Evidence obtained previously suggests that the action of DaNa is independent of $[Ca^{2+}]_o$, that it is antagonized by theophylline and that it reduces the release of Ca^{2+} from intracellular storage sites thereby reducing the steady-state level of $[Ca^{2+}]_i$ at the frog presynaptic terminals[5]. Comparable results have been obtained with the effect of DaNa at the rat adrenal medulla[7]. Since the temperature sensitivity of spontaneous release is not affected by a reduction of $[Ca^{2+}]_o$ (Duncan and Statham[3]), nor is it modified in the presence of DaNa, which might be expected to inhibit the release of Ca^{2+} from intracellular membrane systems[5], we now conclude that the major effect of temperature is probably not to modify $[Ca^{2+}]_i$. It appears more likely that the release mechanism itself is directly and markedly modified by temperature, perhaps associated with a phase-change in the phospholipoprotein system of the plasma membrane and/or the synaptic vesicles at about 16 °C. Such a conclusion is in accord with a) the very different effects of diamide on m.e.p.p. frequency above and below a critical temperature of 16 °C which have been recently described[8] and b) with the observation that stimulation of m.e.p.p. frequency by the ionophore A23187 shows a marked temperature sensitivity in this range, the effect being virtually absent at temperatures below 17 °C (Statham and Duncan[9]). Unfortunately, it is not possible to observe the effects of ionophore treatment on the temperature-frequency curve, as plotted in this communication, since A23187 treatment results in a progressively increasing rate of spontaneous release[9].

It is now clear that there is a complex interrelationship between transmitter release (both spontaneous and evoked), temperature and $[Ca^{2+}]_i$ (Publicover and Duncan[10]); it seems that the particularly high Q_{10} and activation energy shown by the release system above 16 °C is found only under conditions of low $[Ca^{2+}]_i$. Thus, the $Q_{10}^{10\text{-}20\,°C}$ for m.e.p.p. frequency is 10, both under normal conditions and when the terminals are depolarized with 20 mM $[K^+]_o$ in the absence of extracellular Ca^{2+} (conditions in which $[Ca^{2+}]_i$ is low). However, the $Q_{10}^{10\text{-}20\,°C}$ is only 4 for evoked release (as measured by the quantal content of the e.p.p.) and for m.e.p.p. frequency in the depolarized preparation in normal extracellular Ca^{2+} (conditions in which $[Ca^{2+}]_i$ is high)[4].

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2 E. Alnaes and R. Rahamimoff, J. Physiol. *248*, 285 (1975).
3 C.J. Duncan and H.E. Statham, J. Physiol. *268*, 319 (1977).
4 E.F. Barrett, J.N. Barrett, D. Botz, D.B. Chang and D. Mahaffey, J. Physiol. *279*, 253 (1978).
5 H.E. Statham and C.J. Duncan, Eur. J. Pharmac. *39*, 143 (1976).
6 C.J. Duncan and S.J. Publicover, J. Physiol. *294*, 91 (1979).
7 J. Cohen and Y. Gutman, Br. J. Pharmac. *65*, 641 (1979).
8 S.J. Publicover and C.J. Duncan, Eur. J. Pharmac. *70*, 203 (1981).
9 H.E. Statham and C.J. Duncan, Life Sci. *17*, 1401 (1975).
10 S.J. Publicover and C.J. Duncan, Naunyn-Schmiedebergs Arch. Pharmak. *315*, 29 (1980).

Catecholamines in bovine semen

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Summary. In seminal plasma 5×10^{-6} M noradrenaline was found to induce head-to-head association in bull spermatozoa. The sum of noradrenaline and adrenaline in freshly collected semen was 10 ng/ml (5.2×10^{-8} M), i.e., about 100 times lower than previously reported.

With the aid of a colorimetric method Brochart[1] demonstrated the presence of some substance, thought to be adrenaline (A), in semen from 3 mammalian species. In bull semen the concentration was 1.0 µg/ml (5.5×10^{-6} M). Later[2] the substance was considered to be chiefly noradrenaline (NA). The agent was shown to be present in both semen and seminal plasma.

Catecholamines elicit head-to-head association (HHA) in bull sperm washed with Tris buffered medium or Tyrode solution, whereas the oxidation product adrenochrome dissociates spermatozoa associated by washing[3]. The lowest concentrations tested were for A 2×10^{-6}, for NA 5×10^{-5} M, both substances being approximately equally efficient at 5×10^{-5} M. The fact that this effect is inhibited by α- and β-adrenergic blockers and is induced also by exogeneous cAMP seems to indicate the existence of a mechanism triggered by some related compound, to which spermatozoa are more sensitive than to the catecholamines tested. We conceive that the changes brought about in the cell surface by this mechanism, resulting in the artefact HHA, play some role in prefertilization events.

In normal semen HHA occurs only as a result of inactivation by oxidation of a substance located in the sperm surface and seminal plasma[4], preventing sperm from associating by way of another mechanism. Under natural conditions catecholamines do not induce HHA in semen, either because their concentration is too low or because this process is blocked in some way. We have therefore studied whether NA can induce HHA in bull sperm diluted with seminal plasma and have submitted samples of normal bull semen to a sensitive and specific analytical procedure, giving the sum of A and NA, calculated below as NA and symbolized by NA*.

Table 1. Induction of head-to head association in bull spermatozoa by 5×10^{-6} M noradrenaline in seminal plasma

	Association (%)	$\bar{D} \pm SD$	Deviation from zero	
			t	p
Control	0.59	1.20 ± 0.888	4.279	< 0.01
Sample	1.78			

Spermatozoa from 2 ejaculates, from different bulls. $\bar{D}$: mean difference between control and sample counts of 10 determinations; at least 500 cells counted in each determination.

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.

1 M. Brochart, Comp. Rend. Soc. Biol. *142*, 646 (1948).
2 M. Beauvallet and M. Brochart, Comp. Rend. Soc. Biol. *143*, 237 (1949).
3 P.E. Lindahl, Exp. Cell Res. *113*, 421 (1978).
4 P.E. Lindahl and J.E. Kihlström, Fert. Steril. *5*, 241 (1954).
5 P.E. Lindahl, Exp. Cell Res. *87*, 47 (1974).
6 C. Atack and T. Magnusson, Acta Pharmac. Toxic. *42*, 35 (1978).
7 W. Kehr, M. Lindqvist and A. Carlsson, J. Neurol. Transmission *38*, 173 (1976).
8 A. Sosa, B. Ortege-Corona, J. Chargoy and A. Rosado, Fert. Steril. *33*, 235 (1980).

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

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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.