STUDIES ON THE ALKALI METAL REQUIREMENTS OF RAM AND BULL SPERMATOZOA

By I. G. White*

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Summary

Rubidium was as effective as potassium in maintaining the motility of ram and bull spermatozoa washed four times in a sodium phosphate-fructose diluent. Caesium was less active, ammonium ions were ineffective, and lithium was toxic.

The motility of unwashed ram and bull spermatozoa was better maintained in a diluent with sole cation sodium than with potassium. However, the addition of sodium ions did not significantly increase the motility of ram and bull spermatozoa washed four times in the potassium diluent.

Neither 10^{-2}M acetylcholine nor 10^{-5}M physostigmine, singly or in combination, significantly modified the motility of ram and bull spermatozoa washed in the sodium diluent.

Desoxycorticosterone acetate (1 μg/ml) depressed the motility of ram spermatozoa washed in both potassium-free and potassium-containing diluents, but had no significant effect on bull spermatozoa.

I. Introduction

In previous studies it was found that the motility and glycolysis of ram and bull spermatozoa were depressed after repeated washing in a sodium phosphate-fructose diluent (White 1953*a*, 1953*b*). Potassium loss from the spermatozoa was apparently important, as the addition of 0.004M KCl to the diluent almost completely restored the motility and glycolysis of washed ram spermatozoa and increased that of washed bull spermatozoa. Lardy and Phillips (1943), working with bull spermatozoa, have noted a similar stimulation of glycolysis by potassium and the effect on motility has been corroborated for the spermatozoa of both species by Blackshaw (1953*a*, 1953*b*).

Lithium has been shown to depress the motility and glycolysis of human spermatozoa (Macleod, Swan, and Aitken 1949). No studies seem to have been made on the effect of rubidium or caesium on spermatozoa. The ability of rubidium to replace potassium, however, is apparently a general biological phenomenon (e.g. Macleod and Snell 1948; Gallego and Lorente de Nó 1947; Pressman and Lardy 1952).

Ludwig, Greig, and Peterson (1951) have studied the loss of potassium from human erythrocytes suspended in isotonic sodium bicarbonate and claim that it is reduced by acetylcholine and increased by physostigmine, suggesting that intracellular potassium levels are dependent upon cholinesterase activity. If a similar mechanism operates in mammalian spermatozoa the addition of

* Department of Veterinary Physiology, University of Sydney.

acetylcholine and physostigmine might be expected to modify the motility of spermatozoa washed in a potassium-free diluent.

Desoxycorticosterone has a marked effect on tissue potassium *in vivo* (Overman 1951). Little is known about its action at the cellular level; it would seem, however, to modify the permeability of cells, particularly those of the renal tubules. Desoxycorticosterone might therefore be expected also to influence the response of spermatozoa to repeated washing since the permeability of the cells to potassium is clearly involved.

Further studies on the alkali metal requirements of ram and bull spermatozoa are presented in this paper.

II. Materials and Methods

Bull semen was collected by means of the artificial vagina, and ram semen by electrical stimulation as described by Gunn (1936). Only normal ejaculates of good motility were employed.

Suspensions were prepared by diluting 0.5-1.0 ml of semen 1 in 10, and the spermatozoa were washed by spinning this suspension four times at 1500 r.p.m. (about 300*g*) for 10 min.

Spermatozoa suspensions were incubated at 37°C for 4 hr. Motility was scored at hourly intervals by the system of Emmens (1947). In the analyses of variance the sum of the hourly figures has been used as unit observation and the interaction mean square as the error term.

The sodium- and potassium-fructose-phosphate diluents were isotonic and had a pH of 7.0. They were of the following composition:

Sodium diluent: 0.032M $NaH_2PO_4.H_2O$, 0.048M $Na_2HPO_4.12H_2O$, 0.040M NaCl, 0.022M fructose.

Potassium diluent: 0.032M KH_2PO_4, 0.048M K_2HPO_4, 0.040M KCl, 0.022M fructose.

Additional substances were added in concentrations indicated in the tables.

All diluents were freshly prepared each day by appropriate dilution of 0.4M stock solutions of the A.R. salts with glass-distilled water, solid fructose being added to give a concentration of 0.4 per cent (w/v).

