# A QUANTITATIVE ANALYSIS OF CILIARY MOVEMENT BY MEANS OF HIGH-SPEED MICROCINEMATOGRAPHY

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## INTRODUCTION

Studies on the movements of cilia and flagella have recently been developed in various lines: the ultrastructure by electron microscopy, the biochemistry of contractile proteins and energy transformation, and the correlation of mechanical activity to membrane potentials (cf. Sleigh, 1962; Holwill, 1966; Kinosita & Murakami, 1967). There are, however, some peculiar difficulties in analysis of ciliary movement as compared with those of other types of cell motility, since cilia and flagella are very minute in size and move at considerably high frequencies in two or three dimensions.

Among various materials the large abfrontal cilium of the gill of *Mytilus* is a material favourable for descriptive and biophysical studies of the movement because of its relatively large size (up to 100 $\mu$ in length) and of its low frequency of beat (*c.* 2 cyc./ sec.). Of descriptive studies, Gray's (1930) has been well-known. Kinosita & Kamada (1939) have offered a diagrammatic representation based on Gray's and their own records and quantitatively described the movement of the cilium using the basal inclination angle which is formed between the basal part of the ciliary shaft and the tissue surface. The speeds of their cinematographic recordings (not exceeding 24 frames/sec.) were not sufficiently high for detailed analysis of the movement and their photographic images of cilia were not perfectly clear owing to their movement during exposures. Recently, several authors (Gosselin, 1965; Holwill, 1964, 1965; Jahn, Landman & Fonseca, 1964; Machemer, 1969; Miles & Holwill, 1969; Rikmen-spoel, 1964; Sleigh, 1968; Sleigh & Holwill, 1969; Zorgniotti, Hotchkiss & Wall, 1958) have reported the results of high-speed microcinematography of ciliary and flagellar movements. Sleigh (1968) has described the movement of cilia and flagella in terms of the basal inclination and of the position of the bend along the axis.

The present work deals first with a technique which enabled us to record the movement of the single cilium of *Mytilus* gill at 400–500 frames/sec. with single exposures of 1/20,000 sec., secondly with a quantitative description of the ciliary movement in terms of the degree of bending of the ciliary shaft expressed by its curvature at various regions along its axis and of the basal angle, and thirdly with an analysis of the correlation between the degree of bending and the bending moment at the same region, the results of which may throw a light on the active nature of the bending of the ciliary shaft along its entire length during ciliary movement.

## MATERIALS AND METHODS

### Preparation

The large abfrontal cilia of the gills of *Mytilus edulis* were used as materials. A single gill filament cut about 3 mm. long was held between two small pieces of coverslip fixed at the tip of a glass rod supported with a micromanipulator, and bathed in sea water in a trough on a microscope stage (for details, see Baba & Hiramoto, 1970). Under intermittent light, the position and inclination of the gill filament were adjusted by means of the micromanipulator so as to make the image of the cilium clear from base to tip and over the whole course of beating.
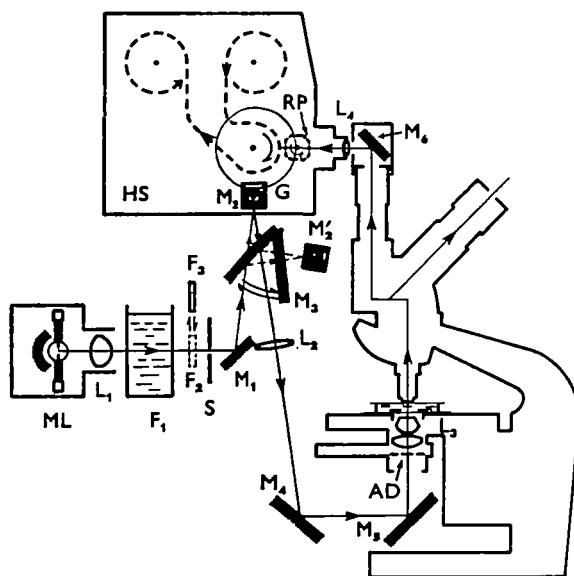


Fig. 1. Optical arrangement for high-speed microcinematography. The light path is indicated by thin lines with arrows. AD, annular diaphragm; $F_1$, $F_2$ and $F_3$, filters; G, gears; HS, high-speed camera; $L_1$, $L_2$, $L_3$ and $L_4$, lenses; $M_1$, $M_3$, $M_4$, $M_5$ and $M_6$, mirrors; $M_2$ and $M_2'$, rotating mirrors; ML, super-high-pressure mercury vapour lamp; RP, rotating prism.

### Optical system

Although the optical system used in the present experiment was reported elsewhere (Baba & Hiramoto, 1970) in detail, it will be described in brief.

The light source used was a super-high-pressure mercury vapour lamp (ML; USH-100D3, Ushio Electric Inc.) operated on direct current. The cilium was illuminated by the light emitted from the source, passing through lenses, filters and a slit, and reflected by mirrors in the following sequence (cf. Fig. 1): a collector lens ($L_1$), a heat-absorbing filter of Mohr's salt solution ($F_1$), a UV-absorbing filter ($F_2$), a slit (S), a mirror ($M_1$), a rotating mirror ($M_2$), a collector lens ($L_2$), mirrors ($M_4$, $M_5$) and a microscope substage condenser ($L_3$). The rays from the light source were focused on the mirror ($M_2$) by the first collector lens ($L_1$) and further on the annular diaphragm (AD) of the substage condenser by the second collector lens ($L_2$), and then became parallel to the optical axis of the microscope and illuminated the object after passing through the condenser lens ($L_3$).

