J. Exp. Biol. (1966), **45**, 113–139
With 2 plates and 14 text-figures
Printed in Great Britain

EFFECTS OF INCREASED VISCOSITY ON THE MOVEMENTS OF SOME INVERTEBRATE SPERMATOZOA*

BY C. J. BROKAW
Division of Biology, California Institute of Technology, Pasadena, California

(*Received* 3 *January* 1966)

INTRODUCTION

In the wave pattern of a flagellum generating planar undulatory bending waves, bending and unbending are represented by rather abrupt transitions between bent and unbent states (Brokaw & Wright, 1963; Brokaw, 1965). The magnitude of these transitions is indicated by the radius of curvature of the bent regions of the wave pattern, but their detailed time course cannot be resolved. The propagated bending waves required for propulsion are generated by the progression of these transitions along the flagellum.

Bending and unbending require internally generated bending moments sufficient to overcome any internal resistances to bending and the external resistance imposed by viscous drag forces as the flagellum moves through its surrounding medium. The bending moment required to overcome the external viscous drag and the work done by this moment can be calculated in terms of wave parameters and viscous drag coefficients (Brokaw, 1965). The present experiments were undertaken to examine the changes in wave parameters resulting from alterations in the viscous drag coefficients, in an attempt to provide a more detailed characterization of the mechanisms for generating and controlling bending and unbending. Spermatozoa of three species of marine invertebrates were examined under the same conditions previously used to study their normal movements, except that the viscosity of the sperm suspensions was increased by the addition of methyl cellulose. To assist the interpretation of the different results obtained with the spermatozoa of different species, additional experiments were carried out in the presence of thiourea, an inhibitor of motility which reduces the beat frequency, and with glycerinated spermatozoa, where the beat frequency can be controlled by the concentration of adenosine triphosphate used for reactivation of their movements.

METHODS

Spermatozoa were collected and photographed in a modified sea-water solution at 16° C., using the methods described in earlier papers (Brokaw, 1963, 1965). For most of the experiments, increases in viscosity were obtained by adding high molecular weight methyl cellulose (4000 cP.; Fisher Scientific Co., no. M-281, lot 722223) to

* This work has been supported in part by a grant from the United States Public Health Service (RG-6965). I am also indebted to Dr Anton Havlik for allowing me to use the Ferranti–Shirley viscometer in his laboratory at the Jet Propulsion Laboratory, Pasadena; to Stuart Goldstein for assistance with the mathematical development in the Appendix; and to Richard Flammang and Gary Ratner for assistance with some of the experimental work.

the sea-water solutions. Unless otherwise indicated, all references to methyl cellulose throughout this paper will refer to this material. A few observations were made with a methyl cellulose of lower molecular weight (100 cP., Fisher Scientific Co., no. M-279, log 723784) which will be referred to as low-MW methyl cellulose. Stock solutions containing 1 or 1·5 g. of methyl cellulose (or 2–3·5 g. of low-MW methyl cellulose) per 100 ml. were made by suspending the dry powder in sea-water solution at approximately 40° C., and then refrigerating, with occasional stirring, until the solutions became clear and appeared to be uniformly viscous. Working solutions were made as required by diluting the stock solution with sea-water solution. All the solutions were refrigerated between experiments, and fresh solutions were made up every 2–3 days.

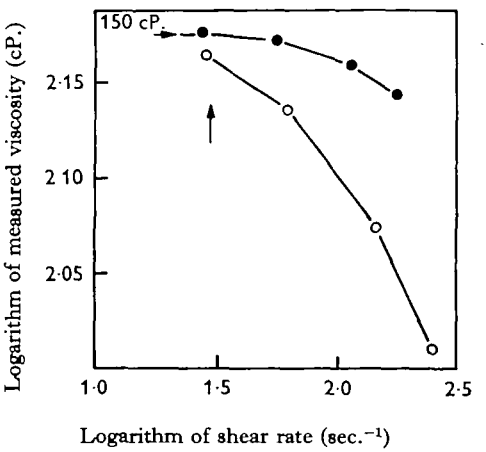
Solutions containing thiourea were made with a diluted sea-water solution, to reduce the possible osmotic shock effects of the relatively high concentrations (up to 0·3 M) of thiourea which were used. A 0·3 M thiourea solution was made by dissolving 0·03 M of thiourea in 85 ml. of sea-water solution and adding distilled water to bring the volume to 100 ml. The pH was readjusted to 8·1 after the thiourea had dissolved. Lower thiourea concentrations were obtained by diluting the 0·3 M solution with sea-water solution. Control solutions, containing urea in place of thiourea, were made in the same manner. The viscosity series with *Lytechinus* spermatozoa was carried out in solutions containing 0·1 M urea.

Glycerinated spermatozoa were prepared by mixing concentrated spermatozoa with an equal volume of sea-water solution, and allowing the mixture to stand for a few minutes at 18° C., before adding it to approximately ten volumes of a 55% (by volume) solution of glycerol containing 0·25 M-KCl, 0·01 M-$MgCl_2$ and 0·02 M of pH 7·8 tris-thioglycolate buffer (Brokaw, 1961) at −10° C. The spermatozoa were stirred gently into the glycerol solution and then kept at approximately −10° C. A fresh preparation was used for each day's experiments. The reactivation solutions contained 0·25 M-KCl, 0·004 M-$MgCl_2$, 0·02 M tris-thioglycolate buffer, 3% polyvinylpyrollidinone, 0·1 M urea, 10^{-4} M adenosine diphosphate and variable concentrations of adenosine triphosphate (ATP) and methyl cellulose. The pH was adjusted to 7·8 before mixing with the proper amount of a concentrated solution of methyl cellulose in distilled water to give the desired final concentrations.

Viscosity measurements were made on the working solutions, before addition of spermatozoa. For the experiments with live spermatozoa, which were generally diluted 100-fold or more in the working solution, the consequent error in estimating the viscosity of the solution in which the sperm were actually swimming was negligible. With the reactivated spermatozoa, a small drop of the suspension of spermatozoa in glycerol was injected with a fine-tipped pipette into a large drop of reactivation solution on a slide and mixed gently with the pipette before adding a cover-glass. The dilution of the glycerol solutions was estimated to be tenfold or greater in all these experiments, which is not great enough to exclude errors in the viscosity estimation. However, this error can be shown to involve at most an overestimate of the viscosity by about 60% at the highest viscosities used in these experiments. The mean error was probably considerably less and was small compared with the other uncertainties in the viscosity measurements and the results obtained with reactivated spermatozoa.

Viscosity determinations

A viscosity measurement was made on each working solution at 16° C. in a standard Ostwald–Cannon–Fenske type of capillary viscometer. The no. 200 size was used at viscosities up to about 10 cP.; the no. 350 size was used at higher viscosities. These measurements provide a unique characterization of each solution, but do not necessarily indicate the effective viscosity for movement of spermatozoa through the solutions, since the viscosity behaviour of methyl-cellulose solutions is complex. Since at least part of this complexity may be expected to arise from a variation in viscosity with the rate of shear in the solution, the viscosity should be measured at the same shear rate which prevails for a spermatozoon moving through the solution.



Text-fig. 1. Measurements of the apparent viscosity of sea-water solutions of methyl cellulose (open circles) or low-MW methyl cellulose (solid circles) at several values of the shear rate, calculated at the surface of the capillary on the assumption that the viscosity is uniform within the solution.

Several methods were tried for measuring the viscosity of typical methyl-cellulose solutions at different shear rates. The most satisfactory method involved simply connecting a wide-bore 10 ml. pipette to the narrow end of a capillary viscometer by about 1 m. of flexible plastic tubing. By supporting the pipette at various levels the pressure driving a constant volume of solution through the tubing and capillary could be varied, to enable measurements over about an eightfold range of shear rates. An example of the results obtained with this arrangement is shown in Text-fig. 1. The results are consistent with measurements at higher shear rates, for which a Ferranti–Shirley cone and plate viscometer was used, and with the published results of Gillespie (1960). These measurements were carried out at ten different concentrations for both methyl cellulose and low-MW methyl cellulose, so that any capillary viscosity measurement could be interpolated to other shear rates within the range studied.

The shear rate, H_L, at the surface of a cylinder moving parallel to its axis with a velocity V_L, through a medium of viscosity η, will be related to the tangential drag coefficient, C_L, as follows:

$$2\pi d\eta H_L = C_L V_L,$$

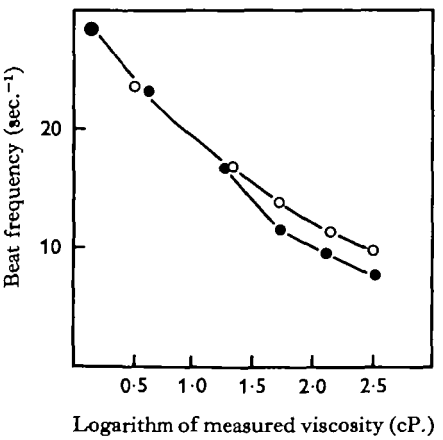
where d is the radius of the cylinder. C_L can be obtained by hydrodynamic analysis of the drag on an elongated cylindrical object (Burgers, 1938) and has been adapted for use in analysis of flagellar movement by Hancock (1953) and Gray & Hancock (1955). Using typical values for a sperm flagellum (Brokaw, 1965) then gives

$$H_L(\text{sec.}^{-1}) = 2{\cdot}0\ V_L\ (\mu/\text{sec.}).$$

By similar arguments the average shear rate at the surface of a flagellum resulting from movement normal to its axis with a velocity V_N can be shown to be

$$H_N\ (\text{sec.}^{-1}) = 3{\cdot}5 V_N\ (\mu/\text{sec.}).$$

These derivations require the usual assumptions involved in a simplified hydrodynamic analysis of flagellar movement, including the assumption that the viscosity of the medium is independent of shear rate. Although there is no assurance that the overall viscous resistances will behave similarly in a capillary and around a flagellum when dealing with methyl–cellulose solutions, in which the viscosity varies with shear rate, these expressions for the shear rate at the surface of the flagellum can be used as a first approximation to estimate the effective viscosity for movement of a flagellum through various methyl–cellulose solutions. As will be seen from the following example, this method does not sufficiently compensate for the non-ideal viscosity behaviour of these methyl–cellulose solutions, and a more accurate adjustment procedure appears to be to adjust the measured viscosities by using twice the logarithmic adjustment factor indicated by measurements of viscosity at various shear rates.



Text-fig. 2. Beat frequencies of *Chaetopterus* spermatozoa as a function of measured viscosity in solutions containing methyl cellulose (open circles) or low-MW methyl cellulose (solid circles). Results from experiments with two different sperm samples have been combined; each point represents 1–4 sets of measurements on twenty spermatozoa.

When *Chaetopterus* spermatozoa swim in a methyl–cellulose solution with a measured viscosity of about 150 cP., V_L is practically equal to the velocity of bend propagation along the flagellum, V_s, which is about 150μ/sec.; H_L is therefore about 300 sec.$^{-1}$. Text-fig. 1 indicates that the logarithmic adjustment factor which should be used to adjust the measured viscosity (measured at a shear rate of approximately 30 sec.$^{-1}$) to a shear rate of 300 sec.$^{-1}$ should be about 0·2 log units; this will give an estimated

actual viscosity of about 95 cP. Text-fig. 2 gives the results of measurements of the beat frequency of *Chaetopterus* spermatozoa in methyl–cellulose and low-MW methyl–cellulose solutions as a function of the measured viscosity. These results indicate that in the range of 100–150 cP. a change in viscosity by 0·2 log units will cause a change of about 10–15% in beat frequency. The beat frequency of spermatozoa at an actual viscosity of 150 cP. should therefore be about 10–15% less than the value measured in a methyl–cellulose solution with a measured viscosity of 150 cP. The beat frequency of spermatozoa in a low-MW methyl–cellulose solution with the same measured viscosity (150 centipoise) should be intermediate. However, the results in Text-fig. 2 indicate that the beat frequency in the low-MW methyl–cellulose solution is actually about 20% less than that in the high-MW methyl–cellulose solution. The difference between the beat frequencies in the two solutions appears to be significantly greater than expected on the basis of the shear rate effect, so that the adjustment factor must be significantly greater than 0·2 log units.

