

EFFECT OF OSMOTIC PRESSURE AND TEMPERATURE GRADIENTS ON COLD SHOCK IN RAM AND BULL SPERMATOOZOA

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Summary

Ram spermatozoa are not affected by brief hypotonicity as judged by permeability to vital stain.

Cooling ram and bull spermatozoa rapidly from 30 to 8°C decreased viability but this was not influenced by the tonicity of the medium. Ram spermatozoa that were diluted during the cold shock procedure were more susceptible than spermatozoa diluted before cold shock.

Increasing the temperature gradient increased the susceptibility of ram spermatozoa to cold shock, and the temperature gradient required to produce cold shock was found to decrease as the initial temperature decreased.

The harmful effects of cold shock cannot be explained in terms of osmotic pressure changes in the medium.

I. INTRODUCTION

Ostasko (1963, 1964) has suggested that the damage to bull spermatozoa caused by cold shock may be due to a sudden decrease in the osmotic pressure of fluid surrounding the spermatozoa, the osmotic pressure inside the cell being maintained by local heat production. In support of this theory, Ostasko (1963) has produced evidence that, provided the drop in temperature is less than 22°C, corresponding to a maximum fall of 0.6 atm in osmotic pressure, hypertonic diluents can lower the osmotic gradient between the surrounding medium and the spermatozoa and thus protect them from cold shock. Lecithin is purported to give protection against cold shock by forming a hydrophobic layer on the cell surface which is not removed by osmotically active substances. This layer is said to decrease membrane permeability and delay osmotic equilibration.

In the present paper experiments similar to those conducted by Ostasko (1963) have been undertaken using ram spermatozoa and diluents with osmotic pressures from 6 to 9 atm. The effect of temperature range and gradient on the severity of cold shock at various osmotic pressures has also been determined. In addition, an attempt has been made to establish the precise temperature conditions needed to produce cold shock in ram spermatozoa.

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II. MATERIALS AND METHODS

(a) Semen

Semen was collected with an artificial vagina except in the final experiment when electrical stimulation was used. Care was taken to prevent sudden changes in temperature of the semen after collection and only samples of good motility were used.

(b) Diluents

The diluents in the first experiment (Figs. 1, 2, and 3) contained 15 mM fructose and 85–113 mM trisodium citrate or 130–173 mM NaCl, giving a range of osmotic pressures at 37°C from 6.5 to 8.5 atm in increments of 0.2 atm. The sodium citrate diluent used in the second and third experiments (Figs. 4 and 5 respectively) consisted of 84 mM trisodium citrate with a range of fructose concentrations from 0 to 118 mM giving osmotic pressures at 37°C of 6–9 atm in increments of 0.3 atm. The theoretical calculation of chemical composition of each diluent was in good agreement with the freezing point depression obtained with a Fiske osmometer.

(c) Viability

Motility was scored on a warm stage at 37°C, full motility being scored as 5 and complete immotility as zero (Ozin 1956). The percentage of cells showing progressive movement was also estimated. The percentage of unstained spermatozoa was determined after staining with Congo red (3 g/100 ml) in 113 mM trisodium citrate buffered to pH 7 with 77 mM sodium phosphate buffer and containing nigrosin (5 g/100 ml) as a background stain.

(d) Freezing Mixtures

Freezing mixtures in the final experiment were obtained using various salt solutions at the eutectic point. The solutions (g/100 ml) maintaining temperatures of -5.7 , -10.8 , -15.5 , and -21.7°C ($\pm 0.5^{\circ}\text{C}$) for at least 10 min were BaCl_2 (22.5), MnSO_4 (32.3), NH_4Cl (18.6), and NaCl (23.3) respectively.

(e) Statistical Methods

Each experiment was replicated three times and the data were then subjected to an analysis of variance using the SILLIAC digital computer.

