Regulation of Calcium Content in Bovine Spermatozoa*

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Plasma membrane vesicles isolated from bovine epididymal and ejaculated spermatozoa have widely different capabilities for transporting Ca$^{2+}$. Spermatozoa were ruptured by nitrogen cavitation, and the plasma membrane fraction was harvested after low speed and sucrose gradient centrifugation; purity was assessed by marker enzyme analyses, electron microscopy, and sedimentation properties. Plasma membrane vesicles isolated from epididymal sperm accumulate Ca$^{2+}$ passively at a faster rate and to a greater extent than vesicles prepared from ejaculated sperm.

Ca$^{2+}$ transport across bovine sperm plasma membranes is an ATP-independent, Na+-dependent process that obligatorily exchanges intravesicular Na$^+$ for extranar Ca$^{2+}$. The rate of Na$^+$/Ca$^{2+}$ exchange is significantly lower in ejaculated sperm vesicles than in those of epididymal sperm. Bovine plasma membranes contain little or no Ca$^{2+}$-dependent ATPase activity.

It is suggested that, at the time of ejaculation, calcium flux into bovine sperm is prevented by the interaction of the plasma membrane with putative factors in seminal fluid that specifically interfere with Na$^+$/Ca$^{2+}$ exchange. We have isolated a protein from seminal plasma that prevents calcium accumulation by bovine epididymal sperm (Rufo, G. A., Jr., Singh, J. P., Babcock, D. F., and Lardy, H. A. (1982) J. Biol. Chem. 257, 4627-4632). A protein with properties resembling those of the seminal calcium transport inhibitor is found on the membrane vesicles from ejaculated sperm but not on membranes from epididymal sperm. We conclude that this protein binds strongly to the plasma membrane of bovine sperm and is responsible for preventing calcium uptake by ejaculated sperm.

In most cells, intracellular calcium is maintained at concentrations orders of magnitude lower than those found in the surrounding extracellular fluid via the so-called calcium pump or (Ca$^{2+}$/Mg$^{2+}$)-ATPase (1-5). While the literature is replete with information regarding the control of calcium extrusion and the voltage-sensitive calcium inflow channels of many excitable cells (1, 2), relatively little is known regarding control of calcium flow into nonexcitable cells.

Sperm plasma membranes have only recently been utilized in the study of calcium transport phenomena. Plasma membranes prepared from boar sperm (6) have been shown to bind calcium with high affinity, especially in the presence of sodium or potassium. In addition, a Na$^+$/Ca$^{2+}$ antiporter has been demonstrated in boar (7) and ram (8) sperm plasma membranes. The studies outlined in this report have been designed to make intraspecies comparisons of calcium transport properties between plasma membrane vesicles isolated from bovine epididymal and ejaculated sperm.

Previous work has shown that washed ejaculated bovine sperm are incapable of accumulating calcium in vitro, whereas epididymal sperm take up calcium rapidly (9). Thus, bovine sperm represent a unique system in which to study the effects of surface modification on Ca$^{2+}$ flux across the plasma membrane. We have isolated a protein from seminal plasma that inhibits calcium uptake by ejaculated sperm (10), and it is of interest to determine the site of action of this inhibitor. The technique of nitrogen cavitation provides sealed, right-side-out plasma membrane vesicles (11) suitable for the study of calcium inflow channels without interference from other cellular organelles.

The studies reported here are the first to demonstrate a sperm plasma membrane Na$^+$/Ca$^{2+}$ antiporter that is amenable to regulation by extrinsic factors present in the seminal plasma.

EXPERIMENTAL PROCEDURES

Preparation of Spermatozoan Plasma Membrane Vesicles—Plasma membrane vesicles were prepared from bovine epididymal and ejaculated sperm essentially by the methods of Gillis et al. (12). Epididymides were obtained within 1 h of slaughter (Oscar Mayer Co., Madison, WI) and cooled to 4°C, and the sperm were expressed according to previously described methods (13). Following two centrifugal washings (600 × g, for 10 min each) in 0.25 M sucrose (Schwarz/Mann), 0.2 mM MgCl$_2$, 10 mM MOPS, pH 7.4, the sperm were suspended in the same buffer to a final concentration of 1-5 × 10$^6$ sperm/ml.

