THE METACHRONAL WAVE OF
LATERAL CILIA OF *MYTILUS EDULIS*

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ABSTRACT

The form of beat of cilia and the structure of the metachronal wave on the lateral gill epithelium of *Mytilus edulis* have been studied on living material by interference-contrast microscopy and stroboscopic illumination, and compared with the same features in rapid-fixed preparations studied by light microscopy and with the scanning electron microscope. The most striking finding is that the beat of the cilia is not planar, as previously assumed, but involves a sideways movement in the recovery stroke Previous reports on nonplanar ciliary beating from protozoan examples describe a planar effective stroke and a counterclockwise rotation in the recovery stroke; in this molluscan example there is a clockwise rotation in the recovery stroke The lateral inclination of the cilia in the recovery stroke is in the same direction as the propagation of the waves, and the orientation of cilia in the recovery stroke is thought to determine whether the waves move to the left or right of the direction of the effective stroke

INTRODUCTION

From the time of the first known description by Sharpey in 1835, the metachronal wave produced by the beating of cilia on the lateral epithelium of gill filaments in bivalve mollusks has intrigued many investigators. A review of the literature undertaken recently in conjunction with the authors' attempt to account quantitatively for the amount of water moved in relation to the movement of the cilia (Sleigh and Aiello, 1972) revealed that the form of beat of these cilia had never actually been determined. One generally accepted description stems from a paper by Gray (1930), in which he showed that one could account for the appearance of a profile view of the wave in *Mytilus edulis* if one assumed that the cilia beat with a certain form and phase relationship The beat pattern which he used to construct the wave was based on the pattern he described for the giant abfrontal cilium, which we now know is composed of about 25 individual cilia beating as one, and whose movement is, in fact, quite atypical (Sleigh, 1968) Photomicrographs of both living and rapid-fixed waves of lateral gill cilia of *Elliptio complanatus* obtained by Satir (1963) are quite similar to those of *Mytilus* obtained by Gray, but Satir's explanation of the wave required reducing the amplitude by about 40% and assigning a much greater proportion of the wave to the effective stroke It has recently become apparent in several organisms that the ciliary beat is not planar, well-established examples of this being the marked counterclockwise rotation of the recovery stroke in the ciliated protozoa *Opalina ranarum* (Tamm and Horridge, 1970) and *Paramecium* (Machemer, 1972). In view of the importance of knowing the true form of the beat in physiological, hydrodynamic, and ultrastructural studies, we undertook a direct investigation of the form of beat of the cilia; at the same time we

studied the detailed structure of the metachronal wave with the expectation that we might also learn something of the wave propagation, whose consistent orientation requires explanation.

MATERIALS AND METHODS

M. edulis was obtained commercially in Bristol or collected on the south coast of England. The mussels were kept in recirculated seawater at 15°C until used. Then the adductor muscles were cut, and the gills were excised into seawater and either left at 10°C for use later or allowed to come to room temperature (18°–22°C) and used immediately. For microscopic observation a piece of gill or a single filament was placed on a glass slide, and a strip of absorbent paper was placed over each end of the tissue; a coverglass was placed on top, the paper strips serving both to hold the tissue in place and to support the coverglass. Seawater was added regularly at one side of the coverglass and drawn off at the other with absorbent paper. In order to activate quiescent cilia or to accelerate the beat, 40 mM KCl or 10^{-7}–10^{-5} M 5-hydroxytryptamine was added to the seawater as required.

Observations were made in several different ways, but most of the data presented below were obtained with a Zeiss photomicroscope using Nomarski interference-contrast optics with a 40× water-immersion objective joined to the upper surface of the coverglass with a drop of distilled water. Photomicrographs of beating cilia were taken using a Zeiss microflash (30 w,1/2000 sec duration) (Carl Zeiss, Inc., N. Y.) and Ilford Pan F 35 mm film (Ilford Ltd., Ilford, Essex,

England). The rate of ciliary beating was determined by stroboscopic synchronization, and to do this the microflash was replaced by a rotating slotted rod which allowed light from the substage illuminator to flash through to the preparation. Direct observation of the stroboscopically stopped wave was also made in this way. Interchanging the stroboscope and the microflash took about 30 sec, and measurements of frequency could be made quite quickly after photographs of the wave had been taken.

In addition to the study of living cilia, we also made observations on ciliary waves that had been fixed rapidly with osmium tetroxide. To do this, the coverglass was removed from a regular preparation and about 0.5 ml of a 2% solution of osmium tetroxide half-saturated with mercuric chloride was dropped onto it from a height of about 10 inches. The coverglass was replaced and observations were made as before. Several such fixed preparations were washed in distilled water and frozen in liquid isopentane cooled in liquid nitrogen. The ice was then sublimated *in vacuo* at −30°C for 72 hr. The dried tissue was mounted on stubs with double-sided tape, coated with gold-palladium in an Edwards high-vacuum coating unit (Edwards High Vacum, Inc., Grand Island, N. Y.), and examined in a Cambridge scanning electron microscope.

OBSERVATIONS AND RESULTS

The distribution of cilia on a gill filament of *Mytilus* is well known, but is shown in Fig. 1 for purposes of nomenclature and orientation. The lateral cilia
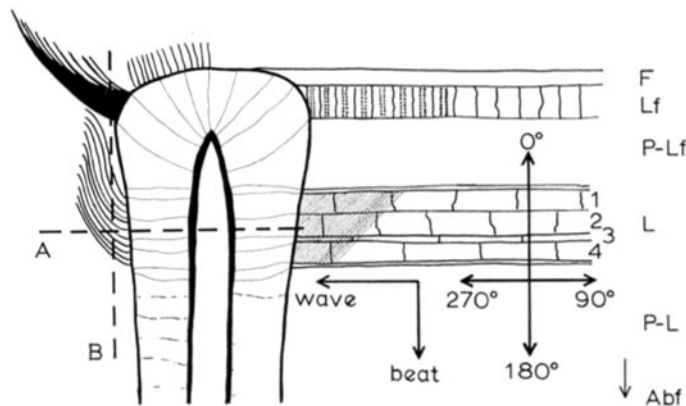

Figure 1 Diagram of part of a section through a gill filament of *M. edulis* and part of the surface of one side of the filament to show the arrangement and orientation of structures described in this paper. The lateral cilia are borne in oblique rows on the four-cell wide band of lateral cells (*L*); the cilia beat downwards, approximately from 0° to 180°, and the metachronal waves travel from 90° to 270°. The line *A* represents the approximate plane of a frontal view of the filament and line *B* the approximate plane of a surface view of the lateral cilia. Other parts of the filament are the frontal cells (*F*), laterofrontal cells (*Lf*), postlaterofrontal cells (*P-Lf*), and postlateral cells (*P-L*); the abfrontal part of the filament (*Abf*) lies below the edge of the diagram.