III. Results

In view of the action of potassium in restoring the motility of washed ram and bull spermatozoa, it was of interest to compare the effectiveness of the other alkali metals. Ammonium ions were also included in these tests, as in some biological systems they are known to substitute for potassium (Boyer, Lardy, and Phillips 1942, 1943; Muntz 1947). The effects of 0.004M potassium, rubidium, caesium, lithium, and ammonia on the motility of washed ram and bull spermatozoa are given in Table 1. Four ejaculates were used with each.

The results were subjected to analysis of variance and the results are summarized in Table 2. It is clear from the data and the analyses of variance that potassium, rubidium, and caesium considerably increased the motility score.

Lithium, on the other hand, was toxic whilst ammonia had no significant effect. Rubidium was as good as, or better than, potassium but caesium was not as effective. In general, ram and bull spermatozoa reacted similarly.

TABLE 1

EFFECT OF 0·004M ALKALI METAL AND AMMONIUM IONS ON THE TOTAL MOTILITY SCORE OF RAM AND BULL SPERMATOZOA WASHED FOUR TIMES IN A SODIUM PHOSPHATE-FRUCTOSE DILUENT

Species	Ejaculate	Ion					
		A Nil	*B* Lithium (LiCl)	*C* Potassium (KCl)	*D* Rubidium (RbCl)	*E* Caesium (CsCl)	*F* Ammonium (NH_4OH)
Ram	1	8·00	5·50	15·00	14·50	8·25	5·75
	2	14·25	10·00	20·00	20·00	17·25	12·75
	3	5·50	3·75	15·25	15·50	10·00	7·25
	4	6·50	4·50	16·00	17·25	8·25	5·50
	Mean	8·50	6·50	16·50	16·75	11·00	7·75
Bull	1	9·00	0·50	14·50	14·75	14·25	9·00
	2	4·75	1·50	9·25	9·75	6·75	10·25
	3	5·25	1·50	8·75	9·00	5·75	3·25
	4	6·75	1·25	8·75	10·00	7·50	5·75
	Mean	6·50	1·25	10·25	11·50	8·50	7·00

TABLE 2

ANALYSIS OF VARIANCE FOR THE DATA IN TABLE 1

Source of Variation	Degrees of Freedom		Variance Ratios			
			Ram		Bull	
Between treatments	5		54·5**		13·4**	
Effect of lithium (*A v. B*)		1		17·6**		29·9**
Effect of potassium (*A v. C*)		1		163·8**		16·3**
Effect of rubidium (*A v. D*)		1		174·2**		21·4**
Effect of caesium (*A v. E*)		1		14·4**		4·9*
Effect of ammonium (*A v. F*)		1		1·5		0·4
Between ejaculates	3		36·2		6·8	
Interaction (error)	15		25		59	

* $P<0·05$.
** $P<0·01$.

The control diluent used in the previous experiment for demonstrating the effect of potassium and the rare alkali metals contained sodium as the only

cation. To test whether sodium itself was important in the functioning of mammalian spermatozoa, motility comparisons were made between spermatozoa washed in:

TABLE 3

EFFECT OF 0·004M NaCl ON TOTAL MOTILITY SCORE OF SPERMATOZOA WASHED FOUR TIMES IN POTASSIUM-FRUCTOSE-PHOSPHATE DILUENT, AND COMPARISON OF THIS DILUENT WITH ONE HAVING Na^+ AS SOLE CATION

Species	Ejaculate	Treatment			
		A. Washed in Potassium Diluent	*B.* Washed in Potassium Diluent+0·004M NaCl	*C.* Unwashed in Sodium Diluent	*D.* Unwashed in Potassium Diluent
Ram	1	7·25	8·00	9·75	3·25
	2	9·25	9·00	16·25	6·50
	3	13·75	13·75	18·75	11·25
	4	13·25	13·75	15·50	10·00
	Mean	11·00	11·25	15·00	7·75
Bull	1	4·00	5·75	17·75	9·25
	2	4·50	6·25	20·00	9·25
	3	3·75	4·00	14·75	3·75
	4	4·50	5·25	19·50	5·50
	Mean	4·25	5·25	18·00	7·00

TABLE 4

ANALYSIS OF VARIANCE FOR THE DATA IN TABLE 3

Source of Variation	Degrees of Freedom		Variance Ratios			
			Ram		Bull	
Between treatments	3		25·0**		86·3**	
A v. B		1		0·1		1·4
C v. D		1		74·4**		130·5**
$(A+B)$ *v.* $(C+D)$		1		0·5		127·1**
Between ejaculates	3		29·5**		4·6*	
Interaction	9		23		30	

* $P<0·05$.
** $P<0·01$.

