Enzymic characteristics of an ecto-cyclic AMP-dependent protein kinase in rat epididymal spermatozoa

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Intact spermatozoa from rat cauda epididymides possess an ecto-(cyclic AMP-dependent protein kinase) activity that causes the transfer of the terminal phosphate group of ATP to the serine residues of all the histone fractions. The enzyme showed a high degree of substrate specificity for the phosphorylation of histones rather than protamine, casein and phosvitin. The cell-external-surface protein kinase requires Mg^{2+} for activity, and other bivalent cations such as Mn^{2+} and Co^{2+} can substitute partially for Mg^{2+} , whereas Ca^{2+} and Zn^{2+} are potent inhibitors of the enzyme. The enzyme has markedly higher affinity for cyclic AMP than for other cyclic nucleotides for its activation, with an apparent K_m value for cyclic AMP of 80 nM. Spermatozoal ecto-kinase activity is not due to contamination of broken cells or any possible cell damage during incubation and isolation of spermatozoa. There was no loss of kinase activity from the cells when washed with 2 mM-EDTA, and the histones phosphorylated by intact spermatozoa were located outside the cells. Protein kinase activity of intact cells was strongly inhibited (approx. 90%) by *p*-chloromercuribenzenesulphonic acid (10 μ M), which is believed not to enter the cells. These data provide further support for the localization of a protein kinase on the external surface of spermatozoa.

A considerable body of evidence has been accumulated to indicate that cyclic AMP may serve as the intracellular second messenger for the action of polypeptide hormones on mammalian tissues (Sutherland, 1972). It is believed that the actions of cyclic AMP are mediated by cyclic AMP-dependent protein kinases, which may then serve to amplify and propagate the initial action of the hormone to various cellular compartments by phosphorylating specific proteins (Kuo & Greengard, 1969; Corbin *et al.*, 1970; Turkington *et al.*, 1973). The observation that the activities of several highly purified biologically active proteins can be modulated by causing their phosphorylation (Walsh & Cooper, 1979; Steinberg & Huttunen, 1972) is consistent with the above view.

Spermatozoa from several mammalian species contain active adenylate cyclase and significant endogenous concentrations of cyclic AMP (Gray *et al.*, 1971; Tash & Mann, 1973; Hoskins *et al.*, 1974). Exogenous cyclic AMP has been demonstrated to stimulate spermatozoal respiration and motility (Garbers *et al.*, 1971; Wyker & Howards, 1977; Hoskins *et al.*, 1978; DeTurner *et al.*, 1978). Cyclic AMP-dependent protein kinases have been demonstrated in bovine epididymal and sea-urchin

spermatozoal cytosolic fraction (Hoskins *et al.*, 1972; Garbers *et al.*, 1973; Lee & Iverson, 1976). Hoskins *et al.* (1972), who observed that the spermatozoal cytosolic protein kinase is a major protein constituent of the cells, suggested that the observed stimulatory effect of exogenous cyclic AMP on spermatozoal motility may be mediated through the spermatozoal cytosolic cyclic AMP-dependent protein kinase.

More recently, evidence has been provided for the localization of a cyclic AMP-dependent protein kinase on the external surface (ecto-protein kinase) of the rat epididymal spermatozoa (Majumder, 1978*b*). The kinase activity of the intact cells catalyses the transfer of the terminal phosphate of exogenous [γ - ^{32}P]ATP to the alkali-labile phosphoester bonds of exogenous calf thymus histones. The time course of the reaction, impermeability of cell membranes to [^{32}P]ATP and the observation that the enzymic activity cannot be removed from the cells by extensive washing with various media support the 'ecto' nature of the enzyme (Majumder, 1978*b*). The present studies provide additional evidence to strengthen the view that spermatozoa possess a cyclic AMP-dependent protein kinase activity on the outer surface. The enzymic charac-

teristics of the cell-bound spermatozoal ecto-protein kinase are described below.

Experimental

Chemicals

Cyclic AMP and other cyclic nucleotides, ATP (horse muscle), calf thymus whole histones, phosvitin, protamine (free base), bovine serum albumin and *p*-chloromercuribenzenesulphonic acid were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Bovine casein was generously supplied by Professor N. C. Ganguli of National Dairy Research Institute, Karnal, India. Sodium orthovanadate was a gift from Dr. I. R. Gibbons of the University of Hawaii, U.S.A. [γ - ^{32}P]ATP was prepared by a simplified procedure described by Majumder & Biswas (1979).