are borne on four main rows of cells at either side of the filament, the basal bodies of the cilia being arranged in oblique rows that extend without interruption across cell boundaries. According to the convention of angular orientation indicated in Fig. 1, the rows of basal bodies are arranged at an angle of 40°–45°; during their effective stroke the ciliary shafts move from a position near that indicated by 0° at the beginning to a position near that indicated by 180° at the end. Metachronal waves pass from right to left, from 90° to 270°, so that, according to the description of laeoplectic metachronism by Knight-Jones (1954), when an observer views the cilia in the direction of wave travel the effective stroke of the beat is towards his left

We have obtained information about the shapes and positions of cilia participating in metachronal waves of the lateral cilia by observing them in profile view and in surface view, for each of which the approximate plane of focus is indicated in Fig 1 A profile view was obtained by laying a piece of gill flat on a slide and viewing it from the frontal surface (Figs. 2, 3) or from the abfrontal surface (Fig. 4). When a filament is viewed from the frontal surface, metachronal waves are seen to travel in opposite directions along the two lateral surfaces; the effective stroke is away from the observer, and the metachronism is always laeoplectic The lateral epithelium on the two sides of the filament is identical and surface views of the wave will always appear alike if the orientation of the filament is consistent. In our figures of surface views, the frontal edge of the filament is towards the top of the page. Such surface views were obtained by placing a single filament on its side and focusing on the upper surface (Fig. 5). Clearer and more informative pictures of the wave were obtained in surface views than in profile views. It was not possible in surface views to see individual cilia in the main part of the wave with bright field or phase optics, but with Nomarski optics, and especially with the 40 × water-immersion objective, it was possible to resolve individual cilia, although the depth of focus was small and only short segments of the cilia could be seen unless the cilia lay in the plane of focus. In order to understand the shape and positions of all cilia in the wave, we began each observation by focusing on the surface of the lateral epithelium and lowered the stage a set amount, usually 3 μm, between each flash photomicrograph exposure, until it was obvious that even the tips of cilia in the effective stroke

were below the plane of focus. The wave could then be reconstructed from the series of five to seven micrographs. Examples from one such series are shown in Figs. 6–8 and exemplify the basic form of beating seen in all our pictures. Because the filament was not lying perfectly flat, the right-hand side is at a deeper focus than the left; this gives a slightly tilted view of the wave, but has the advantage that each picture presents a series of optical sections rising through the wave from right to left. The frontal part of the filament is thicker than the abfrontal part, and as a result the frontal edge of the lateral epithelium is at a slightly deeper focus than the abfrontal edge.

The motion of a lateral cilium can probably best be understood by imagining for a moment that the whole cycle of beat takes place in a single plane, so that the timing of parts of the beat and the position of the main bend in the cilium during stages of the recovery stroke can easily be shown in a diagram of a side view of the beat (Fig. 9A). It is clear from our observations, e.g , Figs 5–8, that, while the effective stroke may be approximately planar, the recovery strike is inclined in the direction of metachronal wave propagation (i e , towards the left in our figures) As the cilium begins to move upwards at the beginning of the recovery strike, the bend in the cilium is so orientated that it draws the cilium towards the side to move approximately in a plane that is inclined at an angle of 45° or less to the cell surface. This bend progresses to the tip of the cilium so that the whole recovery stroke takes place by an unrolling motion in which the ciliary tip moves in a clockwise arc that lies at the left side of the plane of the effective stroke when the ciliated epithelium is seen in surface view (Fig. 9 B) When viewing a photomicrograph of a metachronal wave in the orientation used in our surface views, the stages of a beat cycle may be seen in the normal sequence by following a line of cilia in the direction from 270° to 90°, i.e , in the direction opposite to the propagation of the metachronal waves (as in Fig. 10 B).

Because of the breadth of the band of cilia, there is a difference in the details of the pattern of beat shown by cilia at different parts of the lateral epithelium The cilia at the frontal edge recover in an extended position close to the surface of the postlaterofrontal cells, without interference from laterofrontal cilia, and beat over the more abfrontally placed cilia. Cilia at the abfrontal edge beat down close to the surface of the postlateral
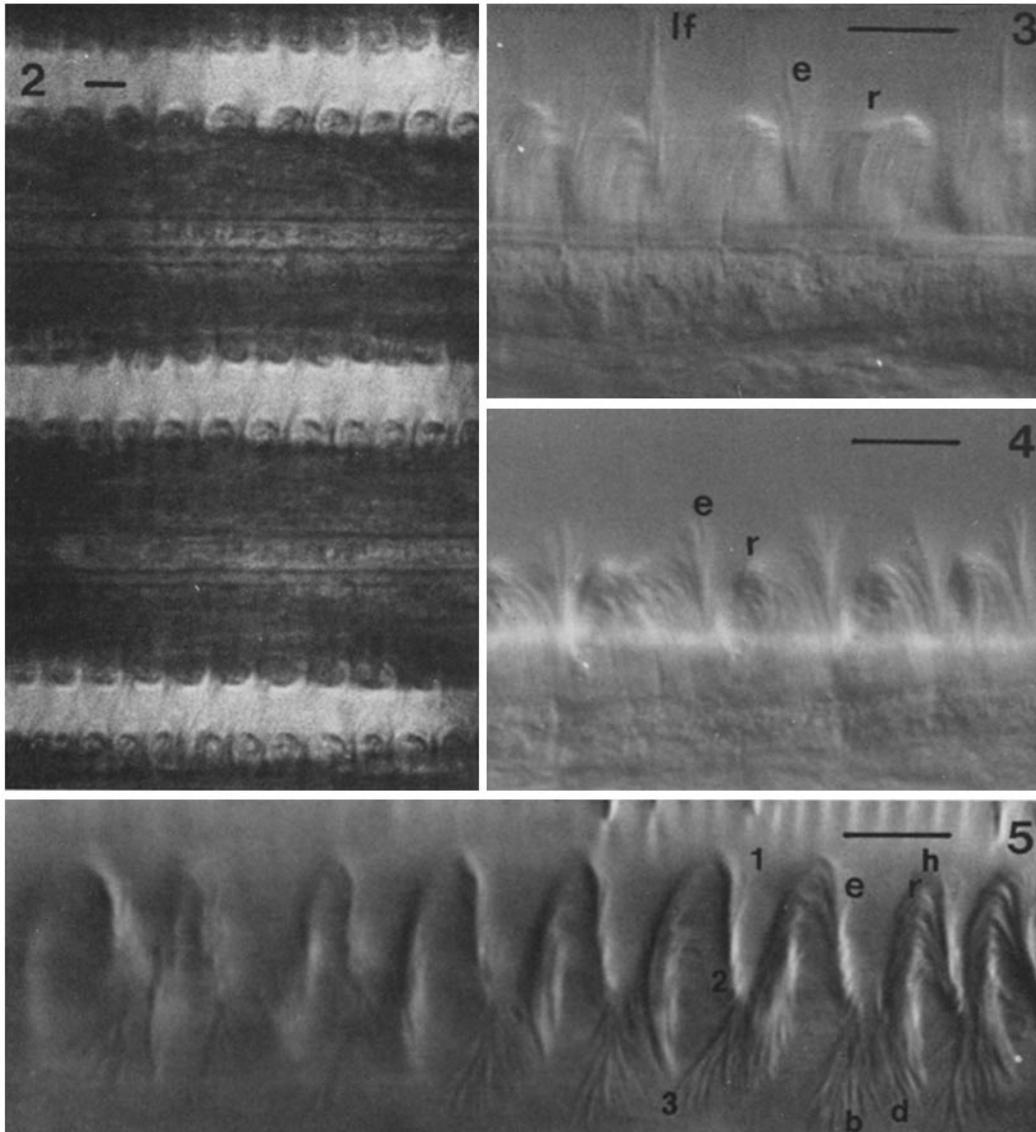
FIGURE 2 Frontal view of several filaments showing a profile view of metachronal waves of lateral cilia on opposite sides of the interfilamentary spaces The effective stroke of the cilia is away from the viewer; waves travel to the left on the upper side of each space and to the right on the lower side $\times$ 520 Scale marker = 10 μm

