

DYNAMICS OF THE HISPID FLAGELLUM OF *OCHROMONAS DANICA*

The Role of Mastigonemes

M. E. J. HOLWILL and PATRICIA D. PETERS

From the Department of Physics, Queen Elizabeth College, London, England. Dr. Peters' present address is Cavendish Laboratory, Free School Lane, Cambridge, England.

ABSTRACT

High speed cinephotographic techniques were used to determine the pattern of fluid flow about the hispid flagellum of *Ochromonas danica* and to investigate the behavior of this flagellum in media of increased viscosity. The fluid currents are consistent with the hypothesis that the mastigonemes are passive, rigid, remain normal to the flagellar surface, and lie in the plane of flagellar undulation during motility.

INTRODUCTION

The mechanism by which a smooth undulating flagellum is able to exert a propulsive force appears to be reasonably well understood (see e.g. Holwill, 1973; Chwang and Wu, 1971) and is such that a freely moving organism will be propelled in the direction opposite to that of wave propagation. Certain organisms with hispid flagella, however, are able to propel themselves in the direction of wave propagation, a phenomenon which appears to be associated with the presence of the flagellar hairs, or mastigonemes. The flagellar hairs are about 15 nm in diameter and are therefore not visible in the light microscope, so that their behavior and configuration during motion cannot be observed directly. The hydrodynamic effect of mastigonemes has been explained qualitatively by Jahn et al. (1964) on the assumption that the mastigonemes are passive, rigid, and remain normal to the flagellar surface and in the plane of the flagellar wave during movement. A quantitative evaluation of this system has been made by Holwill and Sleight (1967) who obtained good agreement

with experimental observations.

Recent electron microscope studies by Bouck (1971, 1972) have led him to suggest that the mastigonemes lie in the plane perpendicular to that of flagellar undulation. Two observations are cited to support his contention: (a) in whole mount preparations of *Ochromonas danica*, the mastigonemes appear to lie in a plane normal to that of the wave shape in which the flagellum is fixed; (b) in transverse sections, the mastigonemes lie in the plane containing the central fibres of the axoneme. It is usually assumed, but has not been generally demonstrated, that the movements of cilia and flagella occur in the plane perpendicular to the central pair of fibrils. The mastigonemes of *O. danica* are attached to the flagellum in the plane of the central fibrils, and this arrangement led Bouck to make the suggestion cited above. In this paper we will present observations of fluid flow about the flagellum of *O. danica* which conflict with Bouck's idea, but support the original mechanism proposed by Jahn et al. (1964).

MATERIALS AND METHODS

A culture of *O. danica* was obtained from the Cambridge Culture Collection of Algae and Protozoa. The organisms were subsequently cultured in 20 cm³ aliquots of an axenic medium containing 0.1% liver infusion, 0.1% tryptone, and 0.1% glucose. The viscosity of the fluid was varied by the addition of methyl cellulose, and viscosities were measured with a Brookfield (Brookfield Engineering Laboratories, Inc., Stoughton, Mass.) viscometer. To obtain information about fluid flow, polystyrene latex particles (diameter $0.246 \pm 0.006 \mu\text{m}$, Dow Chemical USA, Midland, Mich.) were added to the culture medium just before observations were made. The particles are sufficiently light that they moved freely along streamlines in the fluid and sufficiently large that they could be photographed under conditions of dark field illumination. High-speed cinephotomicrographs of flagella and particles were obtained using the technique described by Holwill (1967), but incorporating modifications to the apparatus described by Goldstein et al. (1971). Illumination of the specimens for filming at rates below 150 pictures per second was provided by a xenon flash tube (Chadwick Helmuth Co., Inc., Monrovia, Calif.) synchronized with the camera framing rate, while above this rate a mercury arc lamp was used as the light source.

For technical reasons, observations of particle movements were generally made close to cells which were attached to the surface of the cover slip, although the particle movement generated by free swimming organisms was also examined for comparison purposes. Freely moving organisms were also studied with a view to determining their swimming speeds and flagellar wave parameters.

After processing, the films were analysed frame-by-frame on the translucent screen of a P.C.D. (P.C.D. Ltd., Farnborough, Hants., England.) digital data reader equipped with a Vanguard (Vanguard Instrument Corp., Melville, N. Y.) projection head.