Isolation of epididymal spermatozoa

Spermatozoa were obtained from cauda epididymides of adult albino rats by the procedure of Majumder (1978b), except that the isolation medium (0.25 M-sucrose) was buffered with 10 mM-Tris/HCl, pH 7.2. Freshly extracted epididymal spermatozoal preparation was centrifuged at 800 *g* for 1 min at room temperature (30–34°C) and the pellet was washed twice with 0.25 M-sucrose/10 mM-Tris/HCl, pH 7.2. The cell pellet was finally dispersed in the same medium, and this preparation of spermatozoa was used for these studies unless otherwise stated.

Assay of protein kinase

The activity of spermatozoal ecto-protein kinase was measured by a modification of the method described previously (Majumder, 1978b). The standard assay medium contained 10 μmol of sodium β -glycerophosphate/HCl, pH 7.0, 150 μg of calf thymus histones, 31 nmol of [γ - ^{32}P]ATP (containing 25×10^4 – 8×10^4 c.p.m.), 0.6 μmol of theophylline, 3 μmol of MgCl_2 , 1 nmol of cyclic AMP, 12.5 μmol of sucrose, 60 nmol of EGTA, 40 nmol of dithiothreitol and 50 μl of intact spermatozoal preparation (2.5×10^5 – 6.5×10^5 cells) in a total volume of 0.2 ml. The incubation was carried out at 37°C for 4 min and the reaction was stopped with the addition of 0.2 ml of casein (1%, w/v)/2.5 mM-potassium phosphate/2 mM-ATP and 2 ml of 10% (w/v) trichloroacetic acid. The resulting protein suspension was filtered through a Whatman no. 1 filter-paper disc and then washed with 30 ml of 5% trichloroacetic acid. The discs were dried at 100°C for 15 min before counting for ^{32}P radioactivity in a Packard liquid-scintillation spectrometer in a toluene/0.4% 2,5-diphenyloxazole/0.005% 1,4-bis-(5-phenyloxazol-2-yl)benzene scintillation fluid

(Majumder & Turkington, 1971). Systems lacking spermatozoa served as blanks. One unit of enzyme activity was defined as the amount of enzyme that catalyses the transfer of 10 pmol of ^{32}P from [γ - ^{32}P]ATP to the recovered protein during 4 min under the standard assay conditions.

Preparation of ^{32}P -labelled histones

Exogenous histones were phosphorylated enzymically with the ecto-protein kinase of intact spermatozoa under the standard assay conditions described above, except that the amount of [γ - ^{32}P]ATP in the assay system was 6 nmol and the incubation was carried out at 37°C for 10 min. The reaction was arrested by the addition of 0.2 ml of 0.2 M-NaCl/2 mM-ATP/10 mM-EDTA/histones (2 mg/ml). The suspension was then centrifuged at 800 *g* for 5 min and the pellet was discarded. From the resulting supernatant, ^{32}P -labelled histone was precipitated with 10% trichloroacetic acid. The precipitate was collected by centrifugation and washed successively with 10% trichloroacetic acid (three times), diethyl ether/ethanol (1:3, v/v) and ether (Majumder & Turkington, 1971).

Other analytical methods

^{32}P -labelled histones, prepared by the above procedure, were separated by polyacrylamide-gel electrophoresis (Turkington & Riddle, 1969). After electrophoresis, the gels were stained with Amido Black and destained in 7.5% (v/v) acetic acid. Finally the gels were sectioned and the gel sections were counted for radioactivity directly in Bray's (1960) solution.

^{32}P -labelled histones were hydrolysed in 2 M-HCl in a boiling-water bath for 16 h (Turkington & Riddle, 1969). HCl was removed under vacuum, the hydrolysates were applied to Whatman 3MM filter-paper strips (4 cm $\times$ 54 cm) and then subjected to paper electrophoresis with 8% (v/v) formic acid as the electrophoresis buffer. Electrophoresis was conducted at room temperature for 18 h at a constant voltage of 250 V (Majumder & Turkington, 1972). Unlabelled *O*-phosphoserine, *O*-phosphothreonine and P_i were used as markers during electrophoresis. P_i and phosphorylated amino acids were detected by spraying with Hanes & Isherwood reagent (Martin & Doty, 1949) and ninhydrin solution respectively. The spots were then cut out and counted for ^{32}P radioactivity in a toluene-based scintillation liquid.

