# ELASTIC PROPERTIES OF THE SEA URCHIN SPERM FLAGELLUM

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ABSTRACT The theory of flexural vibrations in thin rods, applied to the movement of flagella, has been extended to include an investigation of the influence of the boundary conditions on the theoretical waveforms. It was found that for flagella which are flexible enough, the flexibility can be estimated solely from the wavelength of the wave traveling in it. This can be expected to hold for those flagella which do not possess a fibrous sheath. The bending moment in flagella in which the ampitude of the wave is maintained as the wave travels distally is almost completely produced by active contractile elements. This means that the active bending moment can be estimated from the radius of curvature of the flagellum and the stiffness. The above findings were applied to the case of the sea urchin sperm flagellum. One finds that the stiffness of the flagellum is caused mainly by the nine longitudinal fibers which must have a Young's modulus of slightly less than $10^8$dyne/cm$^2$. The longitudinal fibers need to develop a tension of $1.6 \times 10^6$dyne/cm$^2$ to account for the bending moment in the flagellum. These two figures are in line with those found for muscle fibers.

## INTRODUCTION

In recent papers (Machin, 1958, Rikmenspoel, 1965) it has been shown that it is meaningful to apply the theory of vibrating thin rods to the problems of the wave motion of flagella. For the flagellum of the bull spermatozoon a very detailed description of the motion was given. In this case a complete set of boundary conditions for solving the differential equation (which describes the motion of the flagellum) could be obtained from the observations. From comparison of the computed wave forms and the observed ones the stiffness of the bull sperm tail could be determined and related to its ultrastructure.

For flagella of sperm of other species the best observations published usually include only frequency, amplitude, and wavelength of the wave motion. In particular this means that the movements which drive the flagellum at its point of attachment (the boundary conditions at the end) are not known in detail.

In this paper it will be shown that in a sufficiently flexible flagellum, the motion of the distal part is determined almost exclusively by the stiffness. It can be said that the influence of the boundary conditions at the point of attachment decays as the wave travels distally. The elastic properties of the flagellum of sea urchin spermatozoa and the forces in the contractile elements are derived solely from data of frequency, wavelength, and amplitude of the wave motion.

## THE ELASTIC MODEL OF FLAGELLA

A flagellum can be considered as a thin tapered rod, which is clamped at the midpiece. Fig. 1 shows a sketch of this model and defines the notation used. The differential equation which describes flexural vibrations in the rod is (Rikmenspoel, 1965):

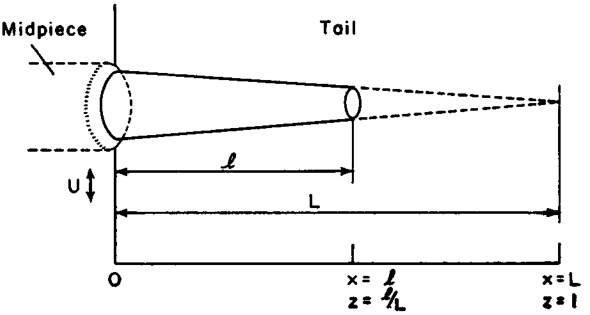

FIGURE 1 Diagram illustrating the model of a tapered flagellum, as used in the calculations. The dimensions in the direction of $U$ are greatly exaggerated. (Fig. 1 reproduced from *Biophysic. J.*, 1965, **5**, 377.)

$$\frac{\partial^2}{\partial z^2}\left[(1-z)^4\frac{\partial^2 U}{\partial z^2}\right] = -c\left(\frac{L}{l}\right)^4(1-z)^2\frac{\partial U}{\partial \tau} \tag{1}$$

with

$$c = \frac{k_0 \omega l^4}{I_0 E} \tag{2}$$

and where $k_0$ = fluid drag coefficient of the rod at the proximal junction,

$$\tau = \omega t$$

$I_0 = \int y^2 ds$, where $ds$ is a surface element in the cross-section at the proximal junction of the tail, and $y$ the distance of $ds$ to the median plane of the tail. $E$ = Young's modulus of the material of the rod.

Equation (1) contains dimensionless quantities, and can be applied to flagella of all dimensions.

Equation (1) describes the motion of a passive flagellum due to a vibration which is forced on it at the junction with the midpiece. The effects of active contractile elements in the flagellum are not taken into account in equation (1). Machin (1963) has argued, however, that in case active contractile elements are present in the tail, "mode-locking" will occur. This means that the wave modes of a passive tail will be preserved in an active one.