RESULTS

Freely swimming cells of *O. danica* move with the flagellum preceding the cell body. Flagellar waves are propagated towards the flagellum tip at about 60 Hz with a wavelength of about $4.5 \mu\text{m}$ and an amplitude of about $1 \mu\text{m}$. During translational movement the organism follows a shallow helical path about the axis of progression. The flagellum carries hairs which are not visible in the optical microscope, but which have been shown by electron microscopy to be about $1 \mu\text{m}$ in length (Bouck, 1971; Peters, 1973).

Fig. 1(a) shows the main features of particle movement in the plane of the flagellar wave of *O. danica*. Near the flagellum, particles are observed

to move from the flagellar tip towards its base, although the flagellar wave is propagated in the opposite direction. Farther from the flagellum, particles move in the direction of the wave, and many were observed to travel in closed paths, as shown in Fig. 1(a). The speed of a particle moving along such a path was greater the closer the particle approached the flagellum.

Fig. 2 represents the detailed movement of a particle near the flagellum, and shows the position of a particle as a function of time. The main features of the particle movements are similar in both stationary and freely swimming organisms. The distances of a particle from the axis [d , Fig. 1(a)] and along the axis [x , Fig. 1(a)] oscillate with the beat frequency, but the mean value of x decreases with time (AA', Fig. 2), indicating a net movement of the particle towards the cell body. The slope of the line AA' gives the mean axial velocity of the particle, while a line of maximum slope (e.g. BB' in Fig. 2) represents the maximum velocity, which is generally about three times the mean value. A line such as CC' with opposite slope to AA' and BB' indicates that the particle is moving distally at the corresponding time.

The mean velocity of particles parallel to the wave axis was found to be greatest about $1 \mu\text{m}$

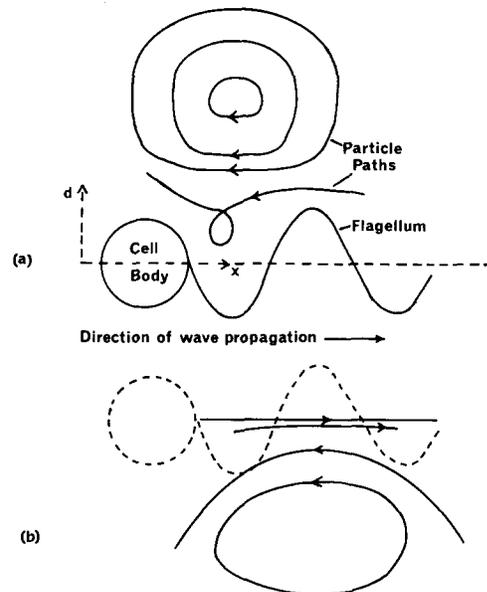


FIGURE 1 Showing the paths of particles near the beating flagellum of *O. danica* (a) in the plane of the wave, and (b) in a plane displaced from, but parallel to, that containing the wave.

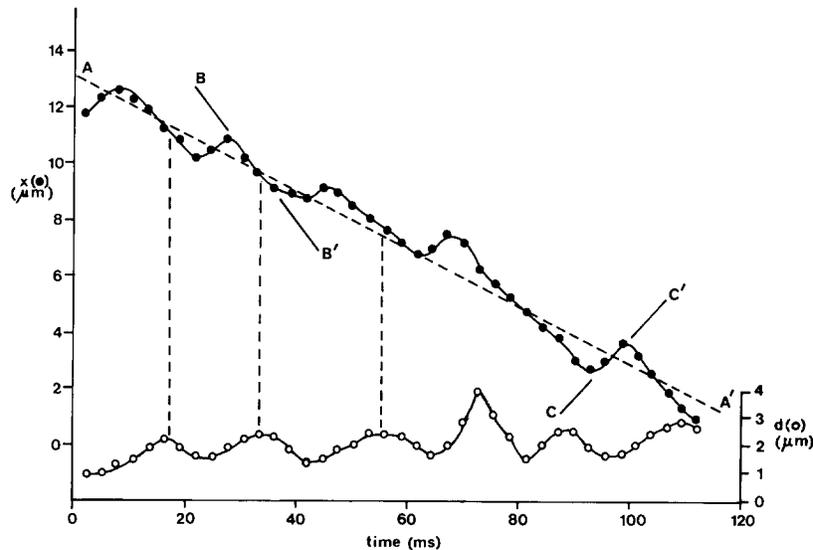


FIGURE 2 Temporal variation of distance along the flagellar axis (x , Fig. 1) and distance from the flagellar axis (d , Fig. 1) for a particle near the beating flagellum of *O. danica*. The particular organism from which this plot was derived was attached to the cover slip and had a flagellar beat frequency of 55 Hz and a wave amplitude of $0.7 \mu\text{m}$.