III. RESULTS

(a) Resistance of Ram Spermatozoa to Hypotonicity

Ram spermatozoa were not affected by brief exposure to low osmotic pressures as judged by staining reaction to Congo red. Thus, when four ejaculates were diluted with nine volumes of sodium chloride of concentrations ranging from 150 to 0 mM and held for 10 min at 30°C before staining, there was no increase in the proportion of stained cells.

(b) Effect of Cooling Ram and Bull Spermatozoa from 30 to 8°C on Motility and Permeability

One drop of ram or bull semen was diluted with nine drops of sodium citrate or sodium chloride diluents of osmotic pressures from 6.5 to 8.5 atm. Motility and staining reaction were determined after 10 min at 30°C and again after the spermatozoa were cold-shocked by placing the tube containing the semen in a water bath at 8°C.

For purposes of comparison, one drop of undiluted semen held at 30°C was mixed with nine drops of diluent held at 8°C and the subsequent viability of the spermatozoa determined.

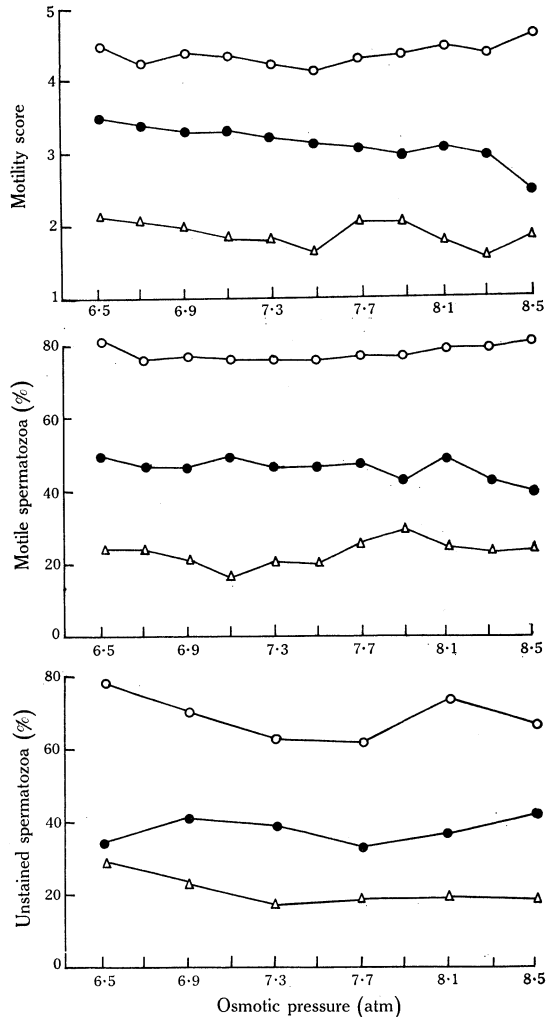


Fig. 1.—Motility of ram spermatozoa diluted 1 : 9 in sodium citrate diluents of varying tonicity. ○ Control (30°C). ● Diluted and then cold-shocked at 8°C. △ Cold-shocked by diluting at 8°C.

Exposure to the low temperature decreased the motility rating and the percentage of motile and unstained spermatozoa (Figs. 1, 2, and 3), but this was not greatly influenced by the osmotic pressure or anion composition of the medium. Ram spermatozoa were more susceptible to cold shock when undiluted semen at 30°C was mixed with diluent held at 8°C. Bull spermatozoa were less susceptible to a sudden decrease in temperature of 22°C and the results were similar irrespective of whether the semen was diluted prior to, or during, the cooling process.

