Origin of a Motility Inhibitor Within the Male Reproductive Tract

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Seminal plasma contains a motility inhibitor of demembranated reactivated spermatozoa. We investigated its origin within the reproductive tract. The highest level of inhibitor was detected in seminal vesicle fluids from the three species investigated (bull, rat, rabbit). Significant levels of inhibitor were also observed in prostatic fluids. Testes and epididymal fluids, as well as bulbo-urethral and coagulating gland homogenates were essentially devoid of inhibitor. On a mg protein basis, the inhibitor in seminal vesicle fluid was about four times less active than the inhibitor of seminal plasma. The high level of inhibitor in seminal plasma can not be explained by the synergistic effect of the combination of seminal vesicle, prostatic and epididymal fluids. Dialysis experiments suggested that the high level of inhibitor in seminal plasma was mainly due to the presence of a dialysable activator. This activator is capable of potentiating up to four-fold the inhibitor present in seminal vesicle fluid.

Key words: sperm inhibitor, demembranated spermatozoa, seminal plasma, seminal vesicle, prostate, epididymis.

J Androl 1984; 5:269–276.

Demembranated reactivated spermatozoa have been used as a model system to study the control of sperm motility at the axonemal level (Gibbons and Gibbons, 1972, 1973; Lindemann and Gibbons, 1975; Mohri and Yanagimachi, 1980; de Lamirande et al, 1983). With this model, motile spermatozoa are immobilized by demembranation with a detergent (Triton X-100), and motility is reinitiated by the addition of magnesium and ATP. While attempting to reactivate demembranated rabbit and human spermatozoa, we observed that seminal plasma hindered the reinitiation of movement (Gagnon et al, 1981). Recently, we reported that seminal plasma from several other species also contained a similar inhibitory factor (de Lamirande et al, 1983). This inhibitor was shown not to be species specific; it was, however, tissue specific, since none of the tissues tested (blood plasma, liver, kidney, brain, and testis) could inhibit the movement of reactivated spermatozoa (de Lamirande et al, 1983). The seminal plasma inhibitor could prevent both the ATP-induced reinitiation of sperm movement and the movement of already motile spermatozoa. These two types of inhibition could be overcome by higher ATP concentrations (de Lamirande and Gagnon, 1983). The seminal plasma inhibitor and aprotinin have many similarities in their physicochemical properties and in their mode of action. However, the seminal plasma inhibitor lacks antitrypsin activity (de Lamirande et al, 1983) and, therefore, cannot be one of the aprotinin-like protease inhibitors present in seminal plasma (Cechova and Fritz, 1976; Vogel, 1979).

In the present report, we have searched for the origin of this factor in the male reproductive tract of several species, including bull, rat, and rabbit. The physicochemical and biological properties of the inhibitors found in seminal vesicles and seminal plasma of bull were compared, and the data

Supported by the Medical Research Council of Canada.

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Submitted for publication August 8, 1983; revised version received December 12, 1983; accepted for publication January 20, 1984.

suggest that the seminal plasma inhibitor originates mainly from the seminal vesicles, and that another tissue may be involved for acquisition of complete potency.

Materials and Methods

Reactivation of Demembranated Spermatozoa

Rabbit ejaculates were collected by means of an artificial vagina from sexually mature New Zealand rabbits. Only the semen with minimal cellular debris and good motility were used. Semen samples were layered on 2 ml of 6% Ficoll (Sigma Chemical Co., NY) in Gey's BSA medium [110 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 2.4 mM $MgSO_4$, 1 mM sodium pyruvate, 10 mM sodium lactate, 15 mM $NaHCO_3$, 10 mM HEPES buffer, and 1 mg/ml bovine serum albumin (BSA)], pH 8.0, and centrifuged at 800 × g for 10 minutes. The seminal plasma (clear upper layer) was collected and saved. The pelleted spermatozoa were gently resuspended in 3 ml Gey's-BSA medium, recentrifuged, and finally resuspended in 0.5 ml of the same medium.