When the mirror ($M_2$) was rotated, the image of the slit rapidly moved in the object plane across the microscopic field, so that the cilium was illuminated for periods of short duration which could be controlled by varying the width of the slit and/or the speed of rotation of the mirror ($M_2$). The image of the cilium was recorded on 16 mm. cine film in a high-speed camera (HS; Himac 16HS, Hitachi) after a magnification of 50 diameters with a phase-contrast objective (Nikon BM40, N.A. 0·65), a 5 × ocular and a relay lens ($L_4$) through a rotating prism (RP).

The rotation of the mirror ($M_2$) was so coupled with gears (G) to the film-driving mechanism of the camera that the cilium might be illuminated when the camera shutter was completely open. Single illumination of 1/20,000 sec. in duration was sufficient to record an image of the cilium of good quality on 16 mm. Kodak plus-X or tri-X cine film with the optical arrangement mentioned above.

By inserting a mirror ($M_3$) and a green filter ($F_3$) into the light path observation without recording was possible. Controlling the speed of rotation of the mirror ($M'_2$) made it possible to reduce the apparent speed of ciliary movement on the principle of the stroboscope, so that the adjustment of focus and orientation of the cilium by the micromanipulator was facilitated.

## Media

The media used were filtered sea water and sea-water solutions of polyvinylpyrrolidone (K-90, Tokyokasei) which will be referred to as PVP, though sea-water solutions of methyl cellulose (MC) were used in some preliminary experiments. Undissolved particles in the solutions were removed by centrifugation of the solutions for 30 min. at 20,000 × $g$ before experimentation. To change the solution the trough containing it was replaced with a new one while the gill filament was supported in its original place with the micromanipulator. This procedure was repeated 2–3 times to prevent contamination by the previous solution.

The viscosities of the solutions were measured with Ostwald viscometers, immediately after recordings of the ciliary movement, at the same temperature in a water bath. Measurements with a pressure or a rotation viscometer revealed that the apparent viscosities of PVP solutions were practically constant over the wide range of shearing stress whereas those of MC solutions varied with shearing stress as Brokaw (1966*b*) pointed out. From the results obtained with the pressure viscometer, for example, the apparent viscosities of PVP solutions (*c.* 19 cP.) were almost constant with standard deviations of 0·4–0·5 cP. in the range of shearing stress (measured at the wall of viscometer) from 15 to 240 dynes/cm.$^2$, whereas those of MC solutions (12–17 cP.) decreased by 30–35 % when the shearing stress rose from 15 to 240 dynes/cm.$^2$ In the present study both sea water and sea-water solutions of PVP were regarded as Newtonian fluids in the analysis of the movement of the cilium, since the shearing stresses of the bathing solution, which were calculated by the drag per surface area, were evaluated to be less than 200 dynes/cm.$^2$ during the movement of the cilium even in the solutions of increased viscosities. Only the results from sea water and PVP solutions are reported in the present paper.

Before and after bathing in either PVP or MC solutions no difference was detected in the movement of the cilium in sea water; thus the toxicity of these solutions may be ignored.

*Viscous resistance of the medium* (cf. Fig. 2)

The force acting on a minute element of the ciliary shaft may be calculated from its length ($ds$), its velocity ($V$) which is obtained from the cinematographic records and the viscosity of the medium ($\eta$). The components of the force ($dF_N$ and $dF_T$) normal to and tangential to the ciliary axis, respectively, exerted on the point ($S$) of the element are given by

$$dF_N = C_N \eta V_N ds \qquad (1)$$

and

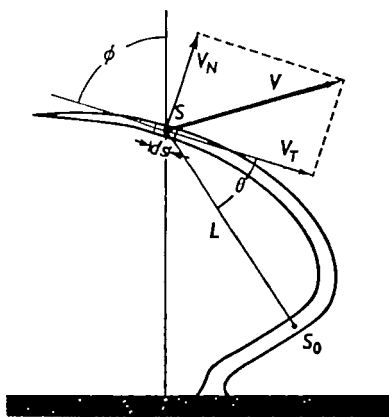$$dF_T = C_T \eta V_T ds, \qquad (2)$$



Fig. 2. Diagram illustrating parameters in calculation of forces and bending moments. The forces due to $V_N$ and $V_T$ are opposite in direction to the corresponding components of the velocity. For details see text.

where $V_N$ and $V_T$ are the normal and the tangential velocities of the element, respectively, and $C_N$ and $C_T$ are dimensionless coefficients independent of $\eta$, $V_N$ and $V_T$. If the element is considered to be a part of a long cylinder,

$$C_T = 0.5\, C_N \qquad \text{(cf. Hancock, 1953)} \quad (3)$$

The absolute value of $C_N$ was determined as follows. According to Yoneda (1962), the torque at the base ($I$) when the cilium beats without changing its shape with an angular velocity ($\omega$) is represented by equation (4) in his paper,

$$I = \omega \eta l^3 \int_0^1 t^2 f(R, t)\, dt \qquad (4)$$

or

$$I = \omega \eta l^3 F(R), \qquad \text{(equation (5) of Yoneda, 1962)} \quad (5)$$

where $l$ is the total length of the cilium, $t$ is the distance from the base to a point of the ciliary axis divided by the total length, $f(R, t)$ is $C_N$ in the present paper and $R$ is Reynolds number. Integrating equation (4) assuming that $C_N$ is constant along the ciliary axis, and equating to equation (5),

