

## MECHANISMS OF CILIARY CO-ORDINATION IN CTENOPHORES

By SIDNEY L. TAMM

*Division of Biological Sciences, Department of Zoology,  
Indiana University, Bloomington, Indiana 47401, U.S.A.*

(Received 15 January 1973)

### INTRODUCTION

The movement of adjacent cilia in many animals is co-ordinated, so that successive cilia along one direction beat slightly out of phase with each other in a regular sequence, resulting in metachronal waves of activity which spread across the ciliated surface. Two types of mechanisms have been proposed to explain ciliary co-ordination. According to the mechanical interaction theory the movement of one cilium mechanically stimulates the next one to beat, by viscous coupling or hydrodynamic drag forces transmitted through the external medium. The opposing view holds that some kind of internal conduction, involving a nerve-like or neuroid process in the tissue itself, is responsible for co-ordination (see recent reviews by Kinoshita & Murakami (1967) and Sleight (1969)). Ctenophores have long been favourite material for experimental studies on ciliary co-ordination because of the relatively large size and separation of their locomotory cilia, or comb plates. Nevertheless, previous work on the problem of ciliary co-ordination in ctenophores has led to both the mechanical interaction and the neuroid theories being applied to the metachronism of comb plates. I propose to show how this long-standing controversy can be resolved, by demonstrating the existence of both types of mechanisms, each characteristic of a particular kind of ctenophore.

The comb plates of ctenophores are the effector organs of swimming. They are arranged in eight rows which run longitudinally from near the apical organ (statocyst) at the aboral pole to the oral end of the body. The effective stroke of the comb plates is directed toward the aboral end during normal swimming; consequently the animal moves mouth-foremost. The activity of the plates is co-ordinated so that they beat in regular sequence starting from the aboral end of the comb row. Metachronal waves of activity therefore pass from the aboral to the oral end of the animal (antiplectic type of metachrony (Knight-Jones, 1954)).

Although ctenophores have an ectodermal nerve net, nerves are not believed to play a role in ciliary co-ordination. Ciliary waves continue to be initiated and transmitted in the presence of excess magnesium ions at concentrations which block typical nervous responses (Lillie, 1908; Horridge, 1965*b*).

Verworn (1890) found that if he completely prevented the movement of one or more plates in *Beroe*, waves did not pass the motionless plates; moreover, if he made a narrow cut between two adjacent plates, waves ran unhindered across the wound. Verworn therefore suggested that the movement of one plate mechanically triggers the beat of the next one.

Parker (1905), on the other hand, showed that in the lobate *Mnemiopsis* waves were transmitted past plates which were prevented from moving. He concluded that co-ordination was due to a nerve-like or neuroid impulse conducted through the tissue itself. Recent electrophysiological investigations of this type of electrical transmission, or epithelial conduction, have been made in other coelenterates (Mackie, 1970).

Child (1933) found that preventing the movement of a comb plate would block passage of waves in *Beroe* and *Pleurobrachia*, but in *Bolinopsis* waves would occasionally pass a stationary plate. Child concluded on the basis of these and other experiments that the movement of a plate does not play a mechanical role in wave transmission, but instead is important in renewing or reinforcing the conduction of an internal impulse from plate to plate along the row. A similar step-by-step neuroid mechanism was later proposed independently by Sleight to explain metachronism of ciliary membranelles in *Stentor* (Sleight, 1957).

Sleight (1963, 1966, 1968) initially believed that the comb plates of *Pleurobrachia* and *Beroe* were co-ordinated by a neuroid mechanism, based on the effects of various drugs and increased viscosity on wave transmission. His most recent work, however, using high-speed ciné analysis, and mechanical interference with ciliary activity has led him to conclude that co-ordination in *Pleurobrachia*, *Beroe* and *Cestus* depends on mechanical interaction (Sleight, 1973).