from the flagellar envelope, and to fall off with distance from the axis in a roughly linear way (Fig. 3). No particles were observed to move consistently in the region between the flagellar shaft and a distance of $1 \mu\text{m}$ from it so the fluid flow in this region could not be analysed. As judged by the random Brownian movement of the particles, the influence of the flagellum in the plane of the wave extended to a distance of about $14 \mu\text{m}$ from the flagellar axis. The same form of movement was found for particles on both sides of the flagellum, but it was found that the velocity of particles at a given distance from the flagellar axis was always higher on one side than on the other. Particles were often observed to cross the axis in a direction which was invariably from the side with the lower velocities to that with the higher velocities.

The movements of particles in the vicinity of the flagellum but in planes other than that of the flagellar wave were also examined [Fig. 1(b)]. Particles near the axis of the wave moved from the flagellar base towards the tip, in contrast to those described earlier, and remained in a plane parallel to, but displaced from, the flagellar wave. At the same time other particles not in the plane of the flagellar wave and not close to its axis moved proximally, and had a velocity profile similar to that of particles moving in the plane of the flagellar wave. The value for d [Fig. 1(a)] for particles which moved distally remained in the range of the

flagellar amplitude, and the nearest part of the flagellum, projected parallel to the axis of d on the d - x plane, was always less than $1 \mu\text{m}$ from such particles.

In media of increased viscosity the particle movement was generally similar to that in normal culture medium, although the velocity of a particle in a given position relative to the flagellum was slower in the former case than in the latter. The region of highest mean axial velocity remained at about $1 \mu\text{m}$ from the flagellar envelope, as for organisms swimming in normal culture fluid. The beat frequency and amplitude of the flagellar wave decreased with increasing viscosity, but the wavelength remained essentially constant (Table I).

DISCUSSION

The description presented here of fluid flow around the flagellum of *O. danica* is consistent with the hypothesis that mastigonemes lie in the plane of the flagellar wave and remain normal to the flagellar surface during movement, as shown in Fig. 4. On this model, fluid velocities are expected to be greatest near the tips of the mastigonemes on the wave crests. Examination of Fig. 2 shows that regions of maximum velocity (e.g. BB') coincide with maxima in the displacement of particles from the flagellar axis (i.e. wave crests) in agreement with the model. Further, Fig. 3 shows a maximum

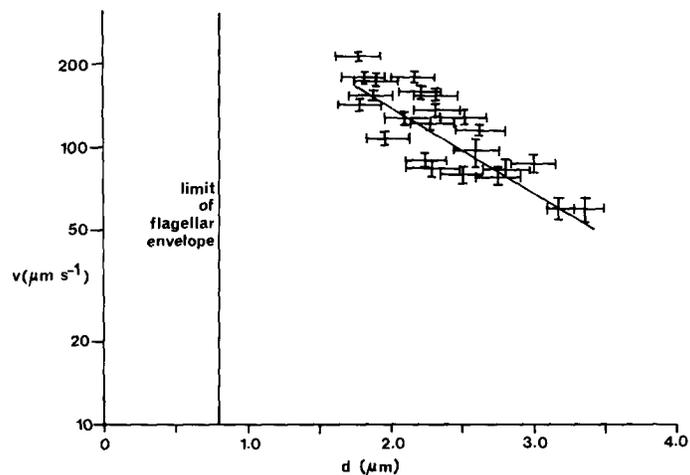


FIGURE 3 Typical example of the dependence of mean axial particle velocity (v) on the distance (d) from the flagellar axis for particles moving in the plane of the flagellar undulation. The organism from which this plot was derived was attached to the cover slip and had a flagellar beat frequency of 51 Hz, an amplitude of $0.8 \mu\text{m}$ and maintained about 1.5 wavelengths on its flagellum.