Viscosity measurements of the type illustrated in Text-fig. 1 indicate that, over the range of viscosities studied, the effect of shear rate on the viscosity of the high-MW methyl–cellulose solutions is about 3 times its effect on the viscosity of the low-MW methyl–cellulose solutions. If the beat frequency is 20% less in a low-MW methyl–cellulose solution with a measured viscosity of 150 cP. than in a high-MW methyl–cellulose solution with the same measured viscosity, it should be about 30% less in a solution with an actual viscosity of 150 cP.; or about 2–3 times the effect originally predicted. The adjustment factor for a methyl–cellulose solution with a measured viscosity of 150 cP. should then be 2–3 times that predicted on the basis of Text-fig. 1, or 0·4–0·6 log units; this will give an estimated actual viscosity of 40–60 cP. For the purposes of this paper, adjusted viscosity values have been obtained by using twice the logarithmic adjustment factor predicted from the measurements of the effect of shear rate on viscosity. This procedure obviously gives only a very rough approximation to the actual viscous resistance encountered by the moving sperm flagellum, but it may be adequate for the needs of this paper. H_L has been used in obtaining the adjusted viscosity values for *Chaetopterus* spermatozoa and in other experiments where a similar type of movement was obtained. H_N has been used in obtaining the adjusted viscosity values for the type of movement found with *Ciona* and *Lytechinus* spermatozoa, where the forward velocity of the spermatozoa becomes very small at high viscosities. This procedural difference has only a slight effect on the adjusted viscosity values.

OBSERVATIONS

Effects of increased viscosity

Stroboscopic and photographic measurements of beat frequencies at various viscosities for spermatozoa of an annelid, *Chaetopterus variopedatus*, a tunicate, *Ciona intestinalis*, and a sea urchin, *Lytechinus pictus*, are summarized in Text-figs. 3–5. Other parameters determined from measurements on photographs are given in Tables 1–3. V_e is the phase velocity of the bending waves with respect to a co-ordinate system fixed in the medium (Brokaw, 1965). Some derived quantities based on these parameters are also given in these tables. $V_s = fL$ is the propagation velocity at which bending and unbending points move along the flagellum. $1-(\lambda V_e/LV_s)$ is a quantity

appearing in the formulae for viscous bending moment and energy expenditure (Brokaw, 1965) which is characteristic of the shape of the bending waves. The viscous bending moment at the bending and unbending points, M_0, and the energy expended per second against the viscous resistance at these points, W, have been calculated without considering any effects introduced by the head of the spermatozoon.

Photographs illustrating the behaviour of these spermatozoa at various viscosities are reproduced in Pl. 1.

Table 1. *Parameters of the bending waves of* Chaetopterus *spermatozoa at increased viscosities*

Measured viscosity (cP.)	1·4	3·2	6·5	13·6	28·3	49	65	127	313
Adjusted viscosity (cP.)	1·4	3·0	6·0	12	19	28	33	46	80
Beat frequency, f, from Text-fig. 3 (sec.$^{-1}$)	26	20·5	18	16	14	12·7	12	11	9·5
Measurements on photographs									
Number of spermatozoa measured	37	22	9	9	3	3	4	4	7
Radius of curvature, ρ (μ)	4·23	3·7	3·2	2·6	2·0	1·6	1·5	1·46	1·23
Wave-length, λ (μ)	19·3	16·5	14·4	13	8·3	6·7	5·8	5·0	4·24
Length, L, of one wave, measured along the flagellum (μ)	25·4	22·8	20·2	18·7	17	15·5	16	14·5	13·2
V_s (μ/sec.)	407	260	200	140	—	40	30	20	—
Calculated quantities									
V_s (μ/sec)	660	470	360	300	240	200	190	160	125
$1-(\lambda V_s/LV_s)$	0·54	0·6	0·6	0·68	(0·8)*	0·93	0·94	0·96	(0·98)
M_0 (10^{-10} dyne cm.)	1·5	2·1	2·6	3·7	4·2	4·5	5·3	5·8	6·4
W (10^{-7} ergs/sec.)	1·2	1·5	1·8	2·6	3·8	4·7	5·3	5·6	6·3

* Values given in parenthesis in Tables 1–4 have been estimated by interpolation or calculated from other measured quantities, in order to obtain a more complete set of values for V_s, M_0 and W.

The wave parameters of *Chaetopterus* spermatozoa decrease uniformly as the viscosity is increased by increasing concentrations of methyl cellulose. At high viscosities (30 cP. or greater) most of the spermatozoa swim smoothly with waves of small radius of curvature, as shown in Pl. 1, figs. 4–6 and in Pl. 2, figs. 4 and 5 of Brokaw (1965). At high viscosities the spermatozoa only infrequently remain near the surface of the slide or coverglass and instead swim freely in the solution. Their continual rotation reveals the nearly planar configuration of the bending waves on the flagellum, in the manner previously described by Gray (1955). Although the measured values indicate a gradual transition to this type of movement from the normal movements at low viscosity, the movements at intermediate viscosities (10–30 cP.) are much less regular. At intermediate viscosities the waves usually decrease in amplitude as they pass along the flagellum, as shown in Pl. 1, figs. 2 and 3. The measured values in Table 1 refer to the proximal portion of the flagellum. In addition there is usually a regular alternation between periods of swimming and periods of inactivity. The transitions between these two states involve gradual changes in wave amplitude at relatively constant frequency. The inactive and active periods usually

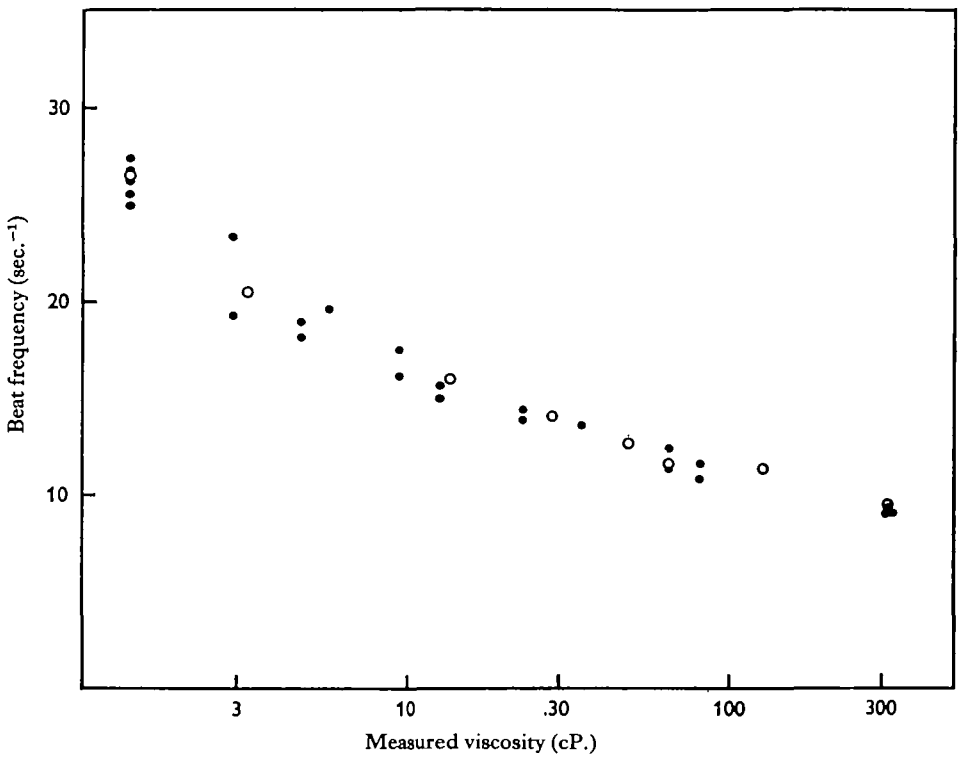
Table 2. *Parameters of the bending waves of* Ciona *spermatozoa at increased viscosities*

Measured viscosity (cP.)	1·4	2·05	2·73	4·26	5·78	13·2	22·6	40	66
Adjusted viscosity (cP.)	1·4	2·0	2·6	4·0	5·3	11·5	18	27	42
Beat frequency, f, from Text-fig. 4 ($sec.^{-1}$)	35	31·5	28·5	25	23	25	20·5	16	13
Measurements on photographs									
Number of spermatozoa measured	11	7	12	22	6	11	9	6	8
Radius of curvature, ρ (μ)	5·5	No significant variation							
Maximum wave amplitude (μ)	4·7	4·3	3·8	3·2	2·7	1·5	1·4	1·3	1·1
Wave length, λ (μ)	22·1	21·6	22·5	21·8	21·0	16	16·3	15·5	14·8
Length, L, of one wave, measured along the flagellum (μ)	30·1	28·1	28·1	26	25	(18)	(17·4)	(16·6)	(16)
V_e (μ/sec.)	665	545	550	445	435	335	—	—	170
Calculated quantities									
V_s (μ/sec.)	1070	860	800	650	575	450	360	270	210
$1-(\lambda V_e/LV_s)$	0·55	0·48	0·45	0·38	0·34	0·25	(0·21)	(0·18)	0·15
M_0 (10^{-10} dyne cm.)	3·8	3·7	4·1	4·0	4·0	3·9	3·8	3·7	2·7
W (10^{-} ergs/sec.)	4·0	3·2	3·4	2·9	2·7	2·5	2·6	1·7	1·3

Table 3. *Parameters of the bending waves of* Lytechinus *spermatozoa at increased viscosities*

Measured viscosity (cP.)	1·4	1·94	2·7	4·7	6·8	7·7	9·1	16·5	41	49
Adujsted viscosity (cP.)	1·4	1·9	2·5	4·4	6·2	7·2	8·4	15	30	36
Beat frequency, f, from Text-fig. 5 ($sec.^{-1}$)	30·1	28·5	26·5	23	21	20	17·5	14	9	9
Measurements on photographs										
Number of spermatozoa measured	35	7	29	21	3	3	12	7	1	1
Radius of curvature, ρ (μ)	5·1	5·1	5·1	5·6	6	6·2	6	—	6	6
Maximum wave amplitude (μ)	4·7	4·6	3·9	3·2	2·5	2·2	1·8	1·4	1·5	0·9
Wave-length, λ (μ)	23·6	23·0	22·3	21·7	21	19	19·2	17·8	20	17·6
Length, L, of one wave, measured along the flagellum (μ)	29·9	29·3	27·6	25·6	24·7	21	20·5	(19)	22	(19)
V_e (μ/sec)	580	585	500	465	425	330	—	—	—	—
Calculated quantities										
V_s (μ/sec)	900	835	730	590	529	420	360	270	200	170
$1-(\lambda V_e/LV_s)$	0·49	0·45	0·45	0·34	0·30	0·29	(0·25)	—	—	—
M_0 (10^{-10} dyne cm.)	2·7	3·0	3·2	4·2	4·0	3·3	3	—	—	—
W (10^{-7} ergs/sec)	2·6	2·7	2·8	2·5	2·3	1·8	1·6	—	—	—

last a few seconds each, and either may be longer than the other. These irregular movements are difficult to measure and photograph, and no quantitative study of the oscillation between activity and inactivity has been made. The degree of irregularity appears to vary considerably between samples of spermatozoa from different animals.



