THE MOTION OF *STRIGOMONAS ONCOPELTI*

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INTRODUCTION

This paper presents the results of a study of the organism *Strigomonas oncopelti* by high-speed cinephotography. Detailed observations of flagellar movement have previously been confined to spermatozoa of the sea-urchin and the bull (Gray, 1955, 1958), although more general descriptions of a variety of flagellates have been given by Ulehla (1911), Lowndes (1936, 1941, 1944) and Zorgniotti, Hotchkiss & Wall (1958). It has generally been assumed that the movement of waves of curvature along a flagellum takes place only from base to tip (Lowndes, 1941, 1944; Brown, 1945). Walker & Walker (1962), however, showed by cinephotomicrography at a low framing rate that waves passed from tip to base along the flagellum of *Trypanosoma brucei*, while a similar inference has been drawn by Afzelius (1962) from observations of spermatozoa from *Myzostomum cirriferum*. The present results show conclusively that such waves occur during the movement of *Strigomonas oncopelti*; other, as yet unpublished, work by the author indicates that a great many of the Trypanosomatidae maintain such waves on their flagella. Waves are also observed to pass in the opposite direction, i.e. from base to tip, along the flagella of most of the organisms so far examined; Afzelius suggests that *Myzostomum* sperm may exhibit a similar phenomenon since they are observed to swim both 'head first' and 'tail first'.

Two main techniques have been used to investigate flagellar motion: the stroboscope and high-speed photography. Although the stroboscopic technique is convenient for the analysis of movements which are repeated at a uniform rate, e.g. 'normal' ciliary and flagellar movements (see, for example, Sleigh, 1955; Brokaw, 1963) it is impossible to detect transient phenomena which may lead to a greater understanding of the mechanisms underlying these activities. High-speed photography eliminates this difficulty when account is taken of the recent advances in camera design. Other problems arise concerning the high intensity of the light source required, but these can be solved by using a suitable experimental arrangement.

Methyl cellulose has often been used to slow down the motion of flagellates in order that they might be followed by eye. It is usually assumed that movements in viscous media have the same form as, but take place at a slower frequency than, movements in the normal medium of the organism concerned, but so far this has been shown to be true for only one organism, *Polytoma uvella* (Brokaw, 1963).

Machin (1958) has suggested that wave propagation along a flagellum might occur mechanically; in such a mechanism a contraction in a particular region of the flagellum is initiated by deformation due to a passively propagated wave from a neighbouring

region. In such a situation he has shown theoretically that the wavelength will be proportional to a certain length l_0 which depends on the physical characteristics of both flagellum and medium; the validity of this relationship is investigated below.

MATERIAL AND METHODS

Strigomonas oncopelti was cultured in 10–20 ml. aliquots of 3 % proteose peptone, 0·5 % glucose and 0·5 % sodium chloride (Newton, 1957). Organisms were prepared for observation after 7–14 days by placing a drop of medium on a slide and gently lowering a coverslip on top.

Cinemicrographs were taken on 16 mm. film using a Stalex high-speed camera and a Zeiss W.L. research microscope under phase-contrast conditions. The image is kept stationary on the continuously moving film by means of a rotating prism similar to that described for the Kodak High-Speed Camera by Zorgniotti *et al.* (1958), while the exact framing rate over a given section of the film can be determined from timing marks made on its edge by a neon lamp flashing at the mains frequency (50 cyc./sec.). Framing rates of 450 frames/sec. were used to record movement in normal medium but this could be reduced to about 100 frames/sec. when methyl cellulose was added. The source of light was a 200 W. mercury arc lamp. During preliminary observations the intensity of illumination was reduced by neutral density filters which also absorbed infra-red radiation. When a film was to be taken these filters were replaced by a water cell which absorbed only the infra-red radiation. A beam splitter incorporated in the apparatus allowed the specimens to be observed during photography.

A series of solutions containing up to 5 % methyl cellulose in water was made. These were centrifuged at 12,000 rev./min. (about 20,000 g) to remove any fibres. A known volume of culture medium (without organisms) was then added to part of each solution and the viscosity of each determined by either a U-tube or a falling sphere viscometer; for the purpose of examination the same proportion of medium containing organisms was added to a smaller volume of methyl cellulose, great care being taken to eliminate air bubbles from the preparation.

OBSERVATIONS

S. oncopelti is a uniflagellate organism with a roughly cylindrical or pear-shaped body measuring about 8·2 μ by 2·6 μ; the average length of the flagellum is 17 μ. Roughly spherical and/or triangular bodies are sometimes seen but these shapes are abnormal. The flagella of resting or morbid organisms are either straight, or, less frequently, slightly curved; a resting flagellum never assumes a sinusoidal shape.

Films taken at lower speeds (about 30 frames/sec.) with an Arriflex cine-camera showed that the velocities and general mode of progression of organisms in a cavity slide were very similar to those in the thinner preparations under a coverslip; it can thus be safely assumed that the latter are sufficiently thick to permit free movement of the organisms about all axes. Furthermore, since these films were made with light of low intensity, a check was provided for determining whether the high light intensity needed for high-speed films sensibly affected movement.