Results

Properties of spermatozoal ecto-protein kinase

The amount of histones phosphorylated by the spermatozoal ecto-protein kinase increased linearly with time for approx. 5 min. Proportional increase in the activity of the enzyme was observed with at least

70 units of the cyclic AMP-dependent protein kinase (approx. 8×10^5 cells). As shown in Fig. 1, the apparent K_m value of the enzyme for ATP is approx. $95 \mu\text{M}$.

Effect of metal ions. Table 1 shows that the activity of ecto-protein kinase of intact spermatozoa is dependent on a bivalent metal ion such as Mg^{2+} , Mn^{2+} or Co^{2+} , the degree of activation being maximal in the presence of Mg^{2+} , whereas Ca^{2+} (10 mM) strongly inhibits the enzyme. Addition of the thiol-specific reagents *p*-chloromercuribenzoate and HgCl_2 strongly inhibits the kinase activity, indicating that thiol group(s) of the enzyme are essential for its activity. NaF (10 mM) and high concentration of salt (NaCl, 0.1 M) have significant inhibitory effects. Spermine (5 mM) cannot replace Mg^{2+} for activating the enzyme. Orthovanadate ($4\text{--}50 \mu\text{M}$) had no appreciable effect on the kinase activity.

Male reproductive organs and their secretions are rich sources of Zn^{2+} (Byar, 1974). However, little is known about the physiological significance of Zn^{2+} in reproductive biology. The effect of various concentrations of Zn^{2+} on the spermatozoal-surface protein kinase was determined (Table 1). Zn^{2+} ($100 \mu\text{M}$) strongly inhibits (approx. 90%) the activity of the enzyme. As shown in Fig. 2, the apparent K_m value of the spermatozoal ecto-protein kinase for Mg^{2+} is approx. 1.2 mM.

Cyclic nucleotide specificity. Several iso-osmotic media were tested for their effectiveness to preserve motility of the freshly extracted epididymal spermatozoa. The spermatozoal motility was best

maintained by modified Ringer's solution (RPS medium, pH 7.0 or 7.4) (Majumder & Biswas, 1979) rather than Medium 199 (Paul, 1955) or 0.25 M-sucrose/10 mM-Tris/HCl, pH 7.2 (results not

Table 1. General properties of intact spermatozoal ecto-protein kinase

Standard assay conditions were used except for the additions or omissions specified below. Each value is the mean of closely agreeing duplicate determinations. Abbreviation: N.D., not detectable.

System	Protein kinase activity (%)
Complete	100
– MgCl_2	4
– MgCl_2 + CoCl_2 (2 mM)	32
– MgCl_2 + CoCl_2 (10 mM)	30
– MgCl_2 + MnCl_2 (2 mM)	34
– MgCl_2 + MnCl_2 (10 mM)	32
– MgCl_2 + CaCl_2 (10 mM)	N.D.
– MgCl_2 + spermine (5 mM)	N.D.
–Dithiothreitol	92
–Dithiothreitol + <i>p</i> -chloromercuribenzoate (0.5 mM)	0
–Dithiothreitol + HgCl_2 (2.5 mM)	0
+ NaF (10 mM)	75
+ CaCl_2 (2 mM)	58
+ CaCl_2 (10 mM)	22
+ Na_3VO_4 ($4 \mu\text{M}$)	102
+ Na_3VO_4 ($50 \mu\text{M}$)	98
+ NaCl (0.1 M)	70
+ ZnCl_2 (20 μM)	62
+ ZnCl_2 (50 μM)	27
+ ZnCl_2 (100 μM)	9

Fig. 1. Effect of ATP concentration on the ecto-protein kinase activity of whole spermatozoa. Protein kinase activity of whole spermatozoa (5.5×10^5) was measured under the standard assay conditions, except for the variation in the amount of ATP. The insert shows the Lineweaver–Burk plot of these data.

Fig. 2. Effect of MgCl_2 concentration on the ecto-protein kinase activity of intact spermatozoa. Protein kinase activity of whole spermatozoa (2.5×10^5) was measured under the standard assay conditions, except for the variation in the amount of MgCl_2 . The insert shows the Lineweaver–Burk plot of these data.