FIGURE 3 Profile view of lateral waves seen from the frontal surface, but with the filament slightly tilted so that several laterofrontal cilia (lf) are in focus. As in Fig 2, the effective stroke (e) is away from the viewer and the wave travels to the right Recovery stroke, r $\times$ 1300. Scale marker = 10 μm.

FIGURE 4 Profile view of lateral waves seen from the abfrontal surface, so that the effective stroke (e) is toward the viewer and the waves travel to the left The filament is slightly tilted so that part of the wave is obscured by the edge of the filament. Recovery stroke (r). $\times$ 1300 Scale marker = 10 μm

FIGURE 5 Surface view of lateral waves, comparable with the diagrammatic side view of Fig 1 The effective stroke is down (toward 180°) and the wave travels to the left (toward 270°). The filament does not lie flat, so that optical sections from near the cell surface to near the ciliary tips are displayed from right to left across the page. The bright lines below e are parts of cilia in their effective stroke The dark lines below r are cilia in their recovery stroke Effective stroke positions 1, 2, and 3 and recovery stroke positions b, d, and h refer to their counterparts in Figs 9 A and 10 B. $\times$ 1300 Scale marker = 10 μm.
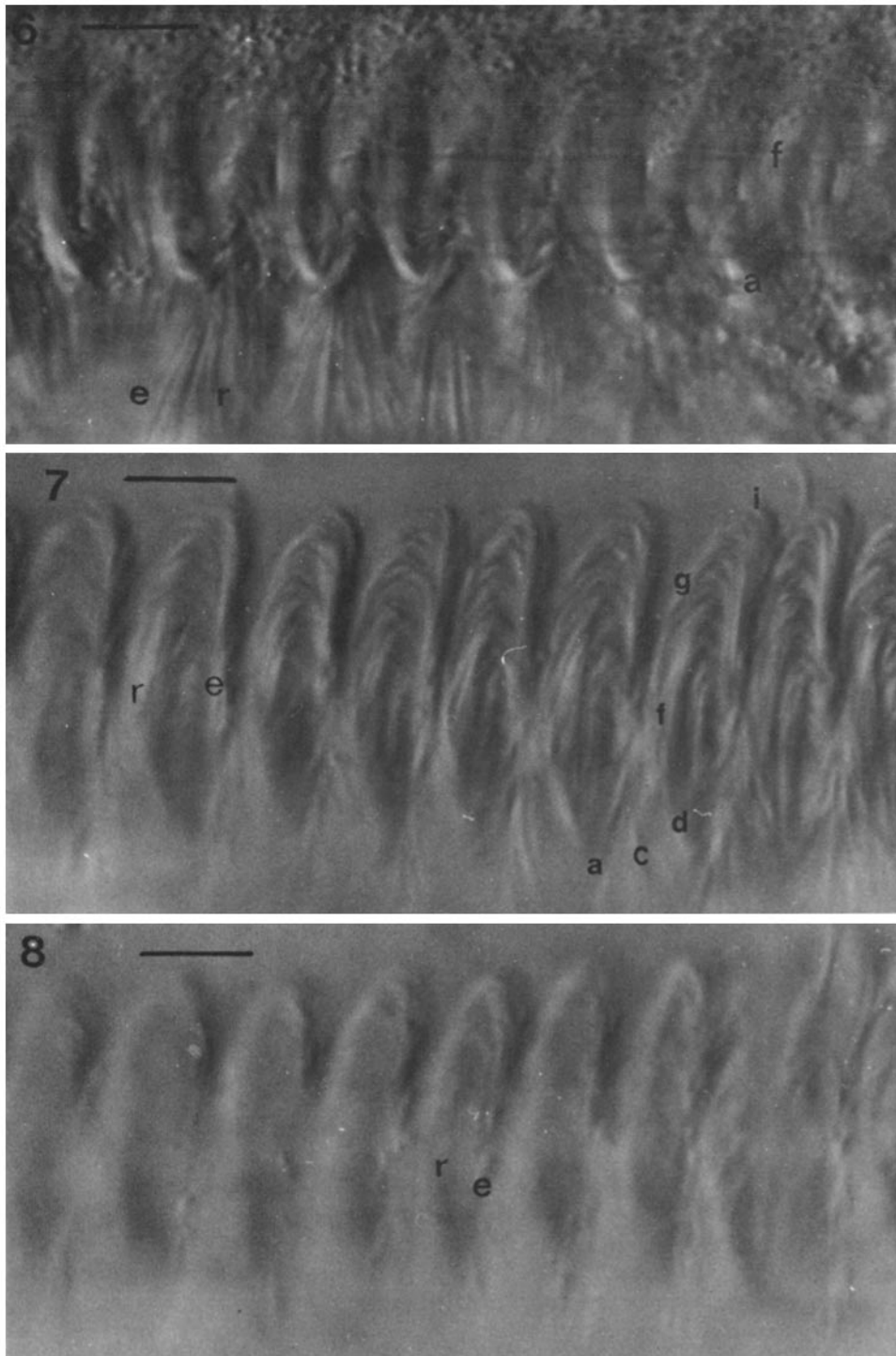
FIGURES 6–8 Surface views of lateral waves The effective stroke is down and the wave travels to the
left in each picture. The plane of focus is slightly lower at the top and right of each picture. Fig 6 shows
the frontal (f) and abfrontal (a) edges of the lateral epithelium; at the end of the effective stroke (e)
and the beginning of the recovery stroke (r) the cilia fan out over the postlateral cells. In Fig. 7 the
middle and late recovery strokes (r) appear as a series of curves as the cilium returns to its starting
position; a, c, d, f, g, and ι correspond to the same lettered positions in Fig. 10 B Several cilia in the
effective stroke (e) appear between recovery waves. Fig. 8 shows cilia in the effective stroke (e), most
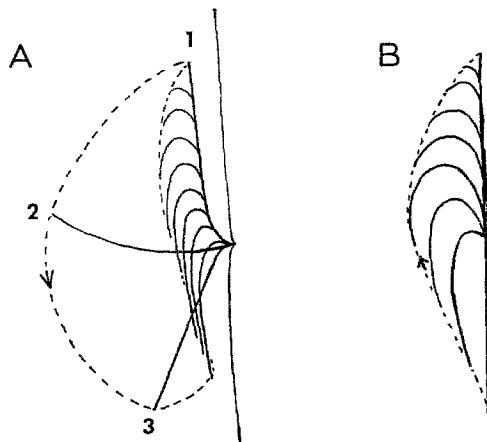of the recovery waves lying below the plane of focus. × 1600. Scale marker = 10 μm.