Movement of the organism with flagellum leading (forward motion)

When viewed under continuous dark-ground illumination a culture of motile *S. oncopelti* appears as shown in Pl. 1, figs. 1, 2; the bright region which precedes the body is generally symmetrical with either a fish-like or ellipsoidal outline. This region is the optical envelope of the flagellar motion and has a length of about 14μ with a breadth varying from 2 to 5μ. One gains the impression that behind this envelope are two bodies intersecting each other at their midpoints with their long axes equally inclined to the axis of progression of the organism. These observations when made by eye are due to persistence of vision as explained by Gray (1955); they can be recorded on film by giving a brief time exposure, in this case $\frac{1}{32}$ sec.
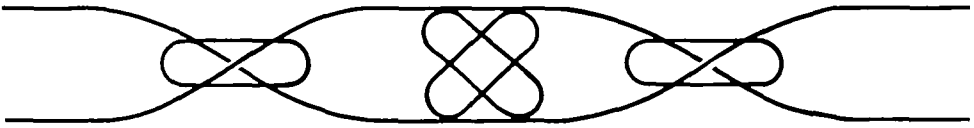
Text-fig. 1. The envelope traced by the body of *Strigomonas oncopelti*. The elliptical outlines represent instantaneous positions of the body to show how the path is formed; at the 'cross-over' point the body is vibrating in a plane perpendicular to the paper, while at the widest point of the path it is vibrating in the plane of the paper.

The movement of the flagellum which produces the envelope is revealed by taking film at high framing rates. During forward movement the organism swims with its flagellum preceding the body, waves of curvature passing along the flagellum from tip to base (Pl. 1, figs. 4–9). The waves originate from a region just behind the tip and pass proximally with an increase in wavelength accompanied in some cases by an increase in amplitude. Towards the distal region of the flagellum the waves have a smaller radius of curvature than in the proximal parts so that not all elements of the flagellum bend to the same extent during their contractile cycles. When the waves are viewed edge-on it is observed that they are predominantly planar (Pl. 1, fig. 3) although the organism rotates about its axis of progression. No correlation was observed between the rotation of the organism and the passage of a wave over a particular part of the flagellum as has been found for bull spermatozoa (Gray, 1955). The rotation occurs once for about every three or four beats of the flagellum. As well as rotating, the body of the organism is caused to oscillate from side to side about an axis which passes through the central region of the body at the same frequency as the flagellar beat. It is this oscillation which gives the impression of two inclined bodies when the organisms are viewed under continuous dark-ground illumination. The path which the body traverses as a result of this oscillation and rotation lies in a form of twist surface and is shown in Text-fig. 1. (A similar surface is easily formed by twisting a strip of paper once or twice about its long axis.) The fact that rotation is not always observed is probably due to the close proximity of the organism to the slide.

The flagellum maintains about 1$\frac{1}{4}$ complete waves along its length with an average wavelength of 14·4μ (taken over 40 organisms) and extreme values of 10 and 16μ. Variations from 8μ at the distal end to 16μ at the proximal end of the flagellum are

often observed on a single organism and the figure of 14·4μ is an average value of the wavelength with respect both to variability between organisms and to variability along a single organism. Values for the amplitude between 1 and 3μ have been recorded with an average value of 2·4μ. Frequencies of beat vary between 10 and 24/sec. having an average value of 16·8/sec. while the average value of the translational velocity of the organism is 17μ/sec. with extremes of 10 and 40μ/sec.

Movement of the organism body-first (backward motion)

S. oncopelti, in common with other members of the Trypanosomatidae, can move body-first by reversing the direction of propagation of waves along the flagellum. These waves assume one of two forms. The first is a sinusoidal wave with an average wavelength of 13μ and amplitude 1·7μ (Pl. 1, figs. 10–13). This wave is very similar in form to the wave during forward motion although the increase in wavelength as a wave progresses is not so pronounced. The frequency of beat is about half that during forward motion of the same organism; typical figures for a single organism are 23·3/sec. for waves propagated from tip to base and 12·0/sec. for waves propagated in the reverse direction. Following the reduction in frequency the translational velocity also falls, in the case cited above from 34 to 20μ/sec.

The second and more common type of wave causing backward movement is shown in Pl. 2; this wave is asymmetric and is propagated at a much lower frequency (about 5/sec.) than the type described above. The amplitudes and wavelengths vary considerably for different organisms; the amplitude has values of between 1·5 and 5·0μ while the wavelength lies in the range 4–8μ. The movement of the body is erratic and the path of the organism usually takes the form of a circle as might be expected from the asymmetry.

Backward movement occurs when either the body of the organism or the tip of the flagellum is obstructed, although such a stimulus is not essential to reverse the direction of propagation of the waves. Reverse propagation does not occur, however, at every obstruction of the body or flagellum tip. Wave propagation from base to tip has also been observed when a central part of the flagellum becomes attached to the slide (see below).

Movement in viscous media

The effect on S. oncopelti of adding methyl cellulose to the medium is similar in many respects to that described for different organisms by other workers, e.g. Brokaw (1963), in that the amplitude, wavelength and, more particularly, the frequency of proximally propagated waves are all reduced while the form of the beat remains unchanged. At viscosities up to about 30 centipoises wave propagation is smooth with an increased number of wavelengths occurring along the flagellum. Movement of the flagellum tends to be more erratic at higher viscosities, although sufficient waves of normal shape are propagated to enable measurements to be made. Table 1 shows the variation of the main physical parameters of the waves with increasing viscosity. For viscosities of 40 cP. and above the waves sometimes die out when they reach the central portion of the flagellum. However, the fact that waves are observed to pass either from tip to base or vice versa, along the whole length of the flagellum at other times is evidence that there is no obstruction causing extinction of the waves.

The amplitude of the body oscillation is also reduced as the viscosity is increased. In normal medium the long axis of the body remains almost tangential to the flagellum at its base, while, at about 30 cP., all oscillation ceases. At a slightly higher viscosity no translational motion of the organism occurs.