Text-fig. 3. Beat frequencies of *Chaetopterus* spermatozoa in solutions of increased viscosity. The results of four experiments with different sperm samples have been combined without adjustment. Each solid circle represents the average of stroboscopic frequency measurements of twenty spermatozoa. The open circles represent averages of measurements on a variable number of photographs.

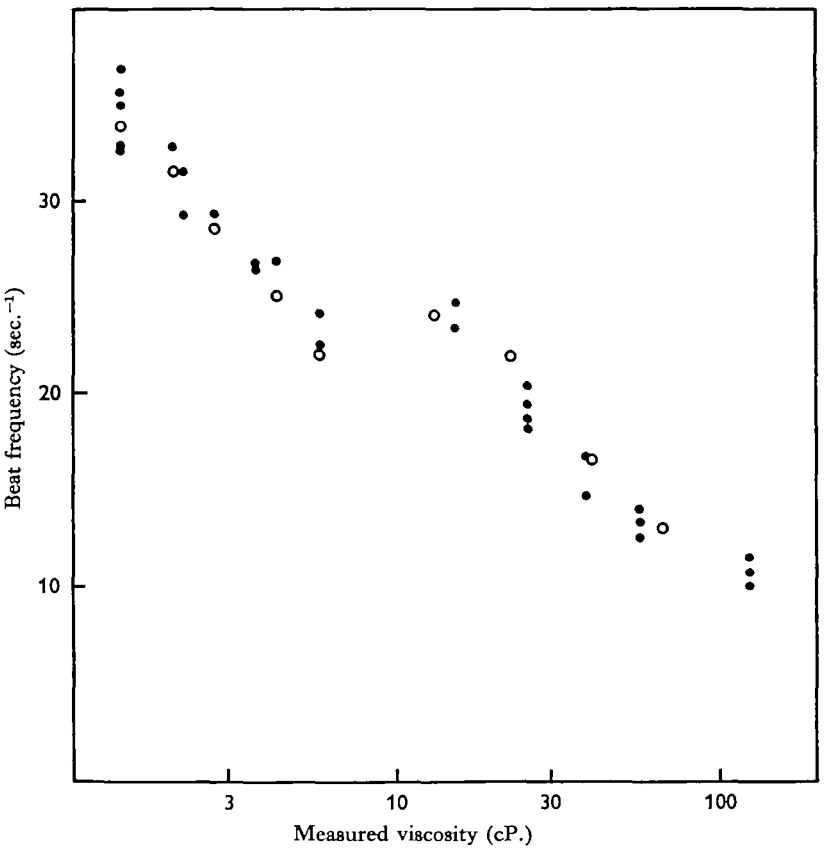
When low-MW methyl cellulose is used to increase the viscosity, the movements of *Chaetopterus* spermatozoa are qualitatively similar, except that the region of irregular movements is extended to viscosities of 100 cP. or more. At viscosities of 30–100 cP. the decrease in amplitude of the waves as they pass along the flagellum is marked, and the latter half of the flagellum may show little or no bending.

The wave patterns of *Ciona* and *Lytechinus* spermatozoa at increased viscosities differ significantly from those of *Chaetopterus*. The principal change with increasing viscosity is a decrease in the amplitude of the waves, while the radius of curvature of the bends remains practically constant. At high viscosities movement is restricted to waves of small amplitude in the proximal region of the flagellum.

At low viscosities, up to about 6 cP., the beat frequency of *Ciona* spermatozoa declines gradually as shown in Text-fig. 4. Bending waves are propagated along the flagellum with nearly constant amplitude, but the amplitude declines to about half its original value as the viscosity increases (Table 2). The wavelength decreases very

slightly, and the radius of curvature is constant within the limits of measurement. Typical waveforms at viscosities in this range are shown in Pl. 1, figs. 7–9.

At higher viscosities, above about 12 cP., the spermatozoa swim regularly and very slowly. The bending waves have maximum amplitude near the base of the flagellum and decrease in amplitude as they are propagated, as shown in Pl. 1, figs. 10–12. The beat frequency declines with increasing viscosity as shown in Text-fig. 4, along a curve which is not continuous with the curve obtained at low viscosities. The radius of curvature is difficult to measure when the wave amplitude is small, but appears to

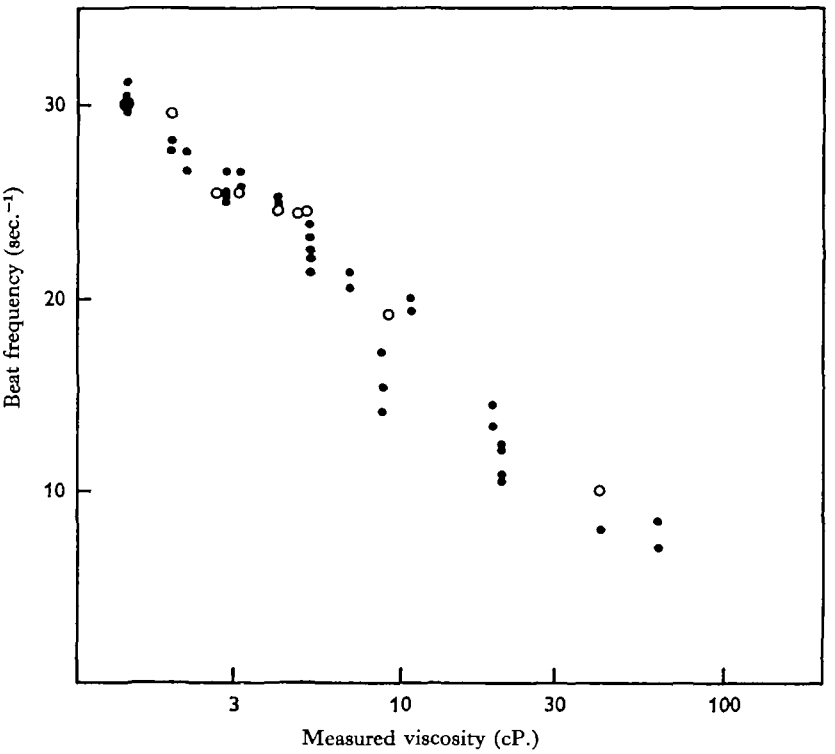


Text-fig. 4. Beat frequencies of *Ciona* spermatozoa in solutions of increased viscosity. The results of four experiments with sperm samples from four different animals have been combined without adjustment. Each solid circle represents the average of stroboscopic frequency measurements of twenty spermatozoa. The open circles represent averages of measurements on a variable number of photographs.

be the same as at low viscosities. The wavelength is reduced in this viscosity range, but decreases only slightly with further increases in viscosity. The combined effect of the discontinuous variation in frequency and wavelength between the low-viscosity and high-viscosity ranges causes the propagation velocity, V_s, to be a smoothly decreasing function of viscosity.

At intermediate viscosities the movements of *Ciona* spermatozoa are very irregular and difficult to measure or photograph. The spermatozoa appear to alternate between

the two types of movement found at lower and higher viscosities. The alternation is usually so rapid (every few seconds) that they do not swim for sufficient time with one type of movement to allow them to become stabilized near a surface where they can be observed accurately. Occasionally a single preparation will contain a few regularly swimming spermatozoa with each type of movement.



Text-fig. 5. Beat frequencies of *Lytechinus* spermatozoa in solutions of increased viscosity. The results of five experiments with sperm samples from five different animals have been combined without adjustment. Each solid circle represents the average of stroboscopic frequency measurements of twenty spermatozoa. The open circles represent averages of measurements on a variable number of photographs.

An additional type of movement is sometimes seen with *Ciona* spermatozoa, usually at high viscosities, but occasionally at lower viscosities. Its prevalence varies greatly between different samples of spermatozoa. This movement is slow, with frequencies around 1/sec. and has a nearly normal waveform. All the spermatozoa showing this type of movement appear to have lost the lateral body, containing mitochondria (Ezell, 1963), which can be seen on most of the rapidly swimming spermatozoa in the sample. Ursprung & Schabtach (1965) also noticed, during studies of fertilization in *Ascidia nigra*, that spermatozoa within the perivitelline space lost their mitochondria and swam very slowly.

Spermatozoa of *Lytechinus* show much greater variability than those of *Ciona* and *Chaetopterus*, both between different sperm samples, between individual spermatozoa in a sample, and with time for particular spermatozoa. In many preparations there appear to be two distinct classes of movement—one considered 'normal' and another

at a lower frequency which is sometimes close to half the frequency of the first class. Individual spermatozoa are frequently observed to switch back and forth between these classes and also to show variations in amplitude at constant frequency. At low viscosities the slower class typically has waves of smaller amplitude and usually shows a noticeable decrease in wave amplitude along the length of the flagellum. At viscosities around 10 cP. the slower class often has waves of greater amplitude than the faster class.

The measurements given in Text-fig. 5 and Table 3 represent attempts to select spermatozoa from the faster, 'normal' class of movement, but even so, these measurements are more variable and not likely to be as meaningful as those on *Ciona* and *Chaetopterus* spermatozoa. As illustrated in Pl. 1, figs. 13–17 and in Table 3, the movements of *Lytechinus* spermatozoa in viscous solutions resemble those of *Ciona* spermatozoa, but the constancy of the radius of curvature of the bends is not as definite.

In the vicinity of 10 cP. the waves are strongly decreased in amplitude, and both the frequency and amplitude vary greatly within each preparation, so that meaningful averages are difficult to obtain. At higher viscosities the wavelength is reduced and bending is restricted to the region near the head of the spermatozoon. Under these conditions, although the movement appears very regular, the small amplitude of the waves renders accurate measurements difficult.

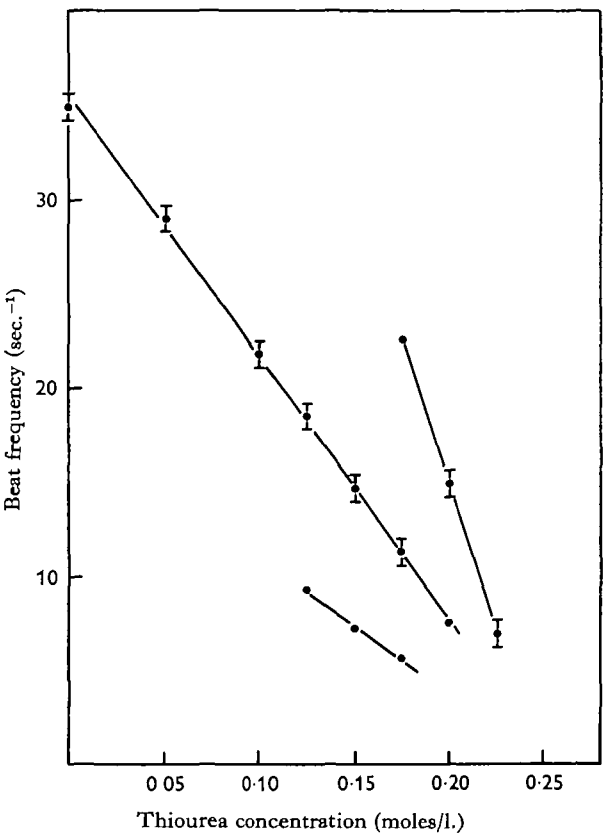
Observations on thiourea-inhibited spermatozoa

Thiourea is an inhibitor of the muscle actomyosin system (Rüegg, Straub & Twarog, 1963) which readily penetrates the membranes of some cells. It appears to penetrate spermatozoa of *Ciona* and *Lytechinus* rapidly, with half-times for effectiveness of 1–2 min. *Chaetopterus* spermatozoa appear to be much less permeable to thiourea; no effects have been studied with these spermatozoa. Some of the results with thiourea have been noted briefly in previous publications (Brokaw, 1964, 1965).