$$F(R) = \tfrac{1}{3} C_N. \qquad (6)$$

Since these equations hold not only for a cilium, but for any model of a geometrically similar figure moving in the manner of a cilium and under similar hydrodynamical situations (which are described by a characteristic Reynolds number), $F(R)$ can be

determined by model experiment. Yoneda (1962) found that $F(R)$ is $1\cdot1$, which is constant within his experimental range of Reynolds number $(R)$. This fact and equation (6) indicate that

$$C_N = 3\cdot3. \tag{7}$$

The bending moment $dM$ around a point $S_0$ on the ciliary axis due to the force at the point $S$ is given by

$$dM = L\cos\theta\ dF_N + L\sin\theta\ dF_T, \tag{8}$$

where $L$ is the distance between $S$ and $S_0$ and $\theta$ is the angle formed by the line $SS_0$ and the tangent to the ciliary axis at $S$. Integrating this from $S_0$ to the tip of the cilium and using equations (1), (2), (3) and (7) give the total bending moment $(M)$ around $S_0$,

$$M = \int dM$$

$$= 3\cdot3\eta \int_{s_0}^{s_t} L(V_N\cos\theta + 0\cdot5\ V_T\sin\theta)\ ds, \tag{9}$$

where variable $s$ denotes the distance of the point $S$ measured from the base along the ciliary axis, $s_t$ is the $s$-value at the tip of the cilium and $s_0$ the $s$-value at $S_0$.

Similarly, the force exerted on the whole cilium by the fluid in the direction parallel to the tissue surface $(F_P)$ is given by

$$F_P = \int dF_P$$

$$= 3\cdot3\eta \int_0^{s_t} (V_N\cos\phi + 0\cdot5\ V_T\sin\phi)\ ds, \tag{10}$$

where $\phi$ is the angle formed by the tangent to the ciliary axis and the normal to the tissue surface. Both integrals (9) and (10) were numerically computed in the present work.

<center>RESULTS</center>

<center>*Description of the movement in sea water*</center>

Pl. 1 shows one beat cycle of a cilium reconstructed from a high-speed cinematographic record taken at 450 frames/sec. by the method mentioned above. Pictures from No. 0 to 80 in the first row (a) were taken every eight frames from the cinematographic strip; No. 84 to 124 in the second (b) every four frames; No. 126 to 148 in the third (c) every two frames; No. 156 to 236 in the fourth (d) every eight frames. The cilium moves very slowly or even stops to move during the first half of the photographs in Pl. 1a and the second half of the photographs in Pl. 1d. From here there is a movement in effective direction (Pl. 1a) followed by a quick backward movement, during which a wave of the bending of the ciliary shaft propagates distally from the base (Pl. 1b). Long before the bending wave reaches the tip of the cilium the proximal region of the ciliary shaft starts the forward movement and the cilium rapidly stands upright (Pl. 1c and the first half of Pl. 1d). Some cilia occasionally rested for a long time at the end of the effective stroke, which was called 'interkinetic period' (Gray, 1930). In the present study the cilia without the interkinetic period were exclusively used.

Referring to the movement of the large abfrontal cilium of *Mytilus*, Gray (1930) published successive photographs taken by microcinematography at 20 frames/sec.,

and Kinosita & Kamada (1939) represented the movement by superimposed drawings of the cilium of Gray's photographs. They also represented the ciliary movement by the change with time of the angle which is formed between the most proximal region of the ciliary shaft and the surface of the ciliary tissue (inclination angle). Sleigh (1968) described the movements of various cilia (including the large abfrontal cilium of *Mytilus*) in terms of the change with time of the inclination angle and of the position of the bend along the ciliary axis. The results of the present study are, as expected, in general agreement with those of previous reports mentioned above, though the present results offer more quantitative data as mentioned below. Kinosita & Kamada (1939) defined the inclination angle so as to *decrease* during effective stroke, whereas Sleigh (1968) defined it so as to *increase* during effective stroke. In the present study the 'basal angle' is defined as angle formed between the proximal region of the ciliary shaft and the *normal* to the tissue surface, being positive when the cilium bends in the direction of the effective stroke and negative when the cilium bends in the reverse direction, since it is considered that no strain (or aberration among the peripheral filaments of the component cilium) exists in the ciliary shaft when the ciliary shaft stands upright on the tissue surface. The basal angles shown in Figs. 3 and 4 are angles formed between the normal to the tissue surface and the tangent to the ciliary axis at the point at $5\,\mu$ from the base owing to technical difficulties in measurement at $0\,\mu$.

Gray (1930) designated the stages shown in Pl. 1$a$ and $d$ as the effective stroke and the stages in Pl. 1$b$ and $c$ as the recovery stroke, whereas Kinosita & Kamada (1939) designated the stages in Pl. 1$a$, $c$ and $d$ as the effective stroke and the stages in Pl. 1$b$ as the recovery (preparatory) stroke. Because of a peculiar movement of the cilium during stages shown in Pl. 1$c$, in the present study the whole cycle was divided into three phases: the effective phase ($a$ and $d$), the recovery phase ($b$) and the preparatory phase ($c$). The effective phase is the phase in which the basal angle changes from zero to the maximum value, the recovery phase is the phase in which the basal angle changes from the maximum value to the minimum value and the preparatory phase is the phase in which the basal angle changes from the minimum value to zero. They are represented by $E$, $R$ and $P$ respectively in Figs. 3, 4 and 6.

