The Effect of Calcium Ions on Cell Volume and Motility of Bovine Spermatozoa (35806)

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Bovine seminal plasma normally contains 6 to 15 m*M* Ca^{2+} (1), but probably only a small portion of this is present as free ionic calcium because of the citrate present (2). Sperm cells have about 27 mg of Ca^{2+}/ 100 g of cells (3). Much of this is rapidly removed by dilution with a calcium-free diluent (4), probably from the surface of the cell (5). Extracellular Ca^{2+} is essential for the maintenance of normal membrane permeability of cells (5), and regulation of the very low intracellular Ca^{2+} level is important because of the stimulatory or inhibitory effect this cation has on cell metabolism (5–7).

Ca^{2+} has been reported to have a stimulatory or inhibitory effect upon sperm motility, respiration, and glycolysis (8–11) depending on the level used and the conditions of incubation. Another indicator of the metabolic and structural status of sperm cells is cell volume (12, 13) and the present report is a study of Ca^{2+} effects on cell volume and motility of bull spermatozoa at high dilution rates.

Materials and Methods. Fresh bull semen was diluted approximately 1:10,000 to facilitate electronic sizing. Salt media buffered with 50 m*M* Tris–HCl at pH 7.0 were prepared to be isosmotic with 0.15 *M* NaCl (12). Calcium was added as the chloride salt. Various treatment combinations of Ca^{2+}, K^+, fructose, CN^-, ouabain, and BSA, were formulated by replacing NaCl isosmotically, and the osmolarity of the media was confirmed by freezing point depression determinations. Details of treatment solution preparation, incubation of spermatozoa, and electronic sizing were as previously described (12). The size of spermatozoa was measured on a scale divided into 25 equal intervals, each interval representing approximately 2.5 μ^3. Data are reported in terms of fourth degree polynomial curves fitted by weighted least squares analysis to the changes in mean volume during the incubation. The mean volume scales on the graphs are in volume intervals.

Results. Figure 1 shows the mean cell volume changes associated with increasing levels of Ca^{2+} in the absence of K^+. The immediate onset of swelling, characteristic of K^+-free media (12), was observed for all treatments; however, the presence of Ca^{2+} markedly shortened the swelling period and greatly reduced the degree of cellular swelling. Differences due to Ca^{2+} levels were significant ($p<0.05$). The two highest levels of Ca^{2+} depressed sperm motility. Under dark-field illumination spermatozoa appeared fuzzy around the border as they became immobilized. Eventually the tail particularly showed evidence of cell disintegration. This effect was observed for all the Ca^{2+} treatments in this report and the time of onset in immotile cells was inversely related to the Ca^{2+} concentration.

Effects of Ca^{2+} in the presence of K^+. Swelling, such as seen in the control (Fig. 1) can be greatly delayed by adding 2.25 m*M* K^+ (12). Therefore, to study the effect of Ca^{2+} in the presence of K^+, 0, 0.05, 0.10, 1.0, 5.0, 15.0, 30.0, and 60.0 m*M* Ca^{2+} were added to buffered saline containing 2.25 m*M* K^+. Results of representative treatments are shown in Fig. 2. Calcium levels below 1 m*M* did not greatly affect the K^+-induced delay in onset of swelling. Higher levels of Ca^{2+} caused immediate moderate swelling followed by shrinkage and at the 60 m*M* level only shrinkage occurred. The four highest levels of Ca^{2+} depressed motility.

Another experiment designed to test the

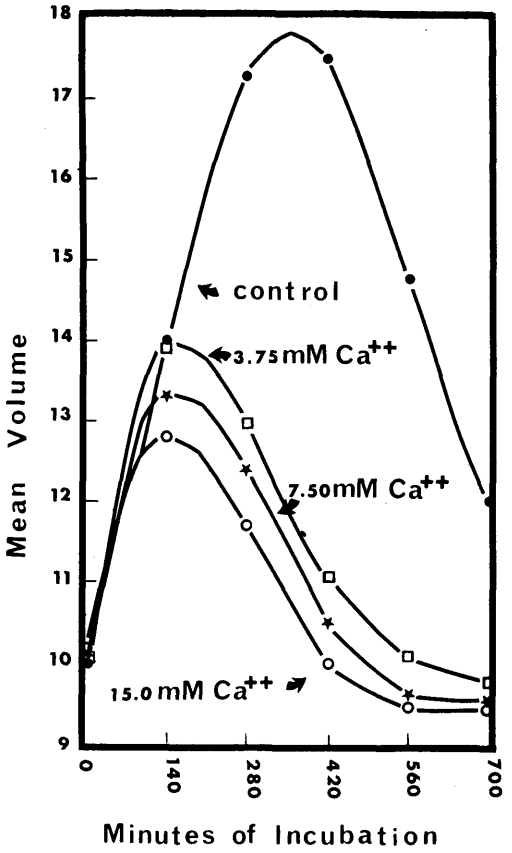

FIG. 1. The mean cell volume of bull spermatozoa incubated at 35° in Tris–HCl buffered NaCl (pH 7.0) containing various levels of $CaCl_2$ replacing isosmotically the NaCl. Each interval on the electronic sizer used to measure mean cell volume represents approximately 2.5 μ^3; thus 10 on this scale $\simeq$ 25 μ^3 (mean of 5 ejaculates).

effects of K^+ or fructose on the response of 15 m*M* Ca^{2+} confirmed the previous results. In addition, it clearly showed that added K^+ or fructose delayed the onset of both swelling and Ca^{2+}-induced shrinkage. The effectiveness of fructose in the absence of adequate K^+ was limited to about 2-hr duration. Further interrelationships of Ca^{2+} with fructose are described in the next experiment.