FIGURE 9 Diagrams representing A, the side view of a cycle of beat of a lateral cilium, showing the downward effective stroke in three numbered positions and the upward recovery stroke; and B, a surface view of a cycle of beating in which the effective stroke is downward in a plane perpendicular to the plane of the page and the recovery stroke is inclined sideways with the result that the cilium moves upward in a clockwise rotation.

cells and recover against the more frontally placed cilia. Most of the cilia lie between these two extremes and are prevented from approaching the cell surface at the ends of either effective or recovery strokes by the surrounding cilia However, in spite of their close spacing, the cilia perform a large amplitude effective stroke with a minimum of interference from other cilia. During the effective stroke the cilia along a transverse (0°–180°) line across the lateral cells move together, but those at the abfrontal end of this line are somewhat in advance of those at the frontal end; the cilia of such a line therefore show a component of symplectic metachronism, and one does not normally see any precise synchrony of the effective stroke. The fanned-out cilia of one transverse line are considerably in advance of those in the next adjacent line to the observer's left (Fig. 10 A), the beginning of the effective stroke of a cilium near the abfrontal end of one line coinciding with the start of the effective stroke of a cilium near the frontal end of the next line There is therefore no well-formed wavefront, in the sense that there is a clearly recognizable line of cilia that are beating in synchrony; the fanned-out cilia of one transverse line effectively constitute a wave front, although the true line of

synchrony is inclined to the transverse line at a small angle of not more than about 5° (i e., at about 185°). The line of synchrony is inclined in the same direction as the rows of basal bodies, but we have not seen the cilia in these rows beat in synchrony. The amount of fanning-out shown by the cilia of a line is variable; in some examples the cilia of a transverse line may be almost synchronous and in other examples the cilium at the abfrontal end of the line may have completed its effective stroke at the time a cilium at the frontal end is about to start its effective stroke

Another feature of the effective stroke that is somewhat variable is its precise orientation We have observed waves where the cilia appear to move along the 0°–180° line in a plane perpendicular to the cell surface and to the axis of the filament. However, in profile views of the wave, while cilia at the middle of their effective stroke form a well-defined line at right angles to the filament axis, those at the beginning of the effective stroke are commonly inclined a little backwards and those at the end of this stroke are inclined a little forwards in the direction of wave travel, this accounts for the divergent tips of cilia in the effective strokes shown in Figs 2–4 The oblique orientation of cilia engaged in effective strokes is also seen in surface views (Figs 5, 7, 8, 11, 12), from which estimates of the direction of the path followed by cilia of between 180° and about 210° have been obtained It is noteworthy that the direction of beat is also oriented towards the same direction as the rows of basal bodies but is never inclined as far as the basal body rows. The line of true synchrony and the plane of beating are very close, but in our observations the former is usually more nearly transverse than the latter.

At the end of the effective stroke the ciliary shafts lie close to the cell surface or against underlying cilia, and are directed abfrontally at an angle of 180°–210° from their insertion (Fig 10 B [a]) As the bend near the base of the shaft develops and begins to pull the cilium back in the recovery stroke, the movement to the left (as seen in surface view) of the proximal part of the cilium imparts a new orientation to the distal part of the cilium, which is drawn upwards at an angle of about 160° (Fig. 10 B [d]). This is the explanation for the fantail appearance of the most abfrontally directed cilia in Figs 5–7, etc. Increase in extent of the bent region of the cilium and its progression up the
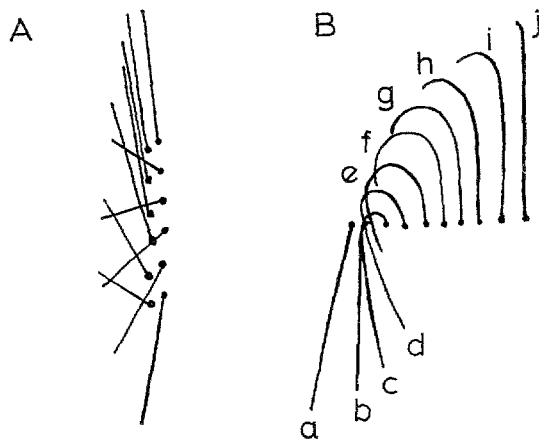
A   B

FIGURE 10 (A), A diagram of an oblique surface view of the positions at an instant during the effective stroke of lateral cilia of two lines running at 0°–180° across the lateral epithelium. The typical fanning-out of the cilia within a line during the effective stroke is shown, and the first (lowest) cilium to move in the left-hand line is at about the same phase of beat as a cilium near the top of the right-hand line. (B), A diagram of a surface view of the positions during the recovery stroke of cilia in a line running at 90°–270° along the lateral epithelium, in which the fan-tail appearance of cilia at the beginning of the recovery stroke (a–d) and the stacking of cilia in the mid-part of the stroke (d–g) are shown. Position a is identical to position 3 of Fig. 9 A.

ciliary shaft draws the ciliary tip upwards and to the left, and the curved recovering cilia are heaped upon one another with the tips of cilia half way through their recovery strokes lying above the bent basal parts of cilia near the beginning of their recovery strokes (Fig. 10 B [e, f]); cilia enter the stack at the base and leave it at the top where the half-extended cilia seem to slip off the frontal edge of the stack and continue to straighten out across the cell surface. During its recovery stroke the cilium lies over in the direction of wave travel, and the initial bend at the ciliary base brings the recovering cilium close alongside the row of cilia in their effective stroke, so that a stack of curved cilia is constantly being built up close behind each advancing front of fanned-out cilia in effective strokes. The space between adjacent effective strokes seems fully occupied by the stack of recovering cilia, implying that there is normally no interkinetic rest period between the end of one recovery stroke and the start of the next effective stroke. In fact, it appears that an effective stroke

may often start before the previous recovery stroke has been completed, while the tip of the cilium is still curved, this may be characteristic of cilia with a faster beat, while slower cilia may extend completely We have examined metachronal waves of cilia beating at frequencies covering more than a threefold range (7.5–25 Hz), and have not observed consistent differences of waveform (Figs 11–16), so that it is clear that both effective and recovery strokes are performed more rapidly in cilia that beat faster, a feature that has also been reported for other cilia (Sleigh, 1972).