Bull spermatozoa were obtained from the cauda epididymidis by retroperfusion with oil through the ductus deferens. The undiluted spermatozoa could be kept at 4 C for three or four days without significant loss of motility. When needed, they were diluted in Gey's-BSA medium for 20 minutes before use to allow them to initiate movement. The cauda epididymal fluid was obtained by centrifugation (12,800 × g, 5 minutes) of undiluted spermatozoa.

Spermatozoa were demembranated in the medium first described by Mohri and Yanagimachi (1980) and subsequently modified to improve the reactivation (de Lamirande et al, 1983). The final medium was: 0.1% Triton X-100, 0.2 M sucrose, 0.025 M potassium glutamate, 1 mM dithiothreitol, and 0.035 M Tris-HCl, pH 8.0. After demembranation and the arrest of movement, 0.5 mM Mg ATP (final concentration made with 0.5 mM ATP and 0.5 mM magnesium sulfate) was added to reinitiate the movement. We confirmed Lindemann's observation (1978) on ejaculated bull spermatozoa that cAMP increased the beat frequency and the percentage of motile demembranated spermatozoa from the bull cauda epididymis. Therefore, cAMP was added (50 μM final concentration) to the demembranation medium when bull spermatozoa were used. No cAMP was added to rabbit spermatozoa, since no improvement in motility was noted (de Lamirande et al, 1983).

Seminal Plasma, Tissue Homogenates

Bull ejaculates were centrifuged at 800 × g for 10 minutes. The seminal plasma was collected and recentrifuged at 11,000 × g for 20 minutes to eliminate any remaining spermatozoa.

Tissues, including seminal vesicles, prostates, testes and epididymides, were collected from rats, rabbits and bulls. Tissues were minced and homogenized in three volumes of 0.3 M sucrose buffered with 20 mM Tris-HCl, pH 8.0. Homogenates were centrifuged at 11,000 × g for 20 minutes, and supernatants were collected and frozen until used.

When tissue fluids were needed, tissues were grossly minced with a small volume of 0.3 M sucrose—20 mM Tris-HCl solution, and incubated at room temperature for 20 to 30 minutes. Fluids were then collected, centrifuged at 11,000 × g for 20 minutes, and frozen until used.

The minimal amount of seminal plasma, of 11,000 × g supernatants of tissue homogenates, and of tissue fluids needed to block completely and instantaneously the movement of demembranated reactivated spermatozoa was quantified. The protein content of these samples was determined according to Lowry et al (1951) using crystalline bovine serum albumin as the standard.

Comparison Between Bull Seminal Plasma and Seminal Vesicle Fluid

The motility inhibitors found in bull seminal vesicle fluid and seminal plasma were submitted to high (100 C, 5 minutes) and moderate (60 C, 20 minutes) temperature, to TCA (5%) precipitation, to alkalinization (pH 13, 20 minutes), and to acetone (30%) precipitation. After these treatments, samples were centrifuged at 11,000 × g for 15 minutes and supernatants collected; pellets were resuspended in 100 mM Tris-HCl, pH 8.0, and recentrifuged. Both supernatants were used to evaluate the remaining potency of the inhibitor treated. When needed, the pH of samples was readjusted and the acetone was evaporated. The effect of dialysis against a water-acetic acid solution, pH 4.0, at 4 C was also tested.

A sample of seminal plasma and of seminal vesicle fluid was analyzed by gel filtration on a 1 × 19 cm Ultrogel ACA-54 column (Pharmacia Co., Piscataway, NJ). The column was equilibrated and run in 100 mM Tris-HCl, pH 8.0, at a flow rate of 2.7 ml/hour. Fractions of 0.27 ml were collected and elution profiles were determined by measuring the absorbance at 280 nm. The inhibitory potency of fractions was tested as described elsewhere (de Lamirande et al, 1983).

Results

Localization of the Seminal Plasma Motility Inhibitor

The origin of the seminal plasma inhibitor of reactivated spermatozoa was investigated in seminal vesicle fluids and homogenates, prostatic fluids and homogenates, testicular homogenates, caput epididymal homogenates, cauda epididymal fluids and homogenates, and coagulating and bulbo-urethral gland homogenates. In the three species investigated, rat, bull, and rabbit, the highest concentration of inhibitor was detected in

seminal vesicle fluid (Table 1). The fluid from the prostate also contained a significant amount of inhibitor. On the other hand, epididymal and testicular homogenates were essentially devoid of inhibitor, since proteins from these tissues at concentrations ranging from 6 to 15 mg/ml did not stop the motility of reactivated spermatozoa (Table 1). No motility inhibitor was found in homogenates of rat coagulating gland and rabbit bulbourethral gland (data not shown).

