CONTRACTILE MECHANISMS IN FLAGELLA

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ABSTRACT The elastic theory of flexural waves in thin rods accurately predicts the velocity of flagellar bending waves over a wide range of viscosities. This shows that flagella behave as a purely mechanical system for the transmission of these waves. An evaluation of the total bending moment reveals that this moment occurs in phase over the entire length of a flagellum. From this it is concluded that each contractile fiber in the flagella is activated simultaneously over its whole length. The magnitude of the bending moment decreases linearly along the flagellum. This is most easily explained by a sliding filament hypothesis in flagella with the elementary 9 + 2 fibers. The expression found for the bending moment explains logically that the wave velocity in flagella is determined by their mechanical properties and the outside viscosity only.

INTRODUCTION

Flagella, when studied with high speed cinemicrography or by stroboscopic techniques, display smooth, periodic waves of bending which travel distally through them. The velocity of these waves in bull sperm and in sea urchin sperm can be adequately explained by the ratio of the stiffness of the flagellum to the external viscosity (Rikmenspoel, 1965, 1966).

Recently data have become available for the wave velocity in the flagellum of several invertebrate sperm (Brokaw, 1966 *a*) and of bull sperm[1] when the viscosity of the surrounding medium is changed. In this paper it will be shown that at higher viscosity the wave velocity in sperm flagella is always the one predicted from the mechanical properties (viscosity and stiffness) of the system only.

It can be shown quite generally that a bending wave in a passive flagellum would dampen out almost completely before reaching the tip of the flagellum (Machin, 1958; Rikmenspoel, 1965). Active contractile[2] elements therefore have to be present, distributed all along the length of the flagellum. These contractile elements must provide the bending moment necessary to keep the wave amplitude up.

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[1] R. Rikmenspoel and A. Jacklet. 1971. Manuscript in preparation.

[2] The term "contractile" is used in this paper to mean "force-producing." It is not intended to imply a choice between a process involving sliding filaments and one in which local shortening occurs.

Sliding filaments have been proposed by Satir (1965, 1968) as the source of the contractile forces. Gray (1955), Machin (1958), and Silvester and Holwill (1965) have favored local contractile activity. Both these mechanisms have recently been reviewed by Brokaw (1968) and Sleigh (1968).

Following a suggestion by Machin (1958) it has been generally assumed that a wave of active bending moment travels along a flagellum with the same velocity as the mechanical displacement wave. The problem obviously arises of how the chemical event of active contraction is synchronized with the mechanical event of bending, in such a way that the right phase between them is maintained. Machin (1963) has proposed that a nonlinearity in the stress-strain relation in flagella could lead to this synchronization. Brokaw (1966 *b*) has assumed that the chemical event leading to the development of the active moment is initiated by the mechanical wave.

However, an actual calculation of the bending moments in the flagella shows that the active contractile event is not a traveling wave, but a *standing* wave. The bending moment is developed simultaneously over the whole length of the flagellum. A synchronization mechanism between the displacement wave and the contractile event is therefore not needed. This leads to a very simple model of an active flagellum in which the contractile fibers over their whole length are sequentially activated·

A preliminary publication of the results of this paper has been made recently (Rikmenspoel, 1971).

EXPERIMENTAL DATA

Brokaw (1966 *a*) has published data on the effects of viscosity on the motility of sperm of three species of marine invertebrates: *Lytechinus*, *Ciona*, and *Chaetopterus*, at raised viscosity of the medium. Among the measurements given were the wavelength λ and the frequency f of the motion of the flagella. The application of elastic theory (next section) requires that the amplitude of the waves in the flagella be $\ll\lambda$. Illustrations given of the wave forms in sperm of *Lytechinus* and *Ciona* show that this condition remains indeed fulfilled at high viscosity. For *Chaetopterus* sperm this is not the case, and the data for this species could therefore not be used. A large amplitude calculation would be needed for the sperm of *Chaetopterus*.

Table I shows the data on λ, f, and the wave velocity $V = f\lambda$ for *Lytechinus* and for *Ciona*.

A calculation of bending moments requires a precise description of the wave forms in the flagella. Brokaw (1965) has made photographs of the three species mentioned, which show up to five positions of the sperm at very short time intervals. The data for our calculations were derived from these photographs.

If we adopt a coordinate system in which x is the running coordinate along the equilibrium position of the flagellum, and $U = U(x, t)$ is its deviation at x from the equilibrium position, a traveling wave can be described (following the notation in

TABLE I

TAIL WAVE FREQUENCY AND WAVELENGTH OF SPERMATOZOA OF *LYTECHINUS*, *CIONA*, AND *CHAETOPTERUS* AT VARIOUS VISCOSITIES, AS MEASURED BY BROKAW (1966 *a*).

Lytechinus				*Ciona*				*Chaetopterus*		
η	f	λ	V_{wave}	η	f	λ	V_{wave}	η	f	λ
centi-poise	*cps*	μ	μ*/sec*	*centi-poise*	*cps*	μ	μ*/sec*	*centi-poise*	*cps*	μ
1.4	30.1	23.6	710	1.4	35	22.1	775	1.0	26.5	19.5‡
1.9	28.5	23.0	656	2.0	31.5	21.6	680	1.4	26	19.3
2.5	26.5	22.3	590	2.6	28.5	22.5	642			
4.4	23	21.7	500	4.0	25	21.8	545			
6.2	21	21.—	442	5.3	23	21.—	482			
7.2	20	19.—	380	11.5	25	16.—	400			
8.4	17.5	19.2	336	18	20.5	16.3	334			
15	14	17.8	249	27	16.	15.5	248			
30	9	20.—	180*	42	13	14.8	193			
36	9	17.6	158*							

* From observation of one single spermatozoan.
‡ From Brokaw (1965).

our earlier papers, Rikmenspoel, 1965 and 1966) as:

$$U(x, t) = A(x) \sin [\omega t + \alpha(x)]. \tag{1}$$

Equation 1 describes a wave which is periodic in time. The nonsinusoidal form of the waves is expressed in the form of the functions $A(x)$ and $\alpha(x)$ for each special case.

