The action of D₂O on the motility of human spermatozoa

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Summary. The effect of 25%, 50%, 75% and 100% D₂O on the survival of human spermatozoa was investigated. More spermatozoa were affected and died more quickly as the concentrations of D₂O increased. Spermatozoa died instantly in 100% D₂O.

Toxic effects of high concentrations of deuterium in mammals and higher plants were studied by Lewis (1934). Moderate levels of deuteration are well tolerated for considerable lengths of time (Katz, Crespi, Hasterlik, Thomson & Finkal, 1957; Bachner, Mackay & Rittenberg, 1964), but it is known that D₂O may act as a male sterilant in mice (see Hughes, Bennett & Calvin, 1959, 1960) and Amarose & Czajka (1962) reported that mice that had drunk D₂O became aspermic when the D₂O content in their body fluids reached 30%.

Rostand (1935) described that spermatozoa of *Rana temporaria* retain their motility and fertilizability in concentrations of D₂O as high as 99% for as long as 12-22 hr. Niedetzky & Dalnoki (1970) reported that 80% of frog spermatozoa remained alive even after 22 hr when suspended in a medium containing 100% D₂O, and 28% of the spermatozoa retained motility even after 120 hr in D₂O.

The present studies were undertaken in the absence of much meaningful data pertaining to the effect of D₂O on the viability and survival of mammalian spermatozoa.

The survival characteristics of human spermatozoa were studied *in vitro* by suspending the spermatozoa in Ringer-Tyrode solution (NaCl, 0.8 g; KCl, 0.02 g; CaCl₂, 0.02 g; NaHCO₃, 0.1 g; NaH₂PO₄, 0.005 g; MgCl₂, 0.01 g; glucose, 0.1 g and 100 ml twice distilled water). This served as the control. Four concentrations of D₂O (25%, 50% and 75% in Ringer-Tyrode solution and 100%) were prepared for experimental studies. Only those samples of semen which showed more than 60% motile spermatozoa were used for the present studies. Semen samples were centrifuged at 3000 rev/min for 10 min. Equal amounts of the concentrated sperm fractions were suspended in 2 ml of the different fluids.

The numbers of motile spermatozoa were counted with a haemocytometer at the start of the experiment, after 15 min, and then every hour. Ten experiments were performed for each D₂O concentration studied and the average values were determined for all the time intervals. The data were evaluated statistically by Student's *t* test. The number of motile spermatozoa decreased as the concentration of D₂O and the time increased (Table 1). All spermatozoa were instantaneously killed when suspended in 100% D₂O. Less than 10% of the spermatozoa in 75% D₂O were motile

Table 1. The percentages of motile human spermatozoa at various times and in different D₂O concentrations

Time (hr)	D ₂ O concentration			
	Control	25%	50%	75%
0	69.43 ± 9.95	69.43 ± 9.95	69.43 ± 9.95	69.43 ± 9.95
1	67.84 ± 7.75	60.09 ± 7.45	49.14 ± 6.95	10.10 ± 6.15
2	62.24 ± 6.90	50.29 ± 5.45	38.26 ± 7.05	8.40 ± 3.50
3	51.40 ± 8.15	42.90 ± 11.45	32.03 ± 7.85	5.50 ± 2.74
4	42.60 ± 11.71	34.46 ± 10.75	26.75 ± 7.80	2.98 ± 2.20
5	34.74 ± 10.55	29.43 ± 9.30	20.57 ± 12.15	1.08 ± 0.95
6	30.74 ± 8.80	24.97 ± 7.85	14.75 ± 7.15	0.30 ± 0.45
7	25.69 ± 5.85	20.10 ± 6.80	7.89 ± 5.09	zero

after 1 hr, but some motility was seen in the other D₂O concentrations even after 6 hr. All the values differed significantly from controls ($P < 0.01$). The present data on human spermatozoa do not, however, accord with the results of Niedetzky & Dalnoki (1970) for frog spermatozoa.

It is well known that hydrogen bonds play an important role in the structure and function of various biopolymers. The replacement of the hydrogen bonds by deuterium results in alteration of the bond strengths so important for proper functioning and stability of biopolymers (see Rabinowitz, Lafair, Strauss & Allen, 1958; Ernst, 1970).

The diffusion coefficient of D₂O is at least 10% less than that of water; the mobility of ions in D₂O media, therefore, is slower than in water and thus could affect the potential differences across the cell membranes (Peng, Ho & Taylor, 1970). Delay in transporting molecules from one intracellular point to another could have serious effects on the metabolism of the cell and endanger its survival.

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