Effects of Prostatic and Cauda Epididymal Fluids on the Seminal Vesicle Inhibitor

Since the seminal vesicle fluid is the richest source of motility inhibitor, the level of this inhibitor in seminal plasma ought to be lower than that of the seminal vesicles. This assumption was not verified for the rabbit nor for the bull, as the level of motility inhibitor in rabbit seminal plasma was not significantly different from that in seminal vesicle fluid, and bull seminal plasma was four times richer in inhibitor than bull seminal vesicle fluid (Table 1). To investigate this discrepancy, we tested the possibility of a synergistic effect between seminal vesicle fluid and fluids from the prostate and the epididymis. When cauda epididymal fluid was mixed with seminal vesicle fluid, the inhibitory potency augmented as the cauda epididymal fluid/seminal vesicle fluid ratio increased (Fig. 1). The maximal stimulatory effect was observed with ratios of 20 and 40 to 1. The prostatic fluid also allowed a significant increase in inhibitory potency (Table 2). When combinations of the three fluids were tested, an increase in inhibitory potency was noted. However, the enhancement was mainly due to the combined action of seminal vesicle and epididymal fluids.

Comparison of Physicochemical Properties of Motility Inhibitors from Seminal Plasma and Seminal Vesicle Fluid

Since bull seminal vesicle fluid and seminal plasma have a very high concentration of motility inhibitor, they were chosen to further characterize the inhibitors. To ascertain that these two fluids contained the same type of inhibitor, physicochemical properties of the inhibitors were compared. Heating to 60 C and 100 C, acid treatment (5% trichloroacetic acid) and acetone precipitation (30% final concentration) induced similar losses of activity in both fluids (Table 3). On the other hand, alkaline treatment reduced by 53% the potency of the seminal plasma inhibitor without affecting the inhibitor from the seminal vesicle fluid. Molecular sieving on Ultrogel ACA-54 revealed very similar patterns of elution for both fluids, with half of the inhibitor migrating with a molecular weight of 12,000, and the other half migrating with the void volume (Fig. 2).

We have shown recently that the inhibition of movement of demembranated reactivated spermatozoa caused by boar seminal plasma could be reversed by Mg ATP (de Lamirande and Gagnon, 1983). The reversibility of the inhibition caused by bull seminal plasma and seminal vesicle fluid was, therefore, tested. With both fluids, the inhibition could be reversed by dilution. Once the inhibition was obtained, the movement could be restored if

TABLE 1. Inhibitory Potency of Seminal Plasma, Tissue Homogenates (H), and Tissue Fluids (F) on Demembranated Reactivated Spermatozoa*

Tissue	Rat	Bull	Rabbit
Seminal plasma	—	0.0115 ± 0.0014	0.662 ± 0.062
Seminal vesicles H	0.187 ± 0.024	0.375 ± 0.107	>10.8
Seminal vesicles F	0.075 ± 0.008	0.043 ± 0.008	0.552 ± 0.084
Prostate H	0.379 ± 0.043	>13.4	>11.0
Prostate F	0.146 ± 0.043	0.564 ± 0.020	0.890 ± 0.187
Testis H	>9.8	>12.4	>15.8
Epididymis, caput H	>7.1	>11.7	>11.4
Epididymis, cauda H	>6.2	>9.0	>11.4
Epididymis, cauda F	—	3.51 ± 0.43	—

* These values represent the minimal protein concentration needed to inhibit completely and instantaneously the motility of reactivated rabbit spermatozoa. They are expressed as mg of sample protein added per ml of reactivation medium. The preparation of tissue samples is described in Materials and Methods. Values are mean ± SEM for three to six experiments, except for rabbit seminal plasma, for which n = 17.

