Active Cation Transport in Dog Spermatozoa

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1. When dog semen is stored at 5° for 24 hr., K⁺ is lost from the spermatozoa and Na⁺ accumulates in the cells. 2. If at the end of the cold-storage period the semen is incubated at 37° in the presence of added glucose there is a rapid uptake of K⁺ and extrusion of Na⁺ from the spermatozoa, the intracellular K⁺ reaching a maximum within 30 min. 3. When the semen is incubated at 20° after cold storage there is an uptake of K⁺ by the spermatozoa over 3 hr. but no change in intracellular Na⁺ concentration. 4. The extrusion of Na⁺ and uptake of K⁺ by dog spermatozoa has been shown to be inhibited by fluoride, iodoacetate, 2,4-dinitrophenol, and cetyltrimethylammonium bromide. 5. Uptake of K⁺ is inhibited by ouabain and half maximum inhibition is obtained with a concentration of 50 m μ M. There is a slight stimulation of K⁺ uptake in the presence of ouabain at about 0.3% of the concentration required for half maximum inhibition.

In a previous study of the distribution of the cations in the semen of the dog, sheep, cow, rabbit, fowl and man, the Na⁺ concentration was found to be lower in the spermatozoa than in the seminal plasma whereas, in general, the K⁺, Mg²⁺ and Ca²⁺ concentrations were higher than in the seminal plasma (Quinn, White & Wirrick, 1965). The distribution of Na⁺ and K⁺ between the cellular and fluid fractions of semen and blood are similar (Quinn & White, 1967) and suggest that a sodium pump may operate in spermatozoa similar to that shown to occur in human and rabbit erythrocytes (Glynn, 1956; Rummel, Pfleger & Seifen, 1961).

Dog semen was chosen for the present investigation of the active transport of cations by spermatozoa, since of all the species studied dog semen has the greatest gradient of K⁺ and Na⁺ between the spermatozoa and seminal plasma (Quinn *et al.* 1965).

MATERIALS AND METHODS

Semen from five mongrel dogs was collected in three fractions by manual manipulation. The pre-sperm fraction was discarded and the sperm-rich and prostatic-fluid fractions were each pooled. The pooled sperm-rich fraction was cooled slowly to avoid cold 'shock' (Wales & White, 1959) by placing the tube containing the semen in a large beaker of water at 25° in a cold room at 5°. After 24 hr. the sperm-rich fraction was diluted with an equal volume of prostatic fluid that contained 5.6 mM-glucose (except in part of the second experiment) plus any inhibitors to be tested. Cation analyses were made on the spermatozoa before and after incubation at 37° or 20°. An equal volume of prostatic fluid containing 5.6 mM-glucose was also added to a sample

of fresh sperm-rich fraction to provide a control in the first experiment.

The method of separating spermatozoa from the supernatant and of estimating Na⁺, K⁺, Ca²⁺ and Mg²⁺ in the spermatozoa by atomic absorption spectroscopy is described by Quinn *et al.* (1965). Cation concentrations are expressed as m-equiv./kg. wet wt. of spermatozoal mass after centrifuging. The statistical significance of the results was assessed by analysis of variance; treatment comparisons were made by *t* tests by using the standard error of the difference between means (S.E.M.) derived from the residual mean square.

RESULTS

Effect of cold storage and subsequent incubation temperature on cation movements. Storing dog spermatozoa for 24 hr. at 5° caused an influx of Na⁺ and an efflux of K⁺ from the cells (Table 1, Expt. 1). Cold storage also caused a slight increase in the concentration of Ca²⁺ and a decrease in the concentration of Mg²⁺ in the spermatozoa. During subsequent incubation for 3 hr. at 37° in the presence of 2.8 mM-glucose there was a reaccumulation of K⁺ in the spermatozoa and a slight extrusion of Na⁺ (Table 1, Expt. 1). The rate of movement of the cations was determined by analysing the spermatozoa after incubation for 15, 30, 60 and 120 min. at 20° and 37° in the presence of 2.8 mM-glucose (Fig. 1). At 37° K⁺ uptake and Na⁺ extrusion occurred mainly within the first 15 min. of incubation and reached a maximum at 30 min. At 20° there was a slower but fairly steady uptake of K⁺ during the 3 hr. period but no net extrusion of Na⁺.

After incubation at 20° or 37° the motility of the

Table 1. *Effect of cold storage and subsequent incubation on the concentration of cations in dog spermatozoa*

Semen was stored for 24 hr. at 5° and subsequently incubated for 3 hr. at 37°. In Expt. 1, all tubes contained 2.8 mM-glucose. In Expt. 2, oxygen was bubbled slowly through the aerobic tubes; evacuated Thunberg tubes containing yellow phosphorus were used for anaerobic incubation. Values are expressed as m-equiv./kg. and are the means of four observations. S.E.M.: Standard error of the difference between means.

