Advances in
MICROBIAL PHYSIOLOGY

edited by

A. H. ROSE

D. W. TEMPEST

Volume 16

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Volume 16

ACADEMIC PRESS

London New

A Subsidiary of Harcou

ACADEMIC PRESS INC. (LONDON) LTD.
24/28 Oval Road
London NW1

United States Edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue
New York, New York 10003

Library of Congress Catalog Card Number: 67-19850
ISBN: 0-12-027716-6

Printed in Great Britain at the Spottiswoode Ballantyne Press by
William Clowes and Sons Limited
London, Colchester and Beccles

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Some Biophysical Aspects of Ciliary and Flagellar Motility

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I. Introduction

Cilia and flagella of eukaryotic cells are motile organelles which serve to produce relative motion between a cell and its environmental liquid. In sessile organisms, such as monads and spónges, flagellar action is responsible for the production of food-bearing currents which flow over the cell surfaces, while many protozoa use cilia or flagella to propel themselves through the fluid in search of food. The patterns of beat of cilia and flagella are varied and variable, as will be described, and yet the ultrastructure of these organelles remains remarkably constant in form and dimension wherever they are found,

be it the single flagellum of a trypanosome or an individual cilium from the multiciliated epithelium of the mammalian lung. There is, in fact, no definitive way to differentiate between cilia and flagella, and the distinction between them has arisen for historical reasons. Various proposals have been made for a collective name for these organelles, but none has been widely adopted so that, in the present chapter, the commonly accepted usage of the two terms will be employed.

During their motion, cilia and flagella form and propagate bends along their length. The energy for this action is derived from chemical processes within the cell, and it is of fundamental interest to enquire how the chemical energy is converted, with no intermediate heating stage, into the mechanical work required to bend the system against internal and external resistances to movement. Study of this mechanochemical system has attracted the attention of scientists from a variety of disciplines, ranging from pure biology through biochemistry and biophysics to applied mathematics. Information obtained by application of the methods and techniques of the several sciences has led to considerable advances in our knowledge and understanding of the basic operation and function of cilia and flagella.

An organelle moving through a fluid dissipates the energy derived from the chemical process responsible for the ciliary or flagellar deformation. The energy can be computed using the equations of hydrodynamics, and sets a lower limit on the chemical energy which must be made available to the organelle during motion. Elucidation of the nature of the chemical reaction is the province of the biochemist, whose techniques have been used to expose the motile machinery to chemical manipulation and hence to obtain useful information about it. Changing the environmental conditions, such as temperature and pressure, of intact cells has also yielded results which have been related to a chemical process occurring within the system. The behaviour of the internal machinery which bends a cilium or flagellum influences the shape adopted by the organelle, and a detailed study of beat patterns, together with ultrastructural studies involving the electron microscope, can provide valuable mechanistic data.

Many aspects of the motion of cilia and flagella were reviewed comparatively recently in an excellent book of some 500 pages (Sleigh, 1975a). In the present chapter, it would be impossible, and unnecessary, to present the information contained therein, but it is the aim of the author to summarize the essential material and indicate the important advances which have been made in the past few years.

II. Structure

Motile cilia and flagella are long, thin organelles (for dimensions, see Table 1) with a circular cross section containing, within a membrane, an array of nine doublet microtubules surrounding a pair of single tubules (Fig. 1), which is maintained by a network of fine

TABLE 1. Dimensions associated with cilia, flagella and their motion

Length (μm)	Diameter (nm)	Beat frequency f (Hz)	Wavelength λ (μm)	Amplitude a (μm)	Propulsive speed (μm s^{-1})
5–200	about 200	1–100	5–100	1–20	1–1000

linkages (e.g. Warner, 1975); the entire assembly is known as the axoneme. The microtubules extend the entire length of a cilium or flagellum and, in cross section, each doublet is oriented so that its axis of symmetry makes an angle of about 10° with the tangent (at the doublet) to a circle drawn through the doublets. Using a high-voltage