spermatozoa were transferred to another petri dish free of inhibitor and containing 3 mM Mg ATP. The competitions ATP-seminal plasma and ATP-seminal vesicle fluid were also confirmed by the following experiments. In the first series of experiments, spermatozoa were first demembranated and reactivated with various Mg ATP concentrations, and then the inhibitory concentration of seminal plasma and seminal vesicle fluid were determined (Fig. 3A and B). As the Mg ATP concentration was increased, the volume of fluids needed to inhibit motility also increased. In the second series of experiments, spermatozoa were incubated with increasing concentrations of inhibitor and the minimal Mg ATP concentration needed for reactivation was determined (Fig. 3C and D). Again, as the amount of inhibitor added to the medium was augmented, the Mg ATP concentration needed to initiate the movement increased. As observed with boar seminal plasma (de Lamirande and Gagnon, 1983), bull spermatozoa seemed to be less sensitive to the motility inhibitor of seminal vesicle fluid and seminal plasma after reactivation (Fig. 3A and B) than when they were exposed to the inhibitor prior to the addition of ATP. For example, when spermatozoa were reactivated with 0.5 mM Mg ATP, it took 15.5 ± 1.5 μg of seminal plasma protein/ml to block the movement (Fig. 3A), but when this amount of seminal plasma was initially added to the spermatozoa, it took more than 1.5 mM Mg ATP to initiate the movement (Fig. 3C). The same comparison holds true for the inhibition by seminal vesicle fluid (Fig. 3B and D). These data suggested

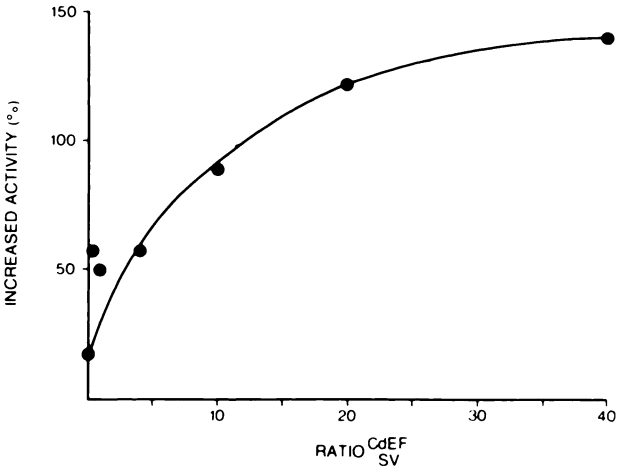

Fig. 1. Effect of various combinations of seminal vesicle and cauda epididymal fluids on inhibitory activity. The ratio CdEF/SV is the ratio of the volumes of cauda epididymal fluid and seminal vesicle fluid mixed. The increased activity is expressed as a percentage (Table 3). Bull spermatozoa from cauda epididymis were used for this experiment.

TABLE 2. Effects on the Motility Inhibitor of Various Combinations of Fluids from Bull Seminal Vesicles, Prostate and Epididymides

Volumes Mixed			Inhibitory Volumes* (μl)		Increase in† Inhibitory Activity (%)
Seminal Vesicles	Prostate	Epididymal Cauda	Expected	Actual	
1				0.3	
	1			78	
		1		78	
10		1	0.33	0.285	15
1		1	0.60	0.41	46
1		10	3.18	1.68	89
1		40	10.68	4.29	141
1	1		0.60	0.48	25
1	5		1.77	0.87	106
5	1		0.36	0.27	30
1	1	1	0.84	0.57	47
1	1	2	1.08	0.54	100
1	2	2	1.29	0.75	72
2	1	2	0.72	0.40	77

* "Expected Volume" is the calculated inhibitory volume considering the inhibitory volume for each of the three fluids and the dilution due to mixing.
"Actual Volume" is the experimental inhibitory volume.
† "Increase in Inhibitory Activity" is calculated from the expected and the actual inhibitory volumes.
Bull spermatozoa from cauda epididymis were used for this experiment.