Solutions of equation (1) can be written (using complex notation) as:

$$U(z,\tau) = G(z)\,e^{i\tau} + G^{*}(z)\,e^{-i\tau} \tag{3}$$

with $G(z) = \sum_{j=0}^{3} \gamma_j g_j(z)$, and where $g_j(z)$ $(j = 0, \cdots, 3)$ are four independent solutions of the $z$ dependent part of equation (1). At the clamped end ($z = 0$), the movement which drives the rod may be written as: $U = a \sin \tau$, $\partial U/\partial z = b \cos(\tau + \varphi)$. Values for $a$, $b$, and $\varphi$ have to be obtained experimentally. The free movement at the distal end is represented by $\partial^2 U/\partial z^2 = \partial^3 U/\partial z^3 = 0$.

The coefficients $\gamma_j$ follow, after applying the boundary conditions, from:

$$\gamma_0 = \frac{a}{2i}\,; \qquad \gamma_1 = \frac{b}{2}e^{i\varphi} = \frac{a}{2}\left(\frac{b}{a}e^{i\varphi}\right) \tag{4}$$

$$\gamma_2 g_2'' + \gamma_3 g_3'' = -\frac{a}{2}\left(\frac{1}{i}\,g_0'' + \frac{b}{a}e^{i\varphi}g_1''\right) \tag{5}$$

$$\gamma_2 g_2''' + \gamma_3 g_3''' = -\frac{a}{2}\left(\frac{1}{i}\,g_0''' + \frac{b}{a}e^{i\varphi}g_1'''\right) \tag{6}$$

Equations (4) through (6) show that for a given value of $b/a$ and $\varphi$ the solutions for $U(z,\tau)$ are proportional to the amplitude of the forced movement, $a$. This means that only two independent parameters occur in the boundary conditions: $b/a$ and $\varphi$.

The wave motion of the rod, given in equation (3), can be written as:

$$U(z,\tau) = A(z)\sin[\tau + \alpha(z)] \tag{7}$$

with

$$A(z) = 2\,|G(z)| \tag{8}$$

and

$$\alpha(z) = \operatorname{arctg}[-\mathrm{Re}\,G/\mathrm{Im}\,G] \tag{9}$$

It can be easily verified that for the slope of $\alpha(z)$ at $z = 0$ it holds:

$$\frac{d\alpha(z)}{dz}(z = 0) = \frac{b}{a}\cos\varphi, \tag{10}$$

independent of the stiffness of the flagellum. In the following section we shall investigate the form of the function $\alpha(z)$ for various values of stiffness and boundary conditions.

Solutions for equation (1) are not very sensitive to the exact value of the truncation factor $l/L$, as long as $1/3 < l/L < 2/3$ (Rikmenspoel, 1965). In all calculations reported below, a fixed $l/L = 0.5$ was therefore used. This value of $l/L$ is a reasonable average for the tapering observed in flagella (compare Afzelius, 1959; Bretschneider and van Iterson, 1947; and Fawcett, 1958).

Values of $\alpha(z)$ were computed on an IBM 7094 computer for various values of $c$, $b/a$, and $\varphi$ with the method described in Appendix II of the paper by Rikmenspoel (1965). For flagella which are flexible enough that $c > 3 \times 10^3$ it was found that the slope of $\alpha(z)$ in the distal 3/4 part of the flagellum is practically independent of the boundary conditions.

Fig. 2 shows, at a value $c = 3 \times 10^3$, the function $\alpha(z)$. The value of $b/a$ was varied through the range of +30 to $-\infty$, that of $\varphi$ from 0 to $-2.6$ rad. For those cases where $(b/a)$ cos $\phi > 0$ it can be observed in Fig. 2 that the wave does indeed start traveling *toward* the proximal junction (in the figure this is shown by $\alpha(z)$ *going downward*). All curves bend toward the same upward slope, however, indicating a *distally* traveling wave in the distal part of the flagellum.

