Bull semen was obtained from the Veterinarian College, Uppsala, and from the A.I. Centre at Kalmar, from where it was transported at 5 °C to the laboratory and used 24 h after collection. All experiments were performed at 22 °C. Sperm density was determined spectrophotometrically, seminal plasma prepared by centrifugation at 100 g for 30 min.

In the association experiments, blank and sample consisted of 90 µl seminal plasma and 10 µl Krebs-Ringer solution or this medium containing 5×10^{-5} M NA. The amount of semen added varied between 0.4 and 0.7 µl according to the density of the semen. Final density was about 1×10^7 spermatozoa per ml. In order to prevent oxidation of NA and association by oxidation of the antiassociating factor, plasma and Ringer solutions were stored under commercial N_2 containing 5% CO_2. Blank and sample were set up immediately before counting and transferred to a hemocytometer after mixing. For details, calculations, and statistical treatment see Lindahl[5].

In less than 5 min after addition to the sperm NA had induced significant HHA (table 1). Thus we conclude that washing of the cells is not a prerequisite for NA-induced association and that seminal plasma does not contain factors blocking the associating effect of 5×10^{-6} M NA. The measures for prevention of association by oxidation had been at least partly successful, since on an average only 0.6% associated cells appeared in the controls. This association had probably taken place already in the semen.

For all analyses of endogeneous NA* and added NA, 1.0 ml semen was extracted by homogenization in ice-cold perchloric acid containing metabisulfite; the extract was chromatographed on a Dowex 50-W column and the catecholamines eluted according to Atack and Magnusson[6]. The NA* containing fraction eluted from the column was divided in 2 parts, one used as blank, the other as sample. The amount of NA* was determined fluorimetrically[7], and for each assay a standard, a reagent blank and an internal standard (NA) were run in parallel with sample and blank, exactly as described by Kehr et al.[7].

No endogeneous NA* could be traced in the extract from samples of semen which had been stored for 24 h at +5 °C. In recovery experiments NA was thoroughly mixed with similarly treated samples immediately before homogenization. Additions of 5.0, 10.0 and 20.0 ng/ml were recovered to about 48%, whereas no NA was detected after addition of 2.5 ng/ml.

In freshly collected semen, however, small amounts of NA* were found. Since the 2 experiments in table 2 were performed with ejaculates originating from the same bull and differing only little as to sperm density, we combined the resulting figures on endogenous NA* in a t-test (table 2). With a recovery of about 48% the concentration of NA* in fresh bull semen can be estimated at about 10 ng/ml (5.2×10^{-8} M), i.e. about a hundredth part of that stated by Brochart. For human semen the corresponding figure of 57 ng/ml was recently published[8].

Table 2. Contents of NA* in freshly collected bovine semen

Content of NA* (ng/ml semen) Ejaculate			
A	B	Mean ± SD	
1.0	16.7	9.69 ± 6.58	
3.0	21.2	Deviation from zero	
7.5	10.6	t	p
4.5	13.2	4.17	< 0.005

Semen from 2 different ejaculates (from the same bull) was submitted to analysis within 10 min after collection, 4 replices being made in each case. Values corrected for recovery. Sperm density in ejaculate A 1.8×10^9 sp/ml, in ejaculate B 1.9×10^9 sp/ml.

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Effects of Ca^{2+} and Mg^{2+} on motility of sea urchin spermatozoa[1]

F. Iwasa, H. Shimizu and H. Mohri[2]

Department of Biology, University of Tokyo, Komaba, Meguro-ku, Tokyo 153 (Japan), and Electrotechnical Laboratory, Optoelectronics Section, Niihari-gun, Ibaragi-ken 305 (Japan), 13 May 1980

Summary. Swimming speed of sea urchin spermatozoa, measured by a light scattering technique, did not change with 0–20 mM Ca^{2+} in the medium. The speed was maximum at the normal concentration of Mg^{2+} (49 mM) in sea water.

Young and Nelson[3] reported that both excess and insufficiency of Ca^{2+} ions depressed the swimming speed of the spermatozoa of the sea urchin *Arbacia punctulata*, the normal concentration in sea water (9 mM) being the optimum. Sano and Mohri[4], on the other hand, found that sperm motility of the sea urchin *Strongylocentrotus intermedius* was not adversely affected by removal of Ca^{2+} or Mg^{2+} ions from the medium. The former investigators determined the swimming speed by sedimenting the spermatozoa in a mild centrifugal field 120 × g). Active spermatozoa sedimented more rapidly than non-motile spermatozoa. In the latter authors' case, the length of tracks of spermatozoa on photographs taken under a dark-field microscope was measured. In the present experiments, the swimming speed of sea urchin spermatozoa was determined by a laser light scattering technique which gives us informations on the statistical properties of motile microorganisms.

Material and methods. Semen of the sea urchin, *Hemicentrotus pulcherrimus,* was obtained by injecting 0.5 M KCl into the coelomic cavity. The composition of artificial sea water (ASW) was as follows: 458 mM NaCl, 9.5 mM KCl, 10 mM $CaCl_2$, 49 mM $MgCl_2$ and 6 mM $NaHCO_3$, pH 8.2. Both $CaCl_2$ and $MgCl_2$ were omitted from Ca^{2+}-, Mg^{2+}-free artificial sea water (CMFASW). Various amounts of 0.5 M $CaCl_2$ and/or 0.5 M $MgCl_2$ were added to CMFASW in order to obtain proper concentrations of Ca^{2+} and/or Mg^{2+}. In some experiments, varying amounts of 0.5 M EGTA or 0.1 M EDTA were added to ASW. The swimming speed of spermatozoa was determined by the laser light scattering technique[5]. The wavelength of the incident laser was 633 nm and the scattering angle was 32°. All measurements were started as quickly as possible after dilution of semen 2×10^3 time with artificial sea water (approximately 1.8×10^7 spermatozoa/ml).

