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IONIC EFFECTS ON THE MOTILITY OF BULL AND CHIMPANZEE SPERMATOZOA

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Summary. The effects of potassium, sodium, chloride and calcium on the percentage progressive motility of samples of chimpanzee and bull spermatozoa were investigated. Inhibitors and stimulators of motility were identified. Three parameters of chimpanzee sperm motility, velocity, frequency and amplitude, were measured. There was no consistent change related to ion concentration. The ionic constituents of chimpanzee and bull seminal plasma were also analysed.

INTRODUCTION

Sperm motility is strongly influenced by the chemical environment. Excessive dilution or severe ionic or osmotic imbalance all cause rapid and irreversible loss of metabolic activity and motility (Tampion & Gibbons, 1963; Lindahl & Drevius, 1964; Nevo & Mohan, 1969). Extremes of potassium concentration were found to depress the motility and viability of bull spermatozoa (Blackshaw, 1953; Cragle & Salisbury, 1959; Salisbury & Lodge, 1962). Sodium is the major bulk cation in maintaining favourable osmotic conditions in seminal plasma of the bull (Mann, 1964), while calcium is customarily excluded from diluents used for extending bull semen because of alleged deleterious effects on the sperm viability and motility. Depending on the concentration and conditions of sample preparation, however, calcium has been reported to have either an inhibitory effect (Bredderman & Foote, 1971) or a stimulatory effect on sperm motility (summarized by Quinn, White & Wirrick, 1965).

Our recent work (McGrady & Nelson, 1972, 1973) has been concerned with the effects of varying ion concentration on motility and membrane potential of single bull spermatozoa. Reports of experimental work dealing with ionic modifiers of monkey sperm motility are sparse. This paper extends our previous results with a systematic study of the effects of potassium, calcium, sodium and chloride ion concentration on the motility of bull and chimpanzee sperm populations.

MATERIALS AND METHODS

Bull semen was provided by the Northwestern Ohio Breeders Association. Chimpanzee (*Pan troglodytes*) semen was obtained from animals at the Toledo Zoological Park, Toledo, Ohio.

Bull spermatozoa were prepared by diluting the semen threefold with Ca^{++}-free medium (Mann, 1964) composed of 500 mg fructose, 23·9 mg $NaHCO_3$, 19·2 mg NaH_2PO_4, 38·4 mg $MgSO.7H_2O$, 42·2 mg KCl, 820·8 mg NaCl per 100 ml glass-distilled H_2O. The pH was adjusted to 7·2 with phosphate buffer or with tris buffer in the Ca^{++}-supplemented media. All experiments were conducted at room temperature. The cell suspension was centrifuged for 10 min at 2000 *g* and the supernatant was discarded. The cells were resuspended in an equal volume of experimental medium. This consisted of the standard Mann's medium in which various concentrations of potassium, sodium, chloride and calcium were present.

For monkey spermatozoa, this procedure was modified since we received only small amounts of chimpanzee semen. The sperm cells were separated from the seminal plasma by centrifugation at 2000 *g* for 15 min and were then suspended in the experimental media. Microscopic examination showed only a small decline in motile activity as a consequence of the preparative procedures.

For cinephotographic examination of sperm behaviour, 0·01 ml of cells in an experimental medium was placed in a chamber to which was added 1 ml of a solution of 1·5% methylcellulose (made up in in the same experimental medium). Methylcellulose reduces cell speed and simplifies high-power observation and cinematography of motile cells. The cells were observed through a Wild inverted phase microscope. Immotile and motile cells in five to six fields (each field containing approximately twenty to twenty-five cells) were counted, then photographed with a Bolex ciné camera. The percentage of cells exhibiting progressive motility was determined on the basis of the examination of approximately 100 cells. Cells suspended in Ca^{++}-free Mann's medium containing 5·5 mM K^+ with 1·5% methylcellulose were taken as the normal or standard with which all other experimentally treated cells were compared. With each sample of bull or chimpanzee semen (approximately forty samples total), controls of 100 cells were run in the standard solution. If the percentage progressive motility differed by more than 12%, no experimental trials were run with that sample.

Three parameters, velocity (μm/sec), frequency of the beat (beats/sec) and amplitude of the flagellar wave (μm), of individual chimpanzee spermatozoa were measured from developed film strips by means of a Lafayette analyser projector.