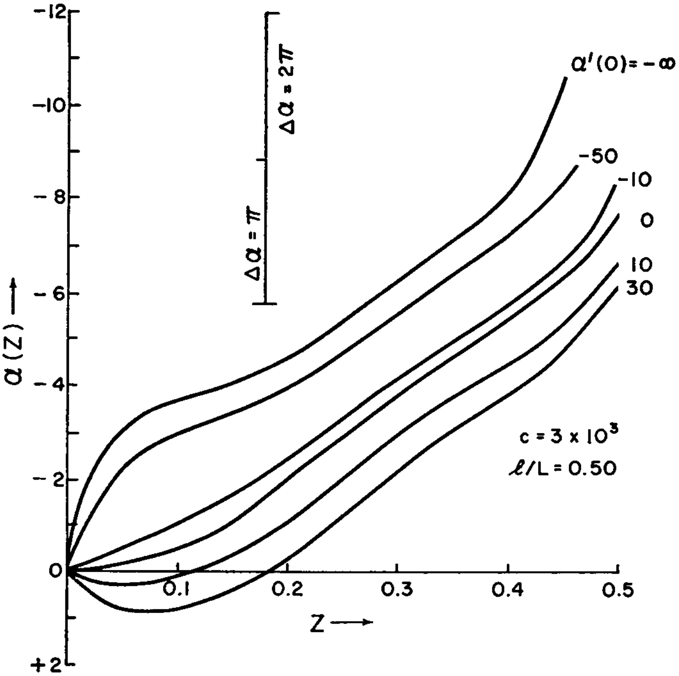

FIGURE 2 Form of $\alpha(z)$ for different values of $(b/a)\cos\varphi$, at a stiffness of the rod $c = 3 \times 10^3$.

As may be expected from equation (10), the influence of varying $b/a$ and $\varphi$ such that the value of $(b/a)\cos\varphi$ remains fixed, is small over the whole length of the flagellum. For this reason only a few representative curves of $\alpha(z)$ are shown in Fig. 2, each curve labeled by the value of $(b/a)\cos\varphi$ only.

The values of $\Delta z$ for which $\Delta\alpha = \pi$ and $\Delta\alpha = 2\pi$ represent the length of one half, and one full wave respectively, in the flagellum. Fig. 3 shows the values of $(\lambda/2)/l$ and $\lambda/l$ for various values of the flexibility, $c$. The computed curves for $\alpha(z)$ are not straight lines. The values of $(\lambda/2)/l$ and $\lambda/l$ are therefore slightly dependent on the choice of the part of the flagellum in which they are determined. In the region near the distal end the accuracy of the computation decreases, due to trunctation error in the series development of the functions $g_j(z)$. The variation due to this cause, and due to varying the boundary conditions is shown in Fig. 3 by the cross-hatched bands.

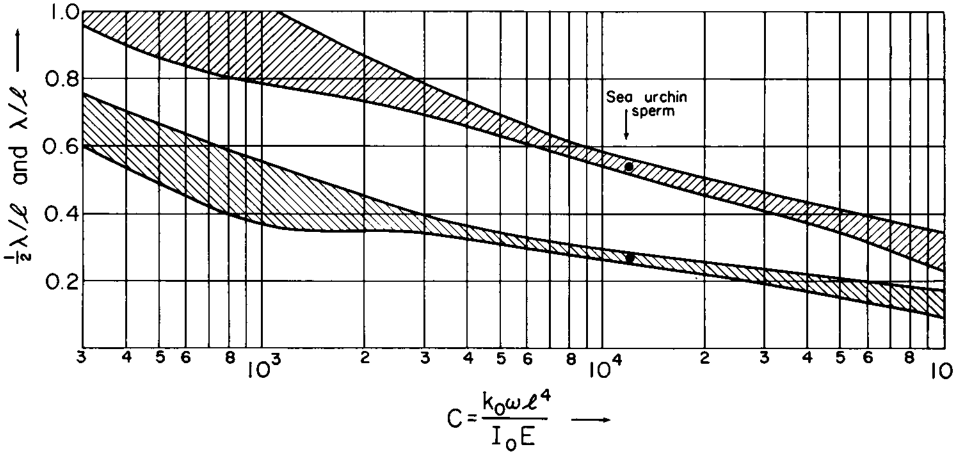

FIGURE 3 Value of $(\lambda/2)/l$ and $\lambda/l$ for flagella as a function of the stiffness, $c$. The cross-hatched band shows the range of $(\lambda/2)/l$ and $\lambda/l$ due to variation of the boundary conditions of equation (1) of the text.

When $c > 10^5$, the series development for the functions $g_j$ is converging so slowly that the method applied for solving equation (1) does not give results of acceptable accuracy. Due to this inaccuracy the cross-hatched bands in Fig. 3 become wider when $c$ approaches $10^5$. However, for $3 \times 10^3 < c < 10^5$, a value for the flexibility of a flagellum, good within a factor of 2, can be obtained solely from the wavelength of the wave in it. For flagella with a stiffness comparable with those of bull sperm ($c \approx 10^3$) this simplified analysis will not be applicable, however, as Fig. 3 shows.

