal & Vanha-Perttula, 1985) using GluNA and AlaNA as substrates. γ-Glutamyl transpeptidase (GGT; EC 3.4.14.5) was measured similarly using γ-GluNA as substrate at pH 8·0 in the presence of 10 mM-GlyGly as the acceptor. Dipeptidyl peptidase IV (DPP IV; EC 3.4.14.5) was assayed at pH 8·0 with GlyProNA as substrate, and angiotensin converting enzyme (ACE; EC 3.4.15.1) with HipGlyGly as described earlier (Vanha-Perttula *et al.*, 1985). The endopeptidase (EndoP; EC 3.4.21) hydrolysing Succ-$(Ala)_3$-*p*-na was assayed according to Laurell *et al.* (1982).

Results

Enzyme activities

Of the enzymes assayed ACE and the endopeptidase hydrolysing Succ-$(Ala)_3$-*p*-na (with and without Zn^{2+}) were not detected either in the fluid or particle fraction of seminal vesicle secretion (Table 1). On the other hand, the Mg^{2+}- and Ca^{2+}-activated, ouabain-resistant ATPase was mainly detected in the particle preparation. AP-A, AAP, DPP IV and GGT were also predominantly in the particulate form with only a minor portion in the supernatant fluid.

Table 1. Distribution of enzyme activities in particles and fluid of the seminal vesicle secretion of bulls

Enzyme	Specific activity (nmol/min mg protein^{-1})	
	Particles*	Fluid†
ATPase (EDTA 1 mM)	0·71 ± 0·42	0
ATPase (Mg^{2+} 10 mM)	3·17 ± 1·23	0·26 ± 0·08
ATPase (Ca^{2+} 10 mM)	2·51 ± 1·03	0·15 ± 0·04
AP-A	360·7 ± 198·7	25·2 ± 12·6
AAP	24·86 ± 11·2	3·48 ± 0·8
GGT	16·7 ± 4·25	2·55 ± 0·9
DAP IV	1288·4 ± 315	96·9 ± 35·9
ACE	0	0
EndoP	0	0

Values are means ± s.d. of duplicates from 5 different samples.
*Particles represent the sediment obtained by centrifugation of the vesicular secretion at 105 000 *g* for 2 h at 4°C.
†The supernatant obtained after centrifugation at 105 000 *g* for 2 h at 4°C.

Structure and formation of secretory particles

After centrifugation of seminal vesicle secretion the pellet preparation displayed particulate material when observed by light microscopy (1 μm section) after toluidine blue staining or in electron microscopy. Two main size categories could be discerned among the particles. Sephacryl S-1000 fractionation resulted in a separation of the particles into the larger (A_1) and smaller (A_2) groups. Both particles were equally mixed in the original pellet (Fig. 1). Most of the particles had usually an almost complete trilaminar unit membrane surrounding floccular or granular contents. Occasionally the particles were surrounded by penta- or other multilaminar membranes.