$A(x)$, the amplitude of the waves as a function of x, can be measured from the envelope of superimposed positions of the sperm.

The derivative $\alpha'(x)$ of $\alpha(x)$ has the meaning of a "local" wave number, and therefore $\alpha'(x) = 2\pi/\lambda$. From the photographs λ can be directly measured at various points along the flagellum, thus yielding $\alpha'(x)$.

Fig. 1 shows the data obtained this way for *Lytechinus*, *Ciona*, and *Chaetopterus*. Photographs of only two sperm of each species were available, but the spread of the data is small enough to make them usable. $\alpha'(x)$ in Fig. 1 can be approximated as $\alpha'(x) = a + bx$, giving $\alpha(x) = C + ax + \frac{1}{2}bx^2$. The constant C was taken to be equal to zero. This yields a smooth curve for $\alpha(x)$, going through zero, in analogy with bull sperm flagella (Rikmenspoel, 1965, and this paper).

Fig. 1 shows that λ depends only weakly on x, which justifies our use of an average λ for the interpretation of the viscosity experiments for the invertebrate sperm.

For bull spermatozoa, $A(x)$ and $\alpha(x)$ were measured at four different viscosities from high speed cinemicrographs as described before (Rikmenspoel, 1965). The

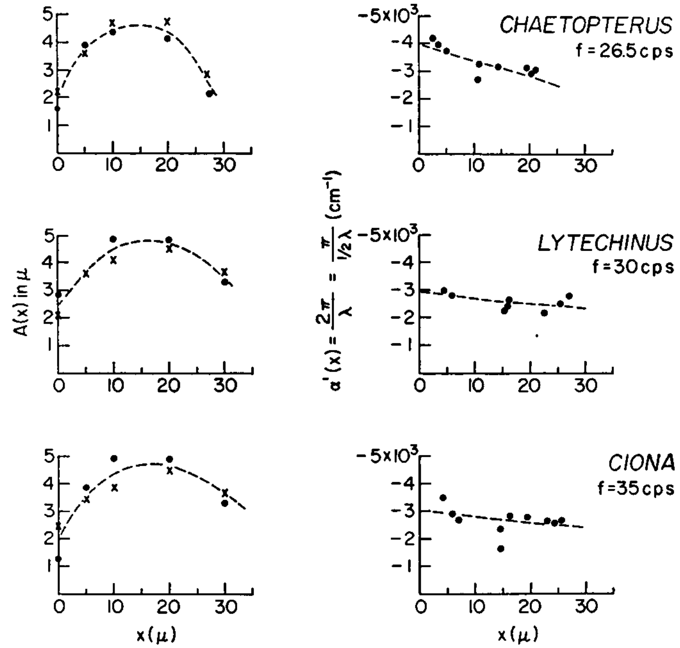
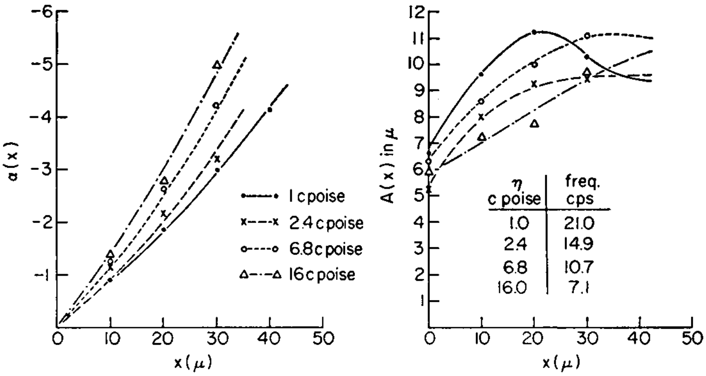


FIGURE 1 The functions $A(x)$ and $\alpha'(x)$ for the invertebrate sperm of *Lytechinus*, *Ciona*, and *Chaetopterus*, as derived from photographs in Brokaw, 1965.



η c poise	freq. cps
1.0	21.0
2.4	14.9
6.8	10.7
16.0	7.1

FIGURE 2 The functions $\alpha(x)$ and $A(x)$ for bull sperm at four different viscosities.[1] The lines shown in the graph for $\alpha(x)$ are computed with the elastic theory.

data shown in Fig. 2 are taken from a forthcoming paper[1] which specifies the details of the experimental circumstances.

ELASTIC THEORY

The theory of flexural waves in a thin elastic rod has been explained in detail before (Rikmenspoel, 1965). Since a further development of the theory will be made in this paper, it seems justified to give a short recount of it.

In a thin rod the moment M_{el} due to its elasticity is given by (Sommerfeld, 1947 *a*):

$$M_{el} = IE\frac{\partial^2 U}{\partial x^2}, \qquad (2)$$

where E is the elasticity modulus of the material of the rod, and I is the second-order moment of the cross section of the rod.

The moment due to the viscous drag of the fluid surrounding the rod M_{visc} is (Sommerfeld, 1947 *b*):

$$M_{visc} = -\int_x^l k(\xi - x)\frac{\partial U}{\partial t}\,d\xi, \qquad (3)$$

where l is the length of the rod, and k is the drag coefficient.

