MOVEMENT OF SEA URCHIN SPERM FLAGELLA

ROBERT RIKMENSPOEL

From the Department of Biological Sciences, State University of New York at Albany, Albany, New York 12222

ABSTRACT

The motion of the sea urchin sperm flagellum was analyzed from high-speed cinemicrographs. At all locations on the flagellum the transversal motion and the curvature were found to vary sinusoidally in time. The curvatures of the flagella increase strongly near the proximal junction. Two sperm are described in transient from rest to normal motion. The full wave motion developed in both sperm within 40 ms.

KEY WORDS sea urchin sperm · flagellum · motion · high-speed film · transients

In recent years the understanding of the force-producing mechanisms in sea urchin sperm flagella has advanced greatly. A sliding filament mechanism, in which cross bridges between the longitudinal fibers are formed by the dynein arms, has been shown to operate in these flagella (23, 10). The molecular understanding of the force-producing mechanisms has led several investigators to develop theoretical models for the processes that control and coordinate the attachment and breakage of the dynein cross bridges (3, 4, 16, 17, 19, 21). The aim of these models is to reproduce the characteristics of the flagellar movements from an equation of motion that contains a prescription for the attachment and detachment of the dynein cross bridges. The somewhat surprising situation exists, however, that a detailed description of the sea urchin sperm flagellar motion has not been presented in the literature. The best data available are from "multiple flash" photographs (1, 6), in which three to five positions of a flagellum are registered on a single still frame. The time interval between the successive positions is of the order of one full period of the flagellar beat. The shape of the flagellar wave is well shown in these photographs, but the time resolution is not sufficient to provide an accurate description of the course of events within a cycle of the flagellar beat.

In this paper a description of the motion of the sea urchin sperm flagellum is given, based on data from high-speed cinemicrographs at 400 frames/s. The filming speed was made possible by the application of a very high-intensity illumination of the specimens.

MATERIALS AND METHODS

Experimental Methods

Sea urchins (Arbacia and Lytechinus) were obtained from N.E. Marine Supply Co. (Woods Hole, Mass.) (Arbacia) and Pacific BioMarine Laboratories, Inc. (Venice, Calif.) (Lytechinus). Sperm shedding was induced by injecting the sea urchins with 1-2 ml of 0.5 M KCl. The spermatozoa were collected and suspended in artificial seawater (Aquarium Systems, Inc., Eastlake, Ohio) to a concentration of approx. 2 × 10⁷/ml. Several hundred sea urchin eggs were added per milliliter of the suspension to prolong the life of the sperm. When the spermatozoa were stored at room temperature, their motility in the suspension was, by visual estimate, approximately constant for a period of several hours.

The pH of the artificial seawater was kept at 7.9. All specimen handling and observations were carried out at a room temperature of 22 ± 1°C.

A few drops of sperm suspension were placed on a microscope slide and covered with a 300-μm thick cover slip. A thin layer of silicon grease sealed the sides of the...
preparations and maintained the thickness of the layer of sperm suspension at approx. 20–40 μm. On the slide, the sea urchin sperm maintained motility for longer than 20 min by visual estimate. Filming was done within 5 min from the moment the slide was made.

**CINEMICROGRAPHY:** During filming, the preparations were placed in a Zeiss photomicroscope under a 10× objective. The filming of sea urchin spermatozoa requires dark field illumination. By trial and error the best combination of intensity of illumination and contrast was found to be obtained using the Zeiss VZ condenser with the low numerical aperture top lens (NA = 0.63), and a specially made central stop of 8.4 mm diameter.

The light source was a 1,000 W xenon arc lamp (no. 982C-1 of Hanovia Lamp Div., Conrad-Hanovia, Newark, N.J.). The arc was imaged 1 to 1 by a four lens quartz condenser onto the point where the filament of the Zeiss high intensity illuminator had been positioned. The band width of the light incident on the preparation was restricted to 420–700 nm by 3 mm GG 420 and 4 mm KG 40 glass filters (Schott and Gen., Mainz, W. Germany). During the few seconds of filming, the filters were removed from the light path which increased the amount of light reaching the film approximately twofold.