	Treatment	Concn. (m-equiv./kg.)			
		Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺
Expt. 1	Fresh	73.5*	43.1**	0.90*	8.70*
	After cold storage	107.0	12.3	2.60	4.96
	After subsequent incubation	89.1	27.2*	2.15	5.91
	S.E.M.	9.0	6.1	0.65	1.25
Expt. 2	After cold storage	111.7	9.6	1.6	10.8
	After subsequent incubation:				
	Glucose, aerobic	78.3*	20.5**	2.3	8.8**
	No glucose, aerobic	88.7	16.9**	3.5	9.4*
	Glucose, anaerobic	82.6*	22.0**	2.9	10.7
	No glucose, anaerobic	121.3	9.4	4.2*	10.3
	S.E.M.	11.5	1.4	1.1	0.5

* $P < 0.05$; ** $P < 0.01$ (significantly different from value after cold storage).

Fig. 1. Changes in the concentration of Na⁺ (—) and K⁺ (----) in dog spermatozoa that had been held in cold storage for 24 hr. and subsequently incubated at 37° (○) and 20° (●) in the presence of 2.8 mM-glucose.

spermatozoa was good and their viability, determined by the percentage of spermatozoa permeable to Congo red (Wales, 1959), was comparable with their viability in fresh semen.

Effect of substrate and oxygen. As glucose was normally added to the semen before incubation, the effect of its omission was studied under aerobic and anaerobic conditions. After 24 hr. at 5° all semen samples in this series were incubated at 37° for 3 hr. and the concentrations of the cations in the spermatozoa were determined (Table 2, Expt. 2). In the presence of glucose there was a significant

accumulation of K⁺ and an efflux of Na⁺ from the spermatozoa under both aerobic and anaerobic conditions. In the absence of glucose there was an influx of K⁺ under aerobic conditions but not under anaerobic conditions. In the absence of both glucose and oxygen, Ca²⁺ accumulated in the spermatozoa against a concentration gradient. There was an efflux of Mg²⁺ when oxygen was present but not in its absence.

Effect of metabolic inhibitors. After 24 hr. at 5°, dog semen was incubated for 1 hr. at 37° in the presence of metabolic inhibitors (sodium fluoride, iodoacetic acid), 2,4-dinitrophenol and the detergent cetyltrimethylammonium bromide. The K⁺ and Na⁺ concentrations of the spermatozoa are given in Table 2 (Expt. 1). Fluoride inhibited the uptake of K⁺ by the spermatozoa. There was also a loss of K⁺ when the spermatozoa were treated with cetyltrimethylammonium bromide. All of the inhibitors and the detergent inhibited the extrusion of Na⁺.

Dog spermatozoa that had been kept in cold storage for 24 hr. were incubated in the presence of ouabain in concentrations from 1 μM to 1 μμM (Table 2, Expt. 2). There was an increasing inhibition of K⁺ uptake with ouabain concentrations between 100 μμM and 1 μμM; inhibition was half maximal at 50 μμM. The movement of Na⁺ was not significantly affected by ouabain.

DISCUSSION

There is an efflux of K⁺ from dog spermatozoa during cold storage, and about half can be regained

Table 2. *Effect of metabolic inhibitors, cetyltrimethylammonium bromide and ouabain on the concentrations of Na⁺ and K⁺ in dog spermatozoa incubated after cold storage*

Semen was stored for 24 hr. at 5° and subsequently incubated for 1 hr. at 37° in the presence of 2.8 mM-glucose. The values are expressed as m-equiv./kg. and are the means of the numbers of observations given in parentheses. S.E.M.: Standard error of the difference between means.

	Treatment	Concn. (m-equiv./kg.)	
		Na ⁺	K ⁺
Expt. 1 (4)	Before incubation	119.1	9.4
	After incubation:		
	Control	67.8*	19.9**
	NaF (40 mM)	159.1	7.9
	Iodoacetic acid (0.5 mM)	87.0	15.6**
	2,4-Dinitrophenol (10 μ M)	83.0	15.8**
	Cetyltrimethylammonium bromide (2.5 mM)	95.6	6.0*
	S.E.M.	20.1	1.2
Expt. 2 (3)	Before incubation	97.8	12.6
	After incubation:		
	Control	84.8	25.9**
	Ouabain (1 μ M)	87.4	28.5**
	Ouabain (100 μ M)	78.3	22.3**
	Ouabain (10 mM)	98.3	20.3*
	Ouabain (1 μ M)	103.9	14.9
	S.E.M.	15.3	2.1

* $P < 0.05$; ** $P < 0.01$ (significantly different from value before incubation).

when the cells are warmed. The accumulation of K⁺ is accompanied by the passage of Na⁺ in the reverse direction, but this is more variable and often less than the movement of K⁺.