(*a*) A sodium-free diluent in which potassium was the only cation; (*b*) The same diluent with 0.004M NaCl added. Motility observations were simultaneously made on unwashed spermatozoa suspended in solutions containing (*c*)

sodium and (*d*) potassium as sole cations, to see which was the more effective diluent.

TABLE 5

TOTAL MOTILITY SCORES OF SPERMATOZOA WASHED FOUR TIMES IN SODIUM-FRUCTOSE-PHOSPHATE DILUENT, 0·01M ACETYLCHOLINE AND 10^{-5}M PHYSOSTIGMINE ADDED SINGLY AND IN COMBINATION (EFFECT OF 0·004M KCl FOR COMPARISON)

Species	Ejaculate	Substances Added				
		A. Nil	*B.* Acetyl-choline	*C.* Physostig-mine	*D.* Acetyl-choline + Physostigmine	*E.* Potassium Chloride
Ram	1	17·50	16·00	18·50	16·75	20·00
	2	10·50	9·50	10·00	9·25	16·25
	3	14·00	11·50	19·00	15·75	20·00
	4	5·25	4·25	5·50	5·25	17·50
	Mean	11·75	10·25	13·25	11·75	18·50
Bull	1	4·50	3·00	3·25	2·75	5·50
	2	3·75	5·75	5·25	3·50	5·50
	3	3·25	3·00	2·25	6·50	10·75
	4	3·25	3·00	1·25	4·25	7·00
	Mean	3·25	3·75	3·00	4·25	7·25

TABLE 6

ANALYSIS OF VARIANCE FOR THE DATA IN TABLE 5

Source of Variation	Degrees of Freedom		Variance Ratios			
			Ram		Bull	
Between treatments	4		8·2**		3·8*	
A v. B		1		0·9		0·1
A v. C		1		0·9		0·0
A v. D		1		0·0		0·7
A v. E		1		8·0**		10·3**
Between ejaculates	3		22·3**		1·1	
Interaction	12		78		48	

* $P<0·05$.
** $P<0·01$.

The results of tests on four ram and four bull ejaculates are set out in Table 3, and the analyses of variance are summarized in Table 4. The motility of unwashed ram and bull spermatozoa was better maintained in a diluent with the sole cation sodium than with potassium. However, the addition of

0.004M NaCl to the sodium-free diluent did not significantly increase the motility score of the washed spermatozoa.

Table 5 gives the results of experiments on four ram and four bull ejaculates, designed to test whether acetylcholine or physostigmine, singly or in combination, affect the motility of spermatozoa washed in a potassium-free diluent. A potassium-containing diluent was included in each experiment for comparison. The analyses of variance for both sets of data are summarized in Table 6. The beneficial effect of potassium is shown by the highly significant difference between diluents *a* and *b*. However, neither 10^{-2}M acetylcholine nor 10^{-5}M physostigmine, singly or in combination, significantly modified the motility score of either the ram or bull spermatozoa.

TABLE 7

EFFECT OF 0·004M KCl AND DESOXYCORTICOSTERONE ACETATE (1 μg/ml) ON MOTILITY OF SPERMATOZOA WASHED FOUR TIMES IN A SODIUM PHOSPHATE-FRUCTOSE DILUENT

Species	Ejaculate	Substances Added			
		A. Nil	*B.* Desoxycorticosterone Acetate	*C.* Potassium Chloride	*D.* Potassium Chloride+Desoxycorticosterone Acetate
Ram	1	7·00	6·25	15·00	9·25
	2	6·00	7·00	13·50	8·50
	3	11·00	3·00	15·00	4·25
	4	12·50	2·75	15·50	4·75
	Mean	9·25	4·75	9·25	6·75
Bull	1	7·00	2·25	12·75	3·50
	2	8·75	10·00	14·50	13·75
	3	4·00	3·25	7·75	7·00
	4	5·00	3·50	9·75	9·25
	Mean	6·25	4·75	11·25	8·50