Waves fixed in osmium tetroxide were similar to waves of living cilia, but appear to contain an excessive proportion of cilia in the mid-recovery position (Figs. 17, 19), and give the general impression that all bent cilia have relaxed somewhat (compare, for example, Fig. 17 with Fig. 7). The symplectic component of metachronism in the effective wave may be seen in Fig. 18. Osmium-fixed waves did not all appear this similar to the living wave, and if fixation was slow, as for example on pieces of filament away from the center of impact of the drop of fixative, all of the cilia came to rest in the fully recovered position Intermediate degrees of fixation were found, and it is possible that more perfect, lifelike fixation could be achieved if an improved technique were used on slowly beating cilia.

Scanning electron micrographs of osmium-fixed gill filaments showed the preservation of a wave (Figs. 20, 21), but close examination revealed that even the best preparations we made would be unreliable regarding details of the form of beat of individual cilia In Fig. 20, cilia in the effective stroke look fairly straight but appear to have stuck to masses of recovering cilia on either side. Fig. 21 shows the inclined stacking of recovery stroke cilia at point ꞏ and the extreme limits of amplitude from the bases of the laterofrontal cilia (lf) in the frontal direction to the surface of the postlateral cells in the abfrontal direction.

As an aid in interpreting our observations, we constructed several models of the ciliated surface of the lateral epithelium, with cilia in various positions. Photographs of a model made from pipe-stem cleaners bent to conform to the observed shape of cilia and set into a block of Plasticine are given in Figs. 22 and 23. Number and letter designations of cilia in various stages of their effective and recovery strokes correspond to those given in

the preceding figures, the resemblance to which becomes clear upon close inspection

DISCUSSION

The form of beat described in this paper for the lateral cilia of *M edulis* is distinctly different from that described for any metazoan, but resembles a mirror image of that described by Tamm and Horridge (1970) for the protozoon *Opalina* Cilia of both species carry out their effective stroke in a plane approximately normal to the surface, but recover in a plane inclined to the surface We wish to suggest that the form of the recovery stroke is normally the most important feature determining the pattern of ciliary metachronism, and that if the plane of recovery in *Mytilus* were normal to the surface as previously supposed, the recovery stroke would provide no mechanical constraint that might cause the metachronal wave to travel preferentially in one direction or the other along the lateral epithelium. Knight-Jones (1954) suggested that the relationship between direction of beat and direction of wave propagation would probably be determined by cytological or stereochemical factors, and a number of mechanisms involving neuroid transmission, nerve impulses, or mechanical interaction have been proposed to explain the wave propagation, as discussed in reviews by Sleigh (1962, 1969) and Kinosita and Murakami (1967)

Following our convention (Fig. 1), the recovery stroke is inclined towards 270° in *Mytilus*, and the proximal part of the cilium rotates clockwise, the recovery stroke in *Opalina* (and also in the ciliate protozoa *Paramecium* and *Didinium*, see below) is inclined towards 90° and the proximal part of the cilium rotates counterclockwise In the lateral cilia of all lamellibranches examined so far, the wave is consistently laeoplectic (Knight-Jones, 1954, Aiello, personal observations) Sleigh (1966) called attention to the fact that in the protozoon *Didinium nasutum* the wave was consistently dexioplectic and that when the animal reversed its direction of swimming both the direction of the effective stroke and the direction of the wave reversed Photographs taken by Parducz (1961) of forward and of backward swimming *Didinium* clearly show that the plane of the recovery stroke is always inclined in the direction of wave propagation (90° in our convention) in specimens studied by the rapid-fixation technique, and, since the inclination of the recovery stroke is faithfully preserved by this technique in *Mytilus*, we regard the evidence for this feature in *Didinium* to be acceptable. In the case of *Paramecium*, Machemer (1972) has shown that the metachronal wave is dexioplectic and the recovery leans towards 90°, he has confirmed by study of living ciliates that the description of the cycle of beat of *Paramecium* cilia by Parducz (1967), based on rapid-fixed material, was substantially correct. The situation in *Opalina*, as shown in scanning electron micrographs by Tamm and Horridge (1970), is distinct from that in the diaplectic examples described above, because in this protozoon the amplitude of the effective stroke is small and the peaks of the symplectic metachronal wave pattern are formed by packed aggregates of cilia in their effective strokes pushing against the laterally inclined recovery strokes of the preceding wave

Consideration of the wave patterns of *Mytilus*, *Didinium*, and *Paramecium* suggests that if the recovery stroke in a diaplectic wave is not in a plane

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FIGURES 11–16 Surface views of lateral waves showing the relative constancy of wave form at different frequencies of beating and on different filaments. The effective stroke is down and the wave travels to the left in each picture. In Fig. 11 the focus was just above the highest point of the recovery wave and through cilia in the effective stroke (*e*); positions *1*, *2*, and *3* correspond to similar positions *1*, *2*, and *3* in Fig 9 A; the beating rate was 20 Hz Fig 12 is from a different preparation, focused midway through the recovery wave; the beating rate was 18.3 Hz. Fig. 13 is from the same preparation as Fig. 12 but at a lower focus Fig. 14 is also from the same preparation as Fig. 12 but taken 20 min later when the cilia were beating at 13.7 Hz. Fig. 15 is from a different preparation where the cilia were beating at 8 7 Hz; the wavelength is measureably shorter but this was not a consistent feature at lower beating rates; there is less fanning-out of the ciliary tips in early recovery but the recovery stroke is still inclined in the direction of wave travel and fills the space available to it between effective strokes. Fig. 16 is from the same part of the filament as Fig. 15, taken a few minutes later when the beating rate was 8.3 Hz. Some of the cilia at the right side of the picture appear to have reached the fully recovered position before starting the effective stroke. × 1350. Scale marker = 10 μm.

