

Sperm Flagella: Autonomous Oscillations of the Contractile System

Abstract. Bull sperm are deactivated, losing all motility, when they are impaled or dissected with a microprobe. Loss of activity is due to the creation of a hole or break in the cell membrane. Uncoordinated contractile activity is retained if external adenosine triphosphate and adenosine diphosphate are present. When these substances are in the medium, coordinated wave motion can be initiated in impaled or dissected sperm by bending a segment of the flagellum.

The regulation of flagellar oscillation has been ascribed to various control elements. Since cilia will respond to electrical stimulation (1), electrical control of flagella is a possibility. The involvement of the centriole as a control element has also been suggested (2). However, in some trypanosomid flagellates more than one wave-initiating site exists (3).

Endogenous cyclic contraction and relaxation of the contractile elements was postulated from earlier studies of glycerinated sperm (4). Although these sperm did indeed sustain oscillations in the presence of adenosine triphosphate (ATP), isolated flagellar fragments that lacked the basal region would not oscillate. We have found that in bull sperm and *Drosophila melanogaster* sperm the centriole is not necessary for coordinated oscillation of the flagellum, and that in bull sperm, internal ATP concentration and mechanical loading are the primary factors necessary for coordinated flagellar waves.

Bull sperm suspended in citrate-egg yolk dilutant (5) were centrifuged and resuspended twice in mineral Krebs medium with 2 mM sodium lactate. Several drops of the final suspension were diluted to 10 ml with Krebs medium, and the pH was adjusted to 7.5. A 1-ml chamber designed for use with a Zeiss 40 \times phase water immersion objective was filled with a portion of the diluted preparation. The washing procedure caused the heads of the sperm to stick securely to the slide while the flagellum remained free and actively waving. The sperm then could be impaled with a glass microprobe. A piezoelectric advancer (6) was used to hold the probe and permit its rapid advance. A Brinkman CP-VI micromanipulator was used to support the advancer and allow manipulation of the probe. Observations were made at $\times 1000$ magnification and filmed (7).

During experiments to record the internal potential of a bull spermatozoon (8), we observed that after impalement with a glass microelectrode the sperm

lost motility. In 200 subsequent trials, the motility of the impaled sperm always ceased. The average time between impalement and deactivation was 30 seconds.

To observe deactivation, we often used only micromanipulation without employing the piezoelectric advancer. No differences in motility were measured between sperm impaled by piezoelectric advance or by micromanipulation. Some vibration always accompanied manual operation of the micromanipulator. This vibration was absent if the piezoelectric advancer was used to thrust the probe the last few micrometers. For this reason sperm impaled piezoelectrically sustained less visible damage.

The effect of impalement was to permit leakage through the cell membrane (8). When both ATP and adenosine diphosphate (ADP) at 5 mM concentration were included in the suspension, impalement resulted in only partial loss of motility. Under these conditions the flagella remained contractile after impalement but did not exhibit co-

ordinated waves. Instead a twitching motion was observed. In a preparation treated with 5 mM ATP, 5 mM ADP, and 4 mM deoxyglucose, the sperm were observed to lose all activity when 40 ml of a 0.1M KCN solution was added to the chamber. If a sperm that was active before KCN inhibition was impaled, the flagellum was partially reactivated and showed a twitching motion. Therefore, ATP does not enter before impalement but does enter after impalement, and this result indicates that a leakage pathway has been created through the membrane.

We conclude that after impalement there is an interchange of chemical constituents between the cell and the external medium. The extent to which this effect contributes to the loss of motility was explored. The ionic constituents of the medium were screened to yield optimal activity of flagella after impalement.

The final medium (Table 1) contained 72 mM K⁺ and 10 mM Na⁺. Almost all of the Cl⁻ was eliminated by the use of methylsulfonate as the main constituent anion. In this medium deactivation of sperm after impalement took longer, up to 3 minutes. In the final medium we consistently observed a characteristic pattern of activity before total loss of motility (Table 1). However, impalement still affected coordination of the flagellar motility.

Several substances involved in control of contractility in other systems were added to the medium to test the

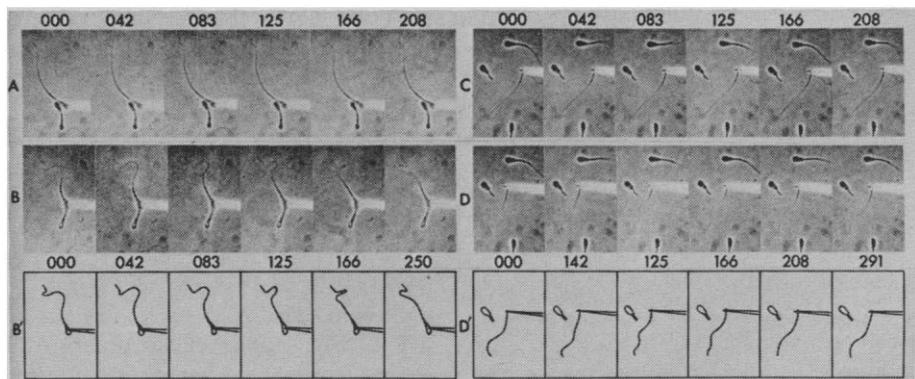


Fig. 1. Reactivation of bull sperm flagella. All preparations contained 0.5 mM ATP and 0.5 mM ADP. Sequences are from 16-mm motion picture film (24 frame/sec); numbers adjacent to each frame indicate time in milliseconds after the first frame in each sequence. (A) An impaled sperm has lost all coordinated wave motion. (B) The same sperm as in A is seen. The distal tip of the flagellum has been fastened to the slide by pressing it with the probe, and a bend has been introduced by pushing the head toward the fastened tip with the probe. Traveling waves are now present. (C) A flagellum has been dissected free of the head and most of the midpiece (visible nearby); all coordinated wave motion is lost. (D) The same sperm as in C is seen. The distal tip of the flagellum has been fastened to the slide by pressing it with the probe and a bend has been introduced by pushing the proximal end toward the fastened tip. Traveling waves are present. Tracings B' and D', made from the prints B and D, delimit the objects of interest.