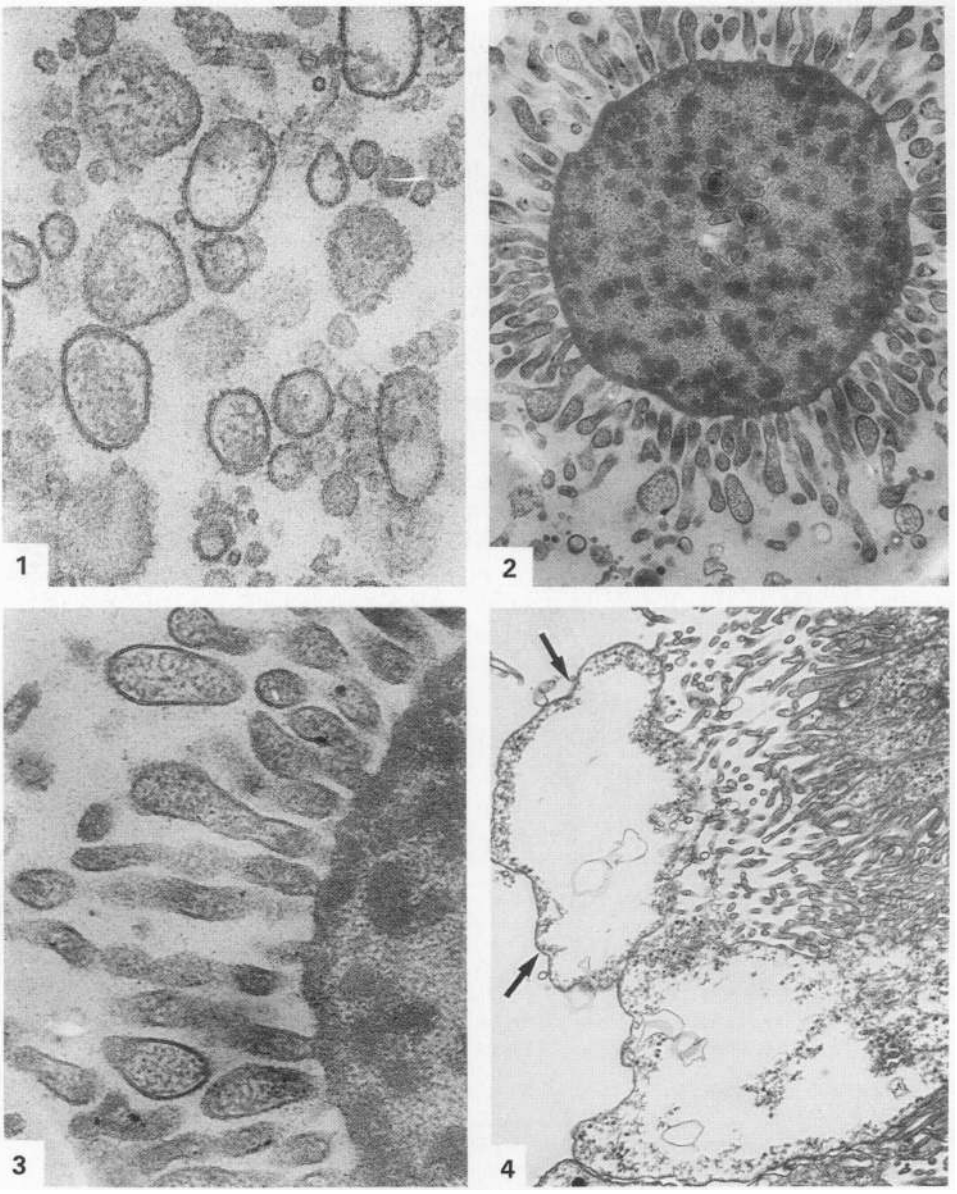

Fig. 1. Electron microscopy of the pellet obtained after centrifugation of the seminal vesicle secretion at 40 000 *g* for 2 h. Most of the particles are surrounded by a trilaminar unit membrane and contain floccular or finely granular material. × 70 000.

Fig. 2. A composite storage body, which contains dense granules partly surrounded by unit membrane in the centre. The surface is covered by microvillar processes. × 12 000.

Fig. 3. Higher magnification of an area in the storage body (Fig. 2) displaying microvillar processes. × 30 000.

Fig. 4. A bleb-like composite vesicle (arrows) on the surface of the seminal vesicle epithelium and another vesicle forming. Both contain granular material as well as some membrane residues. The epithelial surface is covered by elongate microvilli. × 5 000.