Cinemicrographs on Plus X film were made with a Millikan DBM-5C camera (Teledyne Camera Systems, Arcadia, Calif.) at 400 frames/s using a 140° shutter opening. This resulted in an exposure time of each frame of 1 ms. The final magnification of the preparation on the emulsion was 50x.

**DATA ANALYSIS:** Fig. 1 is a positive enlargement of a section of a 16-mm film frame showing the image of a sea urchin sperm. The width of the flagellar image was equivalent to approx. 1 μm in the preparation. At the places on the flagellum which moved at maximal velocity during the 1-ms exposure, the image was broadened to ≈1.4 μm.

After the films were scanned, a number of sperm were selected for detailed analysis. The cells were chosen to show apparently normal, steady, and unhindered motion. The aim was to select cells over the widest possible range of flagellar frequencies. The selected cells were rephotographed on 35-mm film and projected to a final magnification of 5,000. The center line of these projected flagellar images was then traced on paper. Small dust spots fixed to the slide were used as a frame of reference. All subsequent analysis was performed on these tracings.

The width of the flagellar images at the 5,000 magnification used was approx. 5–7 mm. The position of the center of the image could be judged to approx. 0.5 mm, equivalent to 0.1 μm in the preparation. The shape of the flagellum could therefore be determined to an accuracy of around 0.1 μm. Although a subjective judgment is involved in estimating this accuracy, it should be remembered that when the center of a band of 5 mm width is misplaced by 0.5 mm, the widths of the two parts (2 and 3 mm, respectively) show clearly as unequal.

The reference points were generally somewhat irregular in shape. On tracings of subsequent frames the drawn outlines of the reference points repeated to within 1 or 2 mm (SD). The absolute position of a point on the flagella was thus defined to an accuracy equivalent to a few times 0.1 μm.

Near the head-flagellar junction the glare from the image of the head made an accurate tracing difficult, and the errors in the tracings were probably larger than those related above.

The average path of the cells was determined as the center of a number of superimposed tracings. Most of the paths were curved, but a number of cells showed progression along a straight line. Sections of 2 μm length were marked off on each tracing as illustrated in Fig. 2. This defines the running coordinate s along the flagellum. The deviations from the average path at the center of the head and at s = 0, 5, 10, 20, 30, and 40 μm were measured and plotted as a function of time, as illustrated in Fig. 3. In the presentation of the experimental data in the Results section, the running coordi-

![Figure 1](image-url) - Enlarged section of a 16-mm frame of a film at 400 frames/s, showing a sea urchin sperm. The arrows show where the motion of the flagellum caused a widening of the image during the 1-ms exposure. In this print the glare of the head of the spermatozoa has been reduced by partial shading of the image.
RESULTS

General Characteristics

At least 80% of the spermatozoa in the filmed preparations were swimming in circular paths of 10-50 μm radius. The average frequency of the flagellar beat was approx. 35 Hz. The flagellar wave appeared to be planar. This was judged by the absence of movement of the flagella in and out of the plane of focus and by the flat appearance of occasional sperm seen edge on. The rather thick preparations insured that the planar motion was not due to a spatial restriction of the flagellar motions. In most cells approx. 1.5 waves were present in the flagellum. A thin, inert terminal piece 5-8 μm in length was seen at the distal end of all flagella. The amount of light scattered from this terminal piece was of insufficient intensity to be registered on the films.

These characteristics all agree with those in previous descriptions of sea urchin flagella (13, 2, 6, 11). It can therefore be concluded that the preparations used were normal.

No differences were found in the characteristics of motion of spermatozoa of Lytechinus and Arbacia. The results obtained on both species were therefore combined and are presented together below.

Detailed Description

The cells selected for detailed analysis covered a range of flagellar frequencies of 8.2-52 Hz. No cells were found with a frequency of the order of...
20 Hz, and the gap in the data around that frequency in Figs. 7, 8, and 12 could not be filled.

The flagellar motion of the sea urchin spermatozoa was found to be very regular. Fig. 3 shows the deviation from the average path (the "amplitude") as a function of time at various locations on the flagellum of a typical cell. In Fig. 4 the curvature is plotted as a function of time for the same spermatozoon. The accuracy of the curvature measurement was obviously less good than that for the simpler amplitude measurement.