Bovine semen was the generous gift of American Breeders Service, DeForest, WI. Ejaculated sperm were isolated by centrifugation (700 × g for 15 min) at 20°C, then subjected to three washings and diluted as described above. From this point, the procedure for isolating plasma membranes was the same for both types of sperm. All subsequent procedures were carried out at 4°C.

Five-ml aliquots of sperm suspension were placed in a Kontes cell disruption chamber and subjected to nitrogen pressure of 750 p.s.i. for 15 min. The suspension was then slowly extruded into 2.5 ml of buffer to give final concentrations of 0.25 M sucrose, 0.1 M MgCl$_2$, 1 mM EDTA, 10 mM MOPS, pH 7.4. The combined suspensions were centrifuged at 600 × g (Beckman JA-20 rotor) for 10 min, and the pellet was washed twice with an equal volume of the above buffer minus MgCl$_2$. The combined 600 × g supernatant fraction was centrifuged at 6,000 × g for 10 min, and the pellet was washed once with the above buffer. The combined 6,000 × g supernatant was then

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* The abbreviations used are: MOPS, 3-(N-morpholino)propanesulfonic acid; EGTA, ethylene glycol bis(b-aminoethyl ether)-N,N',N'-tetraacetic acid; MES, 2-(N-morpholino)ethanesulfonic acid.
layered on a three-step sucrose gradient (w/v, 1.23, 1.17, 1.05) and centrifuged at 100,000 × g (Beckman SW 27 rotor) for 2 h. Plasma membrane vesicles were collected from the 1.17/1.05 interface by aspiration, washed once with 0.25 M sucrose, 10 mM MOPS, pH 7.4, and collected by centrifugation at 100,000 × g for 1 h. The resulting pellet, consisting mainly of right-side-out plasma membrane vesicles (254), was resuspended in the above buffer to a final protein concentration of 3–5 mg/ml. Protein was determined by the method of Lowry (14) using bovine serum albumin as a standard. Typical yields of membrane protein were 150–300 μg from 10^9 sperm.

**Marker Enzyme Analysis**—The distribution of 5’-nucleotidase (EC 3.1.3.5) and succinate dehydrogenase (EC 1.3.99.1) activities among the several subcellular fractions was determined according to the procedures described by Weaver and Boyle (15) and Pennington (16), respectively. Inorganic phosphate was determined as described elsewhere (17).

**Electron Microscopy**—Purified plasma membranes isolated from epididymal sperm were fixed with 2% cacodylate-buffered glutaraldehyde, pH 7.0, and postfixed in osmium tetroxide. The precipitated membranes were dehydrated with graded ethanol washes, treated with propylene oxide, and embedded in Epon. Thin sections were cut, stained with uranyl acetate, and electron micrographs were obtained using a Hitachi H-500 electron microscope.

**Ca2+ Uptake by Spermatozoan Plasma Membrane Vesicles**—Plasma membrane vesicles prepared from either epididymal or ejaculated sperm were incubated at 30°C for 5 min in 110 mM NaCl, 5 mM KCl, 10 mM MOPS, pH 7.4 (unless noted otherwise). MOPS replaced MOPS in reactions conducted at pH intervals below 7.0 (not illustrated). Reactions were initiated by the addition of CaCl2 labeled with 45Ca (20 μCi/μmol; Amersham Corp.) or by dilution of vesicles into appropriate media containing 45Ca2+. Reactions were terminated by collecting and washing 100 μl (25 μg) of the membrane suspension on Millipore filters (Type HA, 0.45-μm pore size). The filters were washed three times with 5-ml portions of 0.25 M sucrose, 0.1 mM CaCl2, 1.0 mM MOPS, 10 mM MOPS, pH 7.4, and analyzed for radioactivity as previously described (10).