TABLE 3. Effects of Temperature, TCA, Acetone Precipitation, Alkalinization (NaOH) and Dialysis on the Inhibitory Activity of Bull Seminal Plasma and Seminal Vesicle Fluid

Treatment*	Inhibitory Activity (%)†	
	Seminal Vesicles	Seminal Plasma
None	100	100
60 C, 20 min	66	72
100 C, 5 min	33	23
TCA precipitation (5%):		
Supernatant	44	37
Solubilized pellet	54	68
Acetone (30%) precipitation:		
Supernatant	6	10
Solubilized pellet	0	0
NaOH, pH 13, 20 min	92	47
Dialysis, pH 4, 4 C	100	30

* Treatments are described in Materials and Methods. They were performed on bull seminal vesicle fluid and seminal plasma diluted ten-fold with water.

† Percentage of the initial inhibitory activity remaining after treatment. The motility inhibition tests were performed with bull spermatozoa from cauda epididymis.

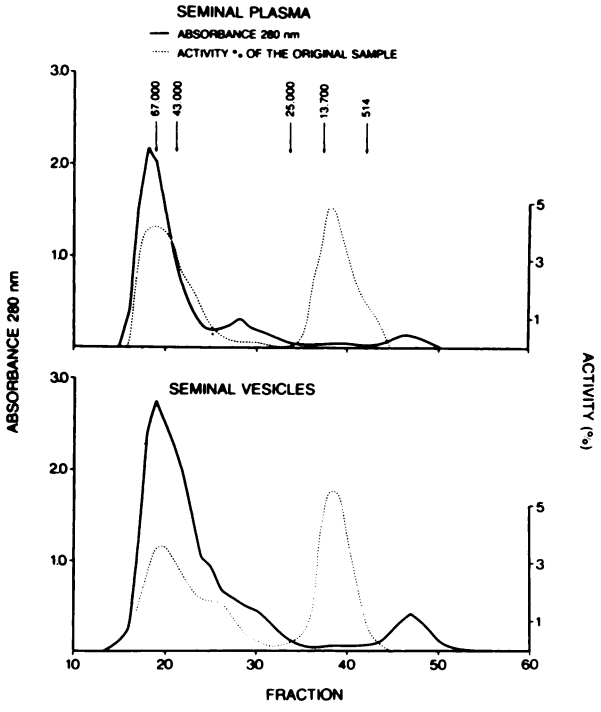

Fig. 2. Gel filtration chromatography of bull seminal vesicle fluid and seminal plasma. The conditions used for the chromatography are described in Materials and Methods. - - - - - Inhibitory activity on demembranated reactivated spermatozoa. Expressed in equivalent (μl) of sample loaded on the column. ——— Absorbance (280 nm) of fractions diluted ten-fold with 100 mM Tris-HCl, pH 8.

that the inhibitor may act on the axoneme. It could also suggest that the inhibitor blocked motility by depleting the ATP pool. ATPase activity of the seminal plasma inhibitor alone, and of preparations of reactivated bull spermatozoa, in the absence or in the presence of seminal plasma inhibitor, were determined (Table 4). The seminal plasma inhibitor had no ATPase activity by itself, as no significant quantity of inorganic phosphorus was detected. In the presence of demembranated spermatozoa, ATP was progressively hydrolyzed. The addition of seminal plasma inhibitor did not increase the ATP hydrolysis, but rather slightly decreased the formation of inorganic phosphorus. Thus, the seminal plasma inhibitor does not act by depleting the ATP pool.

The effect of dialysis at pH 4.0 and 4 C, like the alkaline treatment, showed drastic differential effects on the inhibitors from the two fluids. Upon dialysis, the seminal plasma lost 70% of its inhibitory potency (a 3.3-fold reduction) while the inhibitor from the seminal vesicle fluid was not affected (Fig. 4). The dialysate had no inhibitory activity by itself. This loss, by a 3.3 factor, is very similar to the 3.7 ratio between the inhibitory potencies of the inhibitors in the two fluids (Table 1). To further investigate the possibility that the reduced level of inhibitor in seminal vesicle fluid was due to the absence of a dialyzable factor, bull seminal plasma was dialyzed sequentially. First, the seminal plasma was dialyzed overnight against five volumes of water-acetic acid, pH 4.0, at 4 C. Then the dialysate was collected, and the seminal plasma was redialyzed under the same conditions, but against a very large volume of the water-acetic acid solution. Again, the dialyzed seminal plasma had only 30% of the activity observed before dialysis (Fig. 4). However, when it was mixed with the dialysate in the 1:5 ratio (dialyzed seminal plasma:dialysate), the original activity of the seminal plasma was restored. Furthermore, when the seminal vesicle fluid was mixed with the seminal plasma dialysate, its inhibitory activity was enhanced 3.8-fold, its level becoming similar to that in seminal plasma (Fig. 4). The seminal plasma dialysate, therefore, appears to contain a small molecule that, by itself, has no inhibitory activity, but which can increase four-fold the activity of seminal vesicle fluid or dialyzed seminal plasma.