Concentrations of thiourea up to about 0·2 M reduce the beat frequency of *Ciona* or *Lytechinus* spermatozoa to one-quarter to one-third the normal frequency (Text-fig. 6) with little or no change in the other wave parameters (Pl. 2, figs. 1 and 4). After a few minutes there is a gradual reduction in the amplitude of the bending waves on the distal portion of the sperm flagellum, and eventually the distal portion becomes completely inhibited, as described previously. At thiourea concentrations of 0·2–0·3 M the beat frequency declines gradually with time until the spermatozoa become inactive, possibly as a result of a secondary inhibition of metabolic reactions in the spermatozoa (Dubois & Erway, 1946).

At thiourea concentrations in the range of 0·1–0·2 M the preparations of *Ciona* spermatozoa usually contain spermatozoa which are beating at approximately twice or one-half the predominant frequency, as indicated in Text-fig. 6. Some spermatozoa are observed to switch back and forth abruptly between two of the frequencies. Following these spermatozoa with stroboscopic illumination shows that the ratio between the frequencies is often very close to 2. Pl. 2, fig. 3, shows the waveform characteristic of spermatozoa beating with twice the normal frequency, and may be compared with Pl. 2, fig. 2, which shows the same spermatozoon at its 'normal' frequency a few seconds earlier. In this example, the increase in frequency measured

on the photographs was only about 70%. The radius of curvature is constant or slightly decreased, but the wavelength is substantially decreased, so that the propagation velocity, V_s, only increases by about 10%. The change in wavelength and wave pattern resembles that which occurs when the viscosity is increased from 6 to 12 cP. (Text-fig. 4) where the frequency decreases along a higher curve at the higher viscosities. The movements at higher frequency require about 50% less bending moment and about 20% less energy expenditure than those at the normal frequency.



Text-fig. 6. Effect of thiourea on the beat frequency of *Ciona* spermatozoa. These measurements are all from a single experiment using one sample of *Ciona* spermatozoa. The measured points, for which a range of ±2 S.D. units for the mean is indicated, represent the average of stroboscopic measurements on twenty spermatozoa. The other points indicate the presence of other frequency classes at half or twice the predominant frequency.

The movements found at one-half the normal frequency are much less regular so that no representative photographs are available; usually the bends have somewhat larger radius of curvature than the normal wave pattern, indicating a tendency for the wavelength to increase, thus preventing a large reduction in the propagation velocity.

These effects have not been found with *Lytechinus* spermatozoa. When the viscosity is increased by adding methyl cellulose to solutions containing thiourea, *Lytechinus* spermatozoa, now moving more slowly, respond to viscosity in a manner much more like that ordinarily observed with *Chaetopterus* spermatozoa, as illustrated

in Pl. 2, figs. 4–6. Under these conditions the radius of curvature does not remain constant, as it does in the absence of thiourea, but decreases, to produce bending waves similar to those found with *Chaetopterus* spermatozoa at high viscosities. A series of quantitative measurements of wave parameters at increased viscosities in the presence of thiourea was attempted, but the movements were found to be too unstable to give meaningful results.

The most dramatic demonstration of the effects of thiourea is seen by suspending spermatozoa at high viscosity in solutions containing 0·3 M thiourea and starting to observe them immediately, before an equilibrium concentration of thiourea is reached within the spermatozoa. The bending waves of small amplitude which are seen at first change rather abruptly to bending waves with small radius of curvature, as shown in Pl. 2, figs. 7 and 8, but the frequency continues to decline gradually as the thiourea penetrates, and after a few more minutes the spermatozoa become inactive. The abrupt change in waveform from that shown in Pl. 1, fig. 17, to that shown in Pl. 2, fig. 8, is not associated with any detectable change in beat frequency.

A similar transformation between these two types of movement at high viscosities was sought by lowering the temperature of the microscope stage to about 5° C. Although the actual temperature of the sperm suspension was not determined, a noticeable decrease in beat frequency was observed with no change in the form of the bending waves.

Ciona spermatozoa, in the presence of both thiourea and methyl cellulose, only rarely show this transformation to the type of movemen tcharacteristic of *Chaetopterus* spermatozoa at high viscosities. Normally, at viscosities above 8–10 cP., in the presence of 0·1–0·2 M thiourea, *Ciona* spermatozoa show a completely different form of movement (Brokaw, 1964). The flagellum forms a helix, generated at rates of 1–2 turns/sec., with a pitch of about 20 μ and a radius of about 6 μ. Both left-handed and right-handed helices are common. In addition, a transverse vibration of small amplitude (1 μ or less?) with a frequency of about 10–15/sec. is seen, which appears to represent the passage of small-amplitude waves along the flagellum. Since many spermatozoa attached to the slide by their heads show the same propagated helical waves, the swimming of the spermatozoa with helical flagella cannot be explained by assuming that the rigid helix is propelled by the small-amplitude vibrations.

Observations on glycerinated spermatozoa

The ATP-reactivated movements of glycerinated spermatozoa of *Chaetopterus* resembled those obtained with isolated flagella of *Polytoma uvella* (Brokaw, 1961, 1963). Propagated bending waves and forward propulsion are observed, but the movement is quantitatively much less than that which is characteristic of normal spermatozoa. The response to increased viscosities resembles that described for normal spermatozoa, except that the irregularity of the movements at intermediate viscosities is not seen, suggesting that this irregularity might result from an imbalance between the supply and utilization of energy from ATP, which is avoided when ATP is supplied externally.

Under similar conditions spermatozoa from *Ciona* do not initiate bending waves which pass from the base of the flagellum to the tip, but instead show only a unilateral bending, originating near the mid-region of the flagellum, which sometimes appears

to be propagated towards the tip of the flagellum. The frequency of these movements does not show the uniform increase with increasing ATP concentration which has been found with sea-urchin spermatozoa (Kinoshita, 1958) and other flagella (Hoffmann-Berling, 1955; Brokaw 1961, 1962). Similar non-propagated movements have been described for the ATP-reactivated, glycerinated spermatozoa of some mammals (Bishop & Hoffmann-Berling, 1959).

The ATP-reactivated movements of glycerinated spermatozoa of *Lytechinus* closely resemble the movements of normal spermatozoa, except that they show a pronounced asymmetry similar to that found when normal *Lytechinus* spermatozoa swim in sea-water solutions containing abnormally high concentrations of potassium ion. Sufficient numbers of undamaged spermatozoa are found swimming uniformly in small circles to permit the photographing of their movements under various conditions. However, in any preparation, the reactivated spermatozoa which show nearly normal swimming movements represent only a small fraction of the spermatozoa in the sample. Most of the motile spermatozoa show abnormal movements, often obviously caused by visible mechanical damage or by attachment to the slide or coverglass, and many spermatozoa are completely immotile.

Table 4. *Parameters of the bending waves of glycerinated* Lytechinus *spermatozoa at increased viscosities*

	At 2 mM ATP				At 0·2 mM ATP				
Measured viscosity (cP.)	1·9	4·1	8·6	21·1	1·9	6·5	21	55	157
Adjusted viscosity (cP.)	1·9	3·9	8·2	17	1·9	6·5	18	44	77
Beat frequency, f (sec.$^{-1}$)	20·9	16·0	12·2	10·9	6·9	5·8	4·5	3·5	3·1
Measurements on photograph									
Number of spermatozoa measured	25	28	23	22	18	27	21	17	9
Radius of curvature, ρ (μ)	4·2	4·35	4·45	4·73	5·7	5·0	4·3	2·9	2·0
Maximum wave amplitude (μ)	3·85	3·0	2·2	1·5	5·5	4·6	3·7	3·6	4·0
Wave length, λ (μ)	20·7	20·1	19·1	17·2	21·8	19·2	16·5	11·9	7·3
Length, L, of one wave, measured along the flagellum (μ)	26·2	24·3	22·0	19	29·2	26·2	22·1	18·6	18·1
V_s (μ/sec.)	370	290	200	—	124	90	60	35	8·5
Calculated quantities									
V_s (μ/sec.)	550	390	270	207	200	152	100	65	56
$1-(\lambda V_s/LV_s)$	0·46	0·39	0·34	(0·3)	0·54	0·57	0·56	0·66	0·95
M_0 (10^{-10} dyne cm.)	1·5	1·8	2·1	2·8	1·0	2·2	2·8	3·2	5·2
W (10^{-7} ergs/sec.)	1·2	1·1	1·0	1·0	0·2	0·4	0·5	0·6	1·3

The movements of a reactivated spermatozoon are very constant with time, even at viscosities where the movements of normal spermatozoa show considerable time-variation. The cessation of movement of a particular spermatozoon is usually abrupt.

The effect of viscosity on the reactivated movements of glycerinated spermatozoa of *Lytechinus* was studied at two ATP concentrations, 2 and 0·2 mM, by stroboscopic

measurements and photomicrography. The results are summarized in Table 4, and illustrated in Pl. 2, figs. 9–17. At 2 mM-ATP, the response to viscosity resembles that normally obtained with the spermatozoa of *Lytechinus* or *Ciona*. At 0·2 mM-ATP, where the beat frequencies are significantly lower, the response to viscosity resembles that normally obtained with the spermatozoa of *Chaetopterus* and obtained with *Lytechinus* spermatozoa when the movements were slowed down by thiourea. The effect of thiourea therefore resembles a reduction in ATP concentration; in both cases the altered movement is not accompanied by an inhibition of the ability to dephosphorylate ATP (Brokaw, 1961, 1964, and unpublished measurements).

DISCUSSION

Two distinctly different patterns of response to increased viscosity are shown by *Chaetopterus* spermatozoa and by *Ciona* spermatozoa. Since both patterns can be obtained with *Lytechinus* spermatozoa under appropriate conditions, these two response patterns are probably not indicative of two quite different systems for generating and controlling flagellar bending. The observation of both patterns provides a more substantial basis for detecting some of the properties of mechanisms common to all the spermatozoa. Only a few selected aspects of these observations will be discussed in further detail; the significance of many of the observations is not yet clear.

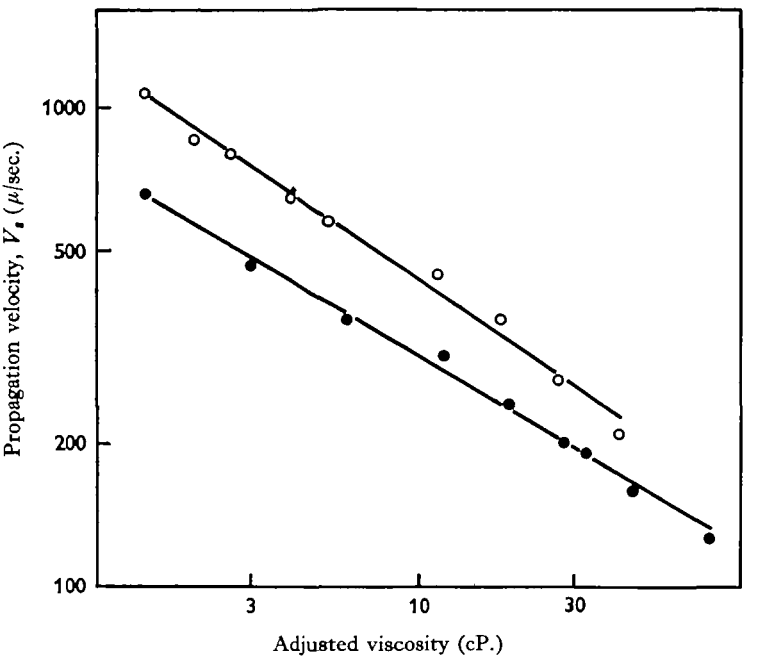
Propagation velocity

Although the detailed pattern of changes in frequency and wavelength with increasing viscosity is different for the spermatozoa of each of the three species studied, the changes are related so that in each case the bend propagation velocity, V_s, decreases uniformly with increasing viscosity. When plotted logarithmically, as in Text-figs. 7 and 8, an approximately linear relationship is obtained in each case, indicating that the product, $\eta^\beta V_s$, where β is an empirical constant, is approximately constant over the range of viscosities studied. Since the values of β are close to 0·5, and since ηV_s^2 is a major part of the expression for the energy expended by the flagellum (Brokaw, 1965), the linearity of these plots might in part reflect a limited range of variation in the energy expenditure.