Ca^{2+} response with fructose, BSA, CN^-, or ouabain. Treatments imposed and their effect on sperm motility are shown in Table I. Corresponding sperm size changes are shown in Fig. 3. All Tris-buffered saline media contained 3.0 m*M* K^+, 10 m*M* Mg^{2+}, and 2.0

TABLE I. The Effect of Ca^{2+} on the Percentage of Motile Spermatozoa.[a]

Treatment	Motile spermatozoa (%); incubation time (hr): 2.5	6.5	10.5	24
Control	45	15	0	0
Ca^{2+}	10	10	0	0
CN^-	0	0	0	0
Ca^{2+} and CN^-	0	0	0	0
Ca^{2+} and ouabain	25	15	10	0
BSA	65	70	65	0+
Ca^{2+} and BSA	70	65	60	0
Ca^{2+}, BSA, and CN^-	0	0	0	0
Fructose	50	40	40	0
Ca and fructose	40	40	40	0
Fructose and CN^-	40	50	45	40
Ca^{2+}, fructose, and CN^-	55	45	50	10
Fructose and BSA	65	65	65	40
Ca^{2+}, fructose, and BSA	60	65	60	15

[a] Mean of four semen samples; see Fig. 3 for the concentrations of ingredients.

m*M* phosphate. The latter two ions were shown previously (13) to produce no significant effect on cell volume or motility. Figure 3a,b shows that CN^- or ouabain had little effect upon the size response curve of sperm

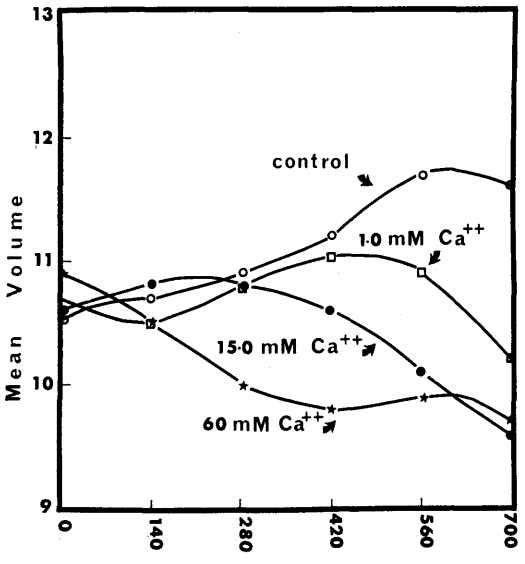

FIG. 2. The mean cell volume of bull spermatozoa incubated at 35° in Tris–HCl buffered NaCl (pH 7.0) containing 2.25 m*M* K^+ and various levels of $CaCl_2$ replacing NaCl in the control as indicated.