11 1 2 3 r e

14

12

15

13

16
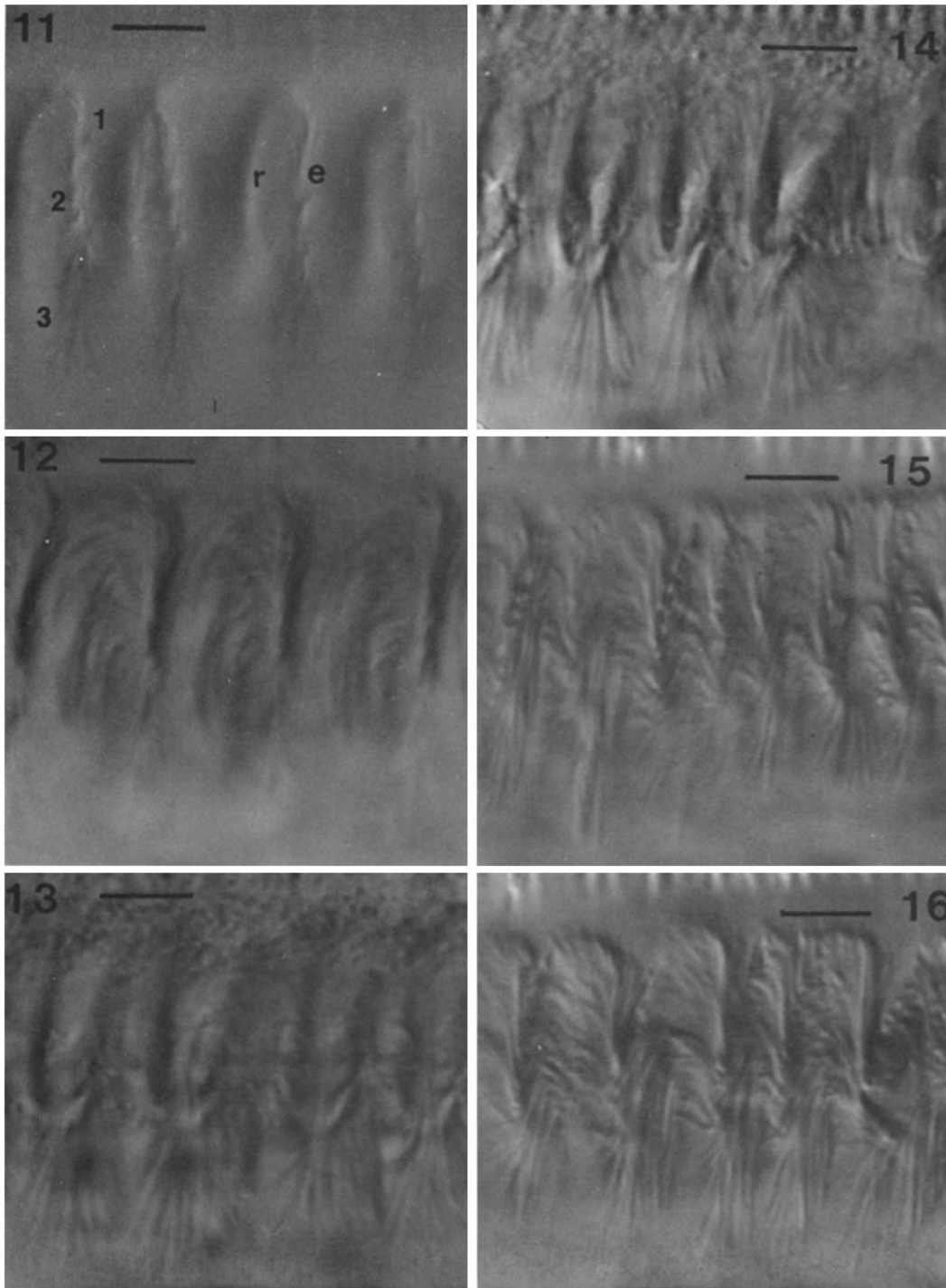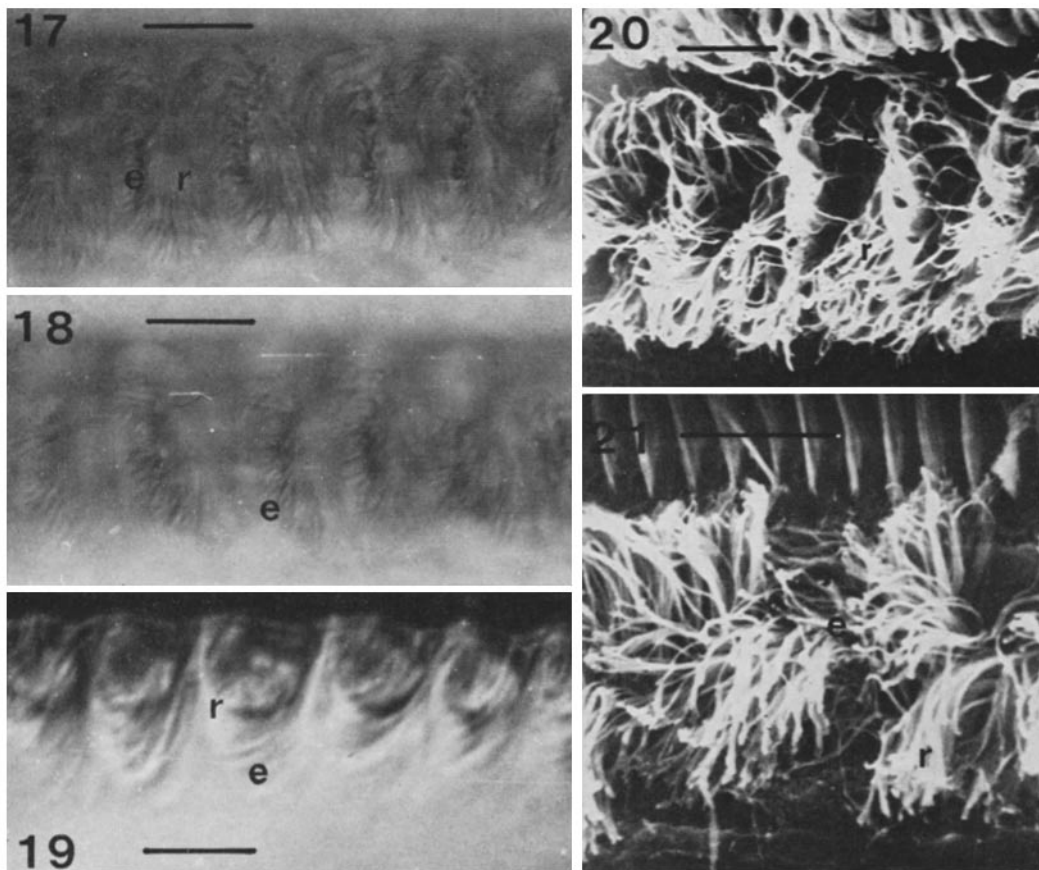
FIGURES 17 and 18 Surface view of osmium-fixed waves; beating rate 11.7 Hz. The effective stroke is down and the wave travels to the left. Fig. 17 shows a thin line of effective strokes passing between the majority of cilia which lie in the midrecovery position. Fig. 18 shows the distal part of slightly curved or inclined effective stroke cilia. As in the living wave, the recovery cilia incline in the direction of wave travel $\times$ 1460. Scale marker = 10 μm.