Table 1. *The variation of wave parameters with change of viscosity*

Viscosity of medium (centipoises)	Wavelength (μ)	Amplitude (μ)	Frequency (cyc./sec.)	Translational velocity (μ/sec.)
1·2	14·4	2·4	16·8	17 0
4·5	9·9	1·4	11·3	14 0
12·9	8·0	1·4	7·6	7·0
32	7·3	1·3	3·2	4·3
80	6·3	0·8	1·8	0
314	5·3	0·7	0 7	0

With the addition of methyl cellulose there is an increase in the proportion of organisms which maintains distally propagated waves along their flagella. The waveform is asymmetric as in the second type of motion described in the previous section; the symmetric type of wave has not been observed in viscous media. The effect of increased viscosity on the frequency is not so marked as in the case of proximally propagated waves; at 1 poise or more the frequency can still be about 3/sec.

In viscous media a distally propagated wave has occasionally been observed to start from a point about half way along the flagellum; subsequent waves have passed from the base and there was no evidence of an obstruction.

A further interesting observation at a viscosity of 80 cP is that both a distally and a proximally propagated wave have been observed simultaneously on a single flagellum (Pl. 3). This particular phenomenon has been recorded only a few times on film. On each occasion the waves were of approximately equal amplitude and at the point where the waves met the flagellum became quiescent. The next wave to be propagated along the flagellum was from tip to base.

Movement of a flagellum attached to the slide

When the tip of the flagellum becomes attached to the slide the waveform is asymmetric with a larger amplitude for waves on one side than the other. The frequency and average wavelength remains the same as for normal motion. Waves are propagated from tip to base and have not been observed to travel in the reverse direction under this condition. Waves propagating in both directions were observed when the tip of the flagellum and the body were fixed to the slide; the waves were erratic and asymmetric, showing no regularity in the reversal of wave propagation. Preparations which have been left for some time under the coverslip frequently have organisms attached to the slide by the mid-portion of the flagellum. Visual observation shows that motion occurs on both sides of the fixed point while analysis of films reveals that waves travel from tip to base on the distal part and from base to tip on the proximal part. Bending is asymmetric along both portions of the flagellum with waves propagated erratically and there seems to be no correlation between the wave motions on the two sides of the attached point.

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DISCUSSION

The situation where the flagellum pulls the organism through the medium, as in the case of *S. oncopelti*, is hydrodynamically no different from that where the organism is pushed through the medium by the flagellum. Equation (xxvi) of Gray & Hancock (1955) describes the propulsive effect of a sinusoidal wave. Substitution of the experimental parameters obtained leads to a value for the forward velocity of $20 \cdot 5 \mu$/sec. if the radius of the sphere which is hydrodynamically equivalent to the body of the organism is taken to be $3 \cdot 8 \mu$. When it is remembered that the wave form along the flagellum of *S. oncopelti* is not strictly sinusoidal, the calculated value is in reasonable agreement with the observed forward velocity of 17μ/sec.

In contrast to what is seen in the spermatozoon *Psammechinus miliaris* (Gray & Hancock, 1955) the presence of the body of *S. oncopelti* considerably reduces the propulsive velocity. If the body were absent the velocity of the then isolated flagellum would be $60 \cdot 5 \mu$/sec. The body reduces this velocity by about two thirds.

The energy needed to sustain waves of the observed frequency and amplitude on the flagellum of *S. oncopelti* can be evaluated from eqn (1) of Rothschild (1961) and is found to be $1 \cdot 8 \times 10^{-8}$ erg. sec.$^{-1}$. From Stokes's law the energy required to propel the body of the organism through the fluid at 17μ/sec. is $2 \cdot 1 \times 10^{-10}$ erg. sec.$^{-1}$; the efficiency is lower than that (10%) for the propulsion of *Polytoma uvella* (Brokaw, 1961) but is nearly the same as the $1 \cdot 6 \%$ calculated for sea-urchin sperm from the data of Gray & Hancock (1955) and Rothschild (1961).

Rotation of the organism could be the result of two factors. First, the flagellum may not beat in a plane, thus imparting a couple to the system, and secondly, the shape of the body may be such as to cause it to rotate when it moves through the medium. No ridges or other structures which could cause the observed rotation have been observed and since the phenomenon is observed in organisms with round or triangular bodies the departure of the flagellar beat from a plane is the more likely cause.

Lowndes has suggested that the inherent instability of an elongated body being pushed through fluid along its greater axis is responsible for the lack of organisms having flagella attached to their posterior ends (apart from sperm with bodies too small for this instability to arise). When *S. oncopelti* was swimming body-first no such instability was observed. The tendency for an organism to rotate so that its long axis is perpendicular to the axis of progression is caused by the viscous couple which acts to turn the body about an axis through the junction with the flagellum. That such an event does not occur during backward movement of *S. oncopelti* indicates that the couple impressed on the body by the flagellum is greater than the viscous couple and that the flagellum is sufficiently rigid to withstand the couple. Brokaw (1961) has found that flagella isolated from *Polytoma uvella* have a sharp bend near the end at which they are usually attached to the organism; it therefore appears that the substructure is such that this sharp curve occurs naturally in the resting flagellum. Thus it is probable that the molecular configuration within the flagellum sets a limit on the maximum curvature exhibited by a wave passing along a flagellum.

The propagation of a wave from tip to base implies the existence of a control mechanism as well as a means for sustaining the wave (Walker, 1961). We will return

to this point after a discussion of the effects of change of viscosity on the wave parameters.

Gray (1958) has given diagrams showing the relationship between the maximum degree of bending on a flagellum and the phase difference between adjacent segments. The curve for maximum radius of curvature $R = 1\cdot25$ in his text-fig 3*b* closely resembles the asymmetric form of a wave propagated from base to tip along the flagellum of *S. oncopelti*. The maximum curvature of such a wave is practically the same as for a wave propagated from tip to base; this indicates a smaller phase difference between adjacent segments than that obtaining when a wave is propagated from the tip of the flagellum. Since the bending is asymmetric the phase differences on the two sides of the flagellum must differ. In the mechanism for wave propagation postulated by Machin (1958) contractile elements along the flagellum are activated when deformed by a passively propagated wave from the immediately proximal region. It should make no difference whether the passive wave came from the proximal or the distal region, since a deformation would still occur; such a mechanism could thus propagate waves in either direction.