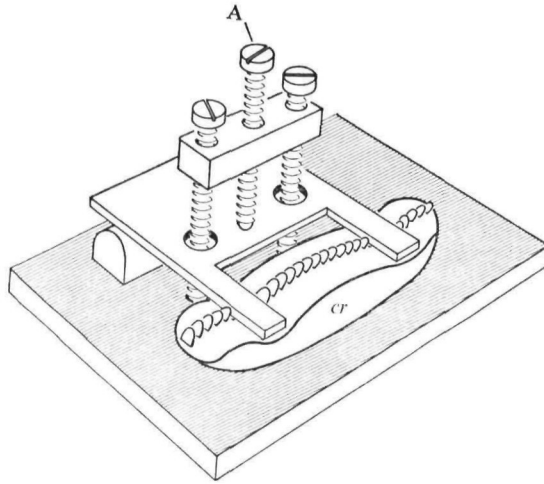
The purpose of this paper is to show how the conflicting results of these investigators can be resolved. The answer lies in the recognition of a ciliated conducting pathway that runs between adjacent comb plates in some ctenophores (lobates) but not in others (cydippids, beroids) (Chun, 1880). By undertaking an experimental analysis of wave transmission in various ctenophores I have found two different mechanisms of ciliary co-ordination, correlated with the presence or absence of this ciliated conducting pathway.

#### MATERIALS AND METHODS

Different genera of ctenophores were studied at the following marine stations: *Mnemiopsis*, at the Marine Biological Laboratory, Woods Hole, Mass.; *Bolinopsis*, at the Gatty Marine Laboratory, St Andrews, Scotland, and at the Friday Harbor Laboratory, Friday Harbor, Wash.; *Eucharis* (= *Leucothea*), at the Stazione Zoologica, Naples, Italy; *Beroe*, at the Gatty Marine Laboratory and Stazione Zoologica; *Pleurobrachia*, at the Gatty Marine Laboratory and Friday Harbor Laboratory; and *Hormiphora*, at the Friday Harbor Laboratory. Ctenophores were collected by carefully dipping them from the surface.

Comb-plate co-ordination experiments were performed on long pieces of comb rows cut out of the animal. These isolated comb rows included the underlying food canal, as well as some mesogloea. Experiments on the ciliated groove were carried out using the aboral end of the ctenophore. The isolated tissue was held stationary by clamping it with a 'micro-vice' mounted in a Lucite chamber filled with sea water (Text-fig. 1). The chamber was placed on the stage of a dissecting microscope and viewed by dark-field or bright-field transmitted light.

Several kinds of micro-tools, operated by a micromanipulator, were used for



Text-fig. 1. Micro-vice apparatus for holding pieces of ctenophores stationary. Part of a comb row (*cr*) is clamped at both ends with a U-shaped Lucite jaw which is tightened by turning screw A. The apparatus is mounted in a Lucite chamber filled with sea water, and viewed through a dissecting microscope. Such preparations show normal metachronal waves, and were used for experiments on ciliary co-ordination.

different experiments. Fine glass needles were made from capillary tubing drawn out in a micro-electrode puller. Breaking off the tips of the needles to varying extents gave glass rods of different diameters.