Differentiating equation 3 twice removes the integral sign, and by putting $\partial^2 M_{el}/\partial x^2 = \partial^2 M_{visc}/\partial x^2$,

$$\frac{\partial^2}{\partial x^2}\left(IE\frac{\partial^2 U}{\partial x^2}\right) = -k\frac{\partial U}{\partial t}. \qquad (4)$$

In a tapered rod, I and k in equation 4 are functions of x. If a flagellum is approximated as a truncated cone, having a diameter d_0 at its base and d_1 at its tip, $I = I(x)$ can be written as

$$I = I_0(1 - [x/L])^4, \qquad (5)$$

where $L = ld_0/(d_0 - d_1)$ is the length of the cone extrapolated to its convergence point.

The amplitude b of the tail wave in sperm is not very small (typically $b \approx \frac{1}{4}\lambda$), and the flagellum is therefore not everywhere perpendicular to its direction of motion. A correction for this, and for the change in diameter of the flagellum with x can be made (Rikmenspoel, 1965) by writing

$$k = k_0\eta(1 - [x/L])^2, \qquad (6)$$

where η is the viscosity of the medium.

Inserting equations 5 and 6 into equation 4, and after the transformation $z = x/L$, $\tau = \omega t$, a dimensionless equation is obtained:

$$\frac{\partial^2}{\partial z^2}\left[(1 - z)^4\frac{\partial^2 U}{\partial z^2}\right] = -\frac{k_0\eta\omega l^4}{I_0 E}\left(\frac{L}{l}\right)^4(1 - z)^2\frac{\partial U}{\partial \tau}. \qquad (7)$$

For a flagellum with a given value of (l/L), solutions for equation 7 are dependent only on the dimensionless parameter $c = k_0\eta\omega l^4/I_0E$.

The boundary conditions are: (*a*) free motion at the tip of the flagellum, represented by $\partial^2 U/\partial z^2 = \partial^3 U/\partial z^3 = 0$ at $z = l/L$, and (*b*) the observed motion at the base: $U(0) = A(0) \sin \tau$ and $\partial U/\partial z = BL \cos (\tau + \varphi)$ for $z = 0$. Values for $A(0)$, B, and φ can be calculated from the data in Figs. 1 and 2.

For the invertebrate sperm at higher viscosity the boundary conditions were taken to be identical with those at $\eta = 1.4$ centipoise. It has been shown (Rikmenspoel, 1966) that for these flagella the solutions of equation 7 are almost independent of the exact boundary conditions at $z = 0$.

The solutions for equation 7 have again the form $U(x, t) = A(x) \sin [\omega t + \alpha(x)]$. The propagation velocity of the wave V is given by $V = f\lambda$, or

$$V = \frac{\omega}{\alpha'(x)}. \tag{8}$$

Once I_0E is known for a given flagellum, the propagation velocity of the wave at any viscosity can then be predicted from equations 7 and 8. This process has been applied to the data of the previous section for invertebrate sperm.

In the case of bull sperm, measurements of $\alpha(x)$ are available which can be compared directly with the computed ones.

The method used for computing solutions of equation 7 has been described in detail (Rikmenspoel, 1965). During the computations for the invertebrate sperm at higher viscosity it was found, however, that such large numbers occurred in the series development of the solutions, that they could not be handled by an 8-digit machine. The computations were therefore carried out on the 14-digit CDC 6600, which gave sufficient accuracy.

Invertebrate Sperm Lytechinus and Ciona

The flagella of *Lytechinus* and *Ciona* sperm are practically cylindrical. A value of l/L of 0.1, corresponding to a ratio of the diameters d_0 and d_l of $d_l/d_0 = 0.9$, was adopted for these sperm throughout.

With the boundary conditions mentioned above, equation 7 defines $\alpha(z)$ as a function of the parameter c. The slope of $\alpha(z)$ gives the value of λ/l as a function of c. This results in a dimensionless plot of λ/l vs. c.

The data in Table I can be plotted as λ vs. ηf. By shifting the two plots, each made on a log-log scale, until the best fit is obtained, values for l and for $c/\eta f = 2\pi k_0 l^4/I_0E$ are obtained. The sperm under consideration here have $k_0 = 1.6$ (Hancock, 1953), and a value for I_0E can thus be found.

This procedure may seem to be unnecessarily complicated, but an accurate value for l of *Ciona* sperm was not available. From the data in Brokaw (1965) a length $l = 39\ \mu$ can be derived; another value of $l = 47\ \mu$ was mentioned later (Brokaw and Benedict, 1968). Due to the occurrence of l^4 in the expression for c, the ultimate value for I_0E is strongly dependent on l.

Table II shows the values for l and I_0E adopted for *Ciona* and *Lytechinus* from

TABLE II

VALUES FOR l AND I_0E FOR THE SPERM FLAGELLA OF *LYTECHINUS*, *CIONA*, AND *CHAETOPTERUS*.

	l From observation	l From fitting	I_0E
	μ	μ	*dyne cm²*
Lytechinus	41.6	41	9.0×10^{-14}
Ciona	39–47.5	42	9.1×10^{-14}
Chaetopterus	32	—	2.5×10^{-14}*

* From observations at 1.0 and 1.4 poise only.

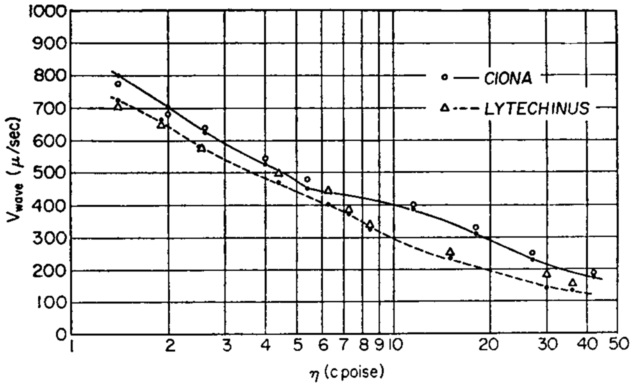


FIGURE 3 Measured values of wave velocity in flagella of *Lytechinus* and *Ciona* (open symbols) and computed values (dots) at various viscosities. The lines drawn through the computed points are for heuristic purpose only.

the procedure described above. The value for l of *Lytechinus* corresponds to the value in the literature, which gives confidence that our procedure was correct.