In addition to these main particle forms, the original pellet contained composite types of particles. One type was formed by an aggregate surrounded by microvillar processes (Figs 2 & 3). It contained condensed areas, which sometimes were surrounded by unit membrane. The other compound particle was frequently found close to the epithelial surface in the lumina of the seminal vesicles. It was a bleb-like membrane-bound structure, which contained numerous small vesicles at its periphery or larger membrane residues (Fig. 4). These were more often encountered within the lumina of the seminal vesicles than in the secretion. Breaks were occasionally encountered in the unit membrane surrounding the large vesicular particles with an obvious release of the contents.

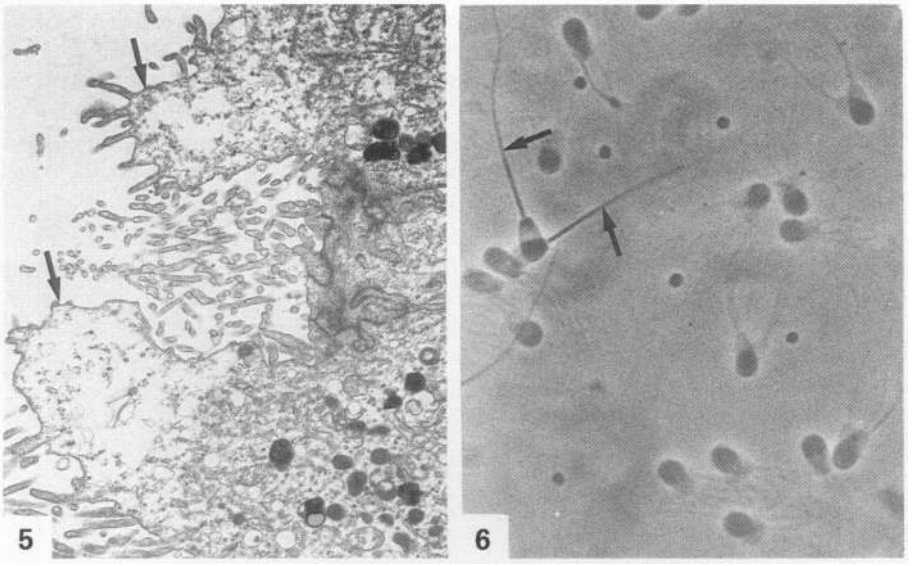

Fig. 5. Two protrusions (arrows) of the seminal vesicle epithelium with cytoplasmic rarefaction. The microvilli have partly or totally disappeared at the site of bulging epithelium and they contain small membrane-bound particles. The adjacent part of the cytoplasm shows numerous dense bodies, which are reminiscent of the secondary lysosomes. × 5 000.

Fig. 6. Spermatozoa from the cauda epididymidis examined by phase-contrast microscopy with stroboscopic illumination. The cells are all adhered to the glass by their heads, but their tails are motile. The tails of non-motile spermatozoa (arrows) are more clearly seen. × 1000.

The luminal surface of bovine seminal vesicle epithelium was normally characterized by the presence of elongate microvilli (Fig. 4). In many places the apical parts of the cells showed cytoplasmic rarefaction with a local gradual disappearance of the microvilli (Fig. 5). These apical parts of the cells appeared to bulge toward the lumen and accumulate vesicular particles, which were closely similar to those found in the vesicular secretion pellet. Areas were found where the apical bulges were detaching from the cells and then corresponded to the vesicular intraluminal bodies containing small particles (Figs 4 & 5). The release of single granules or vesicles were not commonly observed. Close to the cytoplasmic detachment, dense round or ovoid particles were found. The latter may be lysosomes, since they often contained electron-dense residues typical for secondary lysosomes (Fig. 5).

Studies on motility

Spermatozoa harvested from the epididymal caput segments and analysed by phase-contrast microscopy remained immotile when suspended in Medium NKM at 37°C and the addition of vesicular secretion particles or fluid and BSA had no effect. Caput spermatozoa suspended in Medium NKMT at 37°C showed a circular movement with sluggish twitching of tails. BSA caused a slight stimulation of this movement but no progressive motion. However, addition of vesicular secretion particles to caput spermatozoa in Medium NKMT at 37°C caused a forward motility in about 20% (range 15–25%; $n = 5$) of spermatozoa. A greater effect was obtained with spermatozoa obtained from the corpus epididymidis.