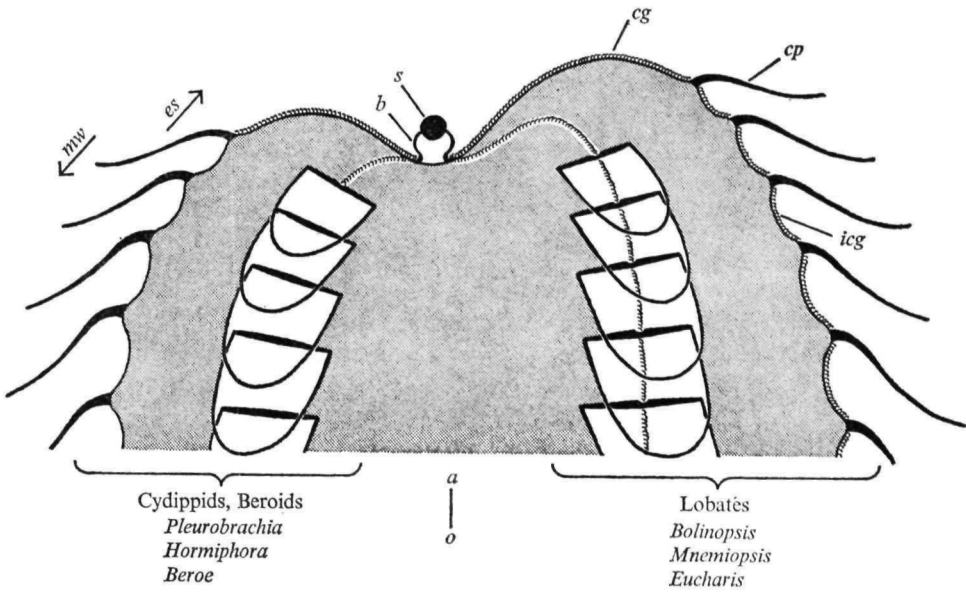
## RESULTS

### *General features of ciliary arrangement and activity*

The arrangement and function of the locomotory cilia of ctenophores has been reviewed recently by Sleigh (1963, 1966, 1968, 1972) and Horridge (1964, 1965*b*, 1966), and will only be described briefly here; relevant features that are less well known are presented in more detail.

The eight rows of comb plates are arranged in pairs in each quadrant of the body (Text-fig. 2). From the aboral end of each row a line of cells bearing small cilia, the ciliated groove, runs toward the aboral pole and joins with its neighbour of the same quadrant. The two ciliated grooves connecting a pair of comb rows terminate at one of four balancer cilia which support the statolith in the apical organ. In cydippids and beroids the ciliated groove ends at the first (i.e. most aboral) comb plate of each row (Text-fig. 2; Pl. 2A). In lobates, however, the ciliated groove continues between successive comb plates throughout the row (Text-fig. 2; Pl. 2B). This difference between ctenophores was recognized long ago by Chun (1880).

A comb plate has the shape of a house-painter's brush, and consists of hundreds of thousands of long cilia which beat together as a unit (Afzelius, 1961). Depending on the species and size of the ctenophore, a comb plate may be 0.5–2 mm long, slightly shorter in width, and 20–40  $\mu\text{m}$  thick at the base. The comb plates are spaced approximately equidistantly, except near the aboral and oral ends of the row where they are closer together, as well as smaller in overall size. Since the distance between



Text-fig. 2. Side view of the aboral end of a 'hybrid' ctenophore, showing the arrangement of locomotory cilia in the two different groups. A pair of comb rows, one member of which is seen from the side and the other from the front, is depicted for each group. In both groups, ciliated grooves (*cg*) connect a pair of comb rows to one of four balancer cilia (*b*) which support the statolith (*s*) in the apical organ. In cydippids and beroids (left side) the ciliated groove ends at the first comb plate (*cp*) of each row. In lobatès (right side) the ciliated groove continues between successive comb plates throughout the row, and is termed the interplate ciliated groove (*icg*). The effective stroke (*es*) of all cilia in the system is directed toward the aboral pole; the sequence of beating starts at the balancer and spreads orally, resulting in metachronal waves (*mw*) which pass from the aboral to the oral end of the animal (*a-o* axis). Note that adjacent comb plates overlap considerably in both groups. For purposes of illustration the relative sizes of the apical organ and the length of the ciliated groove cilia has been exaggerated, and the dome of cilia which encloses the apical organ has been omitted.

plates is usually two-thirds to one-half of their length, adjacent plates at rest overlap to a considerable extent. The cilia of a comb plate are borne on tall, columnar cells (polster cells) which are packed together in a rectangular ridge lying transversely to the comb row (Pl. 1 A).