The data for the variation with time of the amplitude and the curvature gave the impression of being sinusoidal. For each of the cells analyzed, sinusoidal curves were therefore fitted by computer through the data illustrated in Figs. 3 and 4. The method employed for computing these curves involved a least squares fit, as described in the Appendix. The deviations of each datum point from the computed curve, the residuals, were then plotted as a function of their phase in the period of beating. This made it possible to plot the residuals of cells with different frequencies together in one graph.

Fig. 5 shows the residuals for the amplitudes for all cells measured. Except at \( s = 40 \, \mu m \), the residuals are apparently completely random. The transversal motion of each point of the flagella for \( s < 30 \, \mu m \) and of the center of the head is thus sinusoidal in time, and the residuals were caused by small fluctuations in the motion and by measurement errors. Fig. 5 indicates that the fluctuations and errors combined amounted to 0.2–0.3 \( \mu m \). Only at \( s = 40 \, \mu m \) is there some indication of a pattern in the residuals. This pattern shows maxima at phases of roughly 1 and 4 rad, pointing to the existence of a second harmonic component, with an amplitude of approx. 0.3 \( \mu m \). Since the magnitude of this second harmonic component is comparable to the measuring error, no conclusions should be drawn as to its significance. It should be noted that the addition of a second harmonic component of approx. 10% to the main sinusoidal component would give a deformation of the sinusoidal curve that is barely perceptible. We must conclude therefore that at all locations on the flagellum the transversal motion is essentially sinusoidal in time.

The residuals for the curvature are shown in Fig. 6. No patterns are discernible for any of the locations. Fig. 6 shows that close to the head the residuals are quite large. This is to a large extent due to the difficulty of tracing the flagellum accu-
rarely in the vicinity of the head. The head of the spermatozoa caused considerable glare in the low aperture imaging used, as can be seen in Fig. 1. The deviations from a sinusoidal variation with time of the curvature at $s = 1 \mu m$ and $s = 5 \mu m$ are apparently random, however. It should be noted that the average magnitude of the residuals for $s > 5 \mu m$ shown in Fig. 6 is approx. 350 cm$^{-1}$, well above the probable error in measuring the curvature. These residuals represent, therefore, most likely real irregularities in the motion.

The wave shape of the flagella at any given time is not sinusoidal. Brokaw (1) has proposed that the waving sea urchin sperm flagellum shows uniform curvature on the “wave crests,” with straight sections connecting these circular arcs. As the waves travel distally along the flagellum, one would expect stepwise changes in curvature at any given location when the transition of a straight to a circular section passes by. The data of Figs. 4 and 6 do not indicate this. A measurement of the curvature as a function of $s$ for the cells presented in this paper did not reveal the presence of either straight or circular sections. However, the definition of the flagellar shape on the films discussed in the present paper was less good than that of the multiple flash photographs. The original data in reference 1 have therefore been re-analyzed with the procedure described in the subsection “Data Analysis.”

Fig. 7a shows a reprint of a multiple flash photograph (Fig. 1a of reference 1) of a sea urchin sperm flagellum. The very high quality of the photographs made it worthwhile to trace the center of the flagellar images (when projected to a final magnification of 5,000 as described in the subsection “Data Analysis”) with extreme care, insuring a uniform line width of the tracing. The tangents at the ends of the 2-$\mu m$ intervals were drawn as judged by eye. When viewing the tracings under low-power magnification (approx. 5×), the point at which the drawn tangent approached closest to the tracing line could be determined to within a few times 0.1 mm. The segment length $\Delta s$ was corrected in each case if the point of closest approach of the tangent deviated from the marked-off segment endpoint. Since the angle $\Delta \theta$ between two tangents can be measured with negligible error, the accuracy of the curvature $\Delta \theta/\Delta s$ was now determined by the error in $\Delta s$. For the present case, this error was several percents. With the maximal curvatures of $\approx 2,000$ cm$^{-1}$ the accuracy was therefore in the order of 50 cm$^{-1}$. It should be noted that the quality of the flagellar images on the high-speed 16-mm film used in this paper was judged not to be good enough to warrant the described correction of the curvature measurements.