FIGURE 19 Profile view of osmium-fixed wave similar to that used for Figs. 17 and 18 and showing a similar inclination and curving of the effective stroke. The effective stroke is away from the viewer and the wave travels to the left. $\times$ 1460. Scale marker = 10 μm.

FIGURES 20 and 21 Scanning electron micrographs of osmium-fixed waves of lateral cilia, in which the cilia were beating downwards and the waves traveling toward the left; the curved laterofrontal cilia are seen along the upper edge. In Fig. 20 the lateral wave is evident, but the cilia are matted, especially in the late part of the recovery stroke. A few relatively straight effective stroke cilia (e) are present and the fanning-out of the early recovery cilia (r) is evident although they are tangled. The view is from behind and slightly abfrontal. $\times$ 1300. Scale marker = 10 μm. Fig. 21 is a more direct surface view but from slightly behind. Although the deeper parts of the wave are somewhat dim, one can see that the inclined, curving recovery cilia do pile up on top of those further along in the direction of wave travel. The close approach of the tips of later recovery cilia to the base of the laterofrontal cilia, and the proximity of early recovery cilia to the postlateral cell surface indicates the almost 180° amplitude of the beat. $\times$ 2000. Scale marker = 10 μm.

22

23
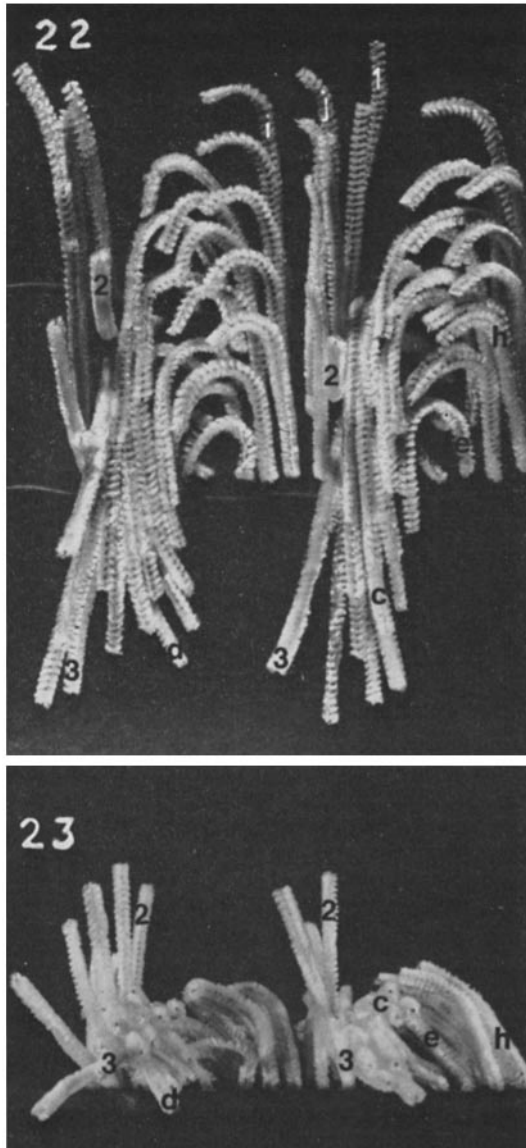
Figures 22 and 23   Photographs of a model of the ciliated surface of the lateral epithelium. In the original model 1 cm = 2 μm; in the photograph 1 cm = 5 μm; the epithelium is 13 μm wide, the cilia 18 μm long, and each of the two waves has a wavelength of 15 μm. The "cilia" are 1 μm in diameter and therefore represent groups of eight or nine cilia. They are relatively sparse compared to the true condition, in which there would be about 260 cilia in a wave of this length. Fig. 22 is a surface view with the wave moving to the left and the effective stroke down, similar to Fig. 5. Fig. 23 is a view from the abfrontal direction with the wave moving to the left and the model turned slightly so that the

normal to the surface, then the wave must travel in the direction of inclination of the recovery stroke in order that the effective stroke shall not be obstructed by the recovery strokes of the immediately adjacent cilia. This is indicated in Fig. 24, where A and B are hypothetical laeoplectic and dexioplectic waves composed of planar effective and recovery strokes, C is the situation in the laeoplectic wave of *Mytilus*, and D the situation in the dexioplectic wave of *Didinium* If laeoplectic or dexioplectic waves were to be formed with the recovery stroke inclined in the direction opposite to the movement of the waves, the movement of the cilia in the effective stroke would be blocked by recovering cilia as shown in Fig. 24 E and F While pattern E can thus be eliminated as a possible structure for the laeoplectic wave in lamellibranchs, it remains to be established whether pattern A ever occurs in the lateral cilia of these mollusks. The transmission electron micrographs of sections through the wave in *Elliptio complanatus* by Satir (1963) show a definite inclination in the direction of the recovery stroke, suggesting a pattern similar to that in *Mytilus*.

The inclination of cilia in the recovery stroke would provide an acceptable answer to the question of the orientation of the central fibrils that was raised by Satir in this paper on *Elliptio* The central fibrils of cilia fixed while quiescent had a fairly consistent orientation, but in those cilia fixed while actively beating the orientation of central fibrils varied by up to 90° in different cilia; Satir concluded that this could mean that the central fibrils may not run straight in active cilia or that the beat is not strictly two-dimensional If a line through the central fibrils must always lie perpendicular to the direction of bending, as concluded by Tamm and Horridge (1970), then a variation in the orientation of the central fibrils must accompany the sideways movement observed in the recovery stroke. While it is clear that twisting within the ciliary axoneme must take place in the recovery stroke to achieve this, the exact location, form, and cause of such a twist is not known. Since the cilia of *Mytilus* consistently bend to one side and the cilia of *Didinium* consistently bend to the other, it is suspected that there is some built-in structural asym-