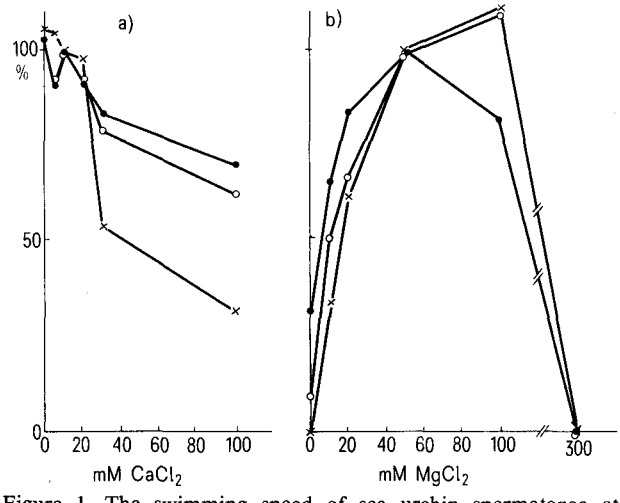

Figure 1. The swimming speed of sea urchin spermatozoa at various concentrations of Ca^{2+} (a) and Mg^{2+} (b). The data are presented as percentage of the average swimming speed in ASW at each time after dilution of semen. ●—●, 2-4 min; ○—○, 6-9 min; × —— ×, 11-13 min after the dilution.

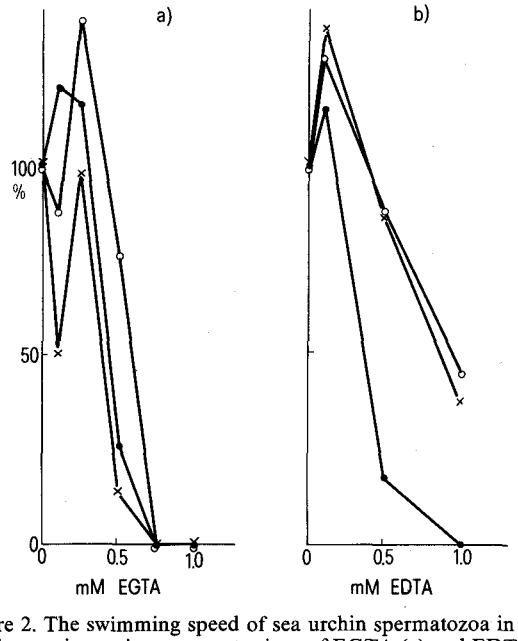

Figure 2. The swimming speed of sea urchin spermatozoa in ASW which contains various concentrations of EGTA (a) and EDTA (b). Details as in figure 1.

Results and discussion. The average swimming speed calculated from the speed distribution was plotted against the concentrations of Ca^{2+}, Mg^{2+}, EGTA and EDTA (fig. 1, 2). In these figures, the average swimming speed obtained in ASW was taken as 100%. The actual values were 210-240 μm/sec and 170-200 μm/sec, 3 min and 15 min after dilution of semen, respectively. In the presence of 49 mM Mg^{2+}, the lack of Ca^{2+} in the medium did not affect the swimming speed. The average speed was almost constant in the concentration range of 0-20 mM, although further addition of Ca^{2+} resulted in a gradual decrease in motility. The result coincides with our previous data[4], but is not consistent with the result obtained with *Arbacia* in which the swimming speed of the spermatozoa in Ca^{2+}-free medium was half that of spermatozoa suspended in ASW[3]. The discrepancy could be caused by a difference in the permeability of the sperm membrane to Ca^{2+}, *Arbacia* spermatozoa being leakier than others. Calcium ions have been known to exert regulatory effects on flagellar and ciliary movement[6-9], but the critical concentration of Ca^{2+} appears to be of the order of μM. The change in external Ca^{2+}concentration would not influence significantly the intracellular Ca^{2+} concentration. In contrast to our previous observation[4], insufficient concentrations of Mg^{2+} (less than 49 mM) adversely affected sperm motility in the presence of 10 mM Ca^{2+}. The average speed in the absence of Mg^{2+} was about one third that in ASW when determined 3 min after dilution of semen, and became almost nil by 15 min after dilution in the case of the experiment shown in figure 1b. Likewise, a large excess of Mg^{2+} depressed sperm motility. Our previous measurements were probably made only on vigorously moving spermatozoa, omitting non-motile ones. $MgATP^{2-}$ is considered as the substrate for dynein ATPase which constitutes the arms attaching to the outer doublets of flagellar and ciliary axonemes and is responsible for sliding between the adjacent doublets[10-12]. The normal concentration of Mg^{2+} in sea water, around 49 mM, seems to be required to maintain the intracellular concentration of Mg^{2+}necessary for optimal functioning of the motile apparatus. Visual assessments of the sperm motility of *Tripneustes gratilla* under a light microscope[13] also indicated a depressant effect due to the removal of Mg^{2+}, but no such effect after removal of Ca^{2+}. Both EGTA and EDTA exerted profound suppressing effects on the swimming speed at concentrations less than 1 mM, as reported for *Arbacia*[3,14]. Since the effective concentrations of these reagents were far less than the normal concentrations of Ca^{2+} and Mg^{2+} in sea water the results would be due to adverse effects other than chelation of Mg^{2+} and/or Ca^{2+}.

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