(c) Effect of Increasing the Temperature Gradient on Motility of Ram Spermatozoa

Ram semen was diluted 1 : 3 in citrate-fructose diluents of osmotic pressures ranging from 6 to 9 atm and held at 37°C for 10 min. Motility of the spermatozoa was estimated before and after placing samples in water baths held at 15, 10, 5, and

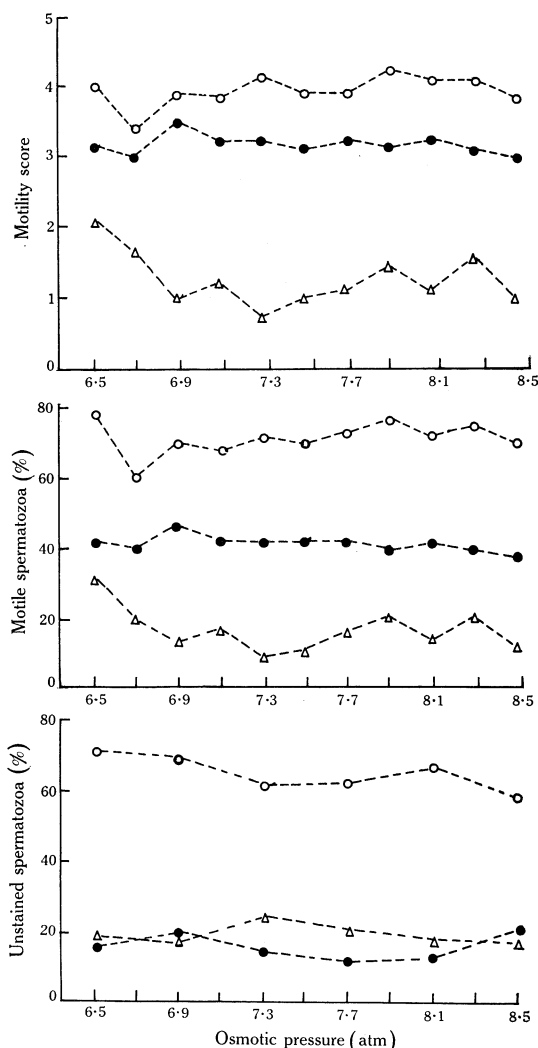


Fig. 2.—Motility of ram spermatozoa diluted 1 : 9 in sodium chloride diluents of varying tonicity. ○ Control (30°C). ● Diluted and then cold-shocked at 8°C. △ Cold-shocked by diluting at 8°C.

0°C for 10 min and the motility ratios (motility score after cold shock : motility score of control) are presented in Figure 4.

Increasing the temperature gradient from 22 to 37°C had an increasingly detrimental effect on motility. However, there were no significant effects of osmotic

pressure on the susceptibility of the spermatozoa to cold shock at any of the temperature gradients.

(d) *Effect on Motility of Ram Spermatozoa of Temperature Range at a Constant Gradient*

The effect of sudden decreases in temperature of 22°C over varying ranges of temperatures was next investigated. Ram semen was diluted as in the experiment