Na+/Ca2+ Exchange Studies—Vesicles were passively loaded with Na+ by incubation in 150 mM NaCl, 10 mM MOPS, pH 7.4, at 4°C for 16–18 h. The Na+-loaded vesicles (60 μg, 300 μM) were incubated at 30°C for 10 min, then diluted to 1.2 ml with initial reaction medium containing 150 mM NaCl, 1.0 mM CaCl2, 10 mM MOPS, pH 7.4, at 30°C. The 45Ca content of the vesicles was monitored for 5 min, after which the initial reaction medium was diluted into K+ or choline-containing medium (see legend to Fig. 4) to promote release of internal Na+. The Ca2+ content was monitored for the next several minutes of incubation. Control experiments were performed in the presence of Na+ or K+-loaded vesicles were diluted into Na+ or K+ medium were conducted in order to examine the dependence of an outwardly directed Na+ gradient on the uptake of Ca2+ by sperm plasma membranes.

**Determination of ATPase Activity**—Spermatozoan plasma membrane vesicles (50 μg) were incubated for 5 min at 37°C in a reaction medium containing 80 mM NaCl, 15 mM KCl, 1.0 mM MgCl2, and 50 mM Tris-HCl, pH 7.6, in a final volume of 1 ml. Reactions were initiated by the addition of 3 mM Na-ATP (P-L Biochemicals) and terminated 10 min later by the addition of 1 ml of 10% trichloroacetic acid. After 15 min at 0°C, the protein precipitate was collected by centrifugation at 10,000 × g for 10 min. The supernatant fraction was assumed for inorganic phosphate as described above. Ca2+-independent ATPase activity was determined in the presence of 1.5 mM EGTA, while Mg2+-ATPase activity was determined in the presence of 1.5 mM EGTA plus 1 mM ouabain (Sigma), (Na+,K+)-ATPase activity was defined as the difference between Ca2+-independent ATPase and Mg2+-ATPase activities. (Ca2+,Mg2+)-ATPase activity was defined as the difference between Mg2+-ATPase activities in the presence of ouabain and 1.5 mM EGTA versus 0.2 mM Ca2+. All assays were performed in duplicate a minimum of three times using three individual preparations of plasma membrane.

**Two-dimensional Gel Electrophoresis of Sperm Plasma Membrane**—This was performed according to the method of O’Farrell (18). Twenty-five μg of sperm plasma membrane protein were separated in isoelectric focusing gels containing 1.6% pH 5.7 and 0.4% pH 3.5–10.0 Ampholine at 400 V for 12.3 h followed by 800 V for 0.75 h. Electrophoresing standards of pl 4.1, 4.9, 6.4, and 8.3 (Calbiochem-Behring) were focused in parallel gels. The proteins were separated in the second dimension in a 0.75-mm thick, 15% sodium dodecyl sulfate-polyacrylamide slab gel at a constant current of 30 mA for 4.5 h. Molecular weight markers and an internal isoelectric focused standard were included in the gel. The slab gels were equilibrated and silver-stained according to the method of Oakley et al. (19).

**RESULTS**

**Preparation of Plasma Membrane Vesicles**—Plasma membranes isolated from epididymal and ejaculated bovine sperm were enriched in 5'-nucleotidase activity by 12- and 6-fold, respectively (Table I). While the specific activity of ejaculated bovine sperm vesicle 5'-nucleotidase was twice that found in epididymal sperm vesicles, the apparent purification of this membrane marker enzyme in the former preparation was only half that in the latter. The most likely explanation for this disparity resides in the fact that bovine seminal fluid is a particularly rich source of soluble 5'-nucleotidase activity (20). Any residual seminal plasma remaining after cell washing or adherence of soluble enzyme to isolated membrane could interfere with the determination of cellular enzyme activity. The low levels of succinate dehydrogenase activity indicate that the final product was almost completely free of contaminating mitochondrial membrane.

