OSMOTIC SHOCK OF RAM SPERMATOZOA

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Since mammalian spermatozoa are generally considered to be less affected by hypertonic than by hypotonic solutions (see Mann, 1964) it was surprising to find that dialysis of ram semen for 6 hr in the continuous flow dialysis apparatus (CFDA) of Dott & Walton (1960) against a hypotonic diluent, did not affect the impedance change frequency (ICF) whereas a hypertonic diluent abolished the ICF within 2 hr (Quinn, White & Voglmayr, unpublished).

The most obvious difference between the CFDA and diluting semen directly in tubes is that high molecular weight substances present in the seminal plasma remain within the dialysis sac and are not further diluted. To test whether the non-dialysable components of the seminal plasma were responsible for the effect, ram semen was diluted with 10 vol. of Krebs-Ringer buffered to pH 7.4 with 50 mM sodium phosphate buffer containing 44 mM-glucose and the spermatozoa washed twice and resuspended at the original concentration. Aliquots of the washed and unwashed spermatozoa were then placed in the CFDA and dialysed against the Krebs-Ringer and an otherwise identical hypotonic diluent (167 m-osmole/l) containing only 18.5 mM-NaCl. Dialysis was continued for 6 hr at 35° C and the ICF/min measured with the impedance bridge and mechanical counter described by Martin & Wallace (1965). Aliquots of the unwashed and washed spermatozoa suspensions were also diluted 1:20 in Krebs-Ringer and hypotonic diluent and incubated for 5 hr at 35° C in specimen tubes. At 30-min intervals, motility was estimated on a warm stage at 37° C using the system of Emmens (1947) and the motility index was obtained by multiplying the motility score (0 to 4) by 4 (since quarter grades were frequently given) and summing over the incubation period. The results (Table 1) show no significant decrease in ICF when the spermatozoa were dialysed against the hypotonic diluent even after washing. The motility of spermatozoa diluted in hypotonic diluent was depressed, particularly after the cells had been washed.

Since the ICF was maintained even after prior removal of high molecular weight substances from the dialysis sac by washing, the possible importance of rate of exposure to hypotonicity was next investigated (Table 2). Aliquots of ram semen were washed in Krebs-Ringer and hypotonic diluent and dialysed for 6 hr at 35° C in the CFDA or diluted 1:20 in both diluents and incubated at 35° C for 5 hr. As in the previous experiment, spermatozoa washed in Krebs-Ringer and dialysed against the hypotonic diluent maintained a high ICF,

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but dilution in this medium depressed motility. Washing in the hypotonic diluent before dialysis greatly reduced the ICF of ram spermatozoa irrespective of the dialysis medium. Dilution in either medium after washing in the hypotonic medium caused some depression of motility but the greatest effect was obtained by diluting a hypotonic medium after washing in Krebs-Ringer.

TABLE 1
EFFECT OF HYPOTONICITY ON THE ACTIVITY OF DIALYSED OR
DILUTED SPERMATOZOA

<i>Spermatozoa</i>	<i>Diluent for dialysis or dilution</i>	<i>Mean ICF/min (dialysed)</i>	<i>Motility index (diluted)</i>
Unwashed	Krebs-Ringer	72	68
	Hypotonic	69	32*
Washed in Krebs-Ringer	Krebs-Ringer	58	68
	Hypotonic	83	11**

Mean values are given for four replicates.

* $P < 0.05$; ** $P < 0.01$. Significantly less than unwashed spermatozoa diluted in Krebs-Ringer.

To confirm that these differences were due to rapid exposure to hypotonicity, rat semen was diluted immediately 1:20 in Krebs-Ringer and hypotonic diluent and the motility during 5 hr at 35° C compared with semen diluted gradually over 30 min and subsequently incubated. The motility index of spermatozoa diluted rapidly in Krebs-Ringer and the hypotonic diluent was 54 and

TABLE 2
THE EFFECT OF RAPID EXPOSURE OF RAM SPERMATOZOA
TO HYPOTONICITY

<i>Washing diluent</i>	<i>Diluent for dialysis or dilution</i>	<i>Mean ICF/min (dialysed)</i>	<i>Motility index (diluted)</i>
Krebs-Ringer	Krebs-Ringer	72	66
	Hypotonic	67	12**
Hypotonic	Krebs-Ringer	13**	51
	Hypotonic	8**	47*

Mean values are given for four replicates.

* $P < 0.05$; ** $P < 0.01$. Significantly less than spermatozoa washed and subsequently dialysed or diluted in Krebs-Ringer.

21, respectively, and for slow dilution the motility index was 54 for both diluents (three replicates). Thus a gradual exposure of ram semen to hypotonicity completely abolished the detrimental effect of rapid dilution.

The final experiment was undertaken to determine the rate at which osmotic shock occurs in spermatozoa and to investigate whether it is reversible. Ram semen was diluted 1:20 in distilled water and after 5, 10, 20 and 40 sec the medium was made isotonic by adding concentrated diluent. The results showed

that the detrimental effect on motility was almost instantaneous in onset and irreversible. We were also unable to restore the motility of spermatozoa incubated in the hypotonic diluent in Table 2. This is in contrast to the observations of Drevius & Eriksson (1966) who reported that 'flagellation' of bull spermatozoa diluted in hypotonic salt solution recommenced as the tonicity of the medium increased. Our motility observations of ram spermatozoa exposed gradually to a hypotonic medium are also inconsistent with any confinement of the tail within a vesicle formed by an osmotically induced swelling of the plasma membrane.

Our results show that the adverse effect of hypotonicity on the motility of ram spermatozoa only occurs when they are exposed rapidly and good motility can be maintained at low toxicities when care is taken to avoid osmotic shock.

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REFERENCES

- DOTT, H. M. & WALTON, A. (1960) Effects of dilution and washing on ram spermatozoa studied by the flow dialysis technique. *J. Reprod. Fert.* **1**, 350.
- DREVIUS, L.-O. & ERIKSSON, H. (1966) Osmotic swelling of mammalian spermatozoa. *Expl Cell Res.* **42**, 136.
- EMMENS, C. W. (1947) The motility and viability of rabbit spermatozoa at different hydrogen ion concentrations. *J. Physiol., Lond.* **106**, 471.
- MANN, T. (1964) *The biochemistry of semen and of the male reproductive tract*. Methuen, London.
- MARTIN, I. C. A. & WALLACE, C. S. (1965) Impedance change frequency of diluted ram semen recorded on a digital scaler. *J. Reprod. Fert.* **10**, 425.