Previously an estimate for the stiffness of *Lytechinus* flagella of $I_0E = 6 \times 10^{-14}$ dyne cm² was reported (Rikmenspoel, 1966), based on a single measurement of wavelength and frequency. This value was claimed to be good within a factor of two. The presently found value of $I_0E = 9.0 \times 10^{-14}$ dyne cm² is more reliable, and can be considered gratifyingly close to the earlier one.

With these adopted values for l and I_0E, the function $\alpha(x)$ was computed for every value of η at which a measurement was reported for *Lytechinus* and *Ciona* sperm. With equation 8 a computed value of the wave velocity V in each case was thus obtained. Fig. 3 shows the measured and the computed values for V for each species. The fit between the measured and the computed points is quite good. It can be seen, however, that at low viscosity the computed points tend to lie higher; at high viscosity they are lower than the measured ones. This reflects the fact that at low viscosity the wave amplitude is larger, resulting in a "virtual shortening" of the flagella.

For *Chaetopterus* sperm I_0E was calculated from the data at 1.4 centipoise given in Brokaw (1966 *a*), and from those in Brokaw (1965) as shown in Fig. 1. In this last case the viscosity was assumed to be that of water: $\eta = 1.0$ centipoise. The values obtained were $I_0E = 2.1 \times 10^{-14}$ dyne cm² and $I_0E = 2.9 \times 10^{-14}$ dyne cm² respectively. The average of 2.5×10^{-14} dyne cm² is significantly lower than that found for the two other species above. We will see later that this probably explains the different wave forms shown by these sperm at high viscosity.

Bull Sperm Flagella

With the values previously found for bull spermatozoa (Rikmenspoel, 1965) of $k_0 = 2.1$, $I_0E = 1.8 \times 10^{-12}$ dyne cm², $l = 50\ \mu$, and a value of the taper $l/L = 0.5$ corresponding to $d_1/d_0 = 0.5$, the function $\alpha(x)$ could be directly computed for the various values of η. The computed lines are inserted in Fig. 2, and fit well to the measured points.

In the above we have thus seen that the flagellum of invertebrate sperm as well as the more complicated flagellum of bull sperm acts as a purely mechanical system in the transmission of the bending waves.

BENDING MOMENTS

It was mentioned in the Introduction of this paper that active force-producing elements are necessary to provide the bending moment which is needed to maintain the amplitude of the wave as it travels through the flagellum. Chemically this can be represented by the transformation

$$c \rightleftharpoons c^*, \tag{9}$$

where c represents the "relaxed" and c^* the "active" state. Reaction 9 may represent the formation of a cross-bridge in a sliding filament model (Satir, 1965, 1968), or a local molecular contraction (Gray, 1955; Machin, 1958; Silvester and Holwill, 1965).

The time course of reaction 9 at different positions on a flagellum is defined by the active bending moment. Before discussing any properties of reaction 9 our first task therefore should be to obtain an expression for the active moment.

In the presence of an active moment M_{act} the balance equation of a flagellum is

$$M_{\text{el}} + M_{\text{act}} = M_{\text{visc}} . \tag{10}$$

With the expressions for M_{el} and M_{visc} in equations 1 and 2, this becomes

$$M_{\text{act}} = -\int_x^l k(\xi - x)\,\frac{\partial U(\xi, t)}{\partial t}\,d\xi - IE\,\frac{\partial^2 U}{\partial x^2} . \tag{11}$$

From the wave forms $U(x, t)$ in Figs. 1 and 2 and with the values for k and IE found in the section entitled Elastic Theory, M_{act} can be evaluated directly for the various invertebrate sperm and for bull sperm. For k the quadratic approximation utilized in the elastic theory was not employed. Instead the more exact correction factor $1 - 0.5\{[A(x)\alpha'(x)]^2 + A'(x)^2\}$, derived in Rikmenspoel (1965), was used. $A(x)$ and $\alpha(x)$ were approximated by simple algebraic functions.

Since $U(x, t)$ is sinusoidal in *time* (though not along x), M_{act} has to be, from equation 11, also sinusoidal in time:

$$M_{act} = M(x) \sin [\omega t + \mu(x)]. \tag{12}$$

Fig. 4 shows the values computed for $M(x)$ and $\mu(x)$ for the sperm of *Lytechinus*, *Ciona*, and *Chaetopterus* at $\eta = 1.0$ centipoise. Fig. 5 gives $M(x)$ and $\mu(x)$ for bull sperm for the four different viscosities for which data are available. It should be kept in mind that the points in Fig. 5 at $x = 40\ \mu$ are obtained from extrapolating the data of Fig. 2 towards the tip of the flagellum.

Figs. 4 and 5 show that $\mu(x)$ is in all cases practically equal to zero. This means that the active moment M_{act} has its maximum value, goes through zero, etc., everywhere in the flagellum at the same time. This is illustrated in Figs. 4 B and 5 B which give the delay time $\Delta t = -\mu(x)/\omega$ of the occurrence of the active moment along the flagellum, compared to that at the base.