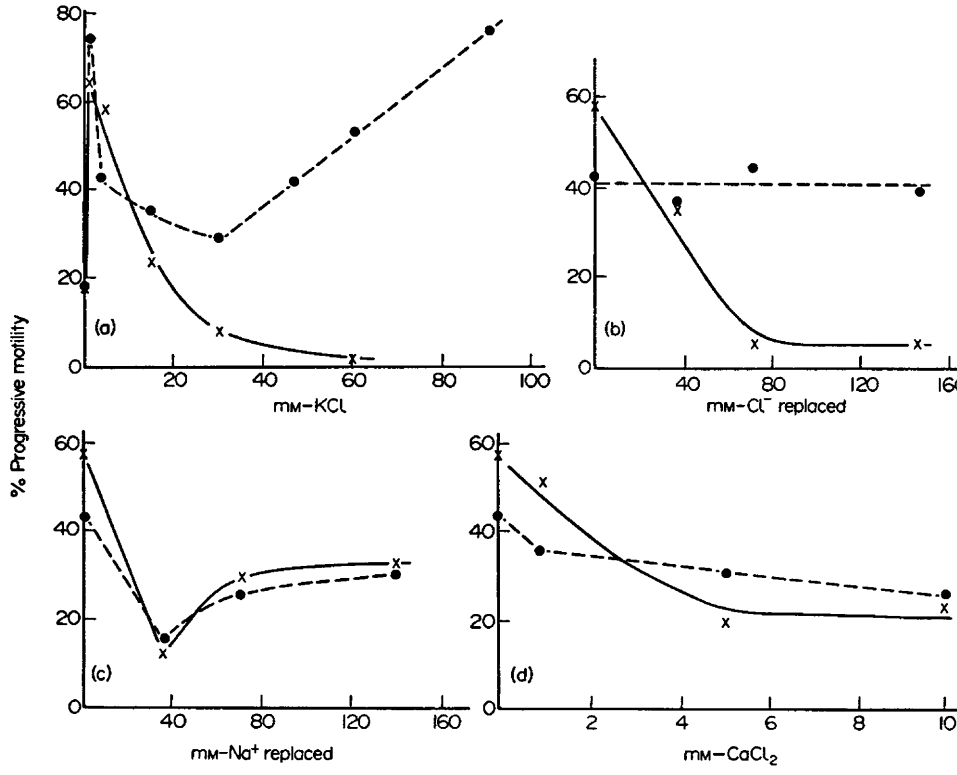
The procedures for flame photometry (K^+, Na^+), spectrofluorometry (Ca^{++}) and titration (Cl^-) of monkey spermatozoa and seminal plasma were the same as reported previously (McGrady & Nelson, 1973).

RESULTS

Cell populations

Potassium. The normal motility of chimpanzee and bull spermatozoa decreased from the standard 44% and 58% in 5·5 mM-KCl medium to 19% and 18%, respectively, in a potassium-free medium (Text-fig. 1a). In 1 mM-KCl, the spermatozoa of both species reached a peak percentage motility: 65% for the

bull and 74% for the chimpanzee. As the concentration of extracellular potassium was increased further up to 30 mM-KCl, there was a decline in progressive motility. Beyond this point, each species reacted differently. The decline for bull spermatozoa continued until only 2% were moving progressively in 60 mM-KCl, whereas an increase in chimpanzee sperm motility occurred at concentrations higher than 40 mM-KCl. At 90 mM-KCl, 74% of the population was progressing normally. Bull sperm cells separated from seminal plasma (without washing) showed 48% progressive motility.



TEXT-FIG. 1. The effect of variations in the extracellular concentrations of (a) potassium, (b) chloride, (c) sodium and (d) calcium on the percentage of progressively moving bull spermatozoa (×) and chimpanzee spermatozoa (●).

Chloride. Text-figure 1(b) illustrates that a decrease in the concentration of extracellular chloride (replaced by methylsulphate) brought about a rapid decrease in the effective motility of bull spermatozoa from 58% in the standard (146 mequiv. chloride/litre medium) to 5% at 73 mequiv./litre. With all chloride in the extracellular medium replaced by methylsulphate, 5% of the bull spermatozoa were still moving progressively.

There was no significant change in the percentage of progressively moving chimpanzee spermatozoa throughout the whole range of chloride concentration.

Sodium. The progressive motility of bull and chimpanzee spermatozoa dropped sharply from the values in the medium containing 141 mequiv.

sodium/litre to 12% and 17%, respectively, when 35 mequiv. sodium/litre was replaced with tris-Cl (Text-fig. 1c). Potassium and chloride were held constant. The sudden decrease was partly reversed as more sodium was replaced by tris-Cl. When all sodium in the suspension medium was replaced by tris-Cl, 32% of bull spermatozoa and 31% of chimpanzee spermatozoa showed progressive motility, still a marked decrease from control levels.

Calcium. As the extracellular concentration of calcium was increased (Text-fig. 1d), the percentage of progressively moving bull spermatozoa decreased from the standard 58% in Ca^{++}-free medium to 20% in the medium containing 10 mM-$CaCl_2$. The addition of extracellular calcium produced a similar gradual decrease in progressive motility of chimpanzee spermatozoa from 44% in Ca^{++}-free medium to 26% in 10 mM-$CaCl_2$.