In the present study mechanical activity of the cilium was described in terms of the change with time of the curvature of the ciliary axis at various regions along the ciliary shaft and of the basal angle mentioned above. If the bending of the ciliary shaft results from the difference in the degree of contraction between two groups of peripheral filaments (microtubules) of the component cilia of this compound cilium, the curvature should be proportional to this difference; and if the bending of the ciliary shaft results from the sliding among the peripheral filaments of the component cilia, the degree of sliding should be proportional to the inclination angle ($\phi$ in Fig. 2) at the point of the ciliary shaft in question or the integral of the curvature along the ciliary axis from the base to the point in question in which the basal angle gives the boundary condition at the base [an equivalent expression has been given by Stair (1965, 1968)]. Therefore, the data of the present study can be used for discussing the mechanism of ciliary movement from the basis of either the contractile filament theory or the sliding filament theory (cf. Brokaw, 1966$a$, 1968; Horridge, 1965; Satir, 1965, 1968; Sleigh, 1962, 1968).

An example from the results of the movement of the single cilium is shown in Fig. 3. In the effective phase, the axis bends slightly convex toward the direction of effective stroke where the sign of curvature is defined to be negative in this paper. This bending is larger and takes place earlier in the proximal region, and propagates distally with increasing velocity. A prominent bending of positive curvature appears
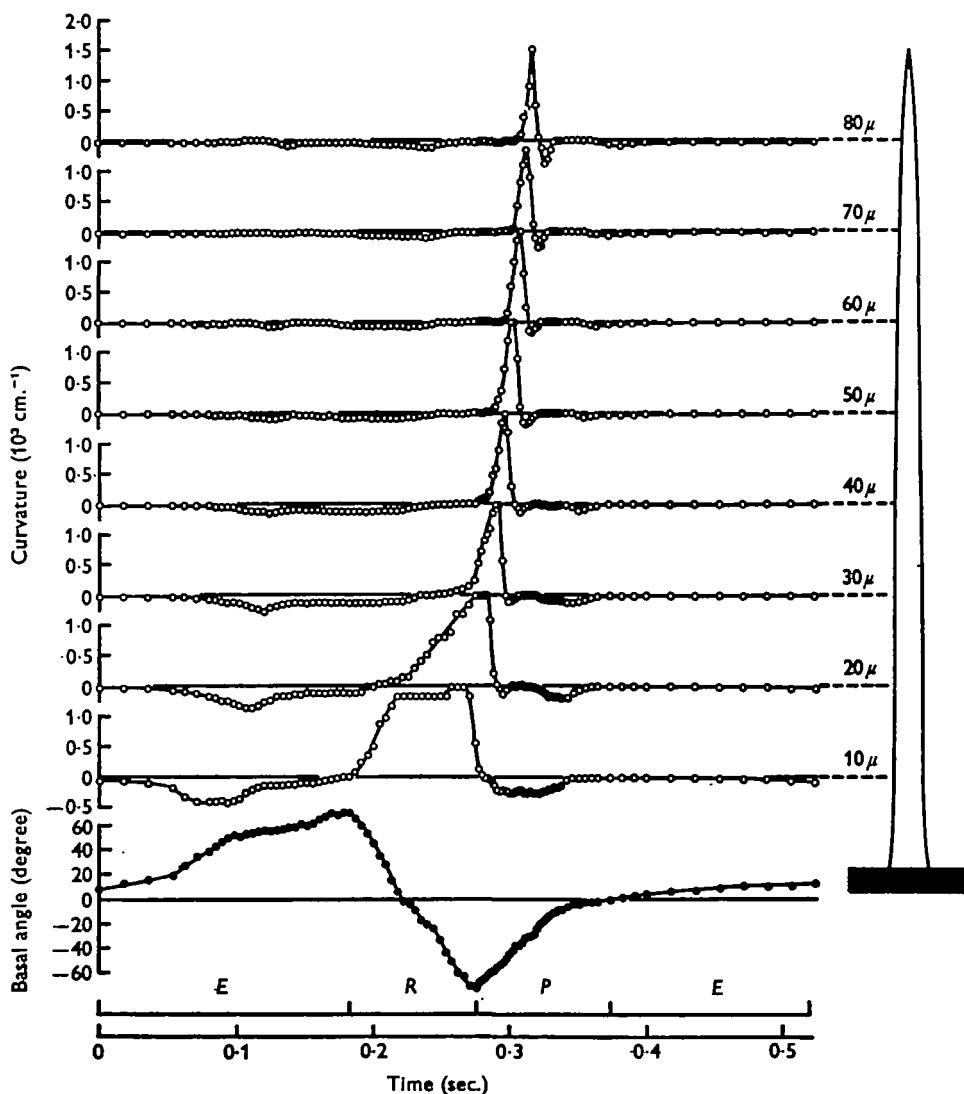


Fig. 3. Changes of the degree of bending (curvature) at various regions (10, 20,...80 $\mu$ from the base) of the shaft of a cilium (90 $\mu$ in length) and of the basal angle during a single beat in sea water. 23·5° C.