Machin has shown that if wave propagation occurs primarily by mechanical means then a length l_0 characteristic of the material which constitutes the flagellum, but dependent also on the frequency, f, and the viscosity μ, of the medium in which the flagellum is beating, is given by

$$l_0 = \sqrt[4]{\frac{QSK^2(2\cdot0 - \ln R)}{2\pi f \cdot 4\pi\mu}}, \tag{1}$$

where Q is Young's modulus of that part of the flagellum which resists bending and SK^2 is the second moment of area of the same portion. R is Reynolds number. The fraction $(2\cdot0 - \ln R)/4\pi\mu$ can be replaced by $[\ln(2\lambda/d) - \frac{1}{2}]/4\pi\mu$, where d is the radius of the flagellum.

Equation (1) can be expressed thus:

$$\frac{8\pi^2 l_0^4}{QSK^2} = \frac{\ln(2\lambda/d) - \frac{1}{2}}{f\mu}. \tag{2}$$

Let us assume $l_0 = \phi(\lambda)$ where ϕ is an undetermined function. The form of ϕ can be found by plotting a graph of log (λ) against $\log\{[\ln(2\lambda/d) - \frac{1}{2}]/f\mu\}$ taking a value of $0\cdot12\mu$ for d (Hopkins, 1964) (see Text-fig. 2).

The graph is linear with a slope of $3\cdot6 \pm 0\cdot5$ (90% certainty) and it is therefore reasonable to assume that $l_0 = C\lambda$, where C is a constant. It is therefore probable that equation (1) applies to the flagellar movement of *S. oncopelti*. It may be as well to point out here that l_0 is a scale length appearing in the theory and cannot be identified with any physical length of the flagellum. The confirmation of equation (1) indicates that waves are propagated along the flagellum of *S. oncopelti* by mechanical means. Since the structure of the flagellum of this organism (Hopkins, 1964) conforms to the almost universal $9 + 2$ pattern of fibrils found in cilia and flagella, it seems probable that a similar means of propagation is common to all ciliates, flagellates, and spermatozoa.

The ratio QSK^2/C^4 can be found from the intercept on either axis of Text-fig. 2 and has a value of $3\cdot5 \times 10^{-12}$ dyne cm.². The value of the second moment of area,

SK^2, will depend on which part or parts of the flagellum act as the compressive element needed to oppose bending; it has been suggested that either the membrane, together with an internal turgor pressure, or the two central fibrils fulfil this function (Harris, 1961). Other possibilities are the nine outer fibrils or the matrix. The membrane is the more favoured by Harris on the basis of a calculation which shows that if the central fibrils provide the necessary rigidity the value for Young's modulus would be 3×10^{11} dyne cm.$^{-2}$; Harris considers this to be an impossibly high value for a biological structure. This view is supported by Afzelius's (1962) observation of a motile sperm which has no central pair of fibres. In contrast, Child & Satir (1964) have succeeded in activating glycerol-extracted cilia from frog epithelia by ATP;
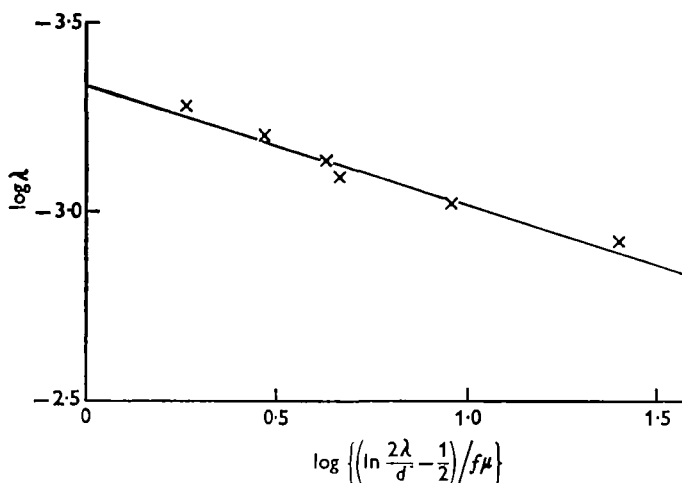
Text-fig. 2. Showing the dependence of $\log_{10}\{[\ln(2\lambda/d)-\frac{1}{2}]/f\mu\}$ on $\log_{10}\lambda$.

examination of these ciliary models in the electron microscope shows that they have disrupted membranes or no membranes at all. In this case at least it therefore appears that the membrane does not act as the compressive element. The observation that certain cilia beat in a plane perpendicular to that containing the two central fibrils (Gibbons, 1961) indicates that these may have a mechanical function as suggested by Fawcett & Porter (1954) and Gibbons (1961). Satir (1961) considers the central pair of fibrils to have a mechanical significance, particularly in maintaining the shape of the cilium, and he finds that they twist relative to each other during the course of a ciliary beat. The asymmetric arrangement of the nine peripheral fibrils is suitable to constrain a cilium to move in a plane and it is possible that they provide the compressive elements (Sleigh, 1962, p. 148).