Table 7 shows the motility scores of ram and bull spermatozoa washed four times in sodium and sodium-potassium diluents, with and without the addition of desoxycorticosterone acetate at a concentration of 1 μg/ml. These experiments, which were replicated on four ejaculates from each species, were of factorial design and were planned to demonstrate the effects of potassium and desoxycorticosterone and any interaction between them. Table 8 summarizes the analyses of variance for each species. The motility of both ram and bull spermatozoa was again much better maintained in the potassium-containing diluent ($P < 0.01$). Desoxycorticosterone, on the other hand, depressed the motility of the ram spermatozoa ($P < 0.01$) but had no significant effect on the bull spermatozoa. There was no evidence of any interaction between potassium and desoxycorticosterone in either species.

IV. Discussion

The studies of the motility of washed ram and bull spermatozoa (Table 1) show an interesting gradation in the biological properties of the alkali metals which can be readily correlated with their sequence in the periodic table. The efficiency of the alkali metals in these experiments increased with increasing atomic number, reached an optimum with potassium and rubidium, and fell off again with caesium.

Table 8

ANALYSIS OF VARIANCE FOR THE DATA IN TABLE 7

Source of Variation	Degrees of Freedom		Variance Ratios			
			Ram		Bull	
Between treatments	3		10·7**		7·3**	
Effect of potassium		1		8·2**		17·2**
Effect of desoxycorticosterone		1		22·1**		4·2**
P/D interaction		1		1·9		0·4
Between ejaculates	3		0·1		7·3**	
Residual (error)	9		112		69	

** $P<0·01$.

Boyer, Lardy, and Phillips (1942, 1943) have shown that potassium is necessary for the conversion of phosphopyruvate to pyruvate by muscle extracts and more recently Muntz (1947) found that it also participates in the phosphokinase reaction of yeast juice. Both reactions are involved in the glycolytic cycle and might be the point at which potassium and other alkali metals affect ram and bull spermatozoa. It should be noted, however, that ammonium ions stimulated the phosphoenolpyruvate and the hexosephosphate reactions to about the same extent, whereas in the experiments reported here, ammonia had no significant effect, while potassium was highly beneficial (Tables 1 and 2). Intact spermatozoa were employed in the present tests, and it is not known with any certainty that ammonium ions are able to penetrate into these cells.

Although the experiments on washed ram and bull spermatozoa reported here and previously (Lardy and Phillips 1943; White 1953*a*, 1953*b*; Blackshaw 1953*a*, 1953*b*) clearly establish that potassium is necessary for their normal functioning, it is obvious from Table 3 that high concentrations of potassium are harmful. The adverse effect of the diluent with potassium the only cation must have been due to the toxicity of potassium rather than the absence of sodium, since the addition of 0.004M sodium chloride to the washed ram and bull spermatozoa did not significantly improve motility. It might be inferred from these experiments that sodium *per se* is not important for ram and bull

spermatozoa, although the inherent toxicity of the potassium diluent may have masked any stimulating effect of the sodium ions.

The motility of ram and bull spermatozoa washed in the sodium diluent was neither improved by acetylcholine nor depressed by physostigmine (Table 5), which suggests that there is no connection between cholinesterase activity and the maintenance of potassium levels in these spermatozoa.

No studies *in vitro* seem to have been made of the effect of desoxycorticosterone on cell permeability. Observations *in vivo,* however, suggest that it decreases the permeability of the renal tubules to potassium and increases that of the tissues generally. If it increased the permeability of spermatozoa to potassium, it might be expected to decrease their motility after washing in a potassium-free diluent. Such an effect on motility is seen with ram spermatozoa in Table 7. However, the motility score was not improved by the addition of potassium to the diluent containing desoxycorticosterone, which makes it unlikely that the toxicity of the hormone was due specifically to an alteration in permeability to potassium.

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