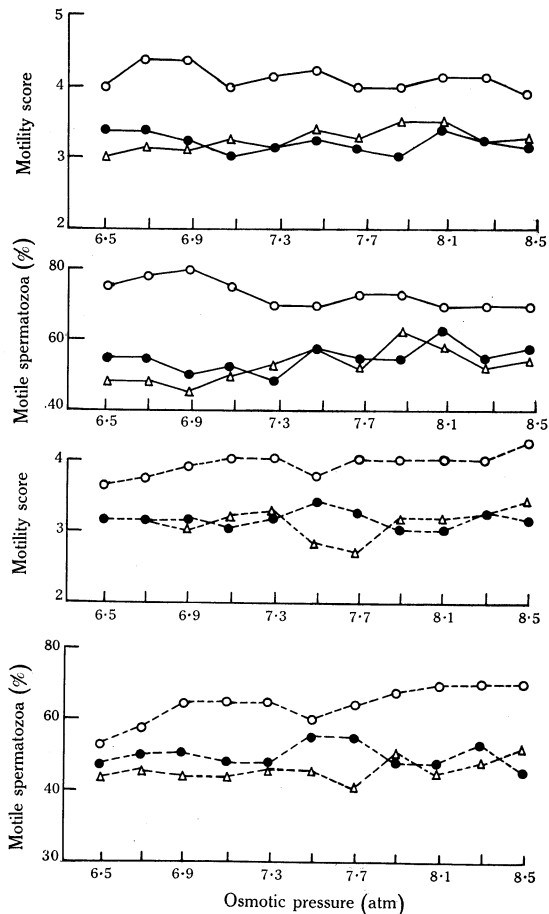


Fig. 3.—Motility of bull spermatozoa diluted 1:9 in sodium citrate (unbroken lines) and sodium chloride (broken lines) diluents of varying tonicity. ○ Control (30°C). ● Diluted and then cold-shocked at 8°C. △ Cold-shocked by diluting at 8°C.

described in Section III(c) and held at 37, 32, 27, and 22°C for 10 min and then transferred to water-baths at 15, 10, 5, and 0°C respectively. The motility ratios (final motility score: motility score of control) of the spermatozoa are presented in Figure 5.

The susceptibility of the spermatozoa to cold shock was considerably less over the higher ranges of temperature than over the lower ranges and again there were no striking effects of osmotic pressure.

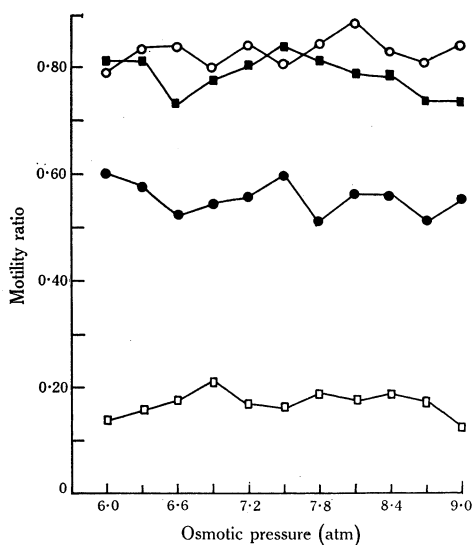


Fig. 4

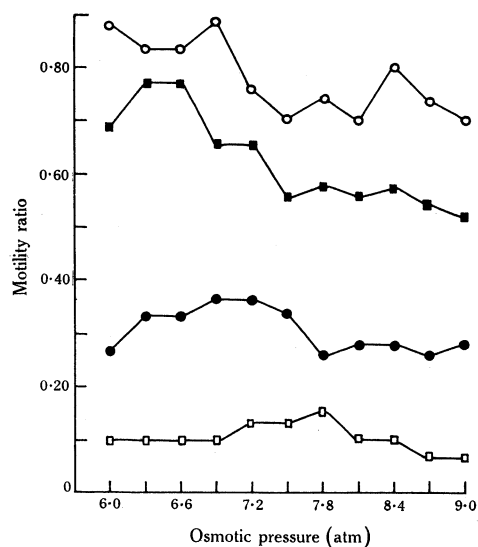


Fig. 5

Fig. 4.—Motility ratios of cold-shocked to control ram spermatozoa in semen diluted 1 : 3 in sodium citrate diluents of varying tonicity. Temperature gradients: ○ 37–15°C. ■ 37–10°C. ● 37–5°C. □ 37–0°C.

Fig. 5.—Motility ratios of cold-shocked to control ram spermatozoa in semen diluted 1 : 3 in sodium citrate diluents of varying tonicity. Temperature gradients: ○ 37–15°C. ■ 32–10°C. ● 27–5°C. □ 22–0°C.