Table 1. The velocity, frequency of beat and amplitude of the flagellar wave of bull and chimpanzee spermatozoa suspended in Mann's medium containing 1·5% methylcellulose

	Bull (*Mean* ± *S.E.M.*)	*Chimpanzee* (*Mean* ± *S.E.M.*)
Velocity (μm/sec)	7·1 ± 0·9	13·5 ± 1·0
Frequency (beats/sec)	0·5 ± 0·05	3·6 ± 0·2
Amplitude (μm)	17·6 ± 1·2	5·0 ± 0·7

Table 2. Ion concentrations in bull and chimpanzee seminal plasma samples in the present study compared with concentrations reported in previous studies

	Bull	*Chimpanzee*
Potassium (mequiv./litre)	35 (5) 39[a] 40[b]	75 (4)
Sodium (mequiv./litre)	108 (5) 125[a] 100[b]	41 (4)
Chloride (mequiv./litre)	38 (5) 40 to 117[a]	18·5 (4)
Calcium chloride (mM)	13·9 (4) 7[a] 12[c] 10[d]	4·9 (4)

Number of determinations shown in parentheses.

[a] Cragle, Salisbury & VanDemark (1958); [b] Quinn & White (1966); [c] Cragle, Salisbury & Muntz (1958); [d] Quinn *et al.* (1965).

Single cells

The velocity, frequency of beat, and amplitude of the flagellar wave of chimpanzee and bull spermatozoa suspended in Mann's medium with 1·5% methylcellulose are compared in Table 1. In chimpanzee sperm cells, the

amplitude of the flagellar wave was lower, while the velocity and frequency of the beat was higher than in bull spermatozoa. When chimpanzee spermatozoa were suspended in media supplemented with sodium, potassium, chloride or calcium, no overall pattern of change emerged in the measurements of velocity, frequency or amplitude. Only when the suspending media contained at least 30 mequiv. potassium/litre did a marked change occur: the velocity decreased to 5·8 μm/sec and the frequency to 0·92 beats/sec.

Ionic analysis of chimpanzee seminal plasma also revealed marked differences in ion concentration compared to bull seminal plasma. Table 2 illustrates that the concentration of potassium was higher in chimpanzee seminal plasma while sodium, calcium and chloride were considerably lower.

DISCUSSION

The major ionic conditions leading to a depression of bull sperm motility were: (1) lack of potassium (K^+-free), (2) deficient chloride (below 100 mequiv./litre), (3) potassium concentration higher than 6 mequiv./litre, (4) calcium chloride concentration in excess of 1 mM, (5) sodium content lower than 100 mequiv./litre. These results concur with those of others (Cragle & Salisbury, 1959; Salisbury & Lodge, 1962) who found that both K^+-free media and high potassium media depressed bull sperm motility. The values reported in Table 1 for bull spermatozoa suspended in Mann's medium with 1·5% methylcellulose represent about a tenfold decrease in velocity and a 20-fold decrease in frequency compared to values reported by Gray (1958) and Rothschild (1961) for cells suspended in seminal plasma at 37°C.

The motility of chimpanzee spermatozoa was stimulated by a high concentration of potassium (above 40 mequiv./litre) and inhibited by (1) increasing the calcium chloride beyond 1 mM, (2) decreasing sodium below 100 mequiv./litre, (3) K^+-free medium, (4) potassium concentration in excess of 6 mequiv./litre but lower than 40 mequiv./litre. Changing the extracellular chloride concentration had no apparent effect.

The value taken as the standard (44% progressively moving chimpanzee spermatozoa in Mann's medium) is comparable to the 30 to 40% reported by Hoskins & Patterson (1967) for cells suspended in Norman Johnson's medium and slightly lower than the value of 54% motility for rhesus monkey spermatozoa suspended in a medium containing egg-yolk, sodium glutamate, and glycerol (Roussel & Austin, 1967).

The results have shown that the effects of ion concentration on the motility of bull and chimpanzee spermatozoa may be enhanced in synthetic media. Bull seminal plasma normally contains concentrations of the four ions tested in ranges similar to those that depress the motility of the washed cells; chimpanzee seminal plasma contains sufficient sodium and calcium to depress motility in the experimental medium, while a potassium concentration similar to that in seminal plasma increased the percentage of progressively moving cells. Ion imbalance in semen or in the female reproductive tract fluids could seriously interfere with fertility. The statistical chance of fertility obviously decreases quickly if more of the ejaculated cells are immotile, or if many of the cells show

abnormal motility patterns. It is suggested, therefore, that since changes in ion concentration may contribute to alterations in sperm motility, the importance of this factor in the evaluation of fertilizing capacity should not be overlooked.

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