in the proximity of the base at the start of the recovery stroke, and during the recovery and the preparatory phases it propagates along the axis up to the tip with increasing rate. The maximum curvature of the bending is almost constant ($1·5 \times 10^3$ cm.$^{-1}$ in Fig. 3) along the axis from base to tip, i.e. the bending wave is decrementless. There is,

however, some difference in duration of the bending at different points; it is longer at the proximal region and becomes shorter as the distance from the base increases. This bending is followed by a bending of the opposite sign which is also propagative. Thus the curvature of each part of the axis has 2–3 maxima and 2–3 minima during one beat cycle.
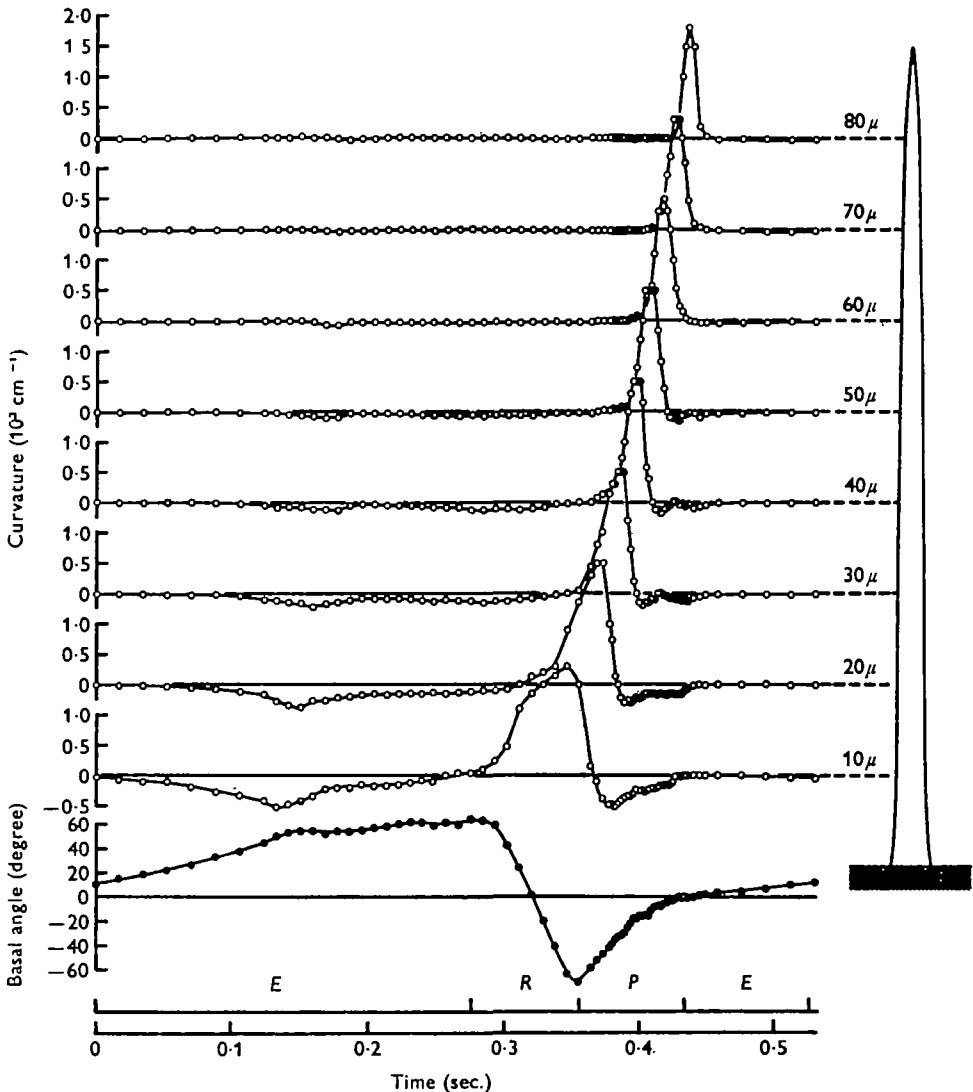


Fig. 4. Changes of the curvature at various regions of the ciliary shaft and of the basal angle of the same cilium in Fig. 3 during a single beat in viscous medium (9·9 cP.). 23·5° C.

### Description of the movement in media of increased viscosities

Fig. 4 indicates the movement of the same cilium as that shown in Fig. 3 in PVP sea water of 9·9 cP. The rate of change of basal angle is much reduced in the effective phase, but the duration of one beat cycle is almost the same, owing to the compensatory reduction of the durations of other phases. In the medium of higher viscosity

(30 cP.) the duration slightly increased (Table 1). The amplitude of the basal angle, namely the difference between the maximum and the minimum angles, decreased with increase in viscosity of the medium (Table 1). When the cilium was put into the media of high viscosities the maximum curvature of the major bending wave in the recovery and the preparatory phases, which propagated without decrement, slightly increased and the propagation velocity decreased. Characteristics in variations of these parameters are presented in Table 1 and in Fig. 5. The propagation velocity ($v$) decreases linearly with logarithm of viscosity, i.e. $v = v_0 - k \log(\eta/\eta_0)$, where $v_0$ is the propagation velocity in sea water ($\eta_0 = 1$ cP.) and $k$ is a constant (mean $\pm$ S.E.: $0.08 \pm 0.006$ cm./sec.).

Table 1. *Effects of viscosity on the duration of a single beat, the*
*amplitude of the basal angle and the maximum curvature of bending*

| Length of the cilium ($\mu$) | Duration (msec.) at various viscosities (cP.) | | | Amplitude of the basal angle (degrees) at various viscosities | | | Maximum curvature* ($10^3$ cm.$^{-1}$) at various viscosities | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 10 | 30 | 1 | 10 | 30 | 1 | 10 | 30 |
| 90 | 520 | 524 | 531 | 144 | 134 | 119 | 1·5 | 2·0 | 2·2 |
| 80 | 488 | 539 | 899 | 123 | 122 | 123 | 1·5 | 2·0 | 2·2 |
| 78 | 425 | 424 | 613 | 138 | 137 | 122 | 1·5 | 1·8 | 2·0 |
| 73 | 250 | 308 | — | 138 | 117 | — | 1·5 | 2·0 | — |
| 62 | 387 | 405 | 570 | 140 | 128 | 120 | 1·8 | 2·6 | 3·0 |

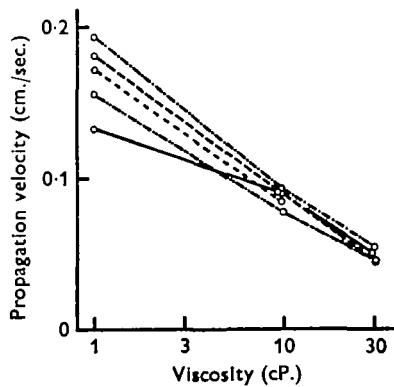* The maximum curvatures were measured at 30 $\mu$ from the base.