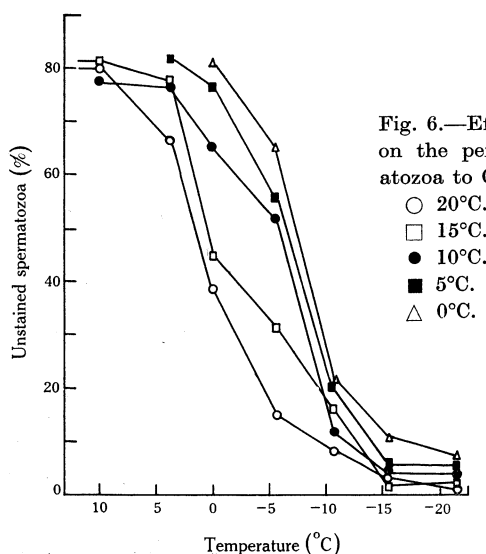


Fig. 6.—Effect of cold shock temperature range on the permeability of undiluted ram spermatozoa to Congo red stain. Initial temperature:

○ 20°C.
□ 15°C.
● 10°C.
■ 5°C.
△ 0°C.

(e) Effect on Permeability of Ram Spermatozoa of Varying Temperature Gradients over Different Temperature Ranges

This experiment was designed to characterize more accurately the temperature range and gradients required to cause permeability changes in undiluted ram spermatozoa with cold shock. The semen was slowly cooled and aliquots placed in constant-temperature baths of 20, 15, 10, 5, and 0°C. Stained smears were prepared after 10 min at these temperatures and again on thawing after sudden cooling to temperatures of 10 to -20°C.

Figure 6 shows that the temperature gradients required to produce cold shock decrease as the initial temperature decreases. At initial temperatures of 10°C, or above, a gradient of at least 20°C is required to reduce the percentage of unstained spermatozoa to 20. At 5°C the gradient is 15, and at 0°C 10.

IV. DISCUSSION

Our results on the effect of osmotic pressure on the susceptibility of spermatozoa to cold shock are in contrast to those of Ostasko (1963). When diluted ram or bull spermatozoa are rapidly cooled through a temperature gradient of 22°C, corresponding to a maximum reduction in osmotic pressure of 0.6 atm in the external medium, there is no reduction of the effects of cold shock in hypertonic diluents. Ram spermatozoa, however, were more severely shocked when undiluted semen was diluted directly in diluents held at the low temperature. Dilution and cold shock may, therefore, have a particularly harmful effect when they occur simultaneously. The possibility of a more rapid cooling of the undiluted semen cannot of course be excluded.

Our results are in agreement with those of Wales and White (1959), showing that increasing temperature gradients are progressively more harmful to spermatozoa cold-shocked from an initial temperature of 30°C. There does not, however, appear to be a strict proportionality between the severity of cold shock and the temperature gradient, as suggested by Ostasko (1963), and the data indicate that the absolute temperatures through which the semen passes are of more importance. Mizuho, Niwa, and Soejima (1963) have also shown that producing cold shock in semen through the same temperature gradient is less harmful to the metabolism and motility of spermatozoa at higher absolute temperatures.

Diluting ram spermatozoa 1 : 9 in distilled water did not cause gross structural damage. This is consistent with the observations of Miescher (1897) and Pursley and Herman (1950). There was, however, an increasing proportion of spermatozoa with coiled tails in solutions of low osmotic pressures, and recently Drevius and Eriksson (1966) have suggested that this is due to osmotic swelling of the spermatozoa which increases their volume three- to fourfold. There is some suggestion that tonicity of the stain influences the permeability of bull spermatozoa to eosin (Swanson and Bearden 1951) but no such effects were detected in ram spermatozoa stained with Congo red. Various authors have found that, in general, osmotic pressures outside the range 6-9 atm are required to depress the motility of ram or bull spermatozoa (Emmens 1947, 1948; Pursley and Herman 1950; Blackshaw and Emmens 1951;

Johnson, Flipse, and Almquist 1956; Wales and White 1958a, 1958b; Cragle and Salisbury 1959; Stevermer, First, and Hoekstra 1964). The metabolic activity of bull spermatozoa is apparently unaffected after rapid exposure to diluents of tonicities ranging from 5 to 11 atm (Cragle and Salisbury 1959).

V. ACKNOWLEDGMENTS

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