The value of the second moment of area, SK^2, for a circular annulus of internal and external diameters r_1, r_2 is $\pi r_2^3(r_2-r_1)$, provided that (r_2-r_1) is small compared with r_a. The membrane thickness of the flagellum of S. *oncopelti* is about 80 Å. (Hopkins, 1964) and is of the same order as that observed in other flagella (see, for example, Gibbons & Grimstone, 1960). The value of SK^2 for the flagellar membrane is thus about 4.3×10^{-21} cm.^{4} which leads to $Q/C^4 = 8.1 \times 10^8$ dyne cm.$^{-2}$. Absolute determination of the couples acting at points along the flagellum is difficult, so we are unable to derive an independent value for Q from such values. It is of interest,

therefore, to consider the value of C in the ratio Q/C^4. According to Machin (1958) the maximum propulsive efficiency occurs when $1/C = 6.3$; if such a value is taken in this case the value for Q would be 5.16×10^5 dyne cm.$^{-2}$. This is rather low compared with the value obtained by Harris for a typical cilium (5×10^8 dyne cm.$^{-2}$) and, although it is not an abnormal value for a biological material it is somewhat lower than the expected magnitude (10^7–10^8 dyne cm.$^{-2}$) for what is presumably, a highly ordered structure.

If Harris's value for Q is taken, the value of $1/C$ is 1.13, which appears to indicate that the flagellum is operating below its optimum efficiency. The propulsive efficiency can be calculated in terms of the wave parameters and, for propulsion by sinusoidal waves, has a rather flat maximum in the region of $\eta k = 1$, where η is the amplitude of the wave and $k = 2\pi/\lambda$ (see Holwill & Burge, 1963, fig. 7). Evaluation of this product from Table 1 shows that any departure from unity is small, so the flagellum of *S. oncopelti* is in fact operating near its most efficient level. Although the waves on the flagellum of this organism are not strictly sinusoidal the value of ηk still provides a measure of the efficiency of propulsion. It should be noted that Machin's (1958) expression for the power expended by a flagellum includes a term for the rate of energy dissipation involved during elastic deformation of the flagellum. In their derivation of the propulsive efficiency of a flagellum Holwill & Burge (1963) do not consider the power expended internally by the flagellum. According to Machin the power required for elastic deformation is

$$P_E = \eta^2 k^4 LfQSK^2,$$

where L is the total length of the flagellum. Numerical evaluation of this expression involves a knowledge of the product QSK^2. Considering the flagellum of *S. oncopelti* the value for P_E is of the same order as the power expended to overcome the external viscous forces if the maximum estimated value for QSK^2 (2×10^{-12} dyne cm.^{2}) is used in the above expression. If smaller values of QSK^2 are used then P_E can be neglected compared to the energy expended externally. Since the values of both Q and SK^2 are uncertain it is impossible to determine the effect of P_E on the propulsive efficiency. For the purposes of the present argument we will adopt the not unreasonable supposition that the flagellum operates at or near optimum efficiency.

Although the evidence seems to be against the two central fibrils acting as the compressive elements it is of interest to compute the values for C and Q on the assumption that they do so act. The fibrils appear tubular with an approximate total diameter of 150 Å. and a wall thickness of 50 Å. (Hopkins, 1964) resulting in a value of $SK^2 = 6.6 \times 10^{-25}$ cm.^{4} for each fibril. Remembering that there are two fibrils the value of Q/C^4 is found to be 2.6×10^{12} dyne cm.$^{-2}$. The value for $1/C$ which results from assuming Harris's value for Q of 5×10^8 dyne cm.$^{-2}$ is 8.5, a value closer to Machin's theoretical one than was found for the membrane.

If the nine outer fibrils provide the resistance to bending the value of SK^2 will be about 13.2×10^{-25} cm.^{4} for each doublet, since their dimensions are similar to those of the central fibrils (Hopkins, 1964). As there are nine fibrils the value for Q/C^4 is 2.9×10^{11} dyne cm.$^{-2}$, giving $1/C = 4.9$ if Harris's value for Q is taken. It is possible that these fibrils are not all resistant to bending at the same time since, as Sleigh (1962) suggests, during contraction they may be influenced by a plasticiser such as

adenosine triphosphate. Thus, depending on the number of fibrils which resist bending at any given time the value of $1/C$ will alter and may approach the theoretical value derived by Machin. It is evident that this value could be attained by adjusting the value of Q by a small amount; an increase of Q to 1.6×10^9 dyne cm.$^{-2}$ in the case of the two central fibrils or a decrease to 1.6×10^8 dyne cm.$^{-2}$ in the case of the nine peripheral fibrils would result in the value for $1/C$ given by Machin.

Although the matrix appears to have no oriented structure when examined in the electron microscope it is nevertheless possible that it could provide the compressive element. The value for SK^2 in this case is 2.2×10^{-20} cm.^{4} leading to $Q/C^4 = 1.6 \times 10^8$ dyne cm.$^{-2}$. Taking Harris's value for Q we obtain $1/C = 0.75$. It is unlikely that an apparently random structure such as the matrix would have as high a value for Young's modulus as 5×10^8 dynes cm.^{2}. If $C = 6.3$ the value for Q is 1×10^5 dynes cm.$^{-2}$ and is thus of the same order as the modulus of the membrane under these conditions. In obtaining the value for $1/C = 6.3$ corresponding to maximum propulsive efficiency Machin (1958) used an expression derived by Taylor (1952) and Hancock (1953) for the velocity of an isolated filament executing sinusoidal waves of small amplitude. Furthermore, the theory used in deriving equation (1) assumes that the amount of bending along the flagellum is small. The curvature of the flagellum of *S. oncopelti* attains a value larger than one which could justifiably be called small. This in itself might provide an adequate reason why the value for $1/C$ derived from the membrane measurements is different from the theoretical value, but the presence of the relatively large body of this organism may be another cause. This as we have seen, significantly affects the translational velocity of the organism.

It is apparent from the work of Holwill & Burge (1963) that the ratio η/λ rather than the value of λ is important in determining the energy requirements of a propagated wave. A strict theoretical treatment of a similar nature to Machin's work for waves of large amplitude is difficult and an approximate graphical method is being undertaken as a first step.