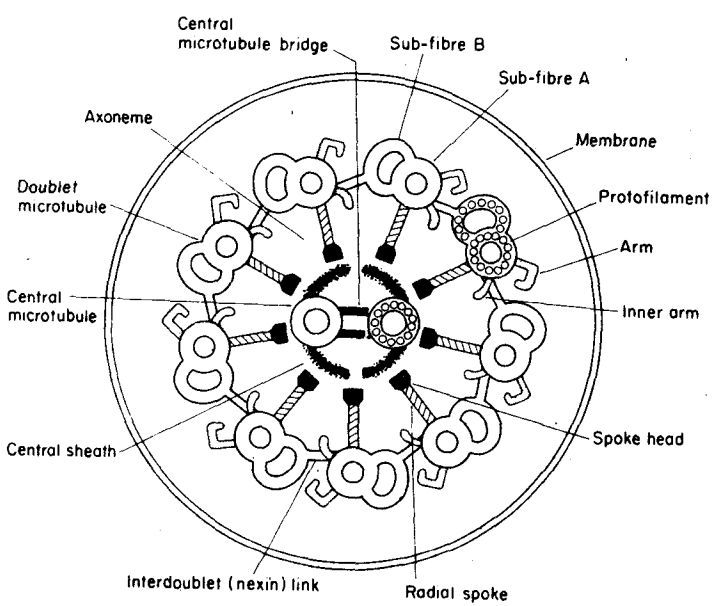

FIG. 1. Arrangement of microtubules and linking structures within a flagellum. After various authors, particularly Satir (1974), Warner and Satir (1974) and Warner (1975).

electron microscope, Gibbons (1975) reports that, in flagella with rigor waves obtained by abruptly removing ATP from a preparation, some twisting of the axoneme is observed, although he indicates that this may be a preparation artefact. A doublet consists of two parts, a complete microtubule (the A sub-fibre or microtubule) containing 13 protofilaments and the B sub-fibre which has fewer protofilaments and shares part of the wall of the A tubule. The skew of the doublets is such that the B sub-fibres contain the outermost points of the axoneme section, these points lying on a circle of diameter about 0.2 μm, while the innermost points are on the A sub-fibre and lie on a circle of approximately 0.15 μm. The spacing between adjacent doublets is between 17.5 and 20 nm and, into this space, extend the paired dynein arms which are attached to the A fibre. Viewed from tip-to-base along the axoneme, the dynein arms, which are responsible for a considerable fraction of the ATPase activity of the axoneme of all organisms so far examined for this feature, point in a clockwise direction. In most electron micrographs, the dynein arms do not extend to touch the neighbouring B sub-fibre but, if a preparation is made with ATP abruptly removed from the system, attachment of the arms to the B-fibre is observed (Gibbons, 1975).

Even when the flagellar membrane is removed by chemical procedures, the 9 + 2 axonemal complex retains its integrity due to the presence of fine linkages between adjacent sub-fibres. The linkages have been described as connecting the inner dynein arm to the neighbouring fibril (e.g. Kiefer, 1970), but Stephens (1975) has shown that axonemes with the dynein arms and B sub-fibres removed retain circumferential linkages, suggesting that the latter join neighbouring A sub-fibres. It appears likely that the interdoublet link (as it has been called by Warner, 1970) and the nexin link (Stephens, 1970) are identical structures which prevent lateral separation of the doublets, and which may be involved in the mechanical process which underlies motility. In longitudinal sections, the dynein arms and the interdoublet linkages have the same periodicity, the repeat distance being 16–20 nm depending on the organism, but insufficient information is available from such micrographs to specify the precise relationship between the two structures. The two rows of dynein arms appear to be in lateral register since, in longitudinal sections sufficiently thin to contain only one row, the same periodicity is observed.

Also attached to each A sub-fibre but directed radially inwards towards the central fibrils is another filamentous structure, the radial

spoke, which is about 38 nm long, 5 nm in diameter and is terminated by an electron-dense head at, or close to, the central sheath, a structure to be discussed later in this review (Section V, p. 28; Warner and Satir, 1974). In longitudinal sections, the spokes are found in repeating groups of two or three along the A sub-fibre, each group spaced an average distance of 86 nm apart. In structures from organisms such as *Elliptio*, where spokes occur in groups of three, it is found that the spoke separations within a group are unequal, with the wider spacing towards the ciliary base. The arrangement of spokes within the axonemal matrix is such that they lie in a helical configuration, but it is important to emphasize that there is no helical structure joining the spokes together. Functional aspects of the radial spokes will be discussed in a later section (p. 28).

In earlier work on ciliary and flagellar fine structure, a helically wound sheath surrounding the central tubules was described by several authors (e.g. Gibbons and Grimstone, 1960; Pedersen, 1970) but, in recent years, observations on certain organisms have revealed an alternative structure, which, however, is still referred to as the central sheath. The structure consists of projections from each of the central fibrils, and the projections may occur in one or two rows along each central tubule. In *Elliptio* cilia, for example, there are two rows of projections to each tubule; within a given row, the projections are parallel to each other and inclined at an angle to the tubule axis (Fig. 2). According to the microtubule observed, the two rows form an erect or inverted chevron pattern, such that the ends of the projections from adjacent microtubules are close together, and may be connected (Figs. 1, 2). In transverse section, the projections appear to be curved, so that, in *Elliptio* cilia, joined chevrons would create a circle inclined to the axoneme axis. The curved nature of the projections has not, however, been established unequivocally. The longitudinal spacing of the projections is 14.3 nm in *Elliptio* cilia, and it is probably of mechanistic importance that this spacing is just one-sixth of the radial spoke-group repeat distance. This point will be discussed in more detail later in this review (p. 28).