Brokaw (1965) has published observations by means of photomicrography on the movement of the sea urchin sperm flagellum. The average values reported for the wavelength $\lambda$ ($22.6\mu$) divided by $l$ ($41\mu$) are inserted in Fig. 3. For the flexibility of the sea urchin sperm flagellum one thus finds:

$$c = \frac{k_0\omega l^4}{I_0E} = 1.2 \times 10^4$$

The value reported for $\omega$ was $2\pi \times 30$ rad/sec, and the value of $l$: $41\mu$. A calculation similar to the one applied to the drag coefficient of the bull sperm flagellum

(Rikmenspoel, 1965) yields the value of $k_0$ for sea urchin sperm: $k_0 = 1.6 \times 10^{-2}$dyne cm$^{-2}$sec. One thus finds:

$$I_0E \approx 6 \times 10^{-14} \text{ dyne cm}^2$$

A cross-section of the sea urchin sperm tail (Afzelius, 1959) is shown in Fig. 4. The nine longitudinal fibers (common in all flagella) are clearly visible in Fig. 4, embedded in matrix. The value of $I_0$ using the dimensions shown in Fig. 4 is: fibers $I_0 = 6 \times 10^{-22}$ cm$^4$, matrix $I_0 = 3 \times 10^{-21}$ cm$^4$.

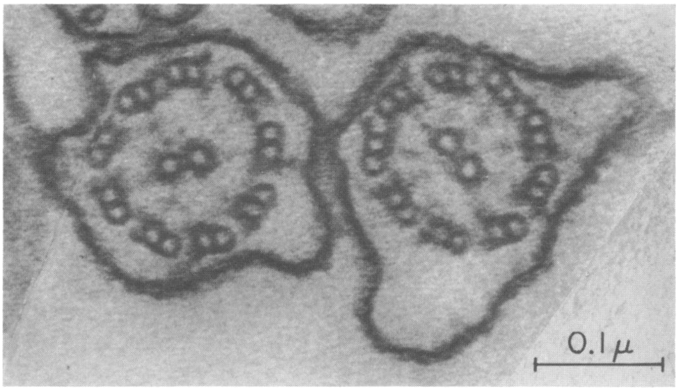

FIGURE 4 Electron micrograph of a cross-section of a sea urchin sperm flagellum. The nine longitudinal fibers (the figure eight–shaped features) each have a cross-section of approximately $250 \times 150$ A$^2$ (Courtesy of Dr. Bjorn Afzelius).

The matrix material shows no observable structure (compare Fig. 4). An upper limit for its Young's modulus $E$ of a few times $10^6$dynes/cm$^2$ is therefore a reasonable figure. The contribution of the matrix material to the stiffness of the sea urchin sperm flagellum accordingly is probably $<6 \times 10^{-15}$dyne cm$^2$, or $<10\%$. This means that the stiffness of the sea urchin sperm flagellum is caused mainly by the longitudinal fibers, which must have a value of $E$ of slightly less than $10^8$dyne/cm$^2$. This latter value is not far from the Young's modulus of muscle fibers of $6 \times 10^7$dyne/cm$^2$ reported by Bozler (1957).

## BENDING MOMENTS IN FLAGELLA

The passive wave described in the two preceding sections is heavily dampened by the fluid drag as it travels distally. For $c > 10^3$, which means that the flagellum is flexible enough to exhibit at least one full wave, the amplitude $A(z)$ at a point 2/3 on the way along the flagellum is reduced to $<0.3A(0)$.

The experimental evidence shows that in sperm flagella the amplitude of the tail wave is at least maintained as the wave travels distally (Gray 1955 and 1958; Rikmenspoel, 1957 and 1965, Brokaw, 1965). This necessitates the assumption of contractile elements in the flagellum, which provide the extra bending moment needed (compare Machin, 1958 and Rikmenspoel, 1965). It has been shown (Rikmenspoel, 1965) that this "active" bending moment $M_{act}(z, \tau)$ may be roughly represented by

$$M_{act}(z, \tau) = p \frac{\partial^2 U}{\partial z^2} \tag{11}$$

where $(1 + p)$ is the amount by which the passively induced moment is amplified by the contractile elements.

When equation (11) is inserted in equation (1), it is possible to solve for that value of $p$ for which the amplitude of the wave 2/3 of the way along the flagellum is the same as at the proximal junction (for a given stiffness). The value of $p$ found in this way is of course dependent on the boundary conditions used. The slope of the function $A(z)$ at $z = 0$ is (Rikmenspoel, 1965, Appendix II)

$$\frac{dA(z)}{dz}(z = 0) = b \sin \varphi$$

independent of the stiffness of the rod. In the present calculations of $p$ the boundary conditions were varied such that $dA(z)/dz$ at $z = 0$ varied from 0.07 to 1.0. This range includes all cases which can be expected to occur.