end effective stroke, position 3, is straight at the viewer. It is similar in orientation to Fig. 4. Numbered and lettered positions of individual cilia refer to approximately the same positions in Figs. 9 A and 10 B.
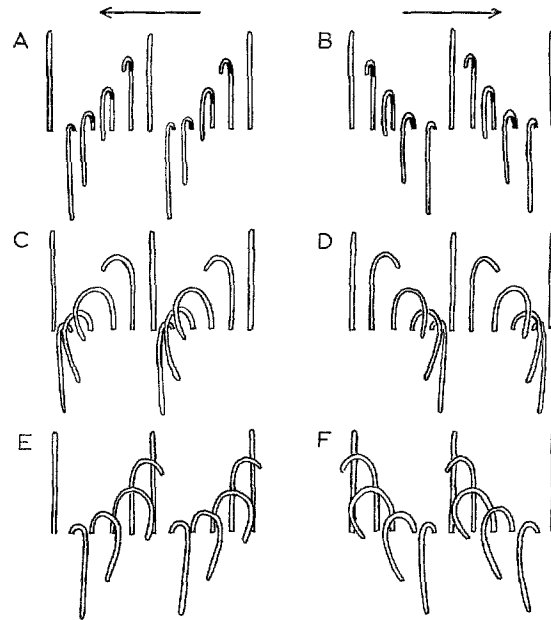
FIGURE 24 Diagrammatic surface views of a line of cilia representing two wavelengths of laeoplectic (A, C, E) and dexioplectic (B, D, F) metachronism, the waves traveling in the directions indicated by the arrows. In each case the cilia beat towards the viewer and downwards in a plane normal to the surface in their effective stroke. In A and B the recovery stroke also lies in a plane normal to the surface in accordance with the conventional interpretation In C the recovery stroke is inclined towards 270° as described in the present paper for *M. edulis* and in D the recovery stroke is inclined toward 90° as in *Paramecium* and *Didinium* (see text). In E and F the recovery stroke is drawn with the opposite inclination to that observed, and it is seen that the cilium that is about to start its effective stroke is blocked by adjacent recovering cilia; the recovery strokes in these cases are therefore inclined in the wrong direction for the form of metachronism indicated. A metachronal wave would normally contain many more cilia than are shown in these diagrams

metry One possible form of this asymmetry could be a difference in the orientation of the arms on the peripheral fibrils of the ciliary axoneme, but in a careful study Gibbons (1961) showed that in a number of examples, including *Paramecium* and a lamellibranch mollusc, *Anodonta*, the arms always pointed in a clockwise direction when the cilium was viewed from the base; corroborative evidence of structural uniformity is available from other examples, including the acoel turbellarian *Convoluta roscoffensis*, whose ciliary structure and laeoplectic metachronism were described by Dorey (1965), and a considerable diversity of ciliated protozoa It looks therefore as if some other source of asymmetry must be sought, but we have at present no suggestions to make

We have proposed here that the orientation of the recovery stroke determines the direction of wave propagation, rather than vice versa, but it would be worthwhile to examine any other

feature that might determine the form and propagation of the waves One such feature could be the orientation of the rows of basal bodies. In all lamellibranchs for which data is available, the rows of basal bodies on the lateral epithelium are inclined towards the direction of wave propagation; in *Mytilus* the rows are at 220°–225° (by our convention), in *Modiolus demissus* they are at 215° (Lucas, 1932), and Atkins (1938) gives evidence for similar orientation in some 29 species, all of which may be deduced from her descriptions to have laeoplectic metachronism (i.e , waves travel to 270°) In *Didinium*, on the other hand, the basal bodies are inclined towards 135°, while the metachronism is dexioplectic (waves travel to 90° [Sleigh, 1966]) There is, however, very little further evidence on this point, because where information on the metachronal waves is available, there is not usually a precise description of the arrangement of the ciliary bases; careful examina-

tion of a number of examples will be necessary to establish how widespread this relationship between metachronism and orientation of the basal body rows may be. In such a case as the lateral cilia of *Mytilus*, the basal bodies are set very close together within the oblique rows, and in the effective stroke, even when this is towards 180° and especially when it is towards 210°, the movement of one cilium would exert some force on the next cilium lying obliquely ahead of it in the row. Thurm (1968) states that if the lateral cilia of *Mytilus* are quiescent, mechanical displacement in the direction of the effective stroke causes them to beat It is clear that close mechanical coupling can exist between cilia borne on the basal bodies of a row, and this may be the reason for the orientation of the wavefront of synchrony being at an angle greater than 180° However, when a cilium beats it is more closely followed by the cilium that lies in the 0° direction, in the next row of basal bodies above, than by the adjacent cilium at 220° in the same row, so one may conclude either that for some reason coupling between cilia in the effective stroke within the 0°–180° line is closer than that in the 40°–220° line, or that the maintenance of rhythm within 0°–180° lines is by tight coupling at another part of the cycle, possibly in the recovery stroke where the curved cilia lie closely packed together. We are led to suggest that the orientation of basal bodies may enhance the coupling within metachronal waves, but that the direction of wave propagation is primarily determined by the inclination of the recovery stroke We are encouraged in this belief by the fact that in both *Paramecium* (with dexioplectic waves) and *Convoluta* (with laeoplectic waves) the basal bodies are arranged in approximate hexagonal array, and no close oblique rows of basal bodies are present In the example of *Paramecium*, the direction of propagation of waves is variable and an oriented close packing could be disadvantageous, where the wave pattern is consistent, then one might expect to find the cilia grouped in the most suitable array for efficient water movement.

This brings us finally to comment on features of the lateral ciliary system of bivalves that seem to us to be functionally important in the propulsion of water. We have pointed out elsewhere (Sleigh and Aiello, 1972) that about half of the water moved by the effective stroke of a cilium lies beyond the ciliary tip, but that in the lamellibranch gill the proximity of the opposite filament limits the space for water movement, although that

filament also provides additional propulsive force acting on the same body of water One of the features limiting the area through which water can flow between filaments is the series of humps formed by the recovery strokes of the lateral cilia (Fig. 2), it is advantageous that these profiles be kept as low as possible by moving the recovering cilia close to the cell surface, and, since there seems to be a minimum radius of curvature of cilia in the recovery stroke, the profile can be kept lower by inclining the cilia to one side and stacking them closely together Increased efficiency is gained in the effective stroke by fanning-out the cilia so that a propulsive force is exerted over a longer period by a given transverse line of cilia, and a more continuous flow of water can be maintained The metachronal wave-length appears to be fairly constant at about 2½–3 times the radius of curvature of the bend in the recovering cilia, which is about as small as it can be since the recovering cilia are accommodated sideways between one effective stroke and the next. It would seem to be advantageous, from the point of view of efficient water propulsion and continuity of flow, to keep the wavelength short and the effective strokes close together.

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