In terms of the occurrence of reaction 9 in the nine contractile fibers, this means that when reaction 9 takes place, it does so over the whole length of a fiber simultaneously within a few milliseconds. Fig. 6 illustrates the arrangement of the fibers. In order to get the sinusoidal time variation of M_{act}, it is necessary that either the fibers at either side (1–4 and 6–9) "fire" in alternation, or that a sequential firing $1 \rightarrow 2 \rightarrow 3 \text{---} \rightarrow 9$ occurs during one period of the motion. The firing has to occur simultaneously over the whole length of a fiber, however.

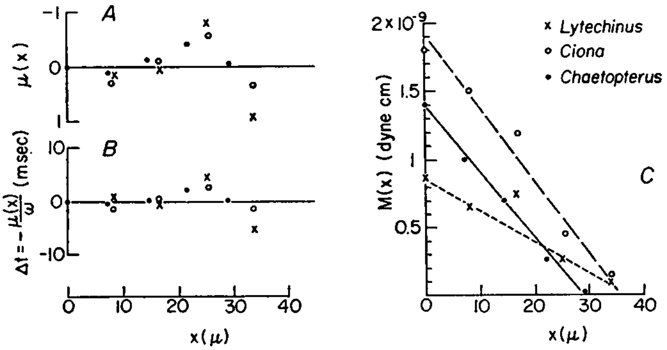


FIGURE 4 (A) The phase $\mu(x)$ of the active moment in the flagellum of *Lytechinus*, *Ciona*, and *Chaetopterus*. (B) Delay time $\Delta t = -\mu(x)/\omega$ in the occurrence of bending moment, relative to the point $x = 0$. (C) Value of the maximum active moment $M(x)$ for the three sperm species as function of x.

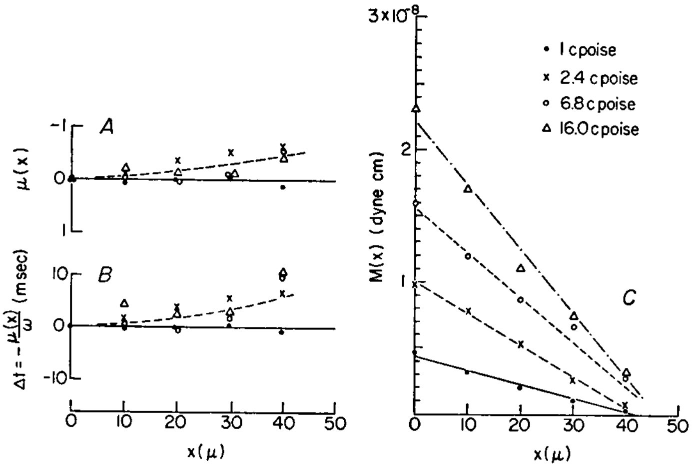


FIGURE 5 (A), (B), and (C). As in Fig. 4, but for the bull sperm flagellum at four different viscosities.

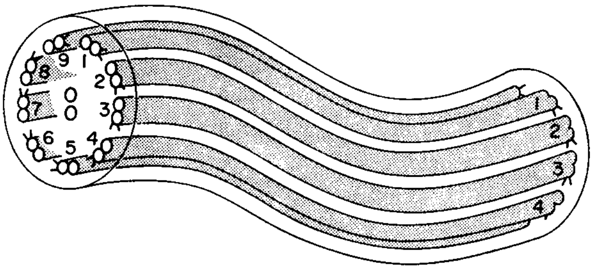


FIGURE 6 Diagram of arrangement of the longitudinal fibers in the invertebrate sperm. The fibers consist of two tubes, out of which pairs of arms project at a spacing of 170 A.

A rotating sequence of firing of the fibers 1 → 2 --- → 9 has the intuitive attraction that it logically explains the helical waves in flagella (see also Gray, 1955). It will be explained in the next section (Equation of Motion) that an alternate side activation of the fibers 1–4, 6–9, etc., also can give rise to helical waves. A choice between these two mechanisms can therefore not be made at present.

Figs. 4 C and 5 C show that $M(x)$ is for both the invertebrate sperm and the bull sperm a linear function of x. For the cylindrical invertebrate sperm this immediately suggests the occurrence of a constant force distribution along the flagellum. For, if a segment dx of a fiber, at a distance ρ from the median (neutral) line produces a force $F\,dx$,

$$M(x) = \int_x^l \rho F\,dx, \qquad (13)$$

or

$$\frac{dM(x)}{dx} = \rho F = \text{constant.} \qquad (14)$$

In the invertebrate sperm with a practically cylindrical flagellum, ρ = constant and therefore F, the force per unit length produced, is a constant. The force-producing units have to be arranged in parallel for the summation of equation 13 to occur. This leads to a mechanical arrangement as in Fig. 7, where independently arranged units act on fiber no. 1. Fig. 7 is in fact a combination of the electron microscope pictures of Gibbons (1963) and Gibbons and Rowe (1965) who identified the dynein molecules as the force-producing sites, and the mechanical model for sliding filaments given by Huxley (1957). There is one difference, however. In Huxley's model all fibers are undergoing a stretching load. In Fig. 7, fiber 1 is loaded for compression, and in order to transmit the distributed force F, produced by the dynein molecules, it should be a stiff structure. This will be elaborated on in the section entitled Equation of Motion.

With the value $(dM/dx) \approx 5 \times 10^{-7}$ dyne and $\rho = 700\text{ A} = 7 \times 10^{-6}$ cm (Afzelius, 1959), one finds for the force at the peak time of the moment:

$$F \approx 7 \times 10^{-2}\text{ dyne/cm.}$$

If only one fiber acts at any time to produce this force, with pairs of dynein molecules spaced 170 A apart (Gibbons, 1965) the force per dynein molecule is roughly 0.6×10^{-7} dyne/dynein. This compares well with the force per actin-myosin cross-bridge in muscle which should be in the order of 10^{-7} dyne/bridge also.