Electron micrographs (Fig. 1) of fixed epididymal sperm plasma membranes illustrate the vesicular nature of the preparation, which is apparently devoid of contaminating organelles. The final product contains some fragmented material along with some vesicles within vesicles, which is characteristic of epididymal sperm.

**TABLE I**

| Fraction | 5'-Nucleotidase | Succinate dehydrogenase |
|----------|-----------------|-------------------------|
| Epididymal | Ejaculated | Epididymal | Ejaculated |
| μmol P, h⁻¹ mg⁻¹ | nmol Int reduced h⁻¹ mg⁻¹ |
| Cavitated sperm | 0.24 ± 0.07 | 0.12 ± 0.39 | 0.59 ± 0.21 | 0.21 ± 0.48 |
| 600 × g pellet | 0.18 ± 0.06 | 0.08 ± 0.13 | 1.00 ± 0.23 | 1.10 ± 0.17 |
| Plasma membrane | 0.79 ± 0.30 | 0.59 ± 0.09 | 0.33 ± 0.19 | 0.37 ± 0.13 |
| Purification | 2.74 ± 0.05 | 5.62 ± 0.19 | 0.03 ± 0.02 | 0.02 ± 0.01 |

* INT, 2-(p-iodophenyl)-3-(p-nitrophenoxy)-S-phenyltetrazolium chloride.

Values given are means ± S.D. of duplicate analyses on four individual preparations of vesicles.

**FIG. 1.** Electron micrograph of plasma membrane vesicles prepared from bovine epididymal spermatozoa. Isolated plasma membranes were prepared for electron microscopy as detailed under “Experimental Procedures.” The preparation contained more than 90% closed vesicles with some vesicles within vesicles. Magnification × 54,000.
istic of similar preparations of plasma membrane from boar sperm (12, 21).

Kinetics of Calcium Uptake by Bovine Spermatozoan Plasma Membrane Vesicles—Rates of \(^{45}\text{Ca}^2+\) uptake were measured in the absence of added energy sources using vesicles prepared from both epididymal and ejaculated bovine sperm. Epididymal membranes sequestered \(^{45}\text{Ca}^2+\) at a faster rate and to a greater extent than those prepared from ejaculated sperm (Fig. 2). Uptake followed saturation-type kinetics (Fig. 2A), and maximal uptake for both types of membrane preparations occurred in 60 min at 30 °C. The effect of varying external calcium concentration from 50–600 \(\mu\text{M}\) is shown in Fig. 2B. The differences in \(\text{Ca}^2+\) uptake between epididymal and ejaculated sperm plasma membranes are similar to those obtained using whole sperm (9) and support the contention that the plasma membrane plays a key role in preventing calcium flux into ejaculated sperm.

Mechanism of Calcium Transport across the Spermatozoan Plasma Membrane—The proposed pathways of calcium influx across the plasma membrane are thought to involve one of two exchange mechanisms: a \(\text{H}^+/\text{Ca}^2+\) antiporter shown to be operative in the plasma membrane of Ehrlich ascites tumor cells (22) or a \(\text{Na}^+/	ext{Ca}^2+\) antiporter found in a number of membrane preparations (23).

When bovine epididymal sperm plasma membranes, equilibrated at pH 7.4, were incubated in buffers of varying pH (5.5–7.9), no appreciable differences were observed in the rate of calcium uptake (Fig. 3). The protonophore carbonyl cyanide \(m\)-chlorophenylhydrazone did not affect calcium uptake at the various pH intervals tested (not shown).