On normal and albuminized microscope slides covered with a coverslip, the spontaneously motile cauda spermatozoa rapidly adhered by their heads. The tails, however, continued to move for many hours at 22°C and 37°C (Fig. 6). This also occurred when the spermatozoa were in saline, Medium NKM or Medium NKMT as well as in the presence of BSA at 22°C and 37°C. However, the addition of vesicular secretion particle preparations (original as well as A_1 and A_2) to the cauda spermatozoa induced vigorous forward motility in all vehicles at 37°C within a few minutes. The adjustment of the pH from 5·8 to 8·0 was compatible with the motility activation. The response was much weaker or negligible at 22°C, when most of the spermatozoa remained stuck by their heads on the slide. Even repeated washing of the vesicular secretion particles with saline did not destroy the motility stimulation. The hyperactivated state continued for at least 3–5 h when the temperature was maintained constant at 37°C. Trifluoperazine (30 μM) appeared to enhance and prolong the hyperactivated motility in the presence of the particles but alone did not elicit any response. In the stroboscopic recording the hyperactivated spermatozoa produced hazy tracks on the film. Identical results were obtained with the dialysed particle pellet preparation, whereas the supernatant induced no activation or was even inhibitory to the motility.

When the effect of the vesicular secretion particles on the motility pattern of cauda spermatozoa was analysed by laser Doppler spectroscopy at 37°C (Table 2), no marked differences were observed in the percentage of motile or progressively motile spermatozoa in the presence and absence of the particles. The mean velocity values of the two groups were also closely similar. A minor enhancement was, however, observed in the velocity of the progressively motile spermatozoa.

Table 2. Effect of particles of seminal vesicle secretion on sperm motility in theophylline-containing Medium NKMT analysed by a laser Doppler spectroscopy technique at 37°C

	Motile (%)	Progressively motile (%)	Velocity (μm/sec)	Progressive velocity (μm/sec)
Medium NKMT	61·8 ± 4·76	37·4 ± 2·30	49·2 ± 1·64	78·6 ± 2·70
Medium NKMT + particles	59·2 ± 6·40	34·6 ± 5·72	51·6 ± 3·90	85·4 ± 1·50

Values are mean ± s.d. for 5 measurements.

Acrosome reaction

The cauda spermatozoa incubated at 37°C in saline, Medium NKM or Medium NKMT did not show the typical acrosome reaction when assessed by staining with naphthol yellow S and erythrocin B. In contrast, the vesicular secretion particles in saline or Medium NKM caused a clear induction of the acrosome reaction within 1 h, when about 40% of spermatozoa had lost the pink staining of the apical head (Fig. 7). With time, the percentage of cells with a complete acrosome

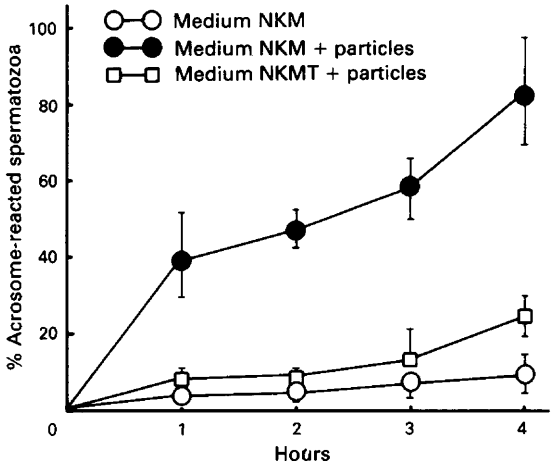

Fig. 7. The percentages of acrosome-reacted spermatozoa at different times of incubation in Media NKM and NKMT without and with particles isolated by centrifugation from the seminal vesicle secretion. The acrosome reaction was assessed after staining with naphthol yellow S and erythrocin B and the results are given as mean ± s.d. of 5 separate analyses.