Table 2. *Effect of various cell-isolation media on the activity of spermatozoal ecto-protein kinase*

Spermatozoa were isolated from cauda epididymides in various media, as indicated below. Protein kinase activities of the cell preparations were measured under the standard assay conditions with and without cyclic AMP ($5\mu\text{M}$). Each value is the mean of closely agreeing duplicate determinations. RPS medium is modified Ringer's solution containing 10mM-glucose and buffered with 16.3mM-potassium phosphate, pH 7.0 or 7.4 (Majumder & Biswas, 1979). 'Medium 199' (Centron Research Laboratories, Bombay, India) is synthetic Medium 199 with Hanks' balanced salt solution (Paul, 1975), buffered with 15mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/NaOH, pH 7.0.

Cell-isolation media	Protein kinase activity (units/ 10^6 cells)	
	-Cyclic AMP	+Cyclic AMP
0.25M-Sucrose/10mM-Tris/HCl, pH 7.2	47	93
RPS medium, pH 7.0 ($-\text{Ca}^{2+}$)	42	82
RPS medium, pH 7.4 ($-\text{Ca}^{2+}$)	38	78
Medium 199	28	96

shown). As shown in Table 2, cyclic AMP-dependent ecto-protein kinase activities of the cells isolated in the various media are similar, indicating that the spermatozoal outer-surface enzymic activity is not an artifact of the isolation media. However, in the absence of exogenous cyclic AMP, the kinase activity is markedly lower in cells isolated in Medium 199 than in other media, indicating that cyclic AMP-mediated activation of the enzyme is maximal when the cells are isolated in Medium 199. Similar results were also obtained when the ecto-kinase activity was measured with various concentrations of cyclic AMP in spermatozoa isolated in 0.25M-sucrose or Medium 199 (results not shown). Fig. 3 shows the effect of different concentrations of cyclic AMP on the ecto-protein kinase activity in spermatozoa isolated in Medium 199. Cyclic AMP at a concentration of approx. $1\mu\text{M}$ causes maximal activation of the ecto-protein kinase. The apparent K_m value of the kinase for cyclic AMP is approx. 80nM. The kinase showed markedly greater specificity for activation by cyclic AMP compared with other cyclic nucleotides, cyclic GMP, cyclic UMP and cyclic CMP (results not shown).

Substrate specificity. The ability of spermatozoal ecto-protein kinase to phosphorylate various protein substrates was evaluated (Table 3). There was a very small amount of endogenous phosphorylation of protein by intact spermatozoa. The spermatozoal-surface kinase phosphorylated histones at considerably faster rates than those for casein and phosvitin. Protamine and bovine serum albumin were relatively inactive as protein substrates of the enzyme. Protein kinase activity was maximal at a histone concentration of approx. 0.5mg/ml. Further increase in histone concentration caused a slow decrease in the enzymic activity. The apparent K_m value of the enzyme for histones is $100\mu\text{g/ml}$ (results not shown).

Fig. 3. *Effect of cyclic AMP concentration on the ecto-protein kinase activity of intact spermatozoa* Protein kinase activity of whole spermatozoa (5.5×10^5) isolated in Medium 199 was measured under the standard assay conditions, except for the variation in the amount of cyclic AMP.

Table 3. *Substrate specificity of intact spermatozoal ecto-protein kinase*

Standard assay conditions were used with 6×10^5 intact spermatozoa in each assay, except that in place of histones the indicated proteins were used as the substrate. Each value is the mean of closely agreeing duplicate determinations.

Protein substrate	Amount (μg)	Protein kinase activity (units)
Nil	—	1.8
Histones	50	78.0
	250	74.5
Protamine	50	2.5
	250	3.2
Casein	50	9.3
	250	18.2
Phosvitin	50	3.6
	250	6.0
Bovine serum albumin	250	3.2

Characterization of enzymically phosphorylated histones. ^{32}P -labelled histone, a product of the reaction catalysed by spermatozoal ecto-protein kinase, was analysed by polyacrylamide-gel electrophoresis. All the histone species that were found to be labelled accounted for nearly 100% of the recovered radioactivity (results not shown). The data clearly demonstrate that exogenous histones, rather than endogenous cellular proteins or contaminating proteins (if any) of the histone preparations, are phosphorylated by the spermatozoal ecto-protein kinase. Amino acid analysis of ^{32}P -labelled histone products of the kinase demonstrated that the enzyme has high affinity for the phosphorylation of serine residues of histones. The ecto-enzyme did not cause any detectable phosphorylation of threonine residues in histones (results not shown).