TABLE I
Effect of Increased Viscosity on the Flagellar Wave Parameters and Propulsion Velocity of Ochromonas danica at a Temperature of 20°C

Viscosity	Frequency	Wavelength	Amplitude	Propulsion velocity
(mN s m^{-2})	(H/z)	(μm)	(μm)	($\mu\text{m S}^{-1}$)
1.00 ± 0.02	59 ± 2	4.5 ± 0.2	0.96 ± 0.12	77 ± 2
2.30 ± 0.05	54 ± 2	4.6 ± 0.2	0.84 ± 0.09	51 ± 2
3.70 ± 0.07	51 ± 2	4.9 ± 0.2	0.75 ± 0.07	34 ± 3
5.6 ± 0.1	50 ± 3	4.76 ± 0.06	0.54 ± 0.07	18 ± 2
7.5 ± 0.2	50 ± 4	4.66 ± 0.08	0.50 ± 0.02	6.4 ± 0.6
10.0 ± 0.2	45 ± 2	4.71 ± 0.08	0.37 ± 0.03	4.2 ± 0.2

(Errors are standard deviations obtained from measurements on at least 20 organisms.)

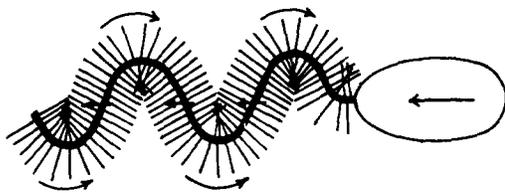


FIGURE 4 Illustrating behavior of mastigonemes which are rigid, passive, remain normal to the flagellar surface, and lie in the plane of flagellar undulation during movement. Reproduced, with permission, from Jahn et al. (1964).

axial particle velocity at a distance from the flagellar envelope of about $1 \mu\text{m}$, which is the approximate length of the mastigonemes. In the wave troughs (Fig. 4) the mastigonemes would be unable to spread out, and would therefore have

little influence on the movement of the fluid. In this region, the advancing flagellar wave is expected to dominate, and fluid is expected to move towards the flagellar tip. This effect is observed experimentally, as shown in Fig. 2 where regions of reversed velocity (e.g. CC') coincide with minimum displacements of particles from the axis (i.e. wave troughs). Similarly, fluid close to the flagellar axis, but not in the wave plane, would, according to the model described by Fig. 4, move distally as is observed.

In the region between the axis and the tips of the mastigonemes, a region of fluid reversal is expected. As noted in the Results it was not possible to obtain experimental measurements of the fluid flow for a distance of $1 \mu\text{m}$ from the flagellar shaft. Since the mastigonemes have this length, it is reasonable to suppose that they interfere with the

particles in this region and prevent them from remaining there. In experiments with smooth flagella, particles appear to touch the flagellar surface (Peters, 1973), so the effect observed for *O. danica* cannot be attributed to hydrodynamic behavior of the fluid near a surface.

Further evidence which supports the idea that mastigonemes are in the plane of the flagellar wave is associated with the movement of particles across the flagellar axis. The different observed velocities on the two sides of the flagellar axis can be attributed to the different mastigoneme densities in the two rows of flagellar appendages (Bouck, 1971; Peters, 1973). It seems probable that the higher velocity is produced by the action of the more densely packed mastigonemes, as can be inferred from the work of Blake (1972) on ciliary movement. The local fluid pressure will be lower on that side of the flagellum where the velocity is higher than on the other side, so that a force will exist tending to move particles to the high-velocity side, as is observed experimentally. This may, however, not be the only effect contributing to the cross-flow of particles. A suitable distribution of mastigonemes on the flagellar shaft could produce the necessary forces.

Since the pattern of fluid movement is qualitatively the same in media of higher viscosities as in normal culture medium, the mastigoneme action is clearly not modified by an increase in viscosity. Although the velocity of the fluid current is reduced, the region of maximum fluid velocity remains at about one mastigoneme length from the flagellar shaft. This strongly suggests that the mastigonemes lie in the plane of the flagellar wave and remain rigid and normal to the flagellum even in fluids of increased viscosity, where the forces acting on them, being approximately proportional to the product of viscosity and frequency, are greater than in culture medium (see Table 1).