During one period of bending the sliding of filaments along each other has to amount to about 800 A (Sleigh, 1968). The work done by one dynein molecule is then approximately 5×10^{-13} erg. Brokaw and Benedict (1968) have shown that in the flagella under consideration from 1 to 2.5 molecules of adenosine triphosphate (ATP) are broken down per period of bending by each dynein molecule, yielding a free energy of 7 to 20×10^{-13} erg. The significance of the closeness of these figures should of course not be overestimated. But its importance lies in the fact that the work required per dynein molecule is the same everywhere along the flagellum, and that the energy can be provided by a few molecules of ATP.

In case more than one fiber is active at one time, the amounts of force and work per fiber mentioned above are of course lowered. The efficiency of conversion of

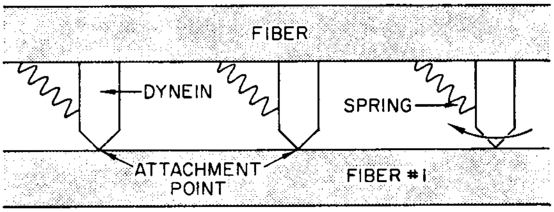


FIGURE 7 Mechanical equivalent of an arrangement of parallel force-producing units in a flagellum.

chemical energy into mechanical work in these sperm has been reported to be around 20%, (Carlson, 1959; Brokaw, 1968). This indicates that the amount of work performed by one dynein molecule during one cycle is 1.5 to 4 × 10^{-13} erg. This makes it probable that several fibers are active at the same time. This could occur during an alternate side firing (fibers 1–4, 6–9, etc.), but also by an overlapping in a sequential firing of the fibers $1 \rightarrow 2 \rightarrow 3 \text{ --- } \rightarrow 9$.

In our previous paper on *Lytechinus* sperm a rough estimate of the active moment was made by putting it equal to the elastic moment. This was done with the hope that the viscous moment would prove to be of approximately the same magnitude as the elastic one. The peak value for M_{el} with the new value for IE found in the present paper is $M_{\text{el}} = 1.8 \times 10^{-10}$ dyne cm (previous value 1.2×10^{-10} dyne cm, Rikmenspoel, 1966). The total active moment turns out to be an order of magnitude larger than M_{el}, however, as shown in Fig. 4 C.

If the contractile forces were generated locally, without the benefit of the summing effect due to the parallel arrangement illustrated in Fig. 7, the active moment $M_{\text{act}} = P\rho$, where P is the force locally developed in the fibers. Our previous value for the moment in *Lytechinus* of 1.2×10^{-10} dyne cm gave a value for P/S of $\approx 1.6 \times 10^{6}$ dyne/cm^2. The present value for M_{act} at the junction of head and flagellum ($x = 0$) in *Ciona* of 1.8×10^{-9} dyne/cm^2 would require maximum tensions of $P/S \approx 2.4 \times 10^{7}$ dyne/cm^2 to be generated at $x = 0$, by four fibers at one side acting simultaneously. This seems an improbably large value indeed. Furthermore, the locally developed force P would have to decrease linearly with x, in order to produce the linearly decreasing bending moment. In view of the cylindrical morphology of the invertebrate flagellum this is not so easily imagined.

As a conclusion we may say that a sliding filament model provides a very satisfactory explanation of the contractile events in invertebrate sperm. A local contraction model does not seem to be attractive.

The bull sperm flagellum, typical in morphology for mammalian sperm, is considerably more complicated than the invertebrate ones. Fig. 8 shows an electron micrograph of a cross section of a bull sperm, taken at this laboratory. The section was made just proximally of the midpiece-tail junction (which corresponds to $x = 0$ in our coordinates), through the midpiece. It shows the doublet inner fibers which are analogous to the fibers found in the invertebrate sperm flagella. Each one of the inner doublets is accompanied by a much larger fiber. These last form the set of the "coarse outer fibers" (Faucet, 1961). ATPase activity has been demonstrated in the coarse fibers. A myosin-like protein (called "spermosin") and an actin-like protein (called "flactin") have been found in these fibers. The total body of biochemical and cytochemical evidence indicating that the coarse fibers are contractile in nature has been reviewed recently by Nelson (1967).

The active moments, and thus the forces, to be produced in bull sperm flagella are at 1 centipoise approximately three times, at 16 centipoise, more than 10 times larger than those needed in the invertebrate species. Brokaw (1966) has concluded that the