\(\text{Ca}^2+\) efflux in exchange for \(\text{Na}^+\) has been demonstrated in several membrane types (23) including the plasma membrane of ram sperm flagella (24) and boar sperm (7). This type of exchange is also thought to operate in the direction of calcium influx (25). To test for the existence of a \(\text{Na}^+-\text{dependent}\) calcium channel in bovine sperm, plasma membrane vesicles were loaded with 150 mM \(\text{Na}^+\) and subjected to conditions that would favor the release of intravesicular \(\text{Na}^+\). \(^{45}\text{Ca}^2+\) uptake was simultaneously monitored using both types of membrane preparations. Fig. 4 reveals that, when diluted into either a \(\text{K}^+\)- or choline-containing medium, \(\text{Na}^+\)-loaded epididymal sperm vesicles sequester external \(^{45}\text{Ca}^2+\) at a significantly greater rate than vesicles from ejaculated sperm. In control experiments, where \(\text{Na}^+\)- or \(\text{K}^+\)-loaded vesicles were diluted into \(\text{Na}^+\) medium (conditions expected to result in no net movement of \(\text{Na}^+\) or to promote inward \(\text{Na}^+\) movement, respectively), no stimulation of the low basal rate of calcium uptake was observed (not shown). Thus, it appears there is a strict requirement for outward movement of \(\text{Na}^+\) as a prerequisite to calcium influx across sperm plasma membranes.

These data distinguish the calcium transport process of sperm plasma membranes as being \(\text{Na}^+\)-dependent and regulated by putative factors present in semen that react with the plasma membrane and serve to restrict the inward flux of calcium into ejaculated sperm. Epididymal sperm vesicles take calcium at a faster rate in \(\text{K}^+\)-containing media than when choline was the prevalent cation. This probably results from the greater permeability of plasma membranes to \(\text{K}^+\) compared to choline. An explanation of the influence of \(\text{K}^+\) is presented under “Discussion.”

ATPase Activities in Bovine Sperm Plasma Membranes—(\(\text{Ca}^{2+},\text{Mg}^{2+}\))-ATPase activity was determined in bovine sperm plasma membranes to assess the role of active transport on maintaining calcium homeostasis in bovine sperm. It has been shown that bovine sperm plasma membranes, containing a \(\text{Ca}^{2+}\)-dependent ATPase that represents 14% of the total membrane ATPase activity (26). In contrast, bovine sperm plasma membranes were found to possess little or no \(\text{Ca}^{2+}\)-dependent ATPase activity. Data in Table I show that this activity represents 2% or less of the total membrane ATPase activity. This specific activity of \(\text{Ca}^{2+}\)-dependent ATPase in bovine sperm plasma membranes is not more than 50% of that observed in ram sperm plasma membranes (26). The lesser activity was not the result of membrane damage, for the \(\text{Ca}^{2+}/\text{K}^+\)-ATPase activity agrees closely with previous determinations (27), indicating that enzyme activity was not affected during isolation procedures. Rendering the membranes permeable with digitonin or Triton X-100 (28) to ensure that

![Fig. 2. Kinetics of \(^{45}\text{Ca}^2+\) uptake by bovine spermatozoan plasma membrane vesicles. Each data point shown in A and B using epididymal sperm vesicles is significantly higher than the corresponding point obtained using vesicles from ejaculated sperm (p < 0.01, n = 3). A, plasma membrane vesicles (125 mg/ml) prepared from either epididymal (○) or ejaculated (□) sperm were incubated in a medium containing 110 mM NaCl, 5 mM KCl, 10 mM MOPS, pH 7.4, at 30 °C. Following a 5-min preliminary incubation, calcium was added to a final concentration of 0.2 mM (20 \(\mu\text{Ci}\) of \(^{45}\text{Ca}^2+\)/\(\mu\text{mol}\)). At the designated time points, duplicate aliquots of vesicle suspension (25 \(\mu\text{g}\)) were assessed for calcium content as described under "Experimental Procedures." Calcium content was constant from 60 to 120 min. Data are expressed as mean values ± S.D. of at least two determinations on five individual preparations of plasma membrane. B, the assay was performed as above except external \(\text{Ca}^2+\) was varied over the concentration range shown and the time of incubation was fixed at 30 min.](http://www.jbc.org/issue/270/34/2549/Figure2.jpg)
45Ca2+ nmol/(mg protein)X(1/min)