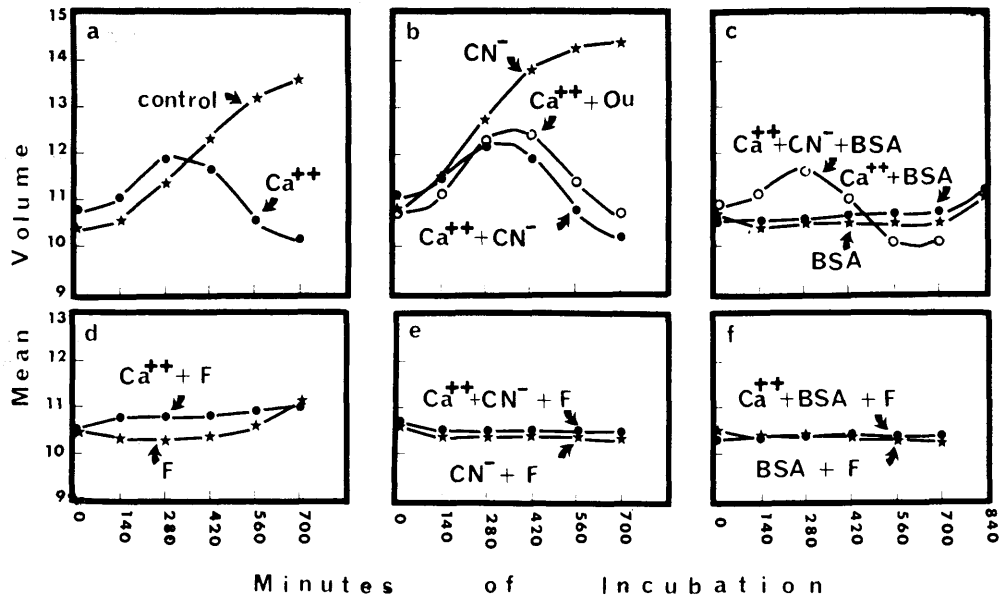

FIG. 3. The mean cell volume of bull spermatozoa incubated at 35° in Tris–HCl buffered NaCl (pH 7.0) containing antibiotic, 2.0 m*M* phosphate, 10 m*M* Mg^{2+}, 3.0 m*M* K^+ and the additives, as shown, in the following concentrations: Ca^{2+}, 5 m*M*; cyanide (CN^-), 4 m*M*; Ouabain (Ou), 0.1 m*M*; bovine serum albumin (BSA), 1 mg/ml; fructose (F), 10 m*M* (mean of 4 ejaculates).

obtained with Ca^{2+}. However, BSA prevented swelling and shrinkage, phenomenon overridden by CN^- (Fig. 3c). Fructose (Fig. 3d–f) stabilized cell volume. The same treatments which were able to stabilize cell volume also prolonged sperm motility (Table I). The effects of Ca^{2+} clearly appear to be energy related, based upon the facts that CN^- abolished the beneficial effects of BSA, and the glycolyzable substrate, fructose, stabilized cell volume, and prolonged motility.

Discussion. When 1.0 m*M* (or less) Ca^{2+} is present along with K^+ in the Tris-buffered incubation medium, it has little effect on the onset of swelling or on motility of bovine spermatozoa. However, as the level of Ca^{2+} is increased, swelling is initiated more rapidly followed by an early reversal of the process leading to marked shrinkage. Swelling is preceded by a general loss of motility and shrinkage is followed by microscopically observable cellular structural changes. The agents which delay Ca^{2+}-induced swelling produce a similar delay in onset of shrinkage, suggesting that the changes that cause shrinkage require a period of time to develop after onset of swelling, the interval being dependent on the Ca^{2+} concentration. Since fructose or BSA maintained sperm motility and prevented the pattern of sperm volume changes otherwise associated with Ca^{2+}, it is likely that the Ca^{2+} effect is dependent on the depletion of the cell metabolic energy.

These Ca^{2+} effects appear to be similar to those observed with human red blood cells (14). Following ATP depletion red blood cells start to swell, then Ca^{2+} enters the cells and they shrink. Bull spermatozoa have been shown to accumulate Ca^{2+} when their viability is reduced (15). The shrinkage phase most likely is indicative of membrane permeability changes as evidenced by the abnormal microscopic appearance of the sperm cell borders and by the ability of Ca^{2+} to reduce the prolonged swelling normally produced by CN^- or ouabain (12). Ca^{2+} is known to compete favorably with K^+ and Na^+ for membrane anionic sites. This could result in marked changes in the properties of the plasma membrane (7, 16). The shrinkage may reflect an increased leakiness of K^+ caused by elevated levels of intracellular Ca^{2+}, as

has been observed for liver cells (17) and erythrocytes (6, 18 19). Ca^{2+} could bring about a reduction in total intracellular osmolarity by binding to intracellular polyanions such as protein and nucleic acids thus reducing their Gibbs-Donnan effects. The 1 for 2 replacement of K^+ with Ca^{2+} as counterions for other intracellular anionic sites would also reduce total intracellular osmolarity (20).

The observed ability of K^+ to delay the swelling and shrinkage caused by Ca^{2+} suggests a possible interrelationship between Na^+, K^+, and Ca^{2+} fluxes, as reported for other cell types (21). In nerve (22), where this relationship has been most thoroughly studied, the efflux of Ca^{2+} appears linked to the influx of Na^+ and the influx of Ca^{2+} is directly related to intracellular Na^+ levels.

Summary. Ca^{2+} was added to a buffered medium containing various combinations of K^+, fructose, CN^-, ouabain, and BSA. Constant osmolarity, pH, and temperature of the incubation media were maintained. Without K^+ in the medium, Ca^{2+} limited the duration of rapid swelling of spermatozoa by causing early onset of shrinkage to about 90% of initial cell volume. Shrinkage occurred sooner as the Ca^{2+} level increased, and was associated with a fuzzy microscopic appearance of the sperm cells. Addition of 2.25 to 3.0 m*M* K^+, and to a lesser extent 1.0 m*M* of fructose, delayed the onset of swelling and subsequent shrinkage associated with Ca^{2+} alone. This delay also was observed in a variety of media which promoted good sperm motility. BSA was able to counteract the effects of Ca^{2+}, but this was abolished by addition of CN^-. Fructose overcame the CN^- effect, indicating that the process is energy related. It appears likely that Ca^{2+} enters the cell only after energy sources are depleted, and its presence there causes structural and permeability changes in spermatozoa.

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