The local curvatures measured as described above on the four flagellar positions are shown in Fig. 7b. It can be seen in Fig. 7b that, of the seven curved sections, only one, designated A, is circular. Details such as the gradual increase in curvature in section B, and the smaller curvature in the center of section C can by close inspection be perceived in Fig. 7a. No straight sections with a zero curvature are indicated in Fig. 7b. All four curves cross the line of zero curvature at the maximum slope. In total, 24 curved sections on three spermatozoa presented in Fig. 1 of reference 1 were analyzed and only three were found to be approximately circular. It appears therefore that the description of the wave shape of the sea urchin sperm flagellum as made up of circular arcs connected by straight sections has to be considered as an approximate one, and that the wave shapes in Fig. 1 of reference 1 are not in conflict with the present treatment.
The results illustrated in Figs. 3 and 5 show that the amplitude $U(s,t)$ at location $s$ on a sea urchin sperm flagellum, at time $t$, can be written as $U(s,t) = A(s) \sin \omega t$, where $A(s)$ is the maximum deviation at location $s$. The different curves in Fig. 3 are shifted in phase with respect to each other due to the progression of the waves along the flagellum. If the phase shift for location $s$ is called $\alpha(s)$, with $\alpha(0) = 0$, all curves in Fig. 3 can be expressed together as:
The functions $A(s)$ and $\alpha(s)$ summarize the information presented in Fig. 3.

Fig. 8 shows plots of $A(s)$ and $\alpha(s)$ for two cells, with a frequency of the flagellar wave of 8.2 and 43 Hz, respectively. It can be seen that the data points for $\alpha(s)$ conform closely to a smooth line. This was observed in all cells analyzed. The derivative $d\alpha/ds$ of $\alpha(s)$ is to be interpreted as a local wave number for the flagellar wave. The wavelength $\lambda$ of a flagellar wave is that value of $s$ for which $\alpha(s) = 2\pi$, as indicated in Fig. 8a.

The amplitude $A(s)$ varies smoothly with $s$, as shown in Fig. 8b. The maximum value of the amplitude, $A$, occurred at approx. 15–20 µm from the proximal junction of the flagellum for all sperm.

In Fig. 9 the value for the wavelength $\lambda$ and the maximum amplitude $A$ as defined above are plotted as a function of the frequency of the flagellar wave. Inserted into Fig. 9 are the values for demembranated sea urchin spermatozoa reactivated at very low external ATP concentrations (7). These sperm showed a flagellar frequency of 1.1 Hz at an external ATP concentration of 0.01 mM and of 2.4 Hz at [ATP] = 0.024 mM. The values for $\lambda$ and $A$ were obtained from the multiple flash photographs of reference 7 by the method described in an earlier paper (19). It can be seen in Fig. 9 that both the amplitude and the wavelength vary in a continuous way over the entire range of frequency of 1.1–52 Hz.

The data for the curvature $\rho$ of the flagella as illustrated in Fig. 4 above can be summarized in a way analogous to that used for the amplitudes in Eq. 1. This leads to an expression $\rho(s,t) = \rho(s) \sin(\omega t + \alpha(s))$, where $\rho(s,t)$ is the curvature at location $s$ at time $t$, $\rho(s)$ is the maximum curvature occurring at location $s$, and $\alpha(s)$ is the phase shift at location $s$ analogous to $\alpha(s)$ used in Eq. 1. The values for the phase $\alpha(s)$ for the curvature data were identical to those found for $\alpha(s)$ from the amplitude data, and they need not be presented.

The values for $\rho(s)$, which represent the maximal curvature at location $s$ on a flagellum, were found not to be correlated with the frequency of
the flagellar wave. Even though the smaller wavelength in flagella with higher frequency (Fig. 9b) would lead one to expect a larger curvature, this is apparently compensated by the smaller amplitude of the waves of flagella at higher frequency. Fig. 10 shows the value of \( \rho(s) \) for five sperm analyzed, which cover the entire range of frequencies of 8.2–52 Hz. Not all sperm were plotted to avoid overlapping of symbols in Fig. 10.