External pH

5.5 6.0 6.5 7.0 7.5 8.0

Fig. 3. Influence of external pH on 45Ca2+ uptake by bovine epididymal sperm plasma membrane vesicles. Plasma membrane vesicles (125 mg/ml) prepared from epididymal sperm were equilibrated at 30 °C in a medium containing 110 mM NaCl, 5 mM KCl, and 10 mM MOPS, pH 7.4. Following a 5-min preliminary incubation, uptake was initiated by the addition of vesicles to media containing 110 mM NaCl, 5 mM KCl, 0.2 mM CaCl2 (20 µCi of 45Ca2+ /µmol) and buffered with either 10 mM MES, pH 5.5, 6.0, and 6.3, or 10 mM MOPS, pH 7.0, 7.4, and 7.9. Duplicate aliquots of membrane suspension (25 µg) were taken at 10 min and assessed for calcium content as described under "Experimental Procedures." Data are expressed as mean values ± S.D. of at least three determinations on three individual preparations of vesicles.

Ca2+-dependent

Mg2+-ATPase

(Na+,K+)-ATPase

(Ca2+ + Mg2+)-ATPase

Table II

ATPase activities in bovine spermatozoan plasma membranes

| Category of activity | Calcium | Ouabain | Epididymal | Ejaculated |
|----------------------|---------|---------|------------|------------|
| mM | mM | µmol P, liberated h-1 |
| Ca2+-independent | 0 | 0 | 2.19 ± 1.12 | 22.2 ± 0.6 |
| Mg2+-ATPase | 0 | 1 | 18.1 ± 2.7 | 19.0 ± 2.3 |
| (Na+ + K+)-ATPase | 0 | 0 | 3.8 ± 1.7 | 3.2 ± 1.8 |
| (Ca2+ + Mg2+)-ATPase | 0.2 | 1.0 | 1.1 ± 0.8 | <0.5 |

*Data are expressed as mean values ± S.D. of at least three determinations on four individual preparations of membrane.
**Calculated as described under "Experimental Procedures."

DISCUSSION

Plasma membrane vesicles have been prepared from bovine epididymal and ejaculated sperm using the method of nitrogen cavitation cell disruption. Membranes prepared by these procedures are thought to originate principally from the acinar, or head, portion of the sperm (7). In support of this view is the observation that cavitation of bovine sperm labeled with fluorescent concanavalin A results in loss of fluorescence from the head region only (2). Enrichment of 5'-nucleotidase activity and the lack of succinate dehydrogenase activity (Table I) indicate that vesicles prepared by the above procedures consist mainly of purified plasma membrane devoid of mitochondrial contamination. The specific activity of 5'-nucleotidase in our membrane preparations agrees well with the value reported for boar sperm plasma membranes (12). The morphology (Fig. 1) and sedimentation properties of bovine sperm plasma membranes resemble similar preparations of plasma membrane from boar sperm (12, 21).

Plasma membrane vesicles isolated from bovine spermatozoa are capable of accumulating exogenously added Ca2+, and the rate and extent of passive Ca2+ influx are significantly reduced in membrane preparations from ejaculated sperm as compared to epididymal sperm. Na+/Ca2+ exchange activity in bovine sperm plasma membranes is considerably lower than that observed in plasma membrane preparations from ram sperm flagella (8) but is comparable to the activity reported for boar sperm head plasma membranes (7). The pH-independent uptake of Ca2+ (Fig. 3) suggests that H+/Ca2+ exchange does not occur in bovine sperm plasma membranes.

Na+/Ca2+ exchange is thought to operate electrogically with the stoichiometry of 3Na+ transported per Ca2+ (23). Na+-loaded epididymal sperm plasma membrane vesicles ac-
cumulate $^{45}\text{Ca}^2+$ at a faster rate and to a greater extent simply masking $\text{Ca}^{2+}$-binding sites on the surface of sperm.