Slightly lower values of β are associated with the two situations where the radius of curvature is reduced at high viscosities, and statistical analysis of the plots suggests that these differences may be significant. However, the relative values of β will be influenced by the method used to obtain adjusted viscosity values, so that the standard deviations indicated for the slopes in Text-figs. 7 and 8 do not encompass all the uncertainty in these values. In any event, the differences between the slopes appear to be small compared to the differences in the behaviour of the radius of curvature, which is approximately constant in three of the experiments, and changes by a factor of approximately three in the other two experiments. The relationship between propagation velocity and viscosity is relatively independent of changes in the radius of curvature, suggesting that the propagation velocity is relatively independent of the radius of curvature. This conclusion also follows from the observation that in spermatozoa swimming with asymmetrical wave patterns, in which the radius of curvature of the bends differs on the two sides of the flagellum (Brokaw, 1965), the propagation

velocities must be essentially the same on both sides. The propagation velocity also appears to be relatively independent of the viscous bending moment, M_0.

The difference between the slopes of the two sets of points in Text-fig. 7, 0·06 log units, may be predicted on the basis of the mechanism of bend propagation developed by Brokaw (1966), if some additional assumptions are made, as described in the Appendix. On this basis, and the interpretation that the β values obtained for *Ciona* and *Lytechinus* spermatozoa under conditions where the radius of curvature does not change indicate a true value of 0·5, the velocity of flagellar bending appears to be inversely proportional to the square root of the viscosity in all the examples tudied. No clear reason for this relationship is apparent.



Text-fig. 7. Log-log plots of propagation velocity (V_s) against adjusted viscosity for spermatozoa of *Chaetopterus* (solid circles) and *Ciona* (open circles). The lines have been fitted by the method of least squares; their slopes and standard deviations, in log units, are $-0{\cdot}40 \pm 0{\cdot}016$ and $-0{\cdot}46 \pm 0{\cdot}01$, respectively.

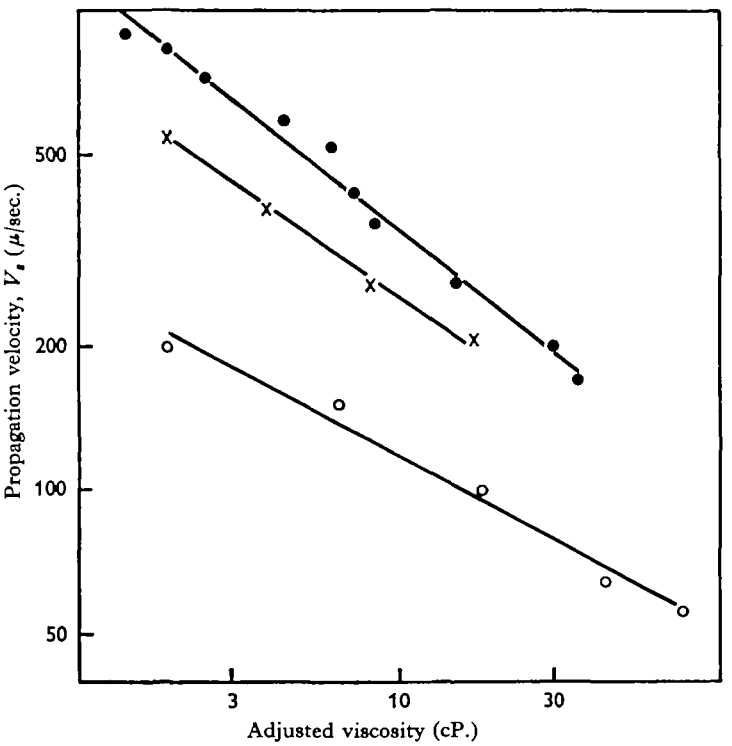
The influence of internal elastic elements

In the original paper on the movements of sea-urchin spermatozoa, Sir James Gray (1955) pointed out that the sperm tail possessed a natural elasticity which caused it to be nearly straight when inactive, and suggested that the energy stored and released when this elastic structure was bent and unbent during normal movement might play an important part in the energetics of propulsion. This idea was pursued by Machin (1958) who proposed a propagation mechanism heavily dependent on the passive wave-propagating properties of an elastic flagellum.

The presence of an elastic resistance to bending implies that an additional active bending moment will be required to cause bending. The magnitude of this additional bending moment will increase as the radius of curvature decreases, and in the simplest

case, where Hooke's law is followed, will be equal to k/ρ, where k is a constant representing the bending resistance, or stiffness, of the elastic structures which resist bending. If the energy stored when these elastic structures are bent is not dissipated by other processes before unbending occurs, an elastic bending moment will be available to assist unbending. There will be some value of k, $k_m = \rho M_0$, such that the sum of the elastic and viscous elements tending to unbend the flagellum will diminish to 0 at the beginning of each unbending transition. Such a condition might trigger the unbending process. From the equation for the viscous bending moment given by Brokaw (1965),

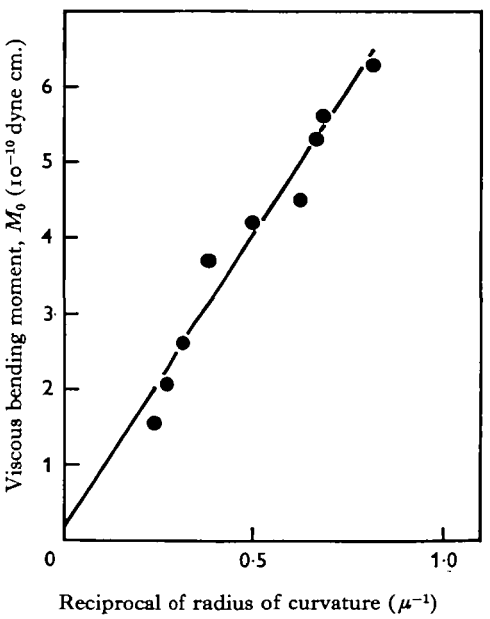
$$k_m = \frac{C_L f}{4} \rho^2 L^2 \left(1 - \frac{\lambda V_e}{L V_s}\right).$$



Text-fig. 8. Log-log plots of propagation velocity (V_s) against adjusted viscosity for spermatozoa of *Lytechinus* in sea water (solid circles) and, after glycerination, with 2 mM ATP (crosses) and 0·2 mM ATP (open circles). The lines have been fitted by the method of least squares; and their slopes and standard deviations, in log units, are $-0{\cdot}53 \pm 0{\cdot}02$, $-0{\cdot}45 \pm 0{\cdot}05$ and $-0{\cdot}36 \pm 0{\cdot}03$, respectively.

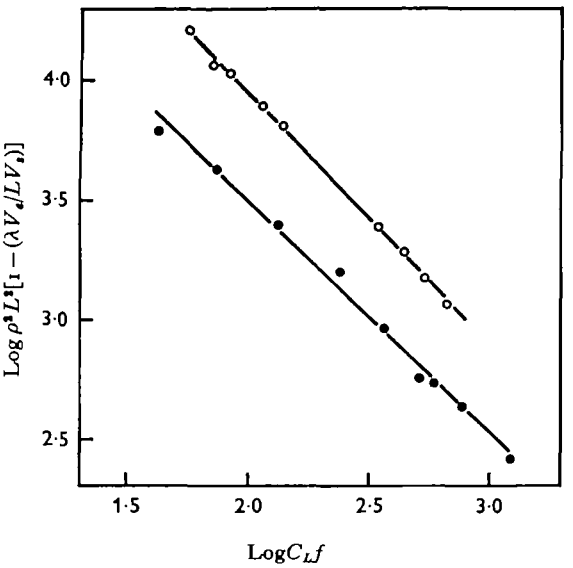
There will be another value of k, k_e, such that the energy stored in the elastic element during bending is just sufficient to do the work which must be done against viscous resistances when the flagellum unbends. For an elastic element which obeys Hooke's law, $k_e = 2k_m$. If $k = k_e$, unbending might be a completely passive process which does not require an active input of energy from chemical reactions. If $k > k_e$, this may still be the case, but some of the energy stored in elastic bending may be wasted, reducing the efficiency of movement. If $k = k_m$, or any other value less than k_e, unbending as, well as bending, will require an active energy input.

One indication that these relationships are significant would be the maintenance of a consistent proportionality between the elastic and viscous bending moments. Such a proportionality will also be characteristic of the propagation of bending waves along a passive elastic filament, as discussed by Machin (1958), but its occurrence does not therefore imply that wave propagation is mechanical, as concluded by Holwill (1965). In the terms of Machin's model for an active flagellum, the viscous and elastic bending moments will be proportional if the response of the active bending elements is adjusted to maintain a fully efficient use of the energy stored by elastic bending of the flagellum, as indicated by the condition for maximum propulsive efficiency given by Machin (1958). The response of the active elements to passive deformation must be adjusted to maintain a constant phase angle between input and output, irrespective of changes in frequency caused by viscosity or other variables. This cannot be done by a system in which there is simply a constant time delay between input and output (Brokaw, 1962). The maintenance of a proportionality between elastic and viscous bending moments may therefore suggest that the flagellum operates in a manner which efficiently uses the elastic energy of bending, but does not indicate how this is accomplished.

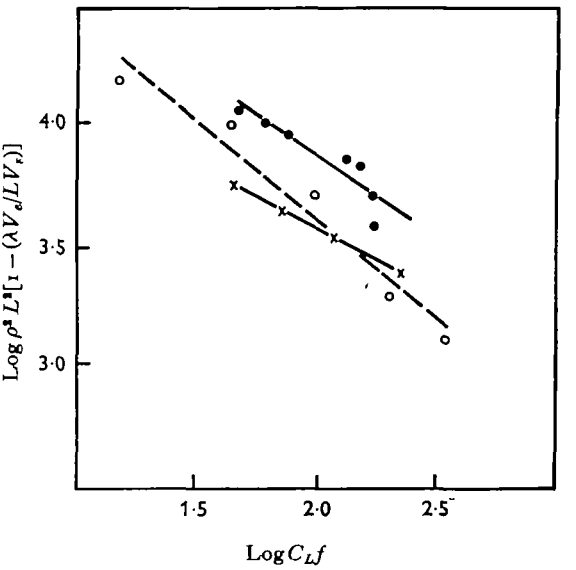


Text-fig. 9. Increase in bending moment with increasing curvature ($1/\rho$) for *Chaetopterus* spermatozoa. The line, fitted by the method of least squares, intercepts the ordinate at $M_0 = 0{\cdot}16 \times 10^{-10}$ dyne cm, and the standard deviation of this intercept is $0{\cdot}4 \times 10^{-10}$ dyne cm, indicating no significant divergence from the origin.

A proportionality between elastic and viscous bending moments is indicated for the movements of *Chaetopterus* and *Ciona* spermatozoa at increased viscosities. In the case of *Chaetopterus* spermatozoa both M_0, the viscous bending moment, and $1/\rho$, which may indicate the magnitude of an elastic bending moment, increase with increasing viscosity, and they are related more or less linearly (Text-fig. 9). In the case



Text-fig. 10. Test of the proportionality between elastic and viscous bending moments with increasing viscosity for spermatozoa of *Ciona* (open circles) and *Chaetopterus* (solid circles). The hypothesis predicts that the points should lie on straight lines with slopes of $-1{\cdot}0$. The lines have been fitted by the method of least squares; their slopes and standard deviations are $-0{\cdot}96 \pm 0{\cdot}04$ and $-1{\cdot}05 \pm 0{\cdot}02$, respectively.