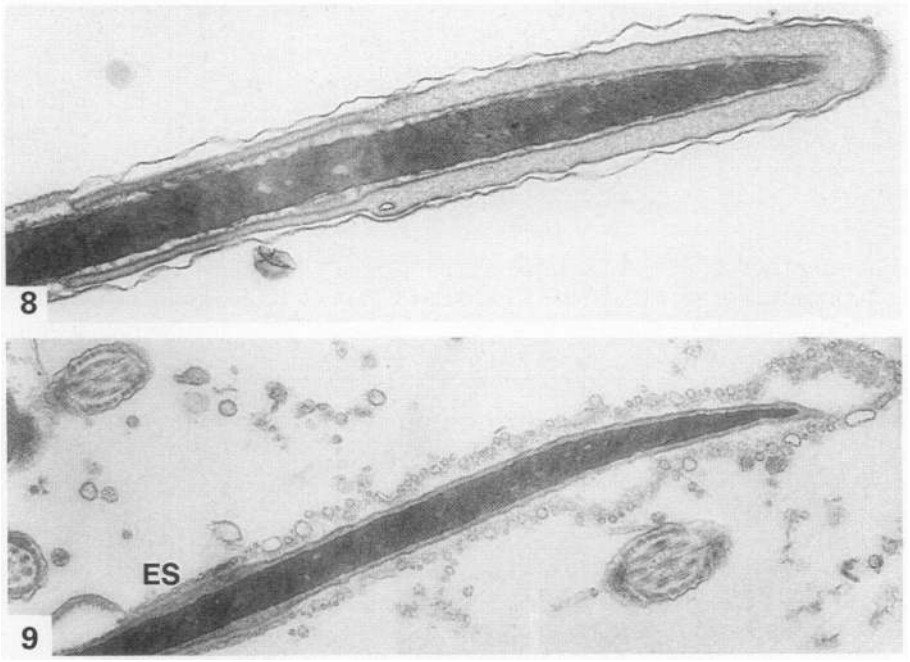

Fig. 8. The apical part of the head of an epididymal spermatozoon which has been incubated in Medium NKM at 37°C for 3 h. Note the distinct plasma and acrosomal membranes. × 20 000.

Fig. 9. Epididymal spermatozoon incubated in Medium NKM with vesicular secretion particles at 37°C for 3 h. Note the vesiculation of the plasma and acrosomal membranes typical for the acrosome reaction. The equatorial segment (ES) shows the formation of small vesicles. × 15 000.

reaction increased and at 4 h about 80% had lost the staining of the acrosome. When, however, the vesicular secretion particles were added with theophylline (Medium NKMT) to the sperm suspension, the induction of the acrosome reaction was markedly suppressed and delayed. The vesicular secretion fluid (supernatant) did not seem to be effective in the induction of the acrosome reaction. Trifluoperazine (30 μM) had neither inhibitory nor activating effect on the reaction. The evaluation of the acrosome reaction in phase-contrast microscopy was consistent with the observations after the staining method. An exact counting of the acrosome reaction-positive and -negative spermatozoa was, however, impossible with the motile cells.

Electron microscopy of spermatozoa incubated in saline, Medium NKM or Medium NKMT consistently showed an intact plasma membrane and acrosome (Fig. 8) during the observation period of 4 h. However, in the presence of the vesicular secretion particles the number of cells with typical vesiculation of the plasma membrane and outer acrosomal membrane (Fig. 9) increased during incubation. The vesicles were larger until the anterior margin of the equatorial segment was reached, but small vesiculation was commonly observed in the latter zone. In the postacrosomal area, midpiece and tail the plasma membrane remained intact in all cases.