The data of Fig. 10 show that towards the head-flagellum junction the maximal curvature increases sharply. Extrapolation to \( s = 0 \) gives a value of \( \rho(0) \) of \( 6-7 \times 10^3 \) cm\(^{-1}\), corresponding to a radius of curvature near the proximal junction of approx. 1.5 \( \mu \)m. This strong curvature near the proximal junction can be observed by close inspection in earlier multiple flash photographs of sea urchin sperm (1, 6, 9, 11). It can also be seen in Fig. 10 that at the end of the flagellum, at \( s = 43 \mu \)m, the curvature does not vanish but has a value of approx. \( 10^3 \) cm\(^{-1}\).

**Asymmetry of the Flagellar Wave**

The spermatozoa swimming in a circular path can be expected to show an asymmetry in the flagellar motion. The curvature data in Fig. 4, which refers to the same sperm as Fig. 3, show a clear offset from the zero line of the curvatures near the head-flagellar junction. This was consistently found for the cells swimming in a circular path. The cells swimming in a straight path did not show this asymmetry. Table I summarizes the data on four sperm swimming in a circular path of approximately equal radius and three sperm swimming in a straight path. It can be seen in Table I that the asymmetry in curvature occurs only near the head-flagellar junction (\( s \leq 5 \mu \)m).

The present data do not show whether the asymmetry is caused by a bent equilibrium position of the flagella, or by an asymmetry in the active force-producing mechanism. However, the fact that motionless cells are most often seen to be straight points to the latter cause as the most probable.

**Transients in Flagellar Motion**

In the course of scanning the films, two spermatozoa were noticed which were initially motionless, but which spontaneously started movement. Since these transients in movement provide probably the sharpest tests for the evaluation of models for sperm flagellar motion the data on both cells will be presented in some detail.

Figs. 11a and 12a present a set of tracings for the two sperm in the process of starting the motion. The deviation of the flagellum from a straight reference line (the amplitude) at various

**Table 1**

| Curvature asymmetry \((\rho_l - \rho_r)/2\) at: |
|-----------------|-----------------|-----------------|
| \( s = 1 \mu m \) | \( s = 5 \mu m \) | \( s < 10 \mu m \) |
| Curved path | 1,800 ± 270 | 520 ± 400 | 200 ± 300 |
| Straight path | 350 ± 350 | 200 ± 300 | 200 ± 300 |

| Average maximal curvature \((\rho_l + \rho_r)/2\) |
|-----------------|-----------------|-----------------|
| \( s = 1 \mu m \) | \( s = 5 \mu m \) | \( s < 10 \mu m \) |
| Curved and straight path | 4,400 ± 500 | 2,300 ± 200 | 1,700 ± 200 |

The asymmetry is expressed as \((\rho_l - \rho_r)/2\), where \( \rho_l \) is the maximal curvature to the “left” and \( \rho_r \) that to the “right.” The average \((\rho_l + \rho_r)/2\) is shown for comparison.

ROBERT RIKMEN Spoel  Movement of Sea Urchin Sperm  317
FIGURE 11  (a) Tracings, at intervals of 2.5 ms, of a sea urchin spermatozoon in the spontaneous transition from rest to full motion.  (b) Amplitudes at various locations on the flagellum as a function of time for the sperm traced in Fig. 11a.  The dotted line at time = 12.5 ms denotes the moment at which motion can be seen to occur at all locations.

locations on the flagellum as a function of time is shown for each sperm in Figs. 11b and 12b, respectively.

The spermatozoon presented in Fig. 11 can be seen from the tracings to start motile activity over the whole length of the flagellum at once. This is confirmed by the amplitude graphs which show motion starting at all locations at approx. 12.5 ms. The spermatozoon of Fig. 12, judged from the tracing alone, would give the impression that the motion started by an increase of the bend near the proximal junction. The amplitude graph in Fig. 12 shows, however, that motion started at every location on the flagellum at approx. 25 ms. In both spermatozoa an apparently normal wave had developed in approx. 40 ms after the onset of motion.