Fig. 5. Effect of viscosity on the propagation velocity of the bending wave (measured at 30 $\mu$ from the base). The results from five different cilia, 90 $\mu$ (---), 80 $\mu$ (— · —), 78 $\mu$(—) , 73 $\mu$ (· · ·) and 62 $\mu$ (— · · —) in length, are shown. 23–24° C.

## Flow of the medium generated by the ciliary movement

The ciliary shaft pushes the medium, causing it to flow during the beat, and at the same time the fluid reacts upon the ciliary shaft with resistance. The time course of the component parallel to the tissue surface of the resistance on the whole cilium is shown in Fig. 6. The cilium encounters a resistance from the medium from the direction of effective stroke during the effective phase and from the opposite direction during the recovery phase. In the first half of the preparatory phase the cilium encounters a

resistance from the direction of the recovery stroke, while in the second half the resistance is opposite in direction. It may be considered that the amount of the medium carried by the ciliary movement per unit time is proportional to the resistance exerted on the cilium, since the latter is the rate of change of momentum given to the medium by the cilium. Therefore, the amount of the medium carried by the cilium in the direction parallel to the tissue surface is proportional to the time-integral of the resistance, i.e. the area enclosed by the curve and the time axis in Fig. 6. The total area above the time axis is $1\cdot91 \times 10^{-4}$ dyne sec., that below the axis is $1\cdot12 \times 10^{-4}$ dyne sec., and their ratio is $1\cdot71$. Similar values of the ratio ($1\cdot74-1\cdot93$) were obtained in other materials. The difference between the areas above and below the time axis corresponds to the net flow, during a beat cycle, in the direction of effective stroke (toward the left in Pl. 1).
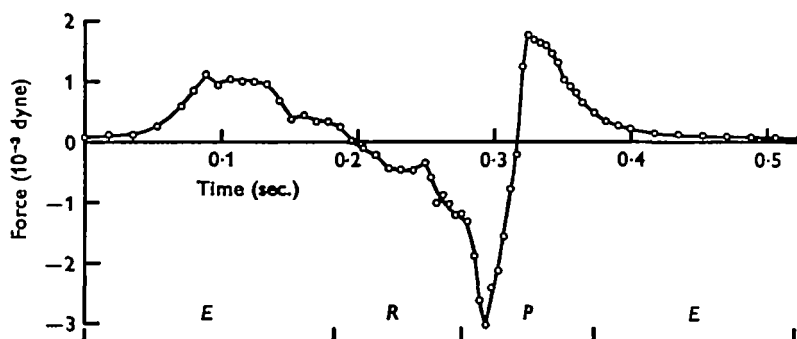


Fig 6. Change in the component parallel to the tissue surface of the force exerted on the whole cilium during a single beat in sea water.

## Time courses of the curvature and the bending moment

It is an important question in relation to the mechanism of ciliary movement whether the bendings of the ciliary shaft at various regions described above are purely passive in nature or whether they involve any active processes in the cilium. Comparison of the curvature with the bending moment at the same point of the ciliary shaft may be a clue to this question, since a definite causal relationship due to the mechanical properties of the ciliary shaft is expected between these quantities if the bending is solely passive in nature. The change with time of the curvature (open circles) of the ciliary axis at the region $30\,\mu$ from the base measured along the axis and the change with time of the bending moment (solid circles) at the same region are shown in Fig. 7. Dotted lines indicate the instances when the curvature reaches its maximum and minimum values. As shown in this figure, the change of the curvature generally occurs in advance of the change of the bending moment, and the time lag between the changes of the curvature and of the bending moment is of the order of several milliseconds.

If the bending were purely passive in nature the change of the bending strain (i.e. the curvature) would be in parallel or lag behind the change of the bending stress (i.e. the bending moment), since the inertia involved in the movement of the ciliary shaft and the surrounding medium is very small and the existence of the viscous component of the mechanical properties of the ciliary shaft would delay the response to the stress. It is, therefore, of much importance that the curvature precedes the bending moment

in the course of the ciliary movement, and this result may indicate that the bending of this region is not a passive one in response to the external force but involves an active process in the cilium.