The line of cells bearing small cilia that runs between adjacent plates in lobate ctenophores will be termed the interplate ciliated groove, because it resembles the ciliated groove connecting balancers to comb rows in all species (Pl. 2). Both ciliated tracts are only 10–20  $\mu\text{m}$  wide throughout most of their length. The ciliated groove forms a triangular widening at its junction with the first comb plate of a row; likewise, the interplate ciliated groove in lobatès undergoes a similar widening at its attachment to the aboral side of each successive plate along a row (Pl. 2). In neither case do the cilia of both ciliated grooves continue through the comb plates; consequently, the cilia of the interplate groove stop at the aboral side and begin again on the oral side of each plate in the row (Pl. 1 A, 2 B).

The cilia of the grooves are about 15  $\mu\text{m}$  long, and at rest assume an upright position with their distal ends bent toward the oral pole (Pl. 1 A). The cells bearing the cilia are typical columnar epithelial cells (Pl. 1 A, B), and do not resemble bipola

ciliated axons, as described by Horridge (1965*b*). Nor are tracts of fine nerve fibres found running beneath the ciliated cells, as was reported by Hertwig (1880) and Heider (1927). However, thin nerve fibres form synapses on the comb-plate cells (Horridge & Mackay, 1964), as well as with the cells of the ciliated grooves (S. L. Tamm, unpublished). All evidence points towards these nerves being part of the ectodermal nerve net responsible for inhibition of ciliary activity when the animal is mechanically disturbed (Horridge & Mackay, 1964).

Preliminary work on *Eucharis* has revealed thin processes that extend under the comb-plate cells from the inner ends of the interplate ciliated groove cells at the aboral widening (Pl. 1 A). These long processes run orally toward the next interplate ciliated groove, but a connexion between adjacent grooves has not yet been traced. Electron microscopy shows that the processes are filled with parallel arrays of microtubules (Pl. 1 C). A possible function of these processes in ciliary co-ordination is considered later (see Discussion).

The statolith in the apical organ is composed of more than 100 cells, each containing a large hard granule which is enclosed in a vesicle that occupies most of the volume of the statolith cell (Tamm, 1973, in preparation). The four sickle-shaped balancers that support the statolith are about 50  $\mu\text{m}$  long, and each balancer consists of 150–200 individual cilia. The distal ends of the balancer cilia lie in grooves of the statolith cells, thus providing tight mechanical coupling between the statolith and balancers (Tamm, 1973, in preparation).

At rest the comb plates lie close to the body surface with their tips directed orally. The active stroke is a vigorous beat toward the aboral end of the animal, followed by a slower return to the resting position. The metachronal waves which travel along the comb rows from the aboral to the oral end begin at the balancer cilia in the apical organ. When a balancer is excited by loading of the statolith on it, a wave of beating passes out orally along the two ciliated grooves connected to it, and thence to the appropriate pair of comb rows (Tamm, 1973, in preparation). In lobate ctenophores the cilia of the interplate ciliated groove participate in the wave of activity which travels down the row. During the passage of a single wave, each cilium in the ciliated groove and each comb plate gives a single beat in succession, with the effective stroke directed toward the aboral pole. The frequency of beating of each pair of comb rows is thus controlled by the balancer to which it is connected by ciliated grooves (Horridge, 1971; Tamm, 1973, in preparation).

### *Experimental design*

The experiments now to be described were usually carried out on at least two genera of ctenophores from each group (those with and those without an interplate ciliated groove), excluding only the cestids. Consistent results were always obtained among ctenophores within a group. The results are summarized by group in Table 1.

Isolated comb rows clamped in the experimental chamber showed normal metachronal waves, with the first plate at the aboral end acting as a pace-maker. During continuous beating at high frequency the ciliary waves travelled too rapidly to follow by eye. However, the progression of single waves, involving only one beat of successive plates followed by a rest, was easy to follow along a row. Consequently, the effects of different experimental treatments on co-ordination were assayed by determining

Table 1. *Results of experiments on ciliary co-ordination in ctenophores with and without an interplate ciliated groove (ICG)*