DISCUSSION

The data presented above reveal some interesting properties of the sea urchin sperm flagellar motion which have not been described before. While it is not within the scope of this paper to compare or evaluate the various models which have been presented for flagellar motion (3, 4, 16, 17, 19, 21), a number of general conclusions can be drawn from the present results. These conclusions either put limitations on the types of theoretical models which could be applied, or could guide the developing of such models.

The deviations from the equilibrium position (the amplitude) and the local curvature at each point on the flagellum were found to vary sinusoidally in time for all sperm. This means that the amplitude, and also the curvature, can be repre-
sent by a single frequency, as in Eq. 1 above. In any model which results in a linear differential equation of motion, the force-producing mechanism (the active contractile moment) should then also be represented by the same single frequency. In other words, the force-producing mechanism should vary sinusoidally in time, with the same period as the period of the flagellar motion. A model in which the active contractile moments do not vary sinusoidally in time (which means that other frequencies representing higher harmonics are present) has to contain nonlinearities, which could suppress the manifestation of the higher harmonic frequencies in the motion of the flagella. Such nonlinear models would likely have to be very complicated.

A sperm flagellum can be considered as an autonomous oscillator (20). This oscillator is a truly mechanochemical one, with the force-producing reactions, e.g., between tubulin and dynein, coupled to the mechanical flagellar motion. The observation that sea urchin spermatozoa which start motion from a resting position can develop a full and apparently normal wave within one period indicates that the mechanochemical oscillation can be completely developed within one period. The oscillatory mechanism is therefore probably of the gated-oscillator or the relaxation type. A resonance oscillator would require a longer swinging-in time than one period to develop the full amplitude (14).

The wavelength and the amplitude of the flagellar motion were found to be smoothly but weakly dependent on the frequency of the flagellar wave (Fig. 9). No discontinuity was found between the intact sperm in the range 8.2-52 Hz and the demembranated, reactivated sperm at very low external ATP concentration (1.1-2.4 Hz). The very slow reactivated sperm were operating at a low, ATP-limited level of contractile activity, well outside the range of normal intact sperm. When Ciona sperm (which are very similar
to sea urchin sperm) are treated with low concentrations of Triton X-100, the frequency of the flagellar motion is much reduced. The wavelength of the flagellar motion changes little, however, even if the flagellar frequency is reduced 10-fold by the Triton inhibition (8). At the lowered frequencies, the force-producing mechanism in the Triton-inhibited sperm functions almost certainly at a much reduced level. All the above observations therefore indicate that the wave-shape in sea urchin sperm flagella is not principally determined by the contractile system in the flagella.

In 1966 this author (18) computed the wavelength of flexural vibrations in a passive, elastic rod as a function of a lumped parameter $c = 2\pi k f l^4/IE$, where $k$ is the fluid drag coefficient of the rod, $f$ is its length, and $IE$ its stiffness; $f$ is the frequency of the vibration. In Fig. 13, which is reproduced from reference 18, the data for the wavelength found in the present paper are inserted. Fig. 13 shows that the wavelengths in sea urchin sperm flagella, as a function of frequency, are compatible with those of a passive rod. It should be noted that Fig. 13 was computed in a small amplitude approximation. The values for the wavelength inserted in Fig. 13 were therefore remeasured from the data as the projection of the wavelength onto the median position. With the values of $k = 1.6 \times 10^{-2}$ dyn cm$^{-2}$ s (18) and $l = 4.3 \times 10^{-3}$ cm, the stiffness $IE$ of the sea urchin sperm flagellum derived from Fig. 13 is $1.1 \times 10^{-13}$ dyn cm$^2$. This value is well in agreement with the value of $1 \times 10^{-13}$ dyn cm$^2$ reported for ciliary axonemes (21), and the value of $1.4 \times 10^{-13}$ dyn cm$^2$ found by Lindemann (15), using a different type of analysis than that applied here.

The above observations seem to indicate that the wavelength of the flagellar wave in sea urchin sperm flagella is not determined by the contractile mechanism, but by the elastic properties of the flagellum. A model for the contractile activity should be formulated so as to cancel its influence on the wavelength of the flagellar motion.