Additional evidence for the outer-surface localization of protein kinase in spermatozoa

Whole spermatozoa catalyse the transfer of the terminal phosphate group of ATP to the exogenously added histones. Localization of the histones phosphorylated by the intact spermatozoa was determined (Table 4). Approx. 90% of the ^{32}P -labelled histones were recovered in the reaction mixture surrounding these cells. The residual small amount (10%) of the labelled histones that was associated with spermatozoa could be dissociated from these cells by increasing the ionic strength of the surrounding fluid (0.3M-NaCl) (results not shown). The data indicate that a small amount of ^{32}P -labelled histones binds loosely to the spermatozoal outer surface. It is thus clear that phosphorylated histone, a product of the reaction catalysed by protein kinase of intact spermatozoa, is located outside these cells.

A small fraction of the membrane protein is loosely bound to the membranes through bivalent bridges (Gulik-Krzywicki, 1975). Treatment of spermatozoa with 2mM-EDTA (4 min at room temperature) did not cause any loss of protein kinase activity from the cells (results not shown), indicating that the spermatozoal protein kinase activity is firmly bound to the cell outer surface and the bivalent cations are not involved in this attachment of the enzyme. These data, together with those reported earlier (Majumder, 1978b), strongly suggest that the spermatozoal ecto-protein kinase may be an integral plasma-membrane protein whose catalytic site(s) are exposed to the outer cell surface.

Spermatozoa lost motility almost completely owing to centrifugations during the isolation of the washed cells. The protein kinase activity of the immobile washed spermatozoa may thus be an artifact of the spermatozoal isolation procedure,

Table 4. *Localization of ^{32}P -labelled histones phosphorylated by intact spermatozoa*

Standard assay conditions were used, with 6.5×10^5 spermatozoa in each assay. The reaction was arrested by the addition of 0.6 ml of 0.15M-NaCl/1mM-ATP/10mM-EDTA, pH 7.0. The cell suspensions were centrifuged at room temperature at 800g for 3 min. ^{32}P -labelled histones present in the resulting cell pellet and the supernatant fluid were precipitated with 10% trichloroacetic acid and then processed for the assay of radioactivity by the procedure described in the Experimental section. Each value is the mean of closely agreeing duplicate determinations.

Fraction	^{32}P -labelled histones	
	(c.p.m.)	(%)
Cell pellet	350	9
Cell-free supernatant	3550	91

Table 5. *Distribution of protein kinase activity in rat epididymal fluid and spermatozoa*

A suspension of highly motile spermatozoa (EF + S) was extracted from rat cauda epididymides with 0.25M-sucrose/10mM-Tris/HCl, pH 7.2. A sample of this preparation was centrifuged at 800g for 2 min to obtain a clear supernatant (epididymal fluid, EF). Portions (50 μl) of motile-spermatozoa suspension (containing 1.6×10^5 spermatozoa and epididymal fluid) and cell-free epididymal fluid were assayed for protein kinase activity under the standard incubation conditions. The values for the motile spermatozoa alone were obtained by subtracting the values for the epididymal fluid from those for the motile-spermatozoa suspension. Each value is the mean of closely agreeing duplicate determinations.

Enzyme fraction	Protein kinase activity	
	(units)	(distribution, %)
Motile sperm suspension (EF + S)	23	100
Epididymal fluid (EF)	7.7	33
Motile sperm [(EF + S) - EF]	15.3	67

since the plasma membrane of these cells may be damaged (undetectable by light microscopy) during centrifugation. The observation that intact motile spermatozoa (uncentrifuged) possess high protein kinase activity (Table 5) indicates that the kinase activity of the intact washed cells is not due to any 'leakiness' of spermatozoa. It is possible that the spermatozoal protein kinase activity may have been derived from the surrounding epididymal fluid as a result of the adsorption of a minor portion of the epididymal-fluid protein kinase activity. The ratio of the enzymic activity of the whole spermatozoa to

that of epididymal fluid (without cells) is approx. 2:1 (Table 5). The relatively high content of the enzymic activity in intact cells compared with that in the epididymal fluid suggests that the spermatozoal protein kinase may not have been derived from the epididymal fluid. These studies also demonstrate the presence of a protein kinase activity in the epididymal plasma which is activated by cyclic AMP (results not shown).

p-Chloromercuribenzenesulphonic acid, a thiol reagent, is believed not to enter the cells (Vansteveninck *et al.*, 1965). This reagent was therefore used as a probe (with omission of dithiothreitol from the standard assay) to evaluate further whether the protein kinase is located on the spermatozoal external surface. *p*-Chloromercuribenzenesulphonic acid (10 μ M) strongly inhibits (approx. 90%) the activity of the spermatozoal kinase (results not shown). Approx. 1.5 μ M of this reagent caused 50% inhibition of the enzymic activity under the standard assay conditions (4 min of incubation). The rapidity of the action of *p*-chloromercuribenzenesulphonic acid at very low concentration to cause inhibition of the spermatozoal enzyme strengthens further the view that spermatozoa possess a cyclic AMP-dependent protein kinase activity on the external surface.