Table 1. Sequence of motility after impalement of bull spermatozoa. The medium contained 0.072M potassium methylsulfonate, 0.163M sucrose, 0.005M MgSO₄, 2 to 5 μM CaCl₂, 0.007M sodium lactate, and 2 percent Na₂HPO₄ buffer at pH 7.5. Concentration of ATP and ADP was varied.

ATP and ADP (mM)	Activity after impalement for:			
	0 to 10 seconds	10 to 50 seconds	50 to 60 seconds	More than 2 minutes
0.0 (control)	2-10 synchronized waves (phase I)	Twitching (phase II)	2-5 waves, very slow (phase III)	No activity (phase IV)
0.1	2-10 synchronized waves	Some jitter superimposed on wave pattern	Continued	Continued
0.5	2-10 synchronized waves	Large-amplitude twitching (4-5 μM)	Continued	Continued
5.0	2-10 synchronized waves	Tight, rapid twitching	Almost inactive, slight jitter	Continued

possibility that they were lost by the sperm after it had been ruptured. Acetylcholine, 0.1 mM and 0.05 mM, had no effect whether or not ATP and ADP were present. Nicotinamide adenine dinucleotide (10 μM), alone and with 10 μM reduced nicotinamide adenine dinucleotide, also failed to change the behavior of the impaled sperm.

Calcium ion in the medium (2 to 5 μM) was removed by chelating Ca²⁺ with 40 μM ethyleneglycol bis(aminomethylether)tetraacetic acid (EGTA). The absence of Ca²⁺ had no effect on sperm after impalement whether or not ATP was present.

Although impalement always affected the coordination, the flagellum would often undergo a period of coordinated motion in potassium methylsulfonate medium just before all activity ceased. By using the microprobe as a micro-knife, it was possible to press the flagellum of an active sperm between the probe and the slide and slice free a given length of flagellum. The detached portion was deactivated with the same pattern of activity and over the same time course as a sperm that was impaled. This was also true of flagella severed distally to the centriole or to the midpiece. The pattern remained unchanged, the flagella often undergoing several coordinated waves before total deactivation.

By using much longer sperm it was possible to allay the effects of diffusion and to isolate the damage produced during dissection to a small portion of the flagellum. The sperm of drosophila, which are 1200 to 1500 μm long, were chosen for this purpose. These sperm possess a centriole and the familiar nine-plus-two tubular structure (9).

Sperm taken from the seminal vesicle were suspended in mineral drosophila medium with glucose (11), and the flagella were dissected into several segments. Each segment that was active originally retained its coordinated wave motion after dissection. Segments 500 to 1000 μm long often would retain their activity for up to 13 minutes.

Both bull and drosophila sperm flagella therefore possess the ability to coordinate waves in the absence of a centriole.

Bull sperm in potassium methylsulfonate medium that contained ATP and ADP (5 mM each) twitched after impalement for an indefinitely long period (more than 5 minutes). If concentrations of both ATP and ADP were lowered to 0.5 mM, the twitching was of larger amplitude. When the flagellum of a sperm in this condition was manipulated into a bent configuration, rhythmic oscillation developed. The coordinated motion was lost if the flagellum was allowed to straighten. This manipulation was repeated as many as ten times on the same flagellum, and each time resulted in the initiation of wave motion. Frequencies in the range of 4 to 6 hz and amplitudes of 6 to 12 μM were observed; these values are within the range of values observed in sperm before impalement. The same manipulation performed on severed flagella in the same medium produced identical results (Fig. 1). Unlike the coordinated motion during the deactivation of sperm in ATP- and ADP-free preparations, the coordinated activity induced by bending in the presence of ATP and ADP (0.5 mM each) lasted for longer than 5 minutes.

The effects of different concentra-

tions of ATP and ADP on coordination of flagellar waves after impalement are shown in Table 1. A range of concentrations suitable for oscillation exists, and altering the system by mechanical bending can extend this range.

Our results are for the most part in accord with the behavior of glycerinated sperm flagella (10); Ca²⁺ is not necessary for oscillation, and Mg²⁺ gives best results at concentrations of 1 to 5 mM. Both of these methods destroy the integrity of the cell membrane, so the cell potential is not likely to be necessary for the observed oscillations. In both techniques, ATP serves as the primary activating agent. However, we observe flagellar motion that appears to consist of normal traveling waves, unlike the reported motion for glycerinated bull sperm. Also, with our technique no part of the midpiece needs to remain attached to the flagellum for oscillation to occur.

The flagellar contractile system has the ability to produce coordinated waves, and ATP is required to sustain these oscillations. At a given ATP concentration, mechanical loading can influence the ability to oscillate. The head, centriole, and midpiece are not necessary for the production of flagellar waves, and a direct involvement of the cell potential seems unlikely.

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