Calculations of the bending moment made at 10 $\mu$ and at 60 $\mu$ from the base led to similar results, i.e. the bending strain preceded the bending stress due to the external force, indicating the existence of intrinsic mechanical activity in distal region as well as in proximal region. The same conclusion was reached from the analysis of the movements of cilia in media of increased viscosities, in which the time lag between the changes of the strain and of the stress was more remarkable than in normal sea water.
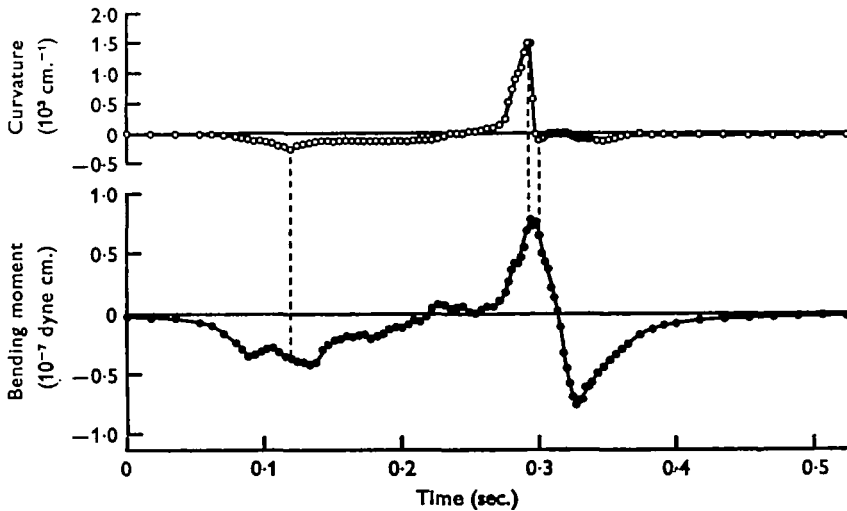


Fig. 7. Changes in the curvature and in the bending moment at 30 $\mu$ from the base during a single beat in sea water.

## DISCUSSION

### Recording system

By the microcinematographic recording technique described in the present paper it was possible to record the movement of single cilia by exposures of 1/20,000 sec. in duration on plus-X film at the crank-speed of 500 frames/sec. using a 40 × phase-contrast objective lens with a high resolving power (N.A. 0·65). Although the large abfrontal cilia of *Mytilus* beat at relatively low frequencies as compared with other cilia and flagella, they exhibit a rapid motion during the preparatory phase, which is comparable with those of other cilia and flagella of higher frequencies (cf. Sleigh, 1968). Brief exposures are consequently required for sharp photographic recordings of the movement of this cilium. Since the maximum speed of this cilium during its beating is about 0·3 cm./sec., the movement of the cilium during a single exposure in the present method is at most 0·15 $\mu$, which is sufficiently small compared with the resolving power of the objective lens.

Although the intensity of illumination was sufficiently strong for photographic recording, no effect was observed on the mode of the ciliary movement as a result of repeated exposures during a series of experiments. This is probably due to the facts

that both heat and ultra-violet rays were absorbed by filters ($F_1$ and $F_2$ in Fig. 1),
that the total duration required for single recording was a few seconds, only 2·5%
of which was the actual illumination time, and that the rays were out of focus at the
object plane.

### Mechanical resistance of the medium on the ciliary shaft

Following Gray & Hancock (1955), the theoretical treatments based on hydro-
dynamic assumptions similar to those in the present study have been given to the move-
ment of cilia and flagella by several authors (cf. Holwill, 1966). In the case of the large
abfrontal cilia of *Mytilus*, in particular, an evaluation of mechanical energy expenditure
has been made during effective stroke in media of various viscosities by Yoneda (1962)
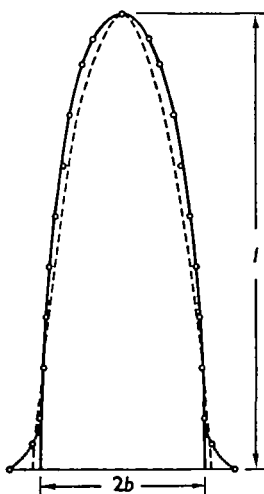and during the whole cycle of beating in sea water by Sleigh & Holwill (1969).



Fig. 8. Shape of a large abfrontal cilium. The circles represent averages of measurements on ten
cinephotographs of the same cilium. Solid line shows a half ellipse which fits well the shape of
the cilium; *l* denotes half of the major axis and 2*b* the minor axis. Broken line indicates the
shape of Yoneda's model. The width (horizontal) is exaggerated, by × 10 in relation to the
length (vertical).

In discussing the mechanical resistance of the medium on the ciliary shaft it is
necessary to know the shape of the cilium used in the present experiment. Fig. 8 shows
the results of determination of the diameters of a large abfrontal cilium at various
regions along the ciliary axis. As shown in this figure, the form of the cilium (repre-
sented by circles) resembles not a cone but a half of a prolate ellipsoid (thick solid line)
except in the region near its base. The axial ratios of the ellipsoids ($l/b$ in Fig. 8) which
approximate to the abfrontal cilia in shape were usually 50–60.

In the case of the movement of a straight or a curved ellipsoid of revolution in a
viscous medium the coefficients of mechanical resistance per unit length ($C_N$ and $C_T$)
are constant along the entire axis in spite of the difference in diameter at different
regions (Happel & Brenner, 1965; Tchen, 1954). Therefore, it is safely assumed that
the coefficients $C_N$ and $C_T$ are constant along the ciliary axis irrespective of the distance
from the base. Inaccuracy in the estimation of the axial ratio of the cilium scarcely
affects the absolute values of the coefficients, since the coefficients change but slowly

with the axial ratio, as expected from theoretical formulas for the resistance of moving ellipsoid (Happel & Brenner, 1965).