Discussion

Previous studies from this laboratory provided several lines of evidence for the localization of a cyclic AMP-dependent protein kinase on the outer surface of spermatozoa (Majumder, 1978*b*). Intact spermatozoa had no detectable activities of plasma-membrane-bound ($\text{Na}^+ + \text{K}^+$)-dependent ATPase and mitochondrial ATPase (Majumder & Biswas, 1979; Majumder, 1981), indicating that the number of damaged or 'leaky' cells in the isolated spermatozoal preparation may not be significant. These data strengthen further the notion that the observed spermatozoal protein kinase activity is not due to 'leakiness' of cells. Association of protein kinase activity with the motile spermatozoa, failure of EDTA treatment to remove the enzymic activity from the cells, strong inhibitory action by the non-penetrating thiol reagent *p*-chloromercuribenzenesulphonic acid, the observation that the kinase activity of the whole spermatozoa is not an artifact of the isolation media and the occurrence of the enzymically ^{32}P -labelled histone products in the extracellular fluid provide further support to the view that the protein kinase activity is located on the outer surface of spermatozoa.

The very small amount of endogenous phosphorylation of spermatozoal external-surface proteins by the ecto-protein kinase (Table 3) may be due to the relatively small number of cells in each assay. Under

physiological conditions, when the spermatozoal concentration in the epididymal fluid is markedly higher, the amount of endogenous phosphorylation of the spermatozoal outer-surface protein(s) may be significant. The presence of exogenous cyclic AMP has been demonstrated in seminal plasma (Gray *et al.*, 1971). At present little is known about the mechanism of action of exogenous cyclic AMP for the stimulation of spermatozoal respiration and motility (Garbers *et al.*, 1971; Wyker & Howards, 1977; DeTurner *et al.*, 1978; Hoskins *et al.*, 1978). Extracellular cyclic AMP of the reproductive fluids may cause rapid activation and release of the protein kinase activity from the spermatozoal surface into the surrounding medium (Majumder, 1978*b*). This interaction of cyclic AMP may perhaps represent the earliest signal to the cell for mediating the various actions of exogenous cyclic AMP on spermatozoa. The activated peripheral kinase may then cause phosphorylation of cell-surface protein(s), which in turn may modulate the functional characteristics of the spermatozoal plasma membrane.

Exogenous ATP has been shown to alter the cell-surface-dependent characteristics of a variety of cells (Jones, 1966; Stewart *et al.*, 1969; Forrester & Lindsay, 1970; Trams, 1974). The presence of exogenous ATP has been shown in the seminal plasma of several species of animals (Brackett & Williams, 1967). The effect of exogenous ATP on spermatozoal physiology has not been adequately studied, but it has been shown to stimulate the uptake of fructose by rat epididymal spermatozoa (Pulkkinen *et al.*, 1978). Extracellular (seminal-plasma) ATP, to which spermatozoal plasma membranes are impermeable, may interact with the spermatozoal ecto-enzymes such as protein kinase and ATPase (Majumder & Biswas, 1979) as the cells pass through the male and female reproductive tracts and thus may serve an important regulatory role on spermatozoal physiology.

Ecto-protein kinase activities have previously been demonstrated in several other cell types (Schlaeger & Fohler, 1976; Mastro & Rozengurt, 1976; Kang *et al.*, 1978, 1979; Chiang *et al.*, 1979). Some of these ecto-enzymes are activated by cyclic AMP and can cause phosphorylation of the endogenous plasma-membrane proteins. The functional significance of this enzyme is not well understood. Human seminal plasma has been shown to be a rich source of protein kinase activity (Majumder, 1978*a*). It is possible that the spermatozoal ecto-protein kinase in conjugation with the epididymal fluid (Table 5) and seminal-plasma protein kinases may cause phosphorylation of specific proteins located in the spermatozoal outer surface as well as in the surrounding male and female reproductive fluids and thus may have an important regulatory role on

spermatozoa during transit through the reproductive organs.

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