The reciprocal movement of K⁺ and Na⁺ in cells incubated at 37° suggests that the movements are linked, as in erythrocytes and the toad bladder (Glynn, 1954, 1956; Koefoed-Johnsen & Ussing, 1958). The accumulation of K⁺ at 20° with no net outward movement of Na⁺ may arise from a situation where the passive influx of Na⁺ exceeds its active extrusion, since at this temperature metabolic activity would be diminished. Swanson (1966) has, however, reported a decreasing sensitivity of a cation-activated adenosine triphosphatase to Na⁺ with decreasing temperature between 37° and 5°. He suggested that an alteration in the configuration of the enzyme at low temperature is responsible for the effect.

The results suggest that Na⁺ and K⁺ gradients can be restored either by glycolytic activity or by respiratory activity in the spermatozoa. Although dog semen does not contain hexose the spermatozoa can metabolize glucose (Bartlett, 1958, 1962; Scott, White & Annison, 1962; Wales & White, 1965), and sugar must be added to cold-stored dog semen if K⁺ and Na⁺ gradients are to be restored under anaerobic conditions. Under aerobic conditions dog spermatozoa can presumably utilize

energy derived from other substrates in the semen for the transport of cations and it is not essential to add glucose to the medium. The identity of the naturally occurring substrate in dog prostatic fluid is in some doubt (Wales, 1965), but studies of the endogenous metabolism of ram spermatozoa suggest that the intracellular material is probably plasmalogen (Hartree & Mann, 1961).

The inhibition of K⁺ and Na⁺ movements in dog spermatozoa by fluoride, iodoacetic acid and 2,4-dinitrophenol provides further evidence that the transport of these ions is dependent on the metabolism of the cell. Fluoride completely inhibits the uptake of K⁺, but in the presence of 0.5 mM-iodoacetate the tricarboxylic acid cycle can presumably supply sufficient energy for the reaccumulation of much of this cation. Although 2,4-dinitrophenol caused a significant decrease in Na⁺ extrusion by dog spermatozoa, concentrations of 1 mM apparently had no effect on the Na⁺-K⁺-carrier-mediating system of erythrocytes (Glynn, 1963; Skou, 1963) and had little effect on the motility of dog spermatozoa (Gonse, 1962). The detergent cetyltrimethylammonium bromide would be expected to cause destruction of the spermatozoal membranes, resulting in loss of K⁺ to the medium.

Cardiac glycosides such as ouabain are specific inhibitors of cation transport (Glynn, 1957; Whittam, 1958) and there is a close correlation

between their inhibitory effects and the Na^+ -plus- K^+ -activated adenosine triphosphatase (Post, Merrit, Kinsolving & Albright, 1960; Dunham & Glynn, 1961; Bonting & Caravaggio, 1963). The slight (5%) increase in the uptake of K^+ by dog spermatozoa in the presence of ouabain in very low concentration ($1\mu\text{M}$) is in parallel with the effects of erythrophleum alkaloids on K^+ transport in erythrocytes (Kahn, 1962a,b) and of cardiac glycosides on the Na^+ -plus- K^+ -activated adenosine triphosphatase from other tissues (Repke, 1961; Lee & Yu, 1963; Bonting, Hawkins & Canady, 1964). As might be expected from the results of these workers, the stimulating concentration of the cardiac glycoside is approx. 0.3% of the concentration required for half maximum inhibition of K^+ uptake by the spermatozoa.

The increase in the Ca^{2+} concentration of dog spermatozoa during cold storage and its further increase when the spermatozoa are incubated anaerobically in the absence of glucose can be explained in terms of an active metabolic-dependent extrusion of Ca^{2+} similar to that reported to occur in liver slices (Judah & Ahmed, 1964). It is necessary to assume, however, that most of the Ca^{2+} that occurs in high concentrations in dog spermatozoa is bound whereas that in the plasma is free, for which there is some evidence in other tissues (Wallach, Reizenstein & Bellavia, 1966).

The decrease in the Mg^{2+} concentration of dog spermatozoa during storage at 5° suggests that, like K^+ , the transport of Mg^{2+} is dependent on the metabolism of the cell. Subsequent incubation of the spermatozoa at 37° in the presence of glucose did not, however, always cause an increase in Mg^{2+} concentration.

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