It might be expected that the Young's modulus for flagellar components would be less than the corresponding ciliary ones when it is considered that each portion of the flagellum is continually changing its radius of curvature, there being no stage at which a relatively long length of the organelle becomes stiff; this is in sharp contrast to the effective stroke of a cilium which exhibits little or no curvature along most of its length. It would be difficult to judge the rigidity of a flagellum with a micro-needle in the same way as Carter (1924) and Yoneda (1960, 1962) have done for cilia. Valuable results, however, might be obtained from micro-manipulations of isolated flagella provided it could be demonstrated that their composition remained unchanged after detachment from the organism.

It is interesting to note that high values have been recorded for Young's modulus of collagen fibres. Although the elasticity varies with extension, maximum values of about 10^{10} dyne cm.$^{-2}$ have been recorded by Conabere & Hall (1946) and Hall (1951), while Cowan, North & Randall (1955) obtain a value near 4×10^{11} dyne cm^{-2}. The increased value of Q suggested earlier to provide the theoretically correct value for $1/C$ in the case of the two central fibrils is, therefore, not impossible.

The fact that the waveform of the posterior flagellum of *Ceratium* can be re-

constructed from circular arcs and straight lines alone has led Brokaw & Wright (1963) to postulate an on–off mechanism for contraction. The mechanically independent contractile elements along the flagellum (as suggested by Gray (1955) and Machin (1958)) are supposed to exist in two states only, fully contracted and fully relaxed. It is suggested by Brokaw & Wright that such a system would require less information than for the generation of a sinusoidal wave. Since no intermediate positions are observed their argument inherently assumes that the transition from a curved segment to a straight one occurs with a time lag which is negligible compared with the period of flagellar beat. The concept of mechanical propagation of waves along a flagellum assumes the period of natural vibration of the system to be of the same order as the observed period of beat. If an on–off contractile system is present in the flagellum of *S. oncopelti*, which appears to propagate waves mechanically, there would be a time lag comparable with the period of beat between the curved and straight positions of a given element. A suitable sequence of circular arcs of differing radii could build up a sine wave or, indeed, almost any form of symmetric wave.

Asymmetric waves could be formed, irrespective of the contractile mechanism or system of propagation, if insufficient numbers of the energy-rich molecules necessary for contraction were distributed along the flagellum.

The observation that under conditions of high viscosity a wave may die out before reaching the base of the flagellum provides evidence that wave propagation may not be entirely mechanical. Alternatively, the flagellum may encounter greater external resistance, either in the form of a physical obstruction or a localized region of increased viscosity, which prevents further propagation of the wave.

It has already been mentioned that some form of control mechanism is necessary in order that a wave may be propagated from tip to base. The control mechanism is necessary for the initiation of a wave from the tip of the flagellum. Afzelius (1962) suggests that the two central fibrils may be responsible since they are absent in the spermatozoa from *Myzostomum cirriferum* which appears to propagate waves in both directions. The presence of these particular fibrils in *Strigomonas oncopelti* (Hopkins, 1964) precludes such an idea. The fact that *S. oncopelti* on meeting an obstruction reverses the direction of its waves indicates that it is probably the membrane which performs this function; the tip of the flagellum of *S. oncopelti* is flattened or rounded (Hopkins, 1964) instead of tapered as in the flagella of other organisms (Gibbons & Grimstone, 1960). The ends of the inner fibrils may therefore be sufficiently close to the flagellar tip to respond to changes in the external conditions. Initiation of a wave half-way along the flagellum also requires a control mechanism as well as a system for propagation.

The presence of waves propagating in both directions on the flagellum of *S. oncopelti* may be the result of non-linearity in the contractile elements as suggested by Machin (1963). Machin's (1963) theory suggests that if two waves co-exist on a flagellum the one with the larger amplitude will predominate. Since the observed waves are of nearly the same amplitude the fact that quiescence is observed at the meeting-point is to be expected.

Further investigation of the movement of *S. oncopelti* with variations in environmental conditions, e.g. temperature, pH, and on flagella isolated from the organism may yield more information about the control mechanism.

SUMMARY

1. The movement of the flagellum of *Strigomonas oncopelti* has been studied using high-speed cinephotography. Waves usually pass along the flagellum from tip to base but under certain conditions waves are propagated from base to tip, reversing the direction of movement of the organism.

2. Increasing the viscosity of the medium reduces the frequency of beat while the shape of the wave remains unaltered. The variations in the wave parameters with increasing viscosity are consistent with a mechanically propagated wave.

3. Calculated values for Young's modulus indicate that the membrane, the matrix, the nine peripheral fibrils or the central pair could act as the compressive elements needed to resist bending. The mechanism controlling the direction of wave propagation appears to lie in the membrane.