The membrane which surrounds the axoneme is an extension of the cell membrane and sometimes carries appendages such as the mastigonemes (thin hair-like processes) of the algal flagellates. Freeze-fracture studies of the flagellar membrane (Gilula and Satir, 1972) have revealed two important patterns of membrane particles, presumably protein in character. In one arrangement, the particles lie in

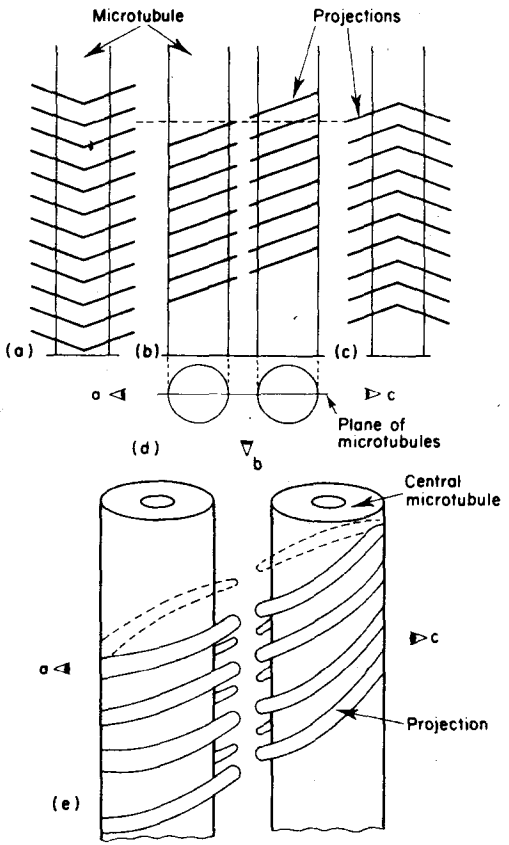

FIG. 2. The "central sheath" structure, showing the arrangement of projections from the central microtubules of *Elliptio* cilia. (a) and (c) show the "chevron" appearance of projections when viewed in the plane of the microtubules; (b) shows a pseudohelical appearance when viewed from a direction normal to the plane of the microtubules; (d) depicts a cross-section through central microtubules to indicate viewing directions for (a), (b) and (c); (e) indicates the three-dimensional impression of structure. After Warner and Satir (1974) and Satir (1974).

longitudinal rows which correspond in position to the underlying doublets, and may indicate some form of connection between the membrane and the doublets, although such a linkage has not yet been directly observed. The other pattern is the ciliary necklace, consisting of between two and six rows of membrane particles, which occurs just distal to the basal plate on which the central pair of tubules end. Linkages are observed to connect the membrane and the A tubule in

the region of the necklace, and the arrangement of particles is consistent with the hypothesis that they represent the membrane-attachment sites of the linkages.

III. Patterns of Movement

The motility of cilia and flagella has been studied by a number of techniques, including high-speed cine-photography, stroboscopic observation and multiple flash photography (see e.g. Holwill, 1967; Baba and Hiramoto, 1970; Brokaw, 1965; Sleigh, 1964; Takahashi and Murakami, 1968). The results of many experiments indicate that the beat patterns of cilia and flagella can be broadly classified in two groups, namely undulatory and tonsate. Typical magnitudes of the various parameters associated with the motion of these organelles are shown in Table 1 (p. 3). A further division of undulatory movement can be made since both two- and three-dimensional waveforms are observed. It is a common practice in dealing with the hydrodynamic aspects of the problem to assume the planar waveform to be sinusoidal (Gray and Hancock, 1955; Holwill and Sleigh, 1967) and the three-dimensional undulation to be helical (Holwill and Burge, 1963; Chwang and Wu, 1971) although even superficial examination of film records indicates that these are poor approximations to the true shape. Critical analysis of the photographs shows that some waveforms can be satisfactorily matched by a pattern consisting of circular arcs linked by straight lines (Brokaw and Wright, 1963; Brokaw, 1965)—the so-called arc-line waveforms. Rather similar in form to the arc-line wave is the meander shape which is adopted by a flexible, elastic rod with freely hinged ends separated by a distance less than the length of the rod. From a mechanistic view point, it is important to establish which, if either, of these patterns is adopted by real cilia and flagella. The existence of the meander wave might suggest that elastic properties of the system are of importance in influencing flagellar motility, while an arc-line pattern could indicate a two-state bending mechanism, i.e. one in which the bending elements are deformed to a specific curvature, the other in which they are straight. As shown in Fig. 3, the arc-line and meander are very similar shapes and, although it is theoretically possible to differentiate between them (Silvester and Holwill, 1972), application of the method to photographic records presents difficulties, because of such factors as poor resolution, and is