The calculation of the resistance of the medium to the *translating* ellipsoid using theoretical formulas (Oberbeck's formulas, cf. Crick, 1950; Happel & Brenner, 1965; Lamb, 1932) indicates that $C_N = 2\cdot46$ and $C_T = 1\cdot53$, when 50 is adopted as the axial ratio. In this case, $C_T$ is close to but larger than a half of $C_N$ ($C_T = 0\cdot62\ C_N$). The torque around the centre of the ellipsoid when it is rotated around the minor axis is calculated to be $0\cdot82\ \eta\omega l^3$ from equations (5) and (6) using the value of $C_N$ mentioned above. On the other hand, the torque from a theoretical formula for the rotation of the ellipsoid (cf. Crick, 1950) is found to be $1\cdot22\ \eta\omega l^3$, which is about $1\cdot5$ times the value mentioned above. [Yoneda (1962) obtained a value of $1\cdot1\ \eta\omega l^3$, being close to the latter, by his experiment using a model whose shape is geometrically similar to that of the large abfrontal cilium (cf. broken line in Fig. 8).] The discrepancy of the results is probably due to the fact that the method for determining resistance mentioned in Materials and Methods is not exactly applicable to the movement accompanying the *rotation* of the body. Because the movement of the ciliary shaft is accompanied by *rotation* as well as by *translation*, a value larger than $2\cdot46$ should be used as $C_N$ in the calculation of the resistance on the ciliary shaft. The value $3\cdot3$ (from Yoneda) used in the present study is adequate in the calculation of the resistance during the effective stroke of the cilium since the hydrodynamic relations of the cilium are similar to those in the model experiment. Although it is not certain whether or not this value is adequate for the calculation of the resistance during the recovery stroke, the resistance calculated using this value are not far from the actual values. The value of $C_T$, $1\cdot65$ (half of $C_N$), used in the present study, which is not substantial in the calculation of the resistance during effective stroke but of much importance in the calculation during recovery stroke, is not so far from the value, $1\cdot53$, obtained from the formula for the translational movement of the ellipsoid.

### Active nature of bending and unbending

The present experiments have shown that the change in the bending strain of the ciliary shaft (curvature) takes place in advance of the change in the bending stress of the same part (bending moment). If the ciliary shaft were purely elastic, the change in the stress would be exactly parallel to the change in the strain; and if viscous components were involved the change in the strain would follow the change in the stress with a lag. This fact, therefore, may imply that the bending and unbending of the ciliary shaft do not result from passive responses to the change in the bending moment but are caused by intrinsic mechanical activity within the cilium. In other words, an active bending-unbending mechanism exists along the cilium from base to tip. The fact that the bending wave propagates without decrement along the cilium from base to tip during recovery and preparatory stroke may support the above conclusion.

Kinosita & Kamada (1939) found that, when the movement of the large abfrontal cilium was arrested with a microneedle at the proximal part of the cilium, the cilium bent at the arrested region, whereas the movement of the cilium was stopped without bending response of the ciliary shaft when the distal part was arrested. Yoneda (1960) obtained a similar result. Although they called the proximal region, which responded to arrest by bending, the *active region*, their results do not conflict with the present

conclusion because they merely indicate that the distal region does not respond to arrest and do not necessarily imply passive nature of the bending of the distal region during normal beating.

The sequence of the mechanical events (changes in stress and strain) mentioned above is readily interpreted on the theory that the peripheral filaments of the cilium respond with contraction after a definite reaction time when they are passively stretched, which was suggested by Machin (1958, 1963) as a result of mathematical analysis of flagellar movement. However, the results of the present study do not provide Machin's hypothesis with strong support, because the time courses of the stress and strain and their intervals are so variable at various regions of the ciliary shaft that causal relation between them can not quantitatively be analysed.

### Effects of viscosity of the medium

The fact that increase of the viscosity of the medium causes decrease not only in the speed of beating but also in the frequency of beat and in the propagation velocity of the bending wave may imply that the increase of viscosity has an influence on the active bending-unbending mechanism of the ciliary shaft as well as on the frequency-controlling mechanism through the mechanical resistance experienced by the ciliary shaft. Probably, both the energy-liberating mechanism for the active bending and unbending and the mechanism for initiation of the beat are directly coupled to the configuration of the cilium at the molecular level as suggested for muscular contraction (cf. Huxley, 1957; Pringle, 1967, 1968).

### SUMMARY

1. An optical arrangement for high-speed microcinematography has been designed so as to record ciliary movement, and the movements of single large abfrontal cilia of *Mytilus* gill have been photographed at 400–500 frames/sec. with brief exposures of 1/20,000 sec.

2. A quantitative description of the movement of the cilium is presented in terms of the changes of the curvature at various regions of the ciliary shaft and of the change of the basal angle.

3. The principle of the description mentioned above is also applied to the movement of the cilium in media of high viscosities, and some parameters of the movement (duration, amplitude of the basal angle, maximum curvature and propagation velocity of the bending wave) are presented.

4. The resistance experienced by the cilium during its beating has been evaluated under some hydrodynamic assumptions, and the flow induced by the cilium and the bending moment of the ciliary shaft due to the viscous resistance have been calculated over a single beat.

5. The change of the degree of bending of the ciliary shaft (curvature) takes place in advance of the change of the bending moment at the same region in the distal as well as in the proximal regions of the ciliary shaft. This fact indicates that active processes are involved in bending and unbending of the ciliary shaft during its beat.

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## EXPLANATION OF PLATE

Movement of a single large abfrontal cilium of *Mytilus* during a single beat. Frames are selected and reconstructed, every eight frames in the first row (*a*), every four frames in the second (*b*), every two frames in the third (*c*), every eight frames in the fourth (*d*), from a high-speed microcinematograph taken at 450 frames/sec. The numbers indicate the sequence of the frames.

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