Fig. 5 shows the value of $p$ needed to maintain the amplitude of the wave at a

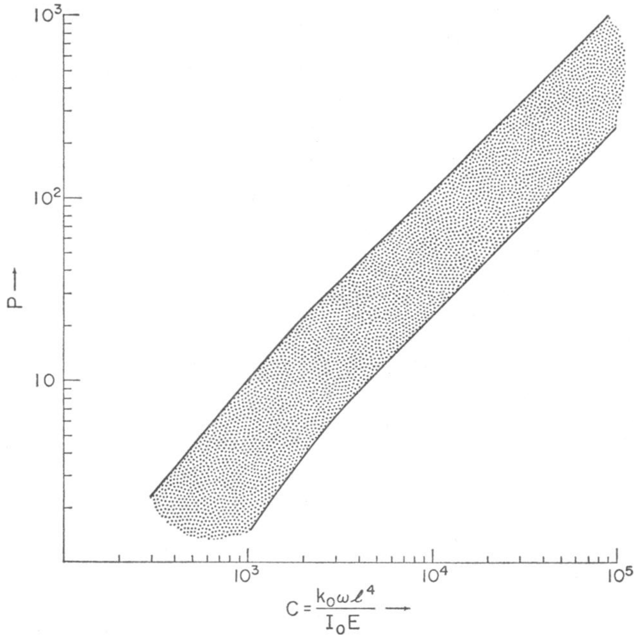

FIGURE 5 Value of $p$ needed to maintain the amplitude of a flagellar wave as it travels distally. The cross-hatched band gives the range of $p$ due to variation of the boundary conditions.

point 2/3 along the flagellum. The dotted band in Fig. 5 represents the variation of $p$ in the range of boundary conditions applied in the calculation. It can be seen from Fig. 5 that if $3 \times 10^3 < c < 10^5$, $p$ is at least $\approx 3$. This means that at least 3/4 of the total moment is provided by active contractile elements. Therefore the "active" moment may be taken to be equal to the total moment with no great error.

If the active bending moment $M_{act}$ at the proximal junction is taken equal to the total bending moment, it follows immediately that

$$M_{act} = \frac{1}{\rho} I_0 E \tag{12}$$

where $\rho$ is the radius of curvature of the flagellum at the proximal junction. For the case of sea urchin sperm Brokaw (1965) has reported $\rho = 5 \times 10^{-4}$ cm. With the value of $I_0E$ found above,

$$M_{act} = 1.2 \times 10^{-10} \text{ dyne cm}$$

By considering the viscous drag forces acting on the sea urchin sperm flagellum, Brokaw estimated the total bending moment to be $2.9 \times 10^{-10}$dyne cm. The fact that the two independent estimates agree within a factor of little more than 2 is in view of the approximate nature of the analysis satisfactory.

If four of the longitudinal fibers at one side of the median plane provide the bending moment of $1.2 \times 10^{-10}$dyne cm, the force $P$ needed is

$$P \approx 6 \times 10^{-6} \text{ dyne/fiber}$$

The tension per unit area of cross-section of one fiber is, with the area $S = 250 \times 150$ A$^2$:

$$P/S \approx 1.6 \times 10^{6} \text{ dyne/cm}^2$$

## DISCUSSION

In the previous sections it has been found that estimates of the stiffness of flagella and of the forces acting in contractile elements can be fairly well estimated from the wavelength of the wave, and from the maximum curvature of the flagellum respectively. This obviates in most cases the need for tedious analysis of the flagellar motion from high speed cinemicrographs, as was applied earlier to the case of bull spermatozoa.

The flagellum of bull sperm for which analysis from cinemicrographs is a necessity (as shown in the section on the stiffness of flagella) receives its large stiffness mainly from the fibrous sheath which surrounds the longitudinal fibers. The conclusion therefore seems warranted that the simplified analysis given in this paper will apply to those flagella in which no fibrous sheath is present.

The longitudinal fibers found in flagella have long been suspected of being contractile elements. Biochemical evidence for a myosinlike protein in the fibers was given by Burnasheva (1958) and Nelson (1961). The value for Young's modulus of the fibers of sea urchin sperm is reported here as close to that of muscle fibers. The estimate of the tension developed in the fibers of bull sperm of 4 × $10^6$dyne/cm$^2$ (Rikmenspoel, 1965) and sea urchin sperm of 1.6 × $10^6$dyne/cm$^2$ (this paper) is in the range of the tension developed by muscle fibers of 1 to 5 × $10^6$dyne/cm$^2$ (Spector, 1956). The identification of the longitudinal fibers in flagella as contractile elements can from this evidence be considered as practically certain.

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