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DISTRIBUTION OF ADENOSINETRIPHOSPHATASE ACTIVITY IN RAM AND BULL SPERMATOZOA

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The active transport of sodium and potassium has been demonstrated in dog spermatozoa (Quinn & White, 1967) and the existence of a cation pump, involving a sodium–potassium activated ATPase has been postulated to account for the gradients of alkali metal ions between the cell and seminal plasma (Quinn, White & Wirrick, 1965). Recently, Uesugi & Yamazoe (1966) have reported the occurrence of a sodium–potassium activated ATPase in boar epididymal spermatozoa, and the present experiments were undertaken to determine the activity and distribution of ATPases in ram and bull spermatozoa.

Semen was diluted with 2·5 vol of 250 mM-sucrose, centrifuged, and the spermatozoa washed twice with 5-ml aliquots of sucrose. Washed spermatozoa, resuspended in sucrose buffered to pH 7·4 (37° C) with 50 mM-tris (tris [hydroxylmethyl] amino methane) and 40 mM-HCl were incubated with 3 mM ATP which was rendered free of cations by eluting the disodium salt (Sigma Chemical Co.) through a column of Zeo Carb 226 (H^+) and restoring the eluate to pH 6·8 with tris. The mixture was incubated at 37° C with combinations of 3 mM-magnesium, 150 mM-sodium and 30 mM-potassium chlorides. After 30 min cold trichloroacetic acid was added to give a final concentration of 10% (w/v), the precipitate was centrifuged down at 0° C and the phosphorus liberated (difference between 0 and 30 min incubation) measured in the supernatant (Fiske & Subba Row, 1925). Checks showed that this was linear for at least up to 60 min.

Both ram and bull spermatozoa exhibited high ATP-splitting activity (Experiment 1, Table 1) in contrast to the last wash fluid from the spermatozoa which had no activity. The activity of the magnesium-dependent ATPase was about 30% higher in whole bull spermatozoa than in ram spermatozoa, and the ATPase activity in the presence of both sodium and potassium was also correspondingly higher in the bull. There was only a slight stimulation of magnesium-dependent ATPase in the presence of either sodium or potassium. When both sodium and potassium were included, ATPase activity increased by 50% over the basal rate, which suggests that a considerable proportion of the ATP is expended in cation transport. The inhibition of cation activation by ouabain parallels a similar effect on the active transport of sodium and potassium by dog spermatozoa (Quinn & White, 1967). Although the ATPase

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TABLE 1

ATPASE ACTIVITY IN WHOLE AND FRACTIONATED RAM AND BULL SPERMATOZOA

Species	*Cation added*	*Experiment 1*	*Experiment 2*						
			Fraction						
		Whole sperm.	1	2	3	4	5	*Sum*	*Whole sperm.*
Ram	–	48**	1·0	1·4*	1·5	1·7	0·4**	6·0	6·7
	Mg	62	1·2	2·7	2·5	2·6	1·0	10·0	8·6
	Mg+Na+K	94**	1·7	3·8**	3·7*	3·5*	1·0	13·7	13·1
	Mg+Na+K+ouabain	74	1·5	3·4**	3·4	2·8	0·8	11·9	10·3
			(1·2)	(2·6)	(2·8)	(2·1)	(0·0)	(8·6)	(10·5)
Bull	–	53**	1·9	1·2	1·0	2·5*	0·3*	6·9	5·2
	Mg	96	2·1	1·6	1·8	4·3	1·3	11·1	9·4
	Mg+Na+K	140**	3·1*	2·6*	3·2	5·2	1·2	15·3	13·7
	Mg+Na+K+ouabain	110	2·8*	2·5**	2·0	4·8	1·1	13·2	10·8
			(2·3)	(2·3)	(3·3)	(2·1)	(0·0)	(9·8)	(10·0)

In Experiment 1 mean values (μg $P_{inorg.}$ liberated/10^8 spermatozoa/hr) are given for six replicates. Values for additional treatments containing Mg+Na only were ram 79, bull 118, and for Mg+K only were ram 67, bull 98.

In Experiment 2 mean values (μmoles $P_{inorg.}$ liberated/mg N/hr) are given for three replicates. The specific activity of Na–K–ATPase expressed as 10^{-9}moles-ATP hydrolysed/10^{-6}moles nitrogen/min is given in parenthesis.

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$ (significantly different from Mg).

activities are twenty to thirty times higher than in epididymal boar spermatozoa (Uesugi & Yamazoe, 1966), the relative activities of magnesium-dependent and cation-activated ATPase are similar. Using the methods outlined by Voglmayr, Scott, Setchell & Waites (1967) the chief product of ATP breakdown has been shown to be ADP. Some AMP, however, was also formed.

A second experiment was undertaken to determine the location of ATPases in sonically disintegrated and fractionated ram and bull spermatozoa. For this experiment the washed spermatozoa were resuspended in tris-buffered sucrose containing 1 mM-ethylenediamine-tetra-acetic acid and disintegrated for 3 min at 20 kilocycles/sec in a Mullard Ultrasonic vibrator. The sonicate was centrifuged at 2000*g* for 15 min and the plug resuspended in 10 ml tris-buffered sucrose, centrifuged again and separated into two fractions—the heavier Fraction 1 consisting mainly of heads and the lighter Fraction 2 containing predominantly mid-pieces (Mohri, Mohri & Ernster, 1965). The pooled sonicate supernatants were centrifuged at 10,000*g* for 10 min and the plug consisting of tail fragments and heavy microsomes was resuspended in tris-buffered sucrose (Fraction 3). The supernatant was further centrifuged at 150,000 *g* for 45 min and the plug (Fraction 4) containing microsomal fragments resuspended in tris-buffered sucrose. Fraction 5 was the soluble supernatant. The identity of the subcellular fractions was checked by photomicroscopy, ATPase activity assayed as for whole cells and protein estimations made by the method of Wales, Scott & White (1961).

The magnesium-dependent ATPase activity predominated in the midpiece, tail and microsomal fractions, with lowest activity in the head. There was a similar distribution of sodium–potassium activated ATPase which, however, was most active in the tail. Ouabain inhibited the cation-activated ATPase of all fractions but the overall inhibition was less than in the whole cells.

The magnesium-dependent ATPase has been shown to be confined almost exclusively to the midpiece and tail of spermatozoa (Nelson, 1954; Mohri, 1964), and to be intimately concerned with the contractile processes of the flagella (see Bishop, 1962). Cation-activated ATPase in the tail could co-ordinate flagellar contractile processes. This co-ordination is absent in glycerol-extracted spermatozoa models (Bishop & Hoffmann-Berling, 1959) which can hydrolyse ATP in the presence of magnesium to produce oscillatory motion but do not have an operative cation transport mechanism because membrane permeability has been disrupted by the extraction procedure.

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