Bovine sperm plasma membranes contain high levels of Mg$^{2+}$-dependent ATPase activity, less than 2% of which is $\text{Ca}^{2+}$-dependent (Table II). This finding contrasts with similar measurements of (Ca$^{2+}$,Mg$^{2+}$)-ATPase activity in plasma membranes isolated from ram sperm flagella (26) and boar sperm head (7) and suggests that active $\text{Ca}^{2+}$ extrusion does not play a significant role in preventing net accumulation of $\text{Ca}^{2+}$ by bovine ejaculated sperm.

Previous studies demonstrating the capability of bovine epididymal sperm, but not ejaculated sperm, to accumulate $\text{Ca}^{2+}$ in vitro (9) and the subsequent isolation of a $\text{Ca}^{2+}$-transport-inhibiting factor from seminal plasma (10), combined with the results of this study, lead us to conclude that the Na$^+$/Ca$^{2+}$ exchanger is responsible for controlling $\text{Ca}^{2+}$ uptake by bovine sperm. Furthermore, the plasma membrane contains the apparatus through which seminal calcium transport inhibitor acts. The presence of a protein having physical characteristics closely resembling those of purified seminal calcium transport inhibitor (Fig. 5) in membrane preparations from ejaculated, but not epididymal, sperm supports our contention that this component of seminal plasma is the factor responsible for preventing calcium transport across the plasma membrane of ejaculated bovine sperm. It may also be concluded that seminal calcium transport inhibitor acts at the level of the plasma membrane and not simply as an extracellular calcium chelator. The finding of Bishop et al. (27) that bovine sperm plasma membrane (Na$^+$.K$^+$)-ATPase is inhibited by seminal plasma indicates that bovine seminal calcium transport inhibitor (10) may act to prevent $\text{Ca}^{2+}$ uptake by interfering with Na$^+$ extrusion from ejaculated sperm. Alternatively, seminal calcium transport inhibitor may act by simply masking $\text{Ca}^{2+}$-binding sites on the surface of sperm cells and thus prohibiting access of $\text{Ca}^{2+}$ to the plasma membrane. The absence of heat lability of $\text{Ca}^{2+}$-dependent ATPase activity in bovine sperm head plasma clearly distinguishes seminal calcium transport inhibitor from the calmodulin-like component of human seminal plasma that stimulates (Ca$^{2+}$,Mg$^{2+}$)-ATPase of erythrocytes (29).

The sperm acrosome reaction is an absolute prerequisite for fertilization (30). It is induced rapidly by ionophore-mediated calcium uptake (31) or more slowly in the presence of calcium but absence of ionophore (32). The failure of ejaculated spermatozoa to take up calcium despite the high concentration in seminal fluid (9) can be explained by the presence of the calcium transport inhibitory protein. Capacitation of spermatozoa in vivo is known to involve the removal or modification of plasma membrane proteins (33). That may release the inhibitory effect of the seminal protein, allow calcium to enter the spermatozoa, stimulate the acrosomal reaction, and accomplish capacitation.

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REFERENCES
1. Baker, P. F. (1978) Ann. N. Y. Acad. Sci. 307, 250–288
2. DiPolo, R., and Beaute, L. (1980) Cell Calcium 1, 147–169
3. Mullins, L. J. (1979) Ann. J. Physiol. 236, C103–C110
4. Schatzmann, H. J., and Burgin, H. (1978) Ann. N. Y. Acad. Sci. 307, 125–147
5. Rasmussen, H., and Goodman, D. B. P. (1977) Physiol. Rev. 57, 421–509
6. Peterson, R. N., Russell, L. D., Bundman, D., and Freund, M. (1979) Biol. Reprod. 21, 583–588
7. Ashraf, M., Peterson, R. N., and Russell, L. D. (1982) Biol. Reprod. 26, Suppl. 1, 37A
8. Bradley, M. P., and Forrester, I. T. (1980) FEBS Lett. 121, 15–18