Text-fig. 11. Test of the proportionality between elastic and viscous bending moments with increasing viscosity for spermatozoa of *Lytechinus* in sea water (solid circles) and after glycerination, with 2 mM ATP (crosses) and 0·2 mM ATP (open circles). The lines have been fitted by the method of least squares; their slopes and standard deviations are $-0{\cdot}69 \pm 0{\cdot}14$, $-0{\cdot}53 \pm 0{\cdot}05$, and $-0{\cdot}84 \pm 0{\cdot}10$, respectively.

of *Ciona* spermatozoa, both M_0 and ρ remain essentially constant at increased viscosities (Table 2). In Text-fig. 10 these relationships are illustrated by log-log plots of $\rho^2L^2[1-(\lambda V_e/LV_s)]$ against C_Lf. This presentation is analogous to Holwill's (1965) log-log plot of λ against C_Lf, based on a sinusoidal model of the bending waves, for the movement of the flagellum of *Strigomonas oncopelti* at increased viscosity. Holwill's results, and the results presented here for *Chaetopterus* and *Ciona* spermatozoa (Text-fig. 10) give strong evidence that at increased viscosities the parameters of the bending waves are adjusted to maintain a consistent proportionality between viscous and elastic bending moments. (Although the statistical analysis of the points for *Ciona* spermatozoa suggests that the slope of the line fitted to the points may be significantly different from $-1{\cdot}0$, these slopes will also be influenced by the manner in which the adjusted viscosity values have been obtained, so that the standard deviations indicated for the slopes do not encompass all the uncertainty in these values.) The results for *Lytechinus* spermatozoa (Text-fig. 11) are much less satisfactory, possibly because of the greater variability in the movements of *Lytechinus* spermatozoa and the difficulty in accurately interpreting the asymmetrical movements obtained with glycerinated spermatozoa. Log-log plots of λ^4 against C_Lf from these results are much less linear, and give slopes of approximately $-1{\cdot}9$ for *Chaetopterus* spermatozoa, $-0{\cdot}8$ for *Ciona* spermatozoa, and $-0{\cdot}5$ for *Lytechinus* spermatozoa, suggesting that a sinusoidal model is not adequate for interpreting these results.

The change in beat frequency between the two ATP concentrations used with the glycerinated spermatozoa of *Lytechinus* is also accompanied by changes in other parameters of bending which tend to maintain the same proportionality between viscous and elastic bending moments at the two ATP concentrations. If this adjustment were complete, the sets of points at the two ATP concentrations (Text-fig. 11) should be colinear; the results are not precise enough to provide any additional support for the hypothesis of proportionality. If the results were more precise the finding of two parallel lines (Text-fig. 11) for the normal spermatozoa and for the glycerinated spermatozoa might suggest that the glycerination treatment changes the stiffness of the flagellum.

Several situations have been encountered in which a proportionality between viscous and elastic bending moments is not maintained. The initial inhibition of *Lytechinus* and *Ciona* spermatozoa by thiourea reduces the beat frequency without significantly altering any of the other wave parameters. This represents a sizeable reduction in viscous bending moment with no apparent change in the elastic bending moment. Similar changes are associated with the very slow movements of *Ciona* spermatozoa lacking the lateral body. In the alternation of thiourea-inhibited *Ciona* spermatozoa between two different frequencies, as illustrated by the spermatozoon shown in Pl. 2, figs. 2 and 3, the higher-frequency movement requires considerably less viscous bending moment, but the elastic bending moment is, if anything, increased. A final example is provided by the absence of changes in wavelength when the beat frequency of *Strigomonas* flagella is altered by changing the temperature (Holwill & Silvester, 1965).

Some, but probably not all, of these discrepancies might be explained by alterations in the stiffness of the flagellum. They argue against the suggestion that the point of unbending might be controlled by the sign of the sum of the elastic and viscous

bending moments. The suggestion that unbending is driven by energy stored by elastic bending does not require that M_0 always $= k/2\rho$, but only that M_0 never exceeds this value. If the results with *Chaetopterus* and *Ciona* spermatozoa at increased viscosities indicate situations where this limitation on M_0 determines the parameters of bending, the other results may represent situations where the flagellum is operating at less than maximum efficiency with respect to use of stored elastic energy during unbending so that M_0 is determined by other factors and need not be proportional to $1/\rho$. Further insight into the possible role of elastic elements in unbending must be sought by other methods.

Radius of curvature and energy expenditure

A partial analysis of the varied patterns of changes in wave parameters with increasing viscosity can be based on the condition that these changes must maintain a constant proportionality between viscous and elastic bending moments. This proportionality requires that the product, $C_L f\rho^2 L^2[1-(\lambda V_e/LV_s)]$ must remain constant as the viscosity changes. To a first approximation, C_L is proportional to the viscosity. This product could be kept constant by changes in f such that $C_L f$ was constant, without requiring any changes in the other wave parameters. In all cases the reduction in f is less than that required to keep $C_L f$ constant. Since f may be established by an oscillator localized at the base of the flagellum (Brokaw & Goldstein, 1965), it may be relatively insensitive to the changes in viscosity which will influence the resistance to movement of the flagellum. Consequently, in all the cases examined, a reduction in $\rho^2 L^2[1-(\lambda V_e/LV_s)]$ is required to maintain the proportionality between viscous and elastic bending moments at increasing viscosities.

If M_0 and ρ cannot change in response to changes in viscosity, as appears to be the case with *Ciona* spermatozoa, $L^2[1-(\lambda V_e/LV_s)]$ must be reduced at increased viscosities, and it can be shown that, for constant ρ, reductions in L^2 must be accompanied by reductions in wave amplitude and the quantity $1-(\lambda V_e/LV_s)$, and vice versa, so that the wave amplitude must be reduced, as is found with *Ciona* spermatozoa. The energy expended by the flagellum will be proportional to

$$(f/\rho^2)\left[C_L f\rho^2 L^2\left(1-\frac{\lambda V_e}{LV_s}\right)\right]$$

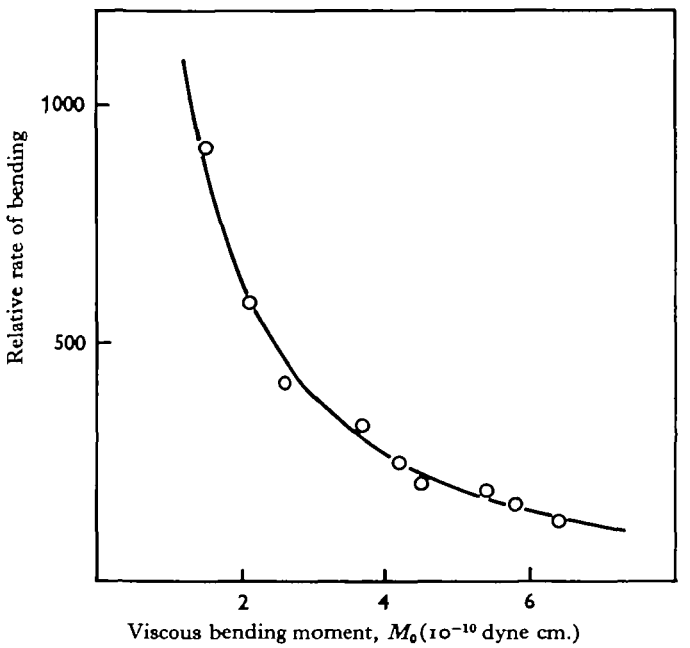
(Brokaw, 1965). If ρ and the second term are constant, and f decreases with increasing viscosity, there must be a decrease in energy expenditure as indicated in Table 2.

If the active bending moment can be increased in response to changes in viscosity, M_0 will increase, and the related decrease in ρ will reduce the amount of decrease required in the term $L^2[1-(\lambda V_e/LV_s)]$. Decreases in ρ will probably cause some decrease in L, and with *Chaetopterus* spermatozoa, the term $1-(\lambda V_e/LV_s)$ increases towards its limiting value of 1·0 as the viscosity is increased (Table 1). If the decrease in ρ were such that ρ^2 was proportional to f, the waveform could be adjusted to maintain constant proportionality between viscous and elastic moments without any change in the energy expenditure. This type of behaviour was never observed. Instead, there is a much greater decrease in ρ with increasing viscosity, so that the term f/ρ^2 increases and there is a sizeable increase in energy expenditure, as indicated in Table 1. Similar behaviour was found with the glycerinated spermatozoa of *Lytechinus* at the lower ATP

concentration. Measurements of the angular velocity of movement of the large compound abfrontal gill cilia of *Mytilus* (Yoneda, 1962) also indicated that the calculated bending moments and energy expenditures increased significantly as the velocity was decreased by increasing the viscosity.

In these instances, where the bending moment increases at increased viscosities, the behaviour of the flagellum may be controlled in a manner similar to that of muscle, where increased tensions and work outputs are found at lower shortening velocities. A measure of the bending velocity (V) of *Chaetopterus* sperm flagella at various viscosities can be obtained by adjusting the values of propagation velocity on the basis of the model discussed in the Appendix. A plot of bending velocity against bending moment for these spermatozoa then has the form shown in Text-fig. 12, and may be compared with typical curves obtained for muscle, by plotting shortening velocity against force (Prosser & Brown, 1961). An essentially linear relationship can be found by plotting bending velocity against the reciprocal of the bending moment, indicating that the points in Text-fig. 12 are adequately fitted by a curve based on the relationship

$$M_0(V + 100\,\mu/\text{sec.}) = \text{a constant.}$$



Text-fig. 12. Plot of relative bending velocity against viscous bending moment for the spermatozoa of *Chaetopterus*. The curve was obtained by fitting a straight line to a plot of relative bending velocity against the reciprocal of the viscous bending moment.

This result differs from the results usually obtained with muscle and expressed by the relationship

$$(P + a)(V + b) = \text{a constant,}$$

in that there is no indication of a constant equivalent to a, and the constant equivalent to b is relatively small.

The above interpretation implies that with *Chaetopterus* spermatozoa increased viscosity causes a decrease in bending velocity, which permits the flagellum to generate a greater active bending moment. This increased active bending moment permits proportional increases in both the viscous and elastic resistances to bending, indicated by increases in M_0 and $1/\rho$.

This type of response to increased viscosity, which involves increases in active bending moment and energy expenditure, may be prohibited under conditions where the system is already using nearly all its available energy. It was found in *Chaetopterus* spermatozoa, which have the lowest normal propagation velocities and energy expenditures, and in thiourea-inhibited and low-ATP movements of *Lytechinus* spermatozoa, where a low beat frequency and a low energy expenditure were obtained without reducing the rate of ATP-dephosphorylation, which is probably the source of energy for movement. It was not found by lowering the temperature with *Lytechinus* spermatozoa, which will presumably decrease the availability of energy as well as the frequency and energy expenditure. However, the absence of clear intermediates between the two types of response to increased viscosity is not fully explained by these considerations. The observations presented in this paper include a number of indications that particular types of wave movement are relatively stable, in comparison to other possible patterns. This is shown clearly by the sharpness of the transition between movements with normal and reduced radius of curvature, when *Lytechinus* spermatozoa are exposed to 0·3 M thiourea. A different type of stability situation occurs with *Ciona* spermatozoa, resulting in a discontinuity in the response of beat frequency to increasing viscosity, and, under some conditions, an alternation between two relatively stable patterns of movement at different frequencies. These observations may indicate important properties of the mechanisms controlling the generation and propagation of flagellar bending waves, but their meaning is not clear at the present time.

SUMMARY

1. Two distinctly different patterns of sperm movement in response to increased viscosity have been found, which appear to be variations in the behaviour of a common mechanism for generating and controlling flagellar bending waves.