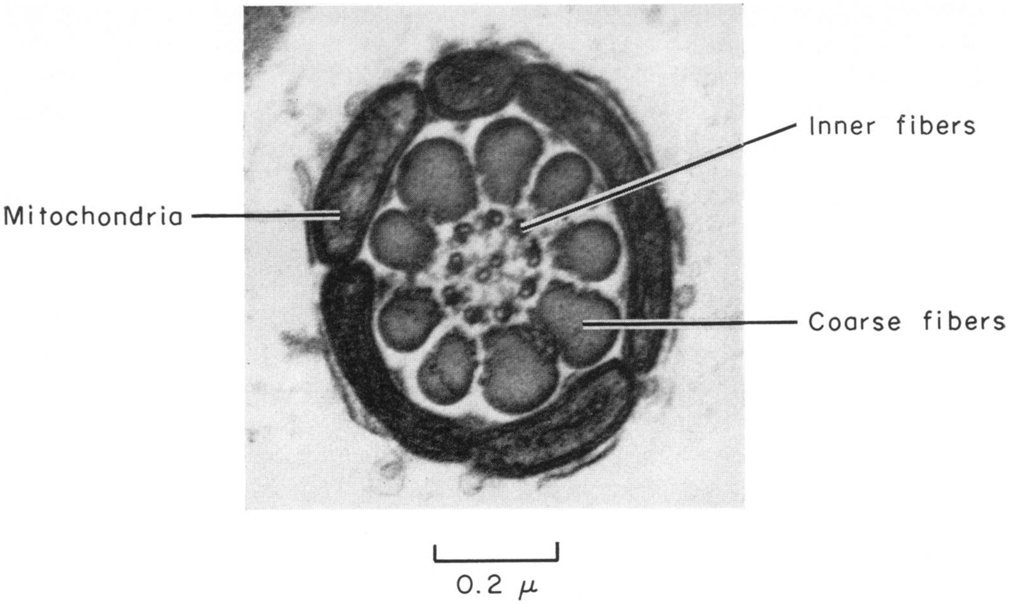


FIGURE 8 Electron micrograph of a cross section of a bull sperm flagellum at the end on the midpiece (just to the left of $x = 0$, in our coordinate system). The mitochondrial sheath, the nine coarse outer fibers, and nine inner fibers are visible. (Courtesy of Mrs. Sandra Sinton).

bending moment developed by the invertebrate sperm is not appreciably increased at higher viscosity. It appears therefore that in bull sperm flagella the bulk of the active moment is to be produced by the coarse fibers.

The diameter of the coarse fibers diminishes rapidly distally. About halfway along the flagellum the first fiber has reduced to zero; before the tip of the flagellum is reached all fibers have gradually disappeared (Faucet, 1961; Bahr and Zeitler, 1964). No structure can be seen in sections of the coarse fibers in bull sperm which is suggestive of a parallel distribution of force-producing sites, as in the invertebrate flagella. The distance of the fibers from the median line, used in equation 14, is not constant along x, which means that a distributed force F, if it existed, would not be constant along x either.

Thus there are no facts available to substantiate an assumption of a sliding filament mechanism in the bull sperm flagellum. It does not mean that it can be absolutely excluded, however.

If the forces are produced locally by the coarse fibers (and are mechanically in series) the maximum force to be developed is

$$P(x) = \frac{M(x)}{\rho}, \qquad (15)$$

and $P(x)$ has to decrease slightly less than linearly with x.

At the midpiece-tail junction ($x = 0$), we previously made a rough estimate of P, as in the case of *Lytechinus*, by taking $M(0) = M_{el}(0)$. In the present sample at $\eta = 1$ centipoise, $M_{el}(0) = 1.6 \times 10^{-9}$ dyne cm (1.2×10^{-9} dyne cm for the sample in Rikemspoel, 1965), and $M_{act}(0) = 4.6 \times 10^{-9}$ dyne cm. This means that the earlier very approximate estimate was a factor of three too low. If only one fiber is active at the time to produce the moment, the maximum force per fiber needed at $x = 0$ is, with $\rho = 1500$ A,

$$\rho \approx 3 \times 10^{-4} \text{ dyne.}$$

The diameter of the fibers in Fig. 8 averages $\approx$1000 A, giving an average cross section S of 7.5×10^{-11} cm^2. The maximum tension per fiber P/S therefore is

$$P/S = 4 \times 10^{6} \text{ dyne/cm}^2.$$

If more than one fiber participates at any given time this figure would be correspondingly lower.

At higher viscosities the moment is increased. At $\eta = 16.0$ centipoise, the moment $M_{act}(0) = 2.6 \times 10^{-8}$ dyne cm. To produce this from one single fiber would require a tension $P/S \approx 2 \times 10^{7}$ dyne/cm^2, which can be considered high. If more than one fiber was active at the same time, this requirement would be relaxed. With four fibers sharing the load, a tension of 5×10^{6} dyne/cm^2 is needed (compared to muscle with 1 to 5×10^{6} dyne/cm^2).

The variation of P with x required by equation 15 can be reasonably accounted for by the decrease of the cross section of the fibers along the flagellum, with a constant value of P/S. As long as no quantitative data are available about the decrease of S with x a more definitive statement cannot be made, however.

In conclusion, we can say that in bull sperm local contractions seem indeed capable of producing the moments needed. As long as no positive indications for a sliding filament mechanism are found, the local contraction mechanism should be considered the most probable.

The values calculated in this section for the forces to be developed by the contractile fibers in the different sperm species have to be treated with some caution. Calculations of M_{act} of equation 11 with simulated sets of data which represented slight variations of the actual data presented in Figs. 2 and 3 were done. From this it was found that the linearity of $M(x)$, and the fact that $\mu(x)$ is small, is not sensitive to experimental error in the data used. A variation of approximately 50% in the value of $M(0)$ can be obtained by a 1 μ variation in the value for the amplitudes. From this we conclude that an uncertainty of up to a facor of two may exist in our figures for the forces produced by the contractile fibers.

EQUATION OF MOTION OF A FLAGELLUM

The equation of motion, equation 11, for an active flagellum can be written, using $M_{\text{act}} = M(x)\cos \omega t$, as:

$$IE\frac{\partial^2 U}{\partial x^2} + M(x)\cos \omega t = -\int_x^l k(\xi - x)\frac{\partial U}{\partial t}. \qquad (16)$$

Equation 16 is fulfilled by the observed wave forms for the invertebrate as well as for bull spermatozoa because M_{act} was derived specifically for that purpose. If the equation is differentiated twice, it will be seen that the "passive" equation 4 appears. $M''(x)$ vanishes, since $M(\text{x})$ is linear in x! Evidently the passive equation 4 is also satisfied by the observed wave forms. We here then see the reason why the passive equation was able to predict correctly the phase of flagellar waves: the active contractile elements have no x-dependent phase factor.