The increase in curvature towards the head-flagellar junction shown in Fig. 10 indicates that the moments in the flagellum do not vanish at this junction. There appears to be general agreement now that the stiffness $IE$ of a single axoneme such as the sea urchin sperm flagellum is approx. $10^{-13}$ dyn cm$^2$ (references 4, 5, 21, and this paper). The curvature $\rho$ of $6-7 \times 10^5$ cm$^{-1}$ at the proximal junction (Fig. 10 above) implies that there is an elastic moment $\rho IE$ at that location of $6-7 \times 10^{-10}$ dyn cm. The viscous moment at the proximal junction, caused by the viscous resistance of the head, is rather small. This is confirmed by the observations that in decapitated sea urchin sperm flagellar waveform is only slightly changed compared to that in intact sperm (2, 6, 9). For a typical intact sperm the viscous resistance moment of the head, approximated as a sphere 2 $\mu$m radius at a distance of 2 $\mu$m from the proximal junction, can be estimated to be $1-2 \times 10^{-10}$ dyn cm. A comparison of the phase of the motion of the center of the head, as shown in Fig. 3, with the phase of the curvature at the proximal junction, as shown in Fig. 4, indicates that the viscous

![Figure 13](image_url)
moment of the head lags about 30° in phase behind the curvature. The total moment (viscous plus elastic) present at the proximal junction is thus $7 - 9 \times 10^{-9}$ dyn cm. An active contractile moment of the same magnitude must exist at this junction.

At the distal tip of the flagellum, the curvature does not vanish but has a magnitude of approx. $10^3$ cm$^{-1}$. The contractile fibers terminate well before the distal end of an axoneme (22), and no active moment can be produced at the distal tip. The elastic bending moment of the order of $10^{-10}$ dyn cm at the distal tip is apparently balanced by the moment caused by the viscous resistance of the inert terminal piece. Equations of motion which form the basis of flagellar models should apparently not be solved with boundary conditions which make all moments vanish at both ends of the flagellum. The existence of an active moment close to $10^{-9}$ dyn cm at the proximal junction and the influence of the inert distal piece must be accounted for in the boundary conditions. It should be remembered that the boundary conditions usually determine the type of solutions to be used in solving equations of motion for flagella.

Goldstein has recently described transients from motionless to active state for sea urchin spermatozoa (12). The sperm were induced to start moving by a change in external pH or the ATP content of the medium. The motion developed gradually in these experiments over a time interval of the order of 1 s. Most probably, the transients reflected the time-course of the changes effected in the medium. The development of waves described in this paper was similar to that found in the present paper. Some sperm could be seen to start motion all along the flagellum, while some appeared to start motion from a bend near the proximal junction.

APPENDIX

Each of the curves shown in Fig. 3 of the text is to be represented by a sine curve. To account for the offset and the average sloping of the curves, a more complete expression has been used:

$$U = at + b + A \sin (\omega t + \phi). \tag{1}$$

With $c = A \cos \phi$ and $d = A \sin \phi$, Eq. 1 becomes

$$U = at + b + c \sin \omega t + d \cos \omega t, \tag{2}$$

which is linear in $a$, $b$, $c$, and $d$. $\omega$ can be estimated with good accuracy from the data illustrated in Fig. 3 of the text. A four dimensional least squares fit was performed on the data for the estimated value of $\omega$, and for values of $\omega$ which were offset by steps of 0.1 rad/s. Each value of $\omega$ yields a value for total square deviations, $S$, over all the data points for a sperm

$$S = \Sigma (U_m - U)^2,$$

where $U_m$ represent the data and $U$ the values according to Eq. 1. That value of $\omega$ which resulted in the smallest value of $S$ was taken as the best fitting $\omega$. For each curve the values for $a$, $b$, $c$, and $d$ obtained with the best fitting $\omega$ were adopted as giving the least squares fit.

The residual $R$ of a datum point $U_m$ is obtained from:

$$R = U_m - U$$

and the phase $\phi$ from:

$$\phi = \arccot \left(\frac{c}{d}\right).$$

The procedure was programmed for a Univac 1110 computer (Sperry Rand Corp., Blue Bell, Pa.). The identical method was used for the data representing the curvature.