2. One type of pattern is found with the normal movements of spermatozoa of *Ciona* and *Lytechinus*, and with glycerinated *Lytechinus* spermatozoa when their movements are reactivated at high (2 mM) ATP concentrations. The radius of curvature of the bends on the flagellum, and the viscous bending moment, remain relatively constant, while the propagation velocity, wave amplitude, and energy expenditure decrease with increasing viscosity.

3. The second pattern of response is found with the normal movements of spermatozoa of *Chaetopterus*, with *Lytechinus* spermatozoa when the beat frequency is reduced by the presence of an inhibitor, thiourea, and with glycerinated *Lytechinus* spermatozoa when their movements are reactivated at low (0·2 mM) ATP concentrations. The decrease in propagation velocity with increasing viscosity is accompanied by substantial reductions in the radius of curvature, and the wave amplitude is not greatly decreased. This response involves an increase in viscous bending moment and energy expenditure as the rate of bending decreases, and may resemble the increased force and work outputs obtainable from muscle at reduced shortening

velocities. This type of response is obtained under conditions where the initial beat frequency and energy expenditure are relatively low, and may not be possible when the initial level of energy expenditure is higher.

4. Although each species shows a unique pattern of changes in frequency and wavelength with increasing viscosity, the relationship between bend propagation velocity and viscosity has nearly the same form in each case, indicating that the velocity of bend propagation is relatively independent of the magnitude of the bend. This result is consistent with at least one proposed mechanism for bend propagation. On the basis of this mechanism, the results suggest that the rate of bending is independent of the magnitude of the bend and inversely proportional to the square root of the viscosity.

5. In some cases, the wavelength and other wave parameters are adjusted so that a constant proportionality between viscous and elastic bending moments will be maintained as the viscosity is increased. This proportionality will also be maintained during the passive propagation of bending waves along an inert flagellum, and for an active flagellum it implies that the wave parameters are adjusted so that energy stored in elastic bending of the flagellum could be efficiently used to effect unbending, without indicating how this adjustment is accomplished. Other cases, in which the proportionality between viscous and elastic bending moments is not maintained, may represent cases where the flagellum is not operating efficiently.

6. Under certain conditions the normal nearly planar bending waves of *Ciona* spermatozoa are replaced by helical bending waves.

APPENDIX

Bend propagation

The propagation mechanism considered here is based on the local passive spread of bending ahead of a region of active bending (Brokaw, 1966), but the mathematical formulation may be general enough so that the conclusions are applicable to other types of locally spreading signals for the activation of bending.

Internal shear within a flagellum will smooth any attempt to produce abrupt transitions between bent and unbent regions along a flagellum. As a result, bending will spread slightly ahead of any point at which such a transition is attempted. If active bending can be triggered by the passively spread bending, the transition may be self-propagating. The spread of passive bending can be expressed by the following equation (Brokaw, 1966):

$$\frac{1}{\rho} = \frac{1}{\rho_0} + \frac{1}{\alpha^2}\frac{d\,(1/\rho)}{ds^2},$$

where s = distance along the flagellum, $1/\rho$ = the actual curvature of the flagellum at any point s, $1/\rho_0$ is the curvature which would be produced as a result of active bending if there were no shear within the flagellum, and α is a constant determined by the structural properties of the flagellum. A solution to this equation was given previously (Brokaw, 1966) for the case where the rate of active bending is infinitely great; a more general case will now be considered.

Let $1/\rho_a$ be the amount of curvature which is sufficient to activate bending. The rate of active bending, $d(1/\rho_0)/dt = 0$ at all points where $1/\rho$ is less than $1/\rho_a$. For

$1/\rho \geqslant 1/\rho_a$, the rate of active bending will be assumed to be a constant, R, until a maximum value of $1/\rho_0 = \kappa$ is reached. If the transitions propagate at a constant velocity, V_s, with constant shape, $1/\rho_0$ will have the form indicated by the solid line in Text-fig. 13. It is convenient to set the point $s = 0$ at the point where $1/\rho_0 = \kappa/2$. A solution to the differential equation given above will then have the following form:

$$1/\rho = Ae^{\alpha s} + Be^{-\alpha s} + Cs + D.$$

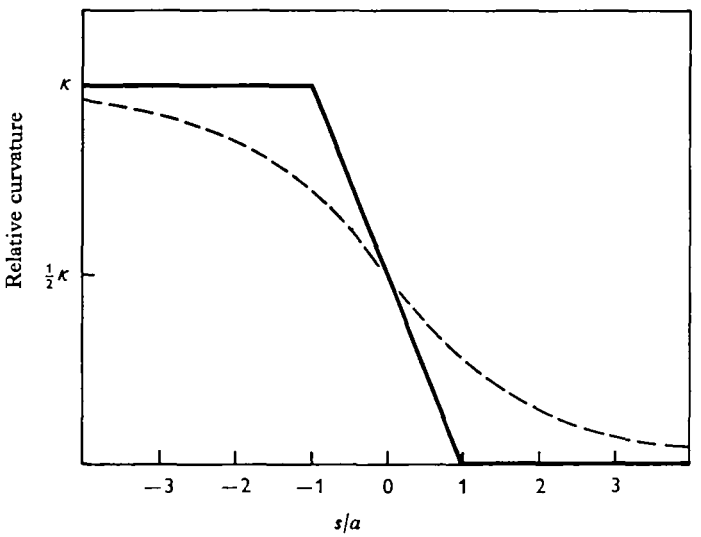
The constants in this expression can be evaluated to satisfy symmetry and boundary conditions, leading to the following complete solution:

$$1/\rho = \kappa[1 - (e^{\alpha a} - e^{-\alpha a})e^{\alpha s}/4\alpha a] \qquad s \leqslant -a$$

$$1/\rho = (1/2)\kappa(1 - s/a) + \kappa(e^{\alpha s} - e^{-\alpha s})e^{-\alpha a}/4\alpha a \qquad -a \leqslant s \leqslant a$$

$$1/\rho = \kappa(e^{\alpha a} - e^{-\alpha a})e^{-\alpha s}/4\alpha a \qquad s \geqslant a,$$

where $a = \kappa V_s/2R$. This solution is illustrated by the dashed line in Text-fig. 13, for the case where $1/\rho_a = 0{\cdot}28\kappa$, which corresponds to the wave pattern of *Chaetopterus* spermatozoa at normal viscosities, if $\rho_a = 15\ \mu$.

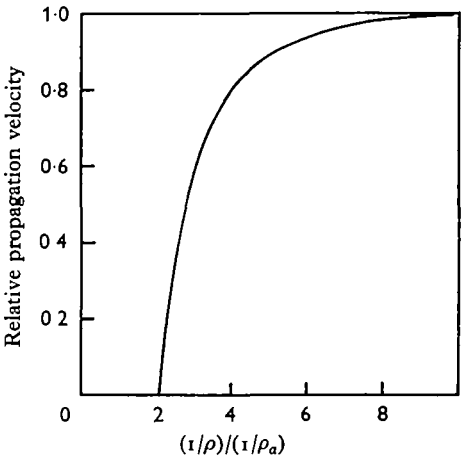


Text-fig. 13. Curvature (dashed line) as a function of distance along the flagellum in the vicinity of a bending transition caused by the active bending indicated by the solid line.

At $s = a$, the point of activation,

$$1/\rho = 1/\rho_a = \kappa(1 - e^{-2\alpha a})/4\alpha a = R(1 - e^{-\alpha k V_s/R})/2\alpha V_s.$$

From this equation the behaviour of V_s as κ varies, R and $1/\rho_a$ being constant, can be obtained, as illustrated in Text-fig. 14. From this curve, values of R corresponding to measured values of V_s can be obtained for the values of κ measured at various viscosities. If this procedure is applied to the results for *Chaetopterus* spermatozoa, on the assumption that $\rho_a = 15\ \mu$, a log-log plot of R against adjusted viscosity yields a line with a slope of $-0{\cdot}48 \pm 0{\cdot}01$ (S.D.) log units, which is now not significantly different from the results obtained with *Ciona* and *Lytechinus* spermatozoa (Text-figs. 7, 8). The results are consistent with the model for bend propagation considered



Text-fig. 14. Effect of the amount of bending on the bend propagation velocity (V_s) for the propagation mechanism discussed here. The abscissa measures the ratio between the curvature of the flagellum and the minimum curvature required to activate bending.

here, but in view of the number of assumptions made they cannot be considered to provide much support for the model. The model predicts that bends cannot be propagated unless their curvature is greater than $2/\rho_a$, which appears to indicate a maximum possible radius of curvature for the propagated bends of *Chaetopterus* spermatozoa of about 7·5 μ. No observations conflicting with this conclusion have been noticed.

REFERENCES

BISHOP, D. W. & HOFFMANN-BERLING, H. (1959). Extracted mammalian sperm models. I. Preparation and reactivation with adenosine triphosphate. *J. Cell. Comp. Physiol.* **53**, 445–66.

BROKAW, C. J. (1961). Movement and nucleoside polyphosphatase activity of isolated flagella from *Polytoma uvella*. *Exp. Cell. Res.* **22**, 151–62.

BROKAW, C. J. (1962). Studies on isolated flagella. In *Spermatozoan Motility*, pp. 269–78. Ed. D. W. Bishop. American Association for the Advancement of Science.

BROKAW, C. J. (1963). Movement of the flagella of *Polytoma uvella*. *J. Exp. Biol.* **40**, 149–56.

BROKAW, C. J. (1964). Thiourea-induced alterations of flagellar waveforms. *J. Cell. Biol.* **23**, 15A.

BROKAW, C. J. (1965). Non-sinusoidal bending waves of sperm flagella. *J. Exp. Biol.* **43**, 155–69.

BROKAW, C. J. (1966). Bend propagation along flagella. *Nature, Lond.* **209**, 161–63.

BROKAW, C. J. & GOLDSTEIN, S. (1965). Activation and adenosine triphosphate diffusion in flagella. *J. Cell. Biol.* **27**, 15A.

BROKAW, C. J. & WRIGHT, L. (1963). Bending waves of the posterior flagellum of *Ceratium*. *Science*, **142**, 1169–70.

BURGERS, J. M. (1938). On the motion of small particles of elongated form, suspended in a viscous medium. In *Second Report on Viscosity and Plasticity, Kon. Ned. Akad. Wet., Verhand.* (Eerste Sectie), **16** (4), 113–84.

DUBOIS, K. P. & ERWAY, W. F. (1946). Studies on the mechanism of action of thiourea and related compounds. II. Inhibition of oxidative enzymes and oxidations catalysed by copper. *J. Biol. Chem.* **165**, 711–21.

EZELL, S. D., JR. (1963). The lateral body of *Ciona intestinalis* spermatozoa. *Exp. Cell. Res.* **30**, 615–17.

GILLESPIE, T. (1960). The viscosity of concentrated polymer solutions. *J. Polymer Sci.* **46**, 383–93.