A theoretical hydrodynamic investigation of the motion of *O. danica* also provides some information about the mastigoneme behavior. Holwill and Sleight (1967) used the approach of Gray and Hancock (1955) to calculate the propulsive velocity of a cell propelled by a hispid flagellum executing sinusoidal waves. By using their equations, modified for the particular arrangement of mastigonemes which occurs on *O. danica*, the variation of propulsive velocity can be obtained as a function of flagellar amplitude and wavelength using the two separate assumptions that the mas-

tigonemes lie in and perpendicular to the plane of flagellar undulation. If the mastigonemes are assumed to lie in this plane the solid curve of Fig. 5 is obtained, whereas if the assumption is made that mastigonemes are perpendicular to the plane of undulation, and remain rigid and normal to the flagellar surface during movement, essentially zero propulsive velocity is predicted for all ratios of amplitude to wavelength. It is instructive to consider the physical basis of this result. For an undulating filament to produce a finite unidirectional force over a period of time it is necessary for the resistance offered by the filament to movement along its axis to be different from that when the filament moves at the same velocity normal to its axis. When the filament has many projections in a single plane, the two resistances are almost equal if the normal movement is made in a plane perpendicular to that containing the projections. This corresponds to a flagellum with mastigonemes perpendicular to the plane of beat, a situation which therefore provides little propulsive thrust. When the normal movement is made in the plane of the projections the two resistances differ. For the flagellum described here, the ratio of the axial to the normal resistance is about 1.9, the figure used in computing the theoretical curve of Fig. 5. Fig. 5 shows that the experimental points agree reasonably well with the theoretical predictions obtained by assuming the mastigonemes to lie in the plane of beating, although better agreement might be obtained if a more rigorous hydrodynamic analysis were used. Such an analysis would include, for example, the interactions between mastigonemes and the effect of the proximity of the flagellum to slide or cover slip surfaces. The effects of these interactions are difficult to estimate in quantitative terms, but will not produce order of magnitude changes in the velocities calculated from the equations used in the present study.

The evidence for the suggestion that mastigonemes lie in a plane normal to that of undulation (Bouck 1971, 1972) is mainly structural and depends on the relative orientation of mastigonemes and the central fibrils of the axoneme. Although the plane of beating appears to be perpendicular to that of the central fibrils in many cilia (e.g. Gibbons, 1961), examples are found among compound cilia where the two appear to be at a small angle (Tamm and Horridge, 1970), and the work of Satir (1963) suggests that great care needs to be taken in the preparation of specimens

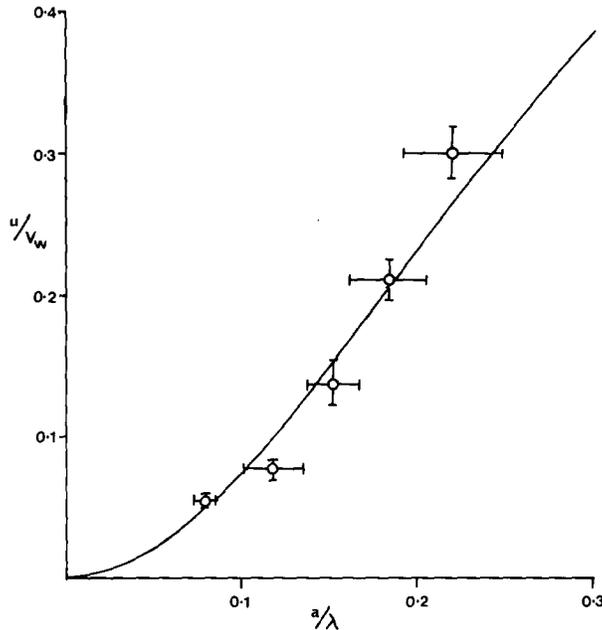


FIGURE 5 Showing the dependence of the ratio of propulsion velocity (u) to wave velocity (V_w) on the wave shape, specified by the ratio amplitude (a) to wavelength (λ), for freely swimming *O. danica*. The open circles represent the experimentally derived values while the solid line is obtained from the equations of Holwill and Sleight (1967) assuming that the ratio of axial resistance to tangential resistance is 1.9 (see text).

for electron microscopy if fine structural details are to be related to aspects of motility. In his work Satir (1963) reports that the planes of the central fibrils of neighboring cilia in a metachronal wave pattern do not coincide, but can have relative orientations of 90° or more. He interprets this to mean either that the central fibrils twist in beating cilia or that the beat is not strictly planar. Fawcett (1968) also expresses the view that great care is needed to determine accurately the relationship between beating and ultrastructural detail in sperm flagella. Thus, even for those organisms which have been studied, there is no definitive evidence that the planes of beating and of the central fibrils are perpendicular. It is clear that further critical studies are necessary to investigate the behavior of the axonemal fibrils during motility.