My thanks are due to Mrs. Sandra Orris for assistance with the experiments and the data analysis.

This work was supported in part by the National Institutes of Health through grant HD-6445.

Received for publication 6 June 1977, and in revised form 19 September 1977.

REFERENCES

1. Brokaw, C. J. 1963. Non-sinusoidal bending waves of sperm flagella. J. Exp. Biol. 43:155-169.
2. Brokaw, C. J. 1966. Effects of increased viscosity on the movements of some invertebrate spermatozoa. J. Exp. Biol. 45:113-139.
3. Brokaw, C. J. 1972. Computer simulation of flagellar movement. I. Demonstration of stable bend propagation and bend initiation by the sliding filament model. Biophys. J. 12:564-586.
4. Brokaw, C. J. 1972. Computer simulation of flagellar movement. II. Influence of external viscosity on movement of the sliding filament model. J. Mechanochem. Cell Motility. 1:203-212.
5. Brokaw, C. J. 1972. Flagellar movement: a sliding filament model. Science (Wash. D. C.). 178:455-462.
6. Brokaw, C. J. 1974. Spermatozoan motility: a
biological survey. The Biology of the Male Gamete. Biol. J. Linn. Soc. 7:433-439.

7. Brokaw, C. J. 1975. Effects of viscosity and ATP concentration on the movement of reactivated sea urchin sperm flagella. J. Exp. Biol. 62:701-709.

8. Brokaw, C. J., and R. Josslin. 1973. Maintenance of constant wave parameters by sperm flagella at reduced frequency of beat. J. Exp. Biol. 59:617-628.

9. Gibbons, I. R. 1974. Mechanisms of flagellar motility. In The Functional Anatomy of the Spermatozoan. B. A. Afzelius, editor. Pergamon Press, New York.

10. Gibbons, B. H., and I. R. Gibbons. 1973. The effect of partial extraction of dynein arms on the movement of reactivated sea urchin sperm. J. Cell Sci. 13:337-358.

11. Goldstein, S. F. 1976. Morphology of developing bends in sperm flagella. In Swimming and Flying in Nature. Vol. 1. T. Y. T. Wu, C. J. Brokaw, and C. Brenner, editors. Plenum Press, New York.

12. Goldstein, S. F. 1976. Bend initiation in quiescent sperm flagella. J. Cell Biol. 70(2, Pt. 2):71a. (Abstr.).

13. Gray, J. 1955. The movement of sea urchin spermatozoa. J. Exp. Biol. 32:775-801.

14. Klotter, K. 1960. General properties of oscillating systems. Cold Spring Harb. Symp. Quant. Biol. XXV:185-188.

15. Lindemann, C. B. 1975. An analytical measurement of the stiffness of intact and demembranated sea urchin sperm during motility. Biophys. J. 15(2, Pt. 2):160a. (Abstr.).

16. Lubliner, J., and J. J. Blum. 1971. Model for bend propagation in flagella. J. Theor. Biol. 31:1-24.

17. Lubliner, J., and J. J. Blum. 1972. Analysis of form and speed of flagellar waves according to a sliding filament model. J. Mechanochem. Cell Motility. 1:157-167.

18. Rikmenspoel, R. 1966. Elastic properties of the sea urchin sperm flagellum. Biophys. J. 6:471-479.

19. Rikmenspoel, R. 1971. Contractile mechanisms in flagella. Biophys. J. 11:446-463.

20. Rikmenspoel, R., A. C. Jacket, and S. E. Orris. 1973. Control of bull sperm motility. Effects of viscosity, KCN and thiourea. J. Mechanochem. Cell Motility. 2:7-24.

21. Rikmenspoel, R., and W. G. Rudd. 1973. The contractile mechanism in cilia. Biophys. J. 13:955-993.

22. Satir, P. 1968. Studies on cilia. III. Further studies of the cilium tip and a “sliding filament” model of ciliary motility. J. Cell Biol. 39:77-94.

23. Summers, K. E., and I. R. Gibbons. 1971. Adenosine triphosphate-induced sliding of tubules in trypsin-treated flagella of sea urchin sperm. Proc. Nat Acad. Sci. U. S. A. 68:3092-3096.