Since the cauda epididymal fluid could increase the inhibitory activity of seminal vesicle fluid (Fig. 1), the possibility that the small molecule could

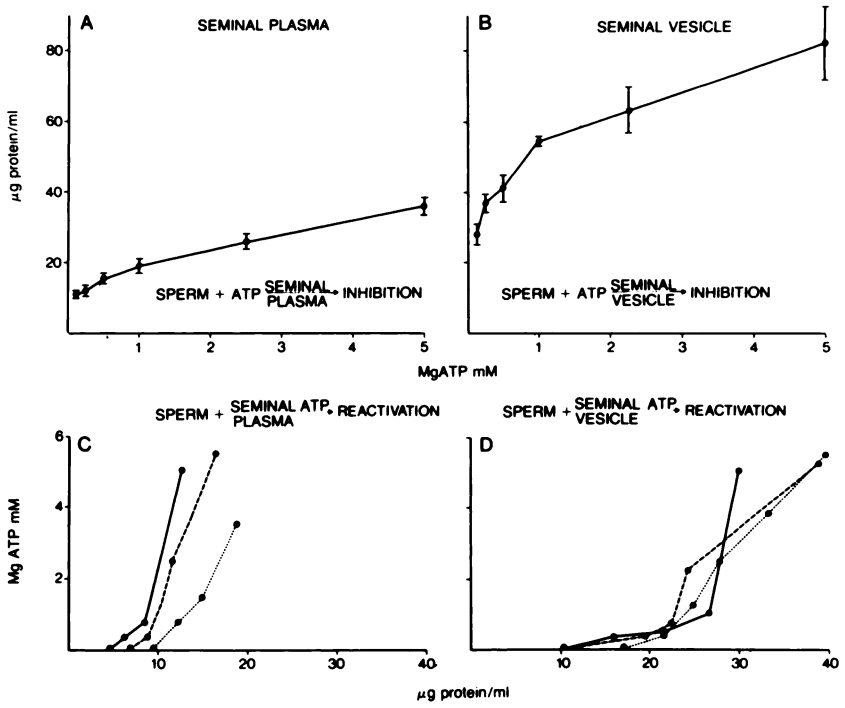

Fig. 3. Interaction between bull seminal plasma and seminal vesicle fluid, and ATP. Upper panel: Spermatozoa were demembranated and reactivated with different Mg ATP concentrations. Then the amount of seminal plasma (A) and of seminal vesicle fluid (B) necessary to inhibit motility was determined for each Mg ATP concentration. Each point represents the mean ± SEM (n = 4). Lower panel: After demembranation, different concentrations of seminal plasma (C) or seminal vesicle fluid (D) were immediately added to spermatozoa and the quantity of Mg ATP required to reinitiate sperm motility was determined. Each curve represents a different seminal plasma or seminal vesicle sample. Bull spermatozoa from cauda epididymis were used for these experiments.

originate in the epididymis was tested. The cauda epididymal fluid was dialyzed under the same conditions as those for seminal plasma. This dialyzed fluid and the dialysate were then tested alone, and together with the seminal vesicle fluid. After dialysis, the cauda epididymal fluid activity was unchanged, and its potentiating effect on the inhibitor of seminal vesicle fluid was not modified. The dialysate had no potentiating effect on the inhibitory activity of the seminal vesicle fluid. Therefore, the low molecular weight factor found in the seminal plasma dialysate does not originate in the epididymis.

