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J. N. MANSBRIDGE*
A. KORNER

Department of Biochemistry,
University of Cambridge.

* Present address: Department of Biochemistry, University of Queensland, St. Lucia, Brisbane.

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Presence of Sodium–Potassium-stimulated ATPase in Boar Epididymal Spermatozoon

SINCE Skou[1] reported that an adenosine triphosphatase (ATPase) stimulated by sodium and potassium in the presence of magnesium (Na–K ATPase) is present in submicroscopic fraction of crab nerve, many investigators have reported the presence of the same ATPase in various tissues[2–6] (see also Hokin and Hokin's recent review[7]), and it was suggested that it plays an important part in the process of active transport of potassium and sodium ions. However, information concerning the presence of such an ATPase in spermatozoa is not, as yet, available. It seems very likely that active ion transport mechanism is also present in the spermatozoon, as its motility is affected by ionic environment. This communication reports our detection of Na–K ATPase activity in boar epididymal spermatozoon.

Spermatozoa obtained from fresh epididymal tissues of a boar were washed twice in 0·25 M sucrose solution and re-suspended in a 0·25 M sucrose solution containing 35 mM *tris*-hydrochloric acid buffer (*p*H 7·4). ATP was rendered free of sodium and potassium by passing it through a 'Dowex 50' cation-exchange resin column in the hydrogen form. The free acid was brought to *p*H 6·8 with 1 M *tris* solution. The reaction mixture contained 3 mM ATP, 3 mM magnesium ions, 35 mM *tris*-HCl buffer (*p*H 7·4) and various concentrations of sodium and potassium ions. Ouabain was added to the medium as required. The total volume of the reaction mixture was kept constant at 2·0 ml. After incubation for 30 min at 37° C, the reaction was stopped by the addition of 1 ml. of 30 per cent trichloroacetic acid, and liberated inorganic phosphorus (P_i) was determined by the method of Fiske and SubbaRow. Activity was expressed as μmoles of inorganic phosphorus/10^{10} sperm/h. The ATPase activity of intact spermatozoa is shown in Fig. 1.

As shown in Fig. 1, ATPase activity of intact spermatozoa increased in the presence of 3 mM of magnesium, and it was stimulated further by sodium ions but not by potassium ions alone. The activity was greatly enhanced by the addition of both sodium and potassium ions in the presence of magnesium ions. Fig. 1 shows also that the ATPase activity in the complete system is inhibited by the addition of 5×10^{-4} M ouabain. The presence of such an ATPase activity in intact spermatozoa suggests that spermatozoa may have a function of active transport of potassium and sodium ions. The following experiments were undertaken to investigate the distribution of Na–K ATPase activity in the spermatozoa.

Washed spermatozoa were suspended in 0·25 M sucrose solution containing 0·2 per cent sodium deoxycholate, 5 mM EDTA, 30 mM histidine–HCl, 30 mM 2-amino-2-methyl-1,3-propanediol, at *p*H 7·0, and subjected to ultrasonic treatment at 10 kc/s for 5 min. The sonicate was homogenized using a 'Teflon' pestle-glass homogenizer at 1,000 r.p.m. for 3 min, and the homogenized sonicate was fractionated by differential centrifugation into four fractions, namely, head, tail, Fraction 1 (which was collected by centrifugation of the supernatant at 10,000*g* for 10 min) and Fraction 2 (which was collected by centrifugation of the supernatant of Fraction 1 at 105,000*g* for 60 min). Each fraction was washed twice in 0·25 M sucrose solution and tested for the Na–K ATPase activity. Typical results of an experiment are shown in Table 1.

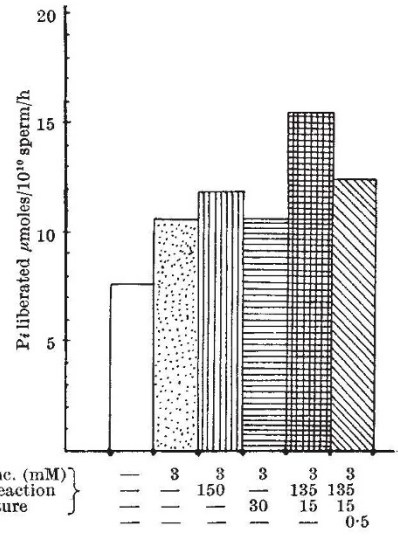

Fig. 1. ATPase activity of intact boar epididymal spermatozoa. The incubation medium consisted of 3 mM ATP, various cations as described above, and 35 mM *tris*-HCl buffer (*p*H 7·4)

Table 1. ATPASE ACTIVITY OF FRAGMENTED BOAR EPIDIDYMAL SPERMATOZOON

Fragmented spermatozoon	Activity (P_i μmoles/N mg/h) Mg	Mg+Na+K	Mg+Na+K/Mg
Head	2·07	2·12	1·02
Tail	17·72	18·90	1·07
Fraction 1	5·53	6·90	1·25
Fraction 2	6·68	8·70	1·30

The incubation medium consisted of 3 mM ATP, 3 mM magnesium chloride, 105 mM sodium chloride, 30 mM potassium chloride, 35 mM *tris*-HCl buffer (*p*H 7·4). The enzyme suspension was added after 5 min temperature equilibration at 30° C, and incubation was continued for a further 30 min. The reaction was stopped by the addition of 1 ml. of cold 30 per cent trichloroacetic acid and aliquots were assayed for inorganic phosphate by the method of Fiske and SubbaRow.

It will be seen from these results that in the presence of magnesium alone ATPase was highest in the tail—which agrees with the results obtained by Nelson[8] for fragmented bull spermatozoa. On the other hand, Na–K ATPase activity was highest in Fraction 2, which contains microsomal sediment, and rather low in other fractions. In other experiments, Na–K ATPase activity of Fraction 2 was inhibited by addition of 10^{-5} M ouabain. Consequently it seems likely that Na–K ATPase activity in intact spermatozoon did not originate from the tail fraction, which consists of a myosine-like substance, but chiefly from Fractions 1 and 2, namely, membranes, mitochondrial and microsomal fractions. It is probable that this sodium–potassium-activated ATPase plays an important part in the enzymatic mechanism of active ion transport involved in the excitation and movement of the spermatozoon.

SHIRO UESUGI
SABURO YAMAZOE

Department of Biochemistry,
School of Medicine,
University of Gunma,
Maebashi, Japan.

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