Discussion

During the journey from the testis to the ejaculate spermatozoa are exposed to a continuously changing microenvironment, which in critical sequence is processing and programming the cells for fertilization of the ovum. The presence of particulate elements in seminal plasma, besides the spermatozoa, has only recently received more attention due to the detailed analyses of secretory granules and vesicles in human seminal plasma (see Ronquist & Brody, 1985, for review). Multiple lines of evidence suggest that, in man, these particulate elements are derived from the prostate, where they are released by a process designated as 'diacytosis'. Based on their origin from the prostate, the human seminal plasma particles have been called 'prostasomes' (Brody *et al.*, 1983). The present study confirmed the early brief remark of Davis (1973) that bovine seminal plasma also contains particulate elements. Substantial evidence was also obtained for their origin from the seminal vesicles. On this basis the term 'vesiculosome' appears an appropriate designation for them.

Our previous studies indicated that the seminal plasma and seminal vesicle secretion of bulls contain 'aggregated' or 'particle-bound' and soluble forms of aminopeptidase A (Agrawal & Vanha-Perttula, 1985) and alanyl aminopeptidase (Agrawal & Vanha-Perttula, 1986a, b). The present findings corroborate these results and specify the location of the particle-bound enzymes in vesiculosomes. Besides these two enzymes, the particles were also highly active for Mg^{2+},Ca^{2+}-stimulated ATPase, GGT and DPP IV, which are all regarded as membrane-bound enzymes in cells and tissues. Significantly, however, the endopeptidases active on Succ-$(Ala)_3$-*p*-na and ACE were not present in the bovine vesiculosomes, although they have been found in the human prostasomes (Laurell *et al.*, 1982; Krassnigg *et al.*, 1985). On the other hand, membrane-bound particles containing Mg^{2+},Ca^{2+}-stimulated ATPase have been found in the bovine cauda epididymidis (Reed & Takahashi, 1973), although their complete enzyme profile and release into the seminal plasma are not known. These findings indicate that the seminal plasma particles in different species may differ from each other not only in the site(s) of origin but also in their detailed biochemical composition.

Electron microscopy of the particulate material in the seminal vesicle secretion disclosed at least three different types of particles. The majority was formed by vesicular structures surrounded by a trilaminar unit membrane and contained unevenly distributed granular or floccular material. These particles can be considered as elementary secretory products. The other two particle types were much larger and can be considered as storage bodies, since they contained numerous elementary particles inside their bordering membranes, which were either smooth or studded with microvillar

processes. The former type was more common and frequently encountered close to the epithelial surface in the bovine seminal vesicles. Such composite vesicular particles could also be found in different stages of development and release at the epithelial surface. Our electron microscopic observations were carried out with material fixed by immersion soon after the slaughter. Such a material easily displays artefacts, which could have been avoided by perfusion fixation. We think, however, that our observations on the secretory process in the seminal vesicle epithelium represents a true phenomenon, since similar findings (protrusions, bleb formations) have previously been reported in both block- and perfusion-fixed male reproductive tissues (Mann *et al.*, 1949; Künzel *et al.*, 1970; Nicander *et al.*, 1974; Mifune *et al.*, 1986).

Our analysis of the motility characteristics of the cauda epididymal spermatozoa with and without vesiculosomes by laser Doppler spectroscopy did not reveal any dramatic changes. However, the visual observation in phase-contrast microscopy disclosed a major change in the sperm motility pattern in the presence of these particles. This discrepancy may be due to the complex movement pattern of hyperactivated spermatozoa, which is not differentiated from the normal pattern with laser Doppler spectroscopy.