Discussion

The motility inhibitor of demembranated reactivated spermatozoa present in seminal plasma of several species was shown to have a certain tissue specificity, since it was not found in tissues outside the reproductive system (de Lamirande et al, 1983). Within the reproductive system, the inhibitor originated mainly from seminal vesicles (Table 1). However, in the bull, where a high concentration of inhibitor is present, the level of inhibitor in seminal vesicle fluid was nearly four times lower than that in seminal plasma. This is contrary to what should be expected, as the inhibitor in seminal vesicle fluid should be diluted with fluids and proteins from other glands. The potentiating effects of cauda epididymal and prostatic fluids on the potency of the seminal vesicle fluid inhibitor provided a possible explanation to the high activity

TABLE 4. ATPase Activity of Seminal Plasma Inhibitor and of Preparation of Reactivated Bull Spermatozoa in the Absence or in the Presence of Seminal Plasma Inhibitor*

Sample	ATPase Activity (nmol/ml) 1 min	10 min
Demembranation medium	0	0
Demembranation medium + Gey's medium	0	0
Demembranation medium + inhibitor	0	0
Demembranation medium + spermatozoa	11.48	52.17
Demembranation medium + spermatozoa + inhibitor	8.99	46.20

* Motile spermatozoa in Gey's medium were demembranated with the reactivation medium described in Materials and Methods. Spermatozoa were then incubated with 0.5 mM (γ-[^{32}P])-ATP (2 mCi/mmol) and 0.05 mM cAMP in the presence and in the absence of partially purified seminal plasma inhibitor (de Lamirande et al, 1983) at a concentration 1.3-fold of that required to stop motility. Controls without spermatozoa were run simultaneously. The hydrolysis of ATP after 1 and 10 minutes of incubation at 25 C was monitored by determining the production of inorganic phosphorus according to Penningroth and Kirschner (1977). Results are expressed in nmole of ATP hydrolyzed per ml of reactivation medium.

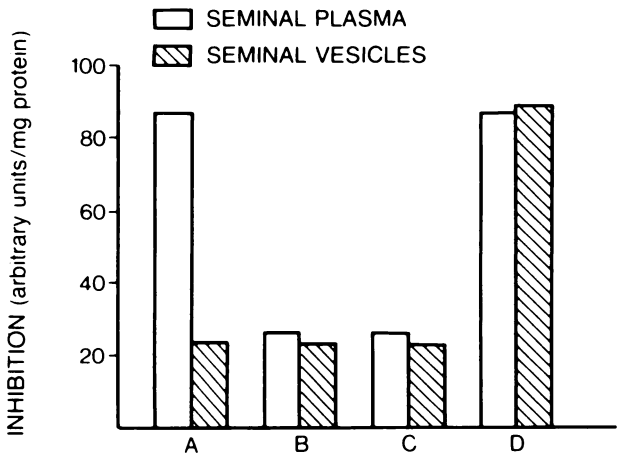

Fig. 4. Effects of dialysis, pH 4.0, 4 C, on bull seminal plasma and seminal vesicle fluid inhibition. Inhibitory potency of seminal plasma and seminal vesicle fluid A: before dialysis, B: after dialysis, pH 4.0, 4 C, C: after dialysis and dilution with H_2O and acetic acid, pH 4.0 (control), D: after dialysis and dilution with seminal plasma dialysate. One inhibition unit is the amount of protein needed to stop the movement of spermatozoa in 1 ml reactivation medium in presence of 0.5 mM Mg ATP.

of inhibitor in seminal plasma. However, the unphysiologic cauda epididymal fluid/seminal vesicle fluid ratio of 20 to 40 needed to generate a potentiating effect (2 to 2.5-fold) is unrealistic (Table 2). The same argument holds true for the prostatic fluid/seminal vesicle fluid ratio of 5 to provide a two-fold increase in the potency of seminal vesicle fluid.

In the bull, the epididymal contribution to the volume of ejaculate accounts for 60% to 65% and consists of spermatozoa and fluid in about equal proportion, while the remaining ejaculatory volume comes from seminal vesicle (25–30%) and prostatic secretions (4–6%) (Dellmann and Wrobel, 1976). The 2:1:2 combination of seminal vesicle:prostate:cauda epididymal fluids was close to the probable physiologic ratio of 45:10:45, but a stimulatory effect of only 77% was observed (Table 2).