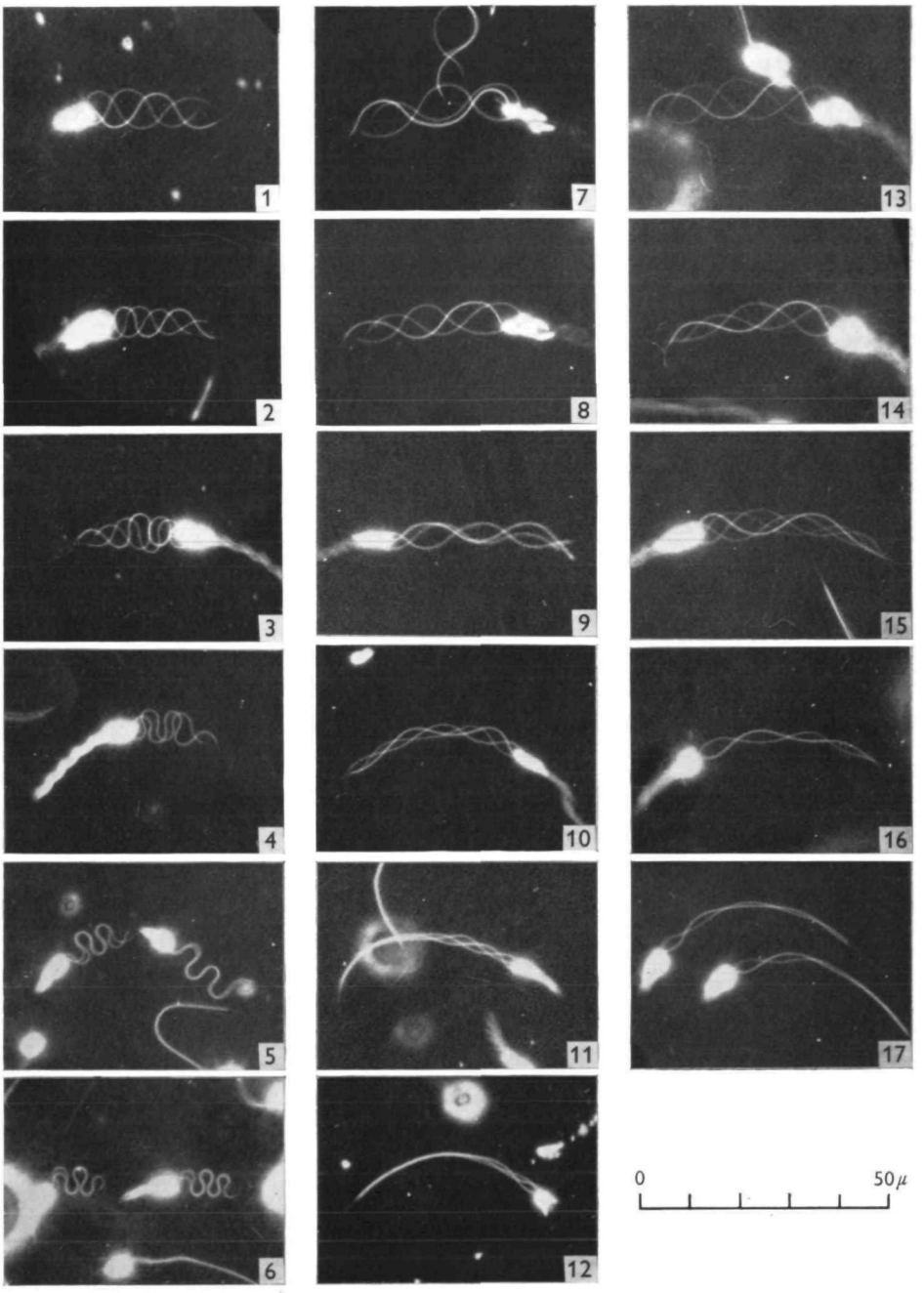
GRAY, J. (1955). The movement of sea-urchin spermatozoa. *J. Exp. Biol.* **32**, 775–801.

GRAY, J. & HANCOCK, G. J. (1955). The propulsion of sea-urchin spermatozoa. *J. Exp. Biol.* **32**, 802–14.

HANCOCK, G. J. (1953). The self-propulsion of microscopic organisms through liquids. *Proc. Roy. Soc.* A, **217**, 96–121.

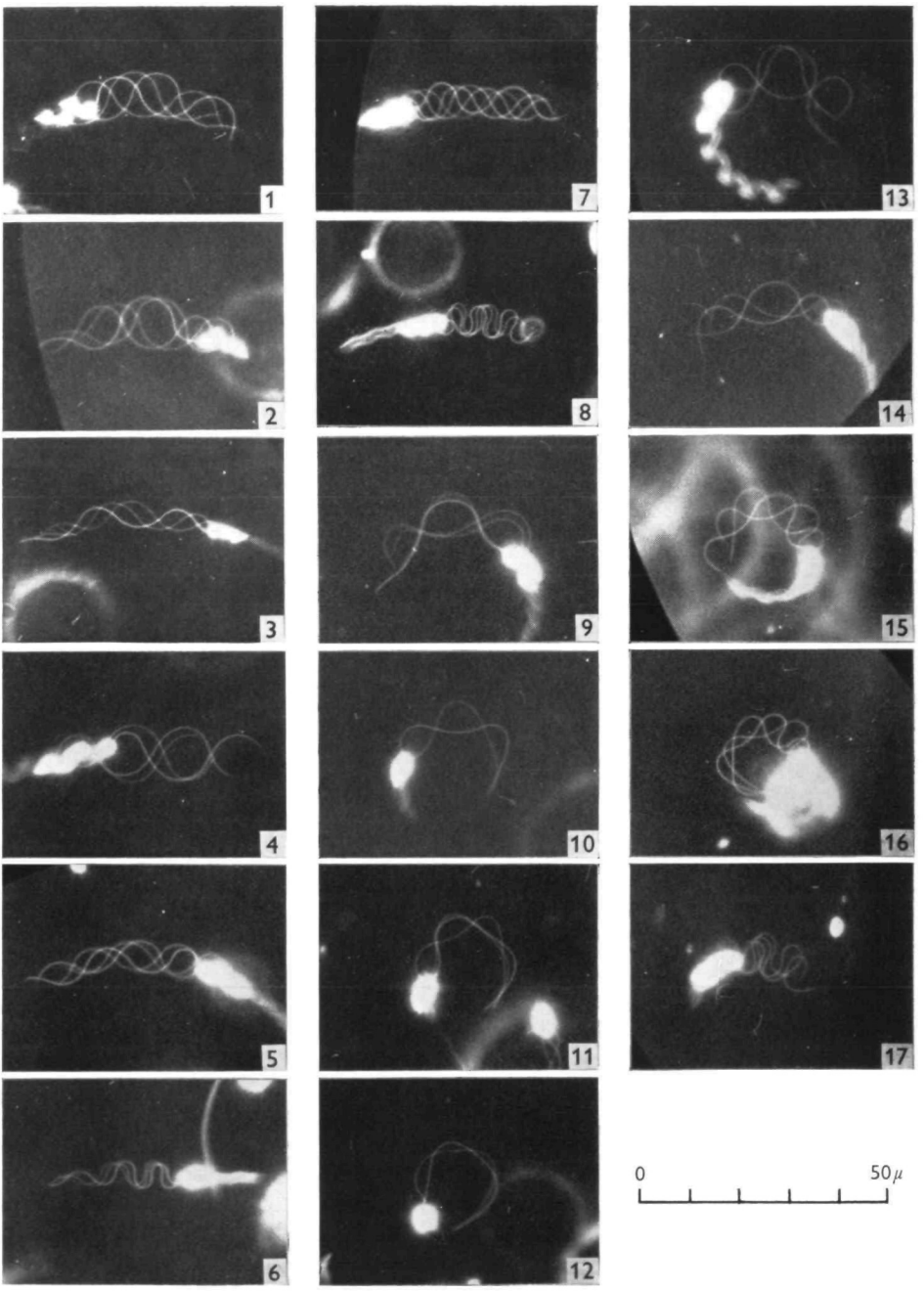
HOFFMANN-BERLING, H. (1955). Geisselmodelle und Adenosintriphosphate. *Biochim. biophys. Acta*, **16**, 146–54.

HOLWILL, M. E. J. (1965). The motion of *Strigomonas oncopelti*. *J. Exp. Biol.* **42**, 125–37.



C. J. BROKAW

(*Facing p.* 138)



C. J. BROKAW

HOLWILL, M. E. J. & SILVESTER, N. R. (1965). The thermal dependence of flagellar activity in *Strigomonas oncopelti*. *J. Exp. Biol.* **42**, 537–44.

KINOSHITA, S. (1958). The mode of action of metal-chelating substances on sperm motility in some marine forms as shown by glycerol-extracted sperm models. *J. Fac. Sci. Tokyo Univ., Sect.* **4**, **8**, 219–28.

MACHIN, K. E. (1958). Wave propagation along flagella. *J. Exp. Biol.* **35**, 796–806.

PROSSER, C. L. & BROWN, F. A. JR. (1961). *Comparative Animal Physiology*, p. 433. London: W. B. Saunders Co.

RÜEGG, J. C., STRAUB, R. W. & TWAROG, B. M. (1963). Inhibition of contraction in a molluscan (*Mytilus edulis*) smooth muscle by thiourea, an inhibitor of the actomyosin contractile mechanism. *Proc. Roy. Soc.* B, **158**, 156–76.

URSPRUNG, H. & SCHABTACH, E. (1965). Fertilization in tunicates: loss of the paternal mitochondrion prior to sperm entry. *J. Exp. Zool.* **159**, 379–83.

YONEDA, M. (1962). Force exerted by a single cilium of *Mytilus edulis*. II. Free motion. *J. Exp. Biol.* **39**, 307–17.

EXPLANATION OF PLATES

PLATE 1

Movement of spermatozoa at increased viscosities. Figs. 1–6 (left column) show spermatozoa of *Chaetopterus*. Figs. 7–12 (centre column) show spermatozoa of *Ciona*. Figs. 13–17 (right column) show spermatozoa of *Lytechinus*. Normal movements (viscosity 1·4 cP.) are illustrated in Pl. 1, figs. 1–6, of Brokaw (1965). The flash frequencies and the measured viscosity for each photograph are listed below. Figs. 5 and 6 show spermatozoa swimming in solutions containing low-MW methyl-cellulose; comparable photographs showing movement in solutions containing high-MW methyl cellulose are shown in Brokaw (1965), Pl. 2, figs. 4 and 7.

Fig.	Flash rate (per sec.)	Measured viscosity (cP.)	Fig.	Flash rate (per sec.)	Measured viscosity (cP.)
1	40	2·9	10	40	14
2	30	14·4	11	20	49
3	25	28·3	12	15	> 100
4	20	65	13	50	2·6
5	15	340	14	50	4·55
6	10	940	15	40	6·8
7	50	2·05	16	40	9·1
8	50	4·26	17	20	41
9	40	6·2			

PLATE 2

Figs. 1–3. Movement of spermatozoa of *Ciona* in solutions containing thiourea. Fig. 1. Flash rate 25/sec.; 0·175 M thiourea. Figs. 2, 3. Flash rate 30/sec.; 0·15 M thiourea.

Figs. 4–8. Effects of thiourea and increased viscosity on the movement of spermatozoa of *Lytechinus*. Flash rates 20/sec. Fig. 4. Measured viscosity 1·4 cP.; 0·15 M thiourea. Fig. 5. Measured viscosity 6·7 cP.; 0·15 M thiourea. Fig. 6. Measured viscosity 120 cP.; 0·15 M thiourea. Fig. 7. Measured viscosity 13·6 cP.; 0·3 M thiourea. Fig. 8. Measured viscosity 145 cP.; 0·3 M thiourea.

Figs. 9–12. Movement of reactivated spermatozoa of *Lytechinus* at an ATP concentration of 2 mM. Fig. 9. Flash rate 40/sec.; measured viscosity 1·9 cP. Fig. 10. Flash rate 30/sec.; measured viscosity 4·1 cP. Fig. 11. Flash rate 25/sec.; measured viscosity 8·6 cP. Fig. 12. Flash rate 20/sec.; measured viscosity 21·2 cP.

Figs. 13–17. Movement of reactivated spermatozoa of *Lytechinus* at an ATP concentration of 0·2 mM.

Fig. 13. Flash rate 14/sec.; measured viscosity 1·9 cP. Fig. 14. Flash rate 15/sec.; measured viscosity 9·4 cP. Fig. 15. Flash rate 12/sec.; measured viscosity 22 cP. Fig. 16. Flash rate 10/sec.; measured viscosity 61 cP. Fig. 17. Flash rate 6/sec.; measured viscosity 164 cP.