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M. E. J. HOLWILL

M. E. J HOLWILL

Plate 3

M. E. J HOLWILL
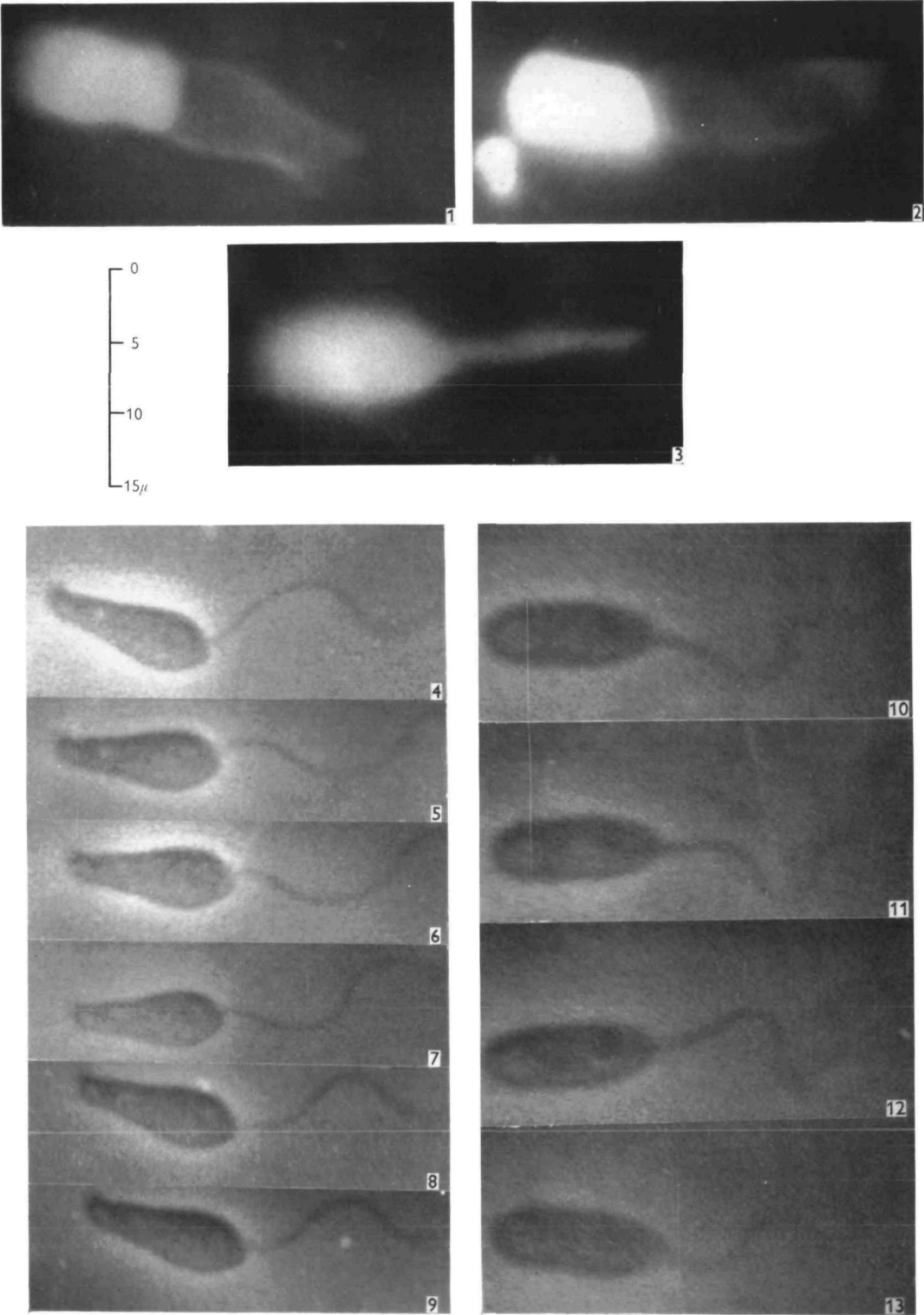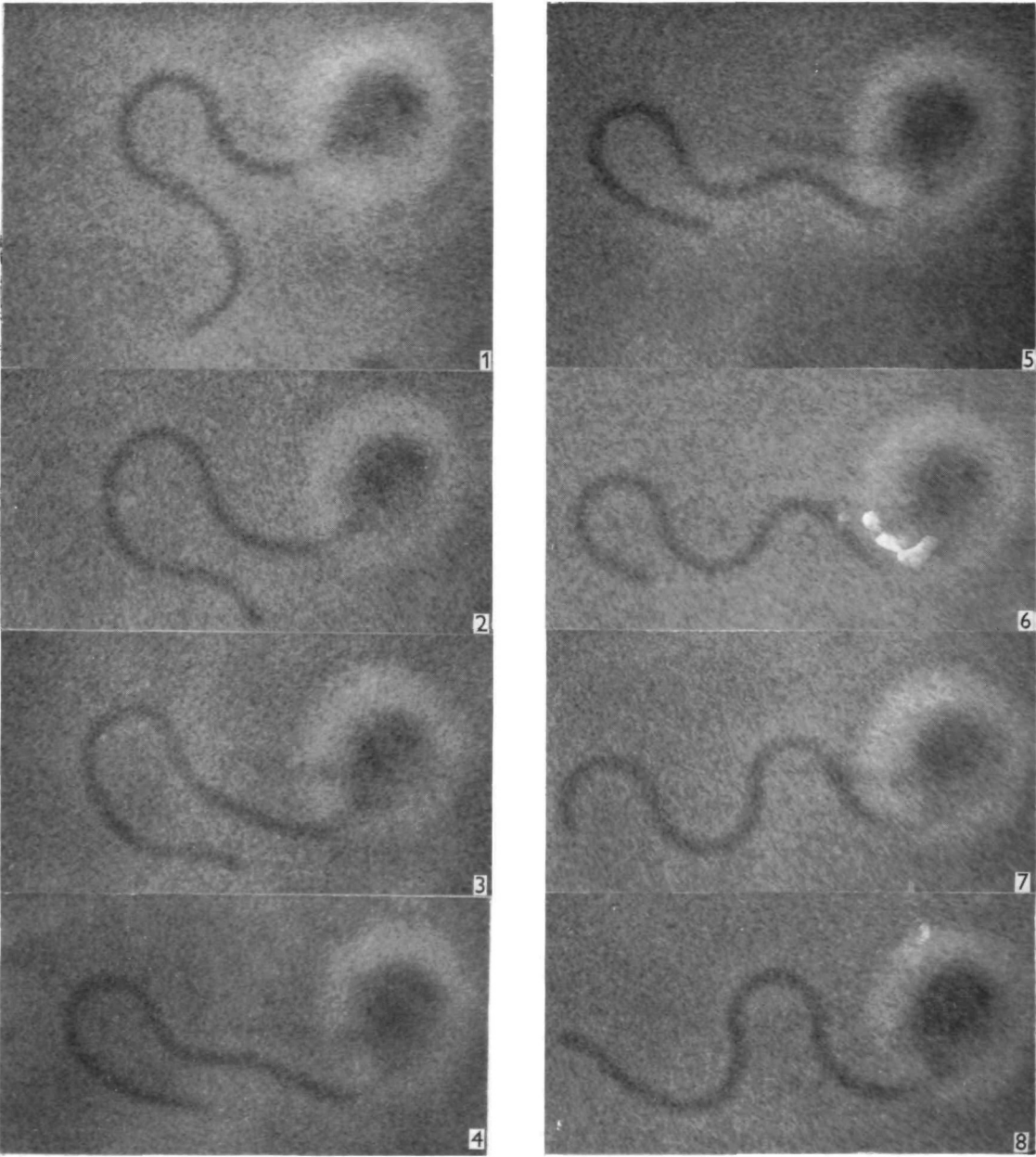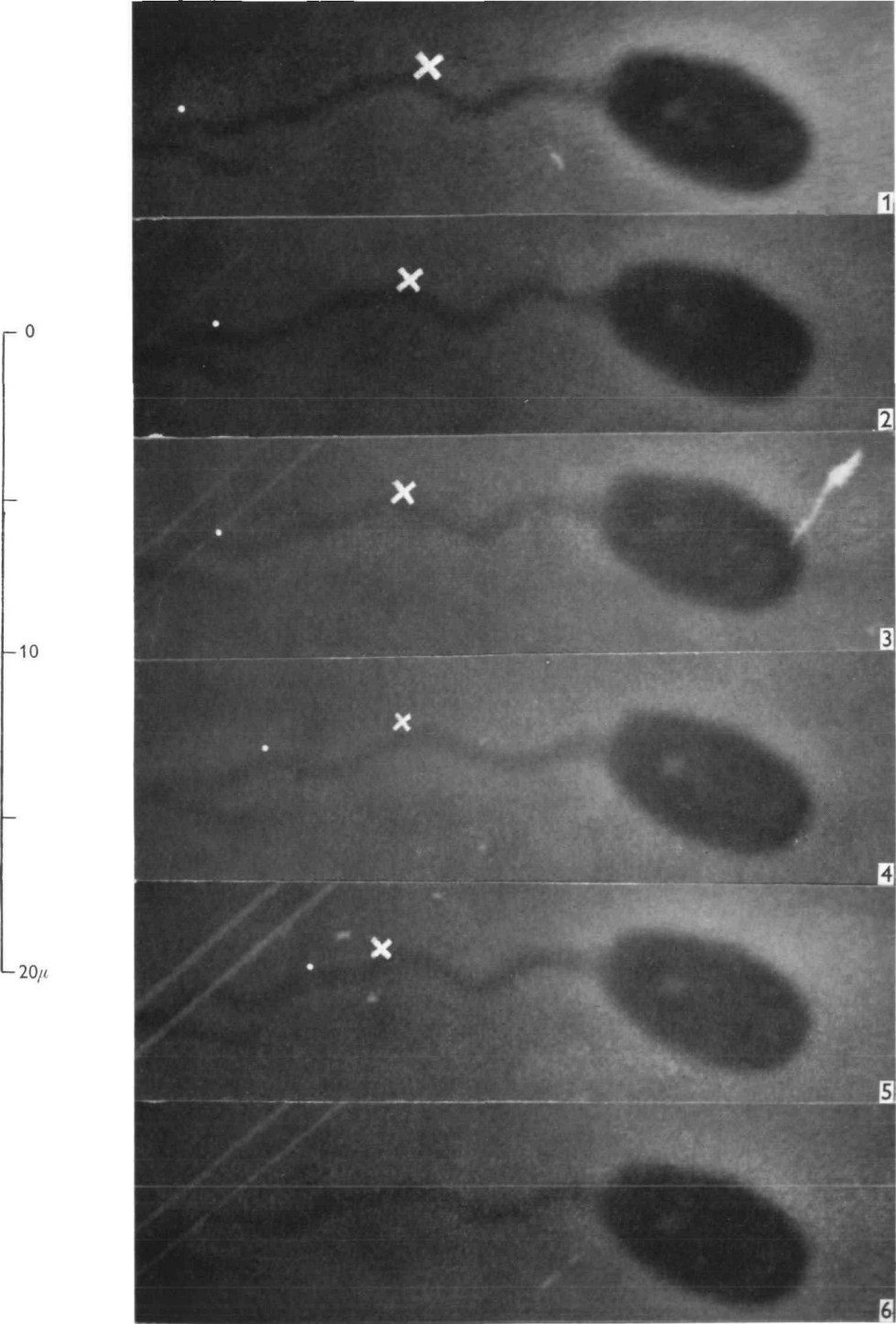
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EXPLANATION OF PLATES

PLATE 1

Figs. 1, 2. Dark-ground photographs showing the envelope of the flagellar motion. Exposure $\frac{1}{32}$ sec.

Fig. 3. Dark-ground photograph showing that the flagellar beat is planar. Exposure $\frac{1}{32}$ sec.

Figs. 4–9. A sequence showing forward motion. Exposure $\frac{1}{2250}$ sec.

Figs. 10–13. A sequence showing backward motion with symmetric waves on the flagellum. Exposure $\frac{1}{2250}$ sec.

PLATE 2

Figs. 1–8. A sequence showing asymmetric waves being propagated along the flagellum from base to tip. Exposure $\frac{1}{400}$ sec.

PLATE 3

Figs. 1–6. A sequence showing two waves travelling in opposite directions. The × and ● indicate the positions of the distally and proximally propagated waves respectively. In Fig. 6 the two coalesce.