We still cannot say however, that the whole problem of the flagellar wave is completely understood. For this it is necessary that a complete solution of equation 16 be obtained, starting from a set of *initial* conditions. In this way the development of a running wave due to the application of the forcing term M_{act}, from time $t = 0$, onto a sperm rest at $t = 0$, should be found. This problem is presently under consideration at this laboratory. It should be noted that the boundary conditions of free movement at $x = l$ are automatically fulfilled in equation 16.

The forces in the contractile fibers in flagella are developed very close to the center line and may therefore be considered as an axial load. It has been known since Euler that axially loaded thin rods are unstable to buckling. Above a certain critical value axial forces, even though well within the linear and reversible range of Hooke's law, give rise to large radial deflections (see e.g. Landau and Lifshitz, 1959).

In the case of a distributed load in a cylindrical rod, such as the invertebrate sperm, the critical load F_{cr} is (Timoshenko and Gere, 1961):

$$F_{\text{cr}} = \frac{7.8IE}{l^3}.$$

With the values for IE found in the section entitled Elastic Theory for *Lytechinus* and *Ciona* sperm, $F_{\text{cr}} = 2.5 \times 10^{-3}$ dyne/cm; for *Chaetopterus*, F_{cr} is slightly less than 1×10^{-3} dyne/cm.

The actual values of F for these species are approximately 10^{-1} dyne/cm. Since this is much larger than the critical value the development of waves is indeed to be expected. Another consequence of the fact that $F \gg F_{\text{cr}}$ is, however, that the small amplitude approximation of equation 16 cannot be expected to predict the exact shape of the wave forms. For this it will be necessary to consider a large amplitude equivalent of equation 16; that is, it will be necessary to determine the *elastica* for

FIGURE 9 Equilibrium forms of a thin wire under axial load far above the critical value (left) and very far above it (right). The forms resemble very much the wave forms in invertebrate sperm at normal viscosities (left) and those of *Chaetopterus* at higher viscosity, respectively (after Timoshenko and Gere, 1961, p. 82).

a thin rod under a pulsating load[3] far above the critical limit. It is promising that equilibrium positions of a very thin rod under a load far above the critical value show indeed the typical configurations of circle segments connected by straight parts, which have been described by Brokaw (1965, 1966 *b*). Fig. 9 illustrates two of these configurations in which the one at the right is at a higher $F{:}F_{cr}$ ratio than the one at the left. The pattern at the right shows a striking resemblance to the wave forms of *Chaetopterus* sperm at high viscosity, in which the ratio $F{:}F_{cr}$ is indeed higher than in *Lytechinus* and *Ciona*. It seems, therefore, that an explanation of the different wave forms will be obtained from stability considerations.

In the tapered bull sperm the critical force P_{cr} can be written as (Timoshenko and Gere, 1961)

$$P_{cr} = \frac{1.5IE}{l^2} = 10^{-7} \text{ dyne.}$$

This shows that the bull sperm flagellum is also unstable ($P > P_{cr}$ except very near to the tip of the flagellum). If an unstable rod is not perfectly symmetrical in cross section, torsional buckling will occur as well as lateral buckling (Landau and Lifshitz, 1959). Since bull sperm flagella especially are not cylindrically symmetric (Faucet, 1961; Nelson, 1967), this means that helical waves in an alternate-side firing pattern (compare the section entitled Bending Moments) cannot be ruled out.

DISCUSSION

Since we have found in the previous sections that the contractile fibers in flagella are activated at once over their whole length, the problem of synchronizing the contractile event with the mechanical bending has disappeared.

In cilia we have recently found that a similar process takes place (Rikmenspoel and Sleigh, 1970). The bending moment in cilia of *Sabellaria* and *Modiolus*, each of which has a distinct but different beating pattern, occurs in phase over the entire length of the cilia. When the moments shown in Rikmenspoel and Sleigh (1970) are replotted as a function of position along the cilium, an approximately linear relation is obtained.

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[3] The load is to be expressed as a moment, of course.

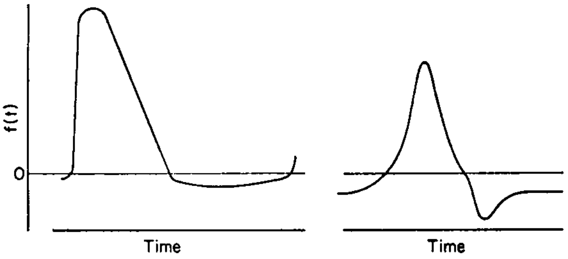

FIGURE 10 Excitation function $f(t)$ for cilia of *Sabellaria* (left) and *Modiolus* (right). Positive and negative values of $f(t)$ refer to excitation of the fibers in these cilia to the left and to the right in their plane of movement. (Derived from Rikmenspoel and Sleigh, 1970).

Cilia have an internal structure which very closely resembles that of the invertebrate sperm flagella treated in this paper. It would seem therefore that also in cilia the sliding filament process discussed before, with the fibers fired over their entire length, occurs.

It can be easily ascertained that cilia are also unstable against buckling when the active moment, as calculated in Rikmenspoel and Sleigh (1970), is considered as an axial load. A crucial test for the validity of the whole model developed in this paper would be therefore the determination of the elastica of cilia in motion. This requires a large amplitude equivalent of equation 16, in which M_{act} is not varying sinusoidally. Instead should be written

$$M_{\text{act}} = m(l - s)f(t),$$

where s is a running coordinate measured along the cilium of length l, and m a proportionality constant. Fig. 10 shows the function $f(t)$ for cilia from *Sabellaria* and *Modiolus*, as derived from Rikmenspoel and Sleigh (1970).

In all of the above considerations, the motion of flagella and cilia has been transformed in fact into a firing pattern of the contractile fibers in these organelles. The control mechanism for this (recently reviewed for cilia by Kinosita and Murakami, 1967) forms an intriguing problem which essentially is still unsolved.

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