Fig. 5. Comparison between protein profiles of membrane vesicles from epididymal and ejaculated sperm. Twenty-five $\mu$g of protein from epididymal (A) and ejaculated (B) sperm membrane vesicles were subjected to two-dimensional electrophoresis. The scale at the bottom corresponds to the migration of isoelectric focusing standards (pl stds.). Molecular weight standards are seen at the far right of B. The standards are phosphorylase a ($M_r = 94,000$), catalase ($M_r = 60,000$), actin ($M_r = 43,000$), vitamin D-dependent Ca-binding protein ($M_r = 27,000$), and lysozyme ($M_r = 14,000$). The arrow in the upper left quadrant of each gel indicates an internal standard of apparent pl 4.2 and $M_r = 47,000$. The arrow in the lower right quadrant of B indicates a heavy concentration of protein with a pl of approximately 8.3 and $M_r = 15,000$. No such protein is present in the epididymal membranes (A).
9. Babcock, D. F., Singh, J. P., and Lardy, H. A. (1979) Dev. Biol. 68, 83-93
10. Rufo, G. A., Jr., Singh, J. P., Babcock, D. F., and Lardy, H. A. (1982) J. Biol. Chem. 257, 4627-4632
11. Russell, L., Peterson, R. N., and Freund, M. (1979) Proceedings of the 4th Annual Meeting of the Society for Andrology, Houston, Texas, Vol. 19, Abstr. 37, p. 37A
12. Gillis, G., Peterson, R. N., Russell, L., Hook, L., and Freund, M. (1978) Prep. Biochem. 8, 363-378
13. Babcock, D. F., First, N. L., and Lardy, H. A. (1975) J. Biol. Chem. 250, 6488-6495
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
15. Weaver, R. A., and Boyle, W. (1969) Biochim. Biophys. Acta 173, 377-388
16. Pennington, R. J. (1961) Biochem. J. 80, 649-654
17. Baginski, E. S., Weiner, L. M., and Zak, B. (1964) Clin. Chim. Acta 10, 378-379
18. O’Farrell, P. H. (1975) J. Biol. Chem. 250, 4007-4021
19. Oakley, H. R., Kirsch, D. R., and Morris, N. R. (1980) Anal. Biochem. 105, 361-363
20. Mann, T., and Mann, C. L. (1981) Male Reproductive Function and Semen, p. 283, Springer-Verlag, New York
21. Lunstra, D. D., Clegg, E. D., and Morre, D. J. (1974) Prep. Biochem. 4, 341-352
22. Hinnen, R., Miyamoto, H., and Racker, E. (1979) J. Membr. Biol. 49, 309-324
23. Carafoli, E. (1981) in Calcium and Phosphate Transport Across Biomembranes (Bronner, F., and Peterlik, M., eds) pp. 9-14, Academic Press, New York
24. Bradley, M. P., and Forrester, I. T. (1980) Proc. Unio. Ooto Med. Sch. 58, 3-4
25. Baker, P. F., Blaustein, M. P., Hodgkin, A. C., and Steinhardt, R. A. (1969) J. Physiol. (Lond.) 200, 431-458
26. Bradley, M. P., and Forrester, I. T. (1980) Cell Calcium 1, 381-390
27. Bishop, D. W., Chakraborty, J., and Whaley, K. J. (1980) J. Androl. 1, 71
28. Tash, J. S., and Means, A. R. (1982) Biol. Reprod. 26, 745-763
29. Forrester, I. T., and Bradley, M. P. (1980) Biochem. Biophys. Res. Commun. 92, 994-1001
30. Bedford, J. M. (1983) Biol. Reprod. 28, 108-120
31. Singh, J. P., Babcock, D. F., and Lardy, H. A. (1978) Biochem. J. 172, 549-556
32. Yanagimachi, R. (1975) Biol. Reprod. 13, 519-526
33. Aonuma, S., Mayumi, T., Suzuki, K., Nogushi, T., Iwai, M., and Okabe, M. (1973) J. Reprod. Fertil. 2, 425-432
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