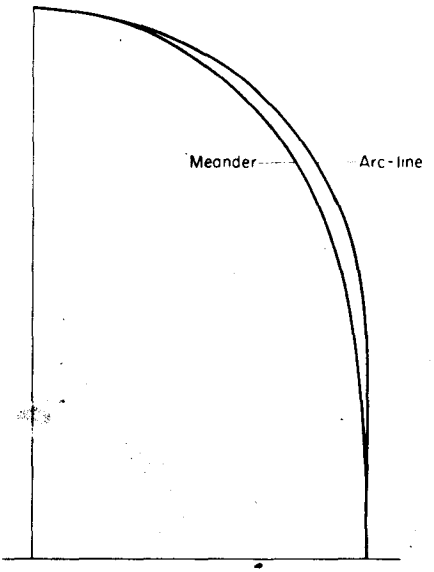

FIG. 3. Arc-line and meander waveforms of the same amplitude and wavelength, showing their close spatial relationship.

only now showing some indications of success (N. R. Silvester, M. E. J. Holwill and D. N. Johnston, unpublished results).

The majority of cells propagate bends from the base towards the tip of an associated cilium or flagellum. Only in a few cases, notably the trypanosomes, is it observed that, during normal movement, waves pass along the flagellum from the tip towards the base (Holwill, 1965, 1966b). In the case of the trypanosomes, and possibly for other organisms also (but in these cases wave analysis has not been undertaken), the direction of wave propagation can, under certain circumstances, be reversed. The reversal usually occurs when the organism encounters adverse conditions, such as an obstruction or an increase in viscosity, and can be regarded as an avoiding reaction. It is interesting to note that there appear to be no gross structure differences between the trypanosome flagellum and that of an organism which propages waves unidirectionally (Burnasheva *et al.*, 1968) although there may be subtle differences in fine structure associated with the ability to reverse the direction of wave propagation.

Waves propagated along a smooth flagellum induce forces which cause a free-swimming organism to move in the direction opposite to

that of wave propagation. In certain cases, notably the ochromonad flagellates, the organism is observed to swim in the direction of the propagated wave. The flagellum of such a cell carries thin hairs or mastigonemes, often arranged in two rows on either side of the flagellum, which are believed to be responsible for the apparently reversed thrust. According to Jahn *et al.* (1964), during motility the flagellum beats in a plane which contains the mastigonemes. Reference to Fig. 4 shows that, as a wave crest passes, the mastigonemes execute a tonsate movement which provides a thrust in the direction opposite to that produced by the flagellar shaft. Provided that the mastigonemes are sufficiently long and rigid, their net induced force will be greater than that of the shaft, so that propulsion of an organism will occur in the direction of the wave. This qualitative explanation of mastigoneme

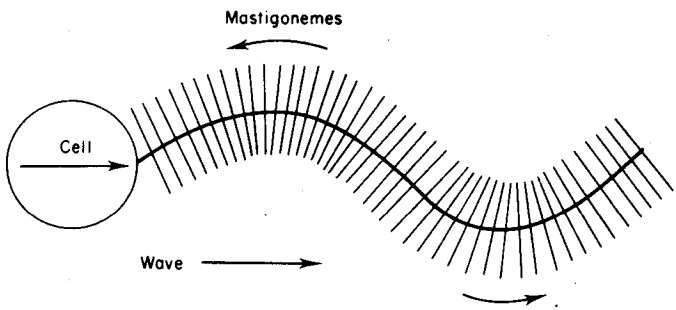

FIG. 4. Mechanism of action of mastigonemes.

action has been shown to give results consistent with experiment when subjected to hydrodynamic analysis (Holwill and Sleigh, 1967), but the mechanism has been challenged by Bouck (1971, 1972) on the basis of electron microscope observations. To be effective, the action described above requires that the mastigonemes lie in the plane of the flagellar wave, whereas Bouck suggests that a more reasonable arrangement, judged from his electron micrographs, is with the mastigonemes perpendicular to the plane of beat. Hydrodynamic analysis of the latter system indicates that reversed propulsion would not occur if the mastigonemes were passive, although Bouck (1972) has suggested a model based on freely hinged, elastic mastigonemes which he claims would produce the observed effect. Discussion of the model by Bouck is, however, rather brief and there is insufficient detail for one to understand precisely how he envisages the behaviour of the system.