The model of mastigoneme activity proposed by Bouck is difficult to reconcile with the observed particle movement, and it is not clear that this model would induce movement of a freely swimming organism in the direction of wave propagation. Bouck (1972) suggests that elastic or freely hinged mastigonemes would be accelerated downwards at a wave crest, and would thereby create a net force opposite to that of the flagellar shaft.

Since the mastigoneme movement would be predominantly perpendicular to the flagellar shaft, it is not easy to visualize how such a force could be developed. However, the discussion of the model given by Bouck is rather brief and it may be that a detailed description would show clearly how he considers the mastigonemes to produce their hydrodynamic effect.

If the points at which the mastigonemes are attached to the flagellum lie in a plane perpendicular to the flagellar undulation, the behavior of the hispid organelle could be explained if the mastigonemes were active or if they bend sharply at their bases to lie parallel to the plane of beating. There is, however, no hydrodynamic or structural evidence to support either of these ideas, and there seems to be little point in considering them further.

In this paper we have presented evidence which indicates that the hydrodynamic effect of the flagellum of *O. danica* is produced by passive mastigonemes which are rigid, remain perpendicular to the flagellar surface during movement, and lie in the plane of flagellar undulation. Since the attachment sites of the mastigonemes lie in the plane of the central fibrils, it seems likely that the flagellum beats in this plane, although carefully

correlated experiments using optical and electron microscopy are required to investigate the behavior of flagellar fibrils during motility.

One of us (Patricia D. Peters) is grateful to Queen Elizabeth College for the award of a research scholarship.

The Vanguard Projection Head and the Brookfield viscometer used in the work described in this paper were purchased with a grant from the Central Research Fund of the University of London, to whom we express our thanks.

Received for publication 12 November 1973, and in revised form 21 March 1974.

REFERENCES

- BLAKE, J. 1972. A model for the microstructure in ciliated organisms. *J. Fluid Mech.* **55**:1.
- BOUCK, G. B. 1971. The structure, origin, isolation, and composition of the tubular mastigonemes of the *Ochromonas* flagellum. *J. Cell Biol.* **50**:362.
- BOUCK, G. B. 1972. Architecture and assembly of mastigonemes. In *Advances in Cell and Molecular Biology II*. E. J. Dupraw, editor. Academic Press, Inc., New York.
- CHWANG, A. T., and T. Y. WU. 1971. A note on the helical movement of micro-organisms. *Proc. R. Soc. Lond. B Biol. Sci.* **178**:327.
- FAWCETT, D. W. 1968. The topological relationship between the plane of the central pair of flagellar fibrils and the transverse axis of the head in guinea pig spermatozoa. *J. Cell Sci.* **3**:187.
- GIBBONS, I. R. 1961. The relationship between the fine structure and direction of beat in gill cilia of a lamelibranch mollusc. *J. Biophys. Biochem. Cytol.* **11**: 179.
- GOLDSTEIN, S. F., M. E. J. HOLWILL, and N. R. SILVESTER. 1971. High speed recording of laser damage. *Visual.* **8**:45.
- GRAY, J., and HANCOCK, G. J. 1955. The propulsion of sea-urchin spermatozoa. *J. Exp. Biol.* **32**:804.
- HOLWILL, M. E. J. 1967. High speed cinephotomicrography of microscopic organisms. Proceedings of the 7th International Congress on High-Speed Photography. Helwich, Darmstadt. 265.
- HOLWILL, M. E. J. 1973. Hydrodynamic aspects of ciliary and flagellar movement. In *Cilia and Flagella*. by M. A. Sleight, editor. Academic Press, Inc., New York.
- HOLWILL, M. E. J., and M. A. SLEIGH. 1967. Propulsion by hispid flagella. *J. Exp. Biol.* **47**:267.
- JAHN, T. L., M. D. LANDMAN, and J. R. FONSECA. 1964. The mechanism of locomotion of flagellates. II. Function of the mastigonemes of *Ochromonas*. *J. Protozool.* **11**:291.
- PETERS, P. D. 1973. Experimental studies of hispid and other flagella. Ph.D. Thesis, University of London.
- SATIR, P. 1963. Studies on cilia. The fixation of the metachronal wave. *J. Cell Biol.* **18**:345.
- TAMM, S. L., and G. A. HORRIDGE. 1970. The relation between the orientation of the central fibrils and the direction of beat in cilia of *Opalina*. *Proc. R. Soc. Lond. B Biol. Sci.* **175**:219.