The adherence of the epididymal spermatozoa on the slides and counting chambers has severely hampered the possibility of using a simple microscopic analysis in the motility studies (Stephens *et al.*, 1981). We took advantage of the natural stickiness of the bovine spermatozoa on normal or albuminized glass slides in our evaluation of the sperm motility. It appeared that the vigorous hyperactivated motility pattern which could be induced in the epididymal spermatozoa prevented the attachment of cells and even released those that were already stuck. This appeared to be due to an altered and vigorous motility pattern and not simply a decrease of adhesiveness. Since the isolated vesiculosome particles, but not the supernatant of the vesicular secretion or bovine serum albumin, were also able to induce forward motility in the theophylline-treated caput spermatozoa, we can conclude that this is a specific effect of the membrane particles. A corresponding stimulation of the motility of human epididymal spermatozoa has been observed with the prostatic component of the split ejaculate (Lindholmer, 1974) and with prostasomes (Stegmayr & Ronquist, 1982). The motility stimulation required a temperature adjustment to 37°C but was possible in different media and in pH values as low as 5·8, which is prevalent in the epididymal fluid. Theophylline activates sperm motility (Hoskins *et al.*, 1975), but in this study it was not able to induce alone the new motility pattern. However, it appeared to facilitate and prolong the response to the vesiculosomes, possibly due to accumulation of cAMP. A similar response was also found with trifluoperazine, which mediates its effect through suppression of the calmodulin-dependent phosphodiesterase.

The isolated vesiculosomes were also able to induce in the epididymal spermatozoa a typical acrosome reaction as confirmed by an established acrosome staining method, phase-contrast microscopy and electron microscopy. By 1 h there were about 40% acrosome reaction-positive spermatozoa and this steadily increased up to 80% at 4 h. Importantly, the seminal vesicle fluid lacked this property. Experimentally, numerous factors are known to elicit the reactions (see Meizel, 1985, for review). In many studies the essential role of Ca^{2+} in these reactions has been emphasized. The observations of Lewis *et al.* (1985) have indicated that bovine seminal plasma contains a protein, caltrin, which by coating the ejaculated spermatozoa is able to prevent the entry of Ca^{2+} ions. This compound might be identical with the decapacitation factor, which has to be removed before the spermatozoon is able to undergo the acrosome reaction. In the present study acrosomal staining or phase-contrast microscopy did not reveal acrosome reactions when the epididymal spermatozoa were treated with seminal vesicle secretion devoid of vesiculosomes. It is possible that the fluid contains the coating protein, which blocks the cell surface changes required for the acrosome reaction to take place. When the spermatozoa were exposed to the vesiculosomes devoid of the fluid compartment, the acrosome reaction was readily induced. This finding supports the concept that caltrin is absent from these particles or they contain factor(s) which more strongly favour the opposite reaction. The large amounts of different peptidolytic enzymes and Ca,Mg-ATPase in the vesiculosomes could be involved in the maturation process of the spermatozoa and

transfer of Ca^{2+} from the vesiculosomes with the final outcome of fenestration and vesiculation of the plasma membrane with the outer acrosomal membrane. With enough caltrin these reactions would be suppressed and so in whole semen premature capacitation and the acrosome reaction would be prevented.

The vesiculosome-induced acrosome reaction was effectively suppressed by theophylline, a well-known inhibitor of cyclic AMP phosphodiesterase. This observation indicates that the accumulation of cAMP in bovine spermatozoa is inhibitory to the capacitation and/or acrosome reaction. In this respect our observations with the bull are consistent with those of Rogers & Garcia (1979) obtained with guinea-pig spermatozoa. Since trifluoperazine was unable to modify the vesiculosome-induced acrosome reaction, the calmodulin-dependent phosphodiesterase is not involved in the process, although the enzyme obviously is present both in the sperm heads and tails (Wasco & Orr, 1984). Further studies are required to establish whether the balance between cAMP and cGMP (yin–yang hypothesis; Goldberg *et al.*, 1975) is an essential factor in the acrosome reaction (Santos-Sacchi & Gordon, 1980). Many other controversies still exist on the role of cyclic nucleotides in capacitation and the acrosome reaction particularly with respect to species differences (see Garbers & Kopf, 1980, for review).

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