To ascertain that the inhibitor in seminal vesicle fluid was related to the inhibitor in seminal plasma, their physicochemical properties were compared. Both inhibitors had similar behaviour when analyzed by molecular sieving (Fig. 2). Half the inhibitor migrated as a molecule having a molecular weight of 12,000 and the other half eluted with the void volume, that is, above 70,000. This high molecular weight form may correspond to adsorbed lower molecular weight inhibitor, since rechromatography of the void volume proteins in identical conditions showed the inhibitor activity eluting after the void volume (data not shown). In addition, when seminal plasma was chromatographed at higher ionic strength (300 mM instead of 100 mM Tris-HCl), the first peak of inhibitory activity, with the molecular weight of over 70,000, completely disappeared, whereas the smaller molecular weight peak was still present. Other similarities between the inhibitors from the two fluids (seminal vesicle and seminal plasma) included their stability at moderate and high temperatures, and the results of TCA and acetone precipitation (Table 3). There were, however, major differences such as alkalinization and dialysis effects, which lowered by 53% and 70%, respectively, the inhibitory capacity of seminal plasma without affecting the inhibitor in seminal vesicle fluid.

Even though the inhibitors in seminal vesicle fluid and seminal plasma have different physicochemical characteristics, they seemed to exert their inhibitory action on sperm motility by similar mechanisms. Their inhibition was reversed by ATP, indicating that the arrest of sperm movement was not due to the action of proteases, and that the inhibitors are not proteases. The inhibition of reactivated sperm movement was also not caused by a sudden depletion of the ATP pool in the medium since the partially purified inhibitor had no ATPase activity by itself (Table 4). These results also suggested a competition between ATP and the motility inhibitor(s) present in seminal plasma and seminal vesicle fluid for a common site on the demembranated spermatozoa. The reversibility curves (Fig. 3) confirmed this competition. These curves were the same type as those obtained with a partially purified preparation of boar seminal plasma inhibitor (de Lamirande and Gagnon, 1983). It must be emphasized that the amount of seminal plasma or of seminal vesicle fluid required to block the movement of already reactivated spermatozoa is higher than when inhibitors are added before ATP (before reinitiation of movement). A similar phenomenon had been observed with the partially purified preparation from boar seminal plasma (de Lamirande and Gagnon, 1983). These data may suggest that the affinity of the inhibitor for a binding site on the axoneme would be higher when the inhibitor is added before the initiation of sperm motility.

The dialysis-recombination experiments provided a possible explanation for the discrepancy in

the level of inhibitor in seminal vesicle fluid and seminal plasma. Upon dialysis, the seminal plasma lost 70% of its original activity, falling to a level similar to that of seminal vesicle fluid. However, when the dialysate was returned in the right proportion to the dialyzed seminal plasma, or combined in the same proportion with seminal vesicle fluid, both dialyzed seminal plasma and seminal vesicle fluid showed a 3.5 to 4-fold increase in inhibitory activity, which was a level similar to that of undialyzed seminal plasma.

In conclusion, the motility inhibitor of bull seminal plasma comes from a weakly active macromolecule fully potentiated by a dialyzable factor. The macromolecule originates in the seminal vesicle fluid, whereas the dialyzable factor has an unknown origin. This small molecule could perhaps be present in seminal vesicle fluid, but in a form not suitable for potentiating the macromolecular component of the inhibitor.

The physiologic importance of this inhibitor is presently unknown. Preliminary results seem to indicate that an inverse relationship exists between the concentration of the motility inhibitor in seminal plasma and the time during which spermatozoa from different species (rabbit, man, boar, bull) remain motile in their own seminal plasma (unpublished results). However, the testing of its effects on intact spermatozoa must await its purification. The results reported here and elsewhere (de Lamirande et al, 1983; de Lamirande and Gagnon, 1983) indicate that this inhibitor and demembranated sperm models provide useful tools to study the mechanism controlling the sliding of doublet microtubules.

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Supportive Organizations Acknowledged

The ASA Acknowledge the Following Organizations for their Support of the Society's 9th Annual Meeting, March 13–16, 1984, in Los Angeles, California.

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* In support of poster session wine and cheese party.
† In support of workshop: Male Genital Infection and Fertility.