Experiment	Shown in Text-fig. no.:	Transmission (+) or interruption (-) of metachronal waves in ctenophores	
		With ICG (lobates)	Without ICG (cydippids, beroids)
1. Prevent movement of comb plate(s) by:			
(a) Pressing down one plate	3(a)	-/+	-
(b) Holding one plate upright	3(b)	+	-
(c) Pressing down several plates	3(c)	+	-
2. Cut between comb plates	3(d)	-	+
3. Amputate comb plates	3(e)	+	-
4. Increase the distance between plates	3(j)	+	-
5. Prevent movement of the ICG	3(f)	-	-
6. Cut the ICG	3(g)	-	-
7. Prevent movement of the ciliated groove	3(h)	-	-
8. Cut the ciliated groove	3(i)	-	-

whether or not single waves passed across the treated region. Because any cilium in the system can act as a pacemaker, waves were frequently present on the oral side of a block which interrupted co-ordination. In this case, however, the waves originating at the oral side of the block had a new rhythm with a different frequency from those on the aboral side.

#### *Preventing movement of comb plates*

The normal motion of a single comb plate was prevented by either pressing it flat against the body surface with a rod (Text-fig. 3a) or holding it upright by pushing against its base on the oral side (Text-fig. 3b). In the first case motion of the plate is completely prevented, whereas in the second the upright plate can still move aborally in a restricted power stroke. Alternatively, several adjacent plates could be completely prevented from moving by pressing them against the body surface with a piece of Lucite (Text-fig. 3c).

In *Pleurobrachia*, *Hormiphora* and *Beroe* the effect of restricting plate movement by any of these methods is the same: forward waves coming from the aboral end of the comb row are not transmitted past the restrained plate(s). The effect is reversible: upon releasing the plate(s), co-ordination is resumed immediately throughout the row.

In lobate ctenophores the same experiments give the opposite results. For example, holding a single plate upright, or pressing several adjacent plates close to the body surface, does not interrupt wave transmission in *Bolinopsis*. In *Eucharis* also, waves pass across a region where the movement of several plates is completely prevented. In this situation it can be seen that the interplate ciliated grooves between the non-beating plates participate in the transmission of the wave. Single waves of beating entering the blocked region are conducted down successive ciliated grooves between non-beating plates, emerging as normal waves (involving plate beating) at the oral end of the block. The cilia of the interplate ciliated grooves can still beat under these conditions because they lie on a level slightly below the projecting ridges of the comb-plate cells. Since the comb plates overlap one another, pressing down several with

piece of plastic merely forms a 'roof' over the ciliated grooves in the depressions between them.

Pressing down a single plate against the body surface with a glass rod, however, sometimes stops wave transmission in lobate ctenophores. Apparently, this method of restricting plate movement also interferes with the movement of the underlying ciliated groove (see below).

#### *Cutting between comb plates*

A narrow cut was made with iridectomy scissors through the epithelium and underlying food canal approximately midway between two adjacent comb plates (Text-fig. 3*d*). Care was taken not to disturb the spacing between the plates, or to damage the plates themselves.

In *Pleurobrachia* this operation did not interrupt wave transmission along the row: single waves passed across the cut region as if the tissue were intact. The same experiment on *Mnemiopsis*, however, prevented propagation of waves across the cut.

#### *Amputating comb plates*

A single comb plate, or a series of several adjacent plates, were amputated near the base with iridectomy scissors (Text-fig. 3*e*). The remaining stubs, approximately one-fifth the original length, beat rapidly and continuously immediately following the amputation. After a few minutes the amputated plates no longer beat continuously, and it was possible to determine the effect on metachronal transmission.

In *Pleurobrachia* waves stopped after the amputated plate, or after the first one if more than one plate had been amputated. In the case of a single amputated plate this stub beat when a wave came from the aboral end of the row, but the unamputated plate oral to it did not. Where a series of plates were amputated, the first stub at the aboral end of the group participated in forward waves, but waves were not transmitted to the other stubs lying oral to it.

The same experiments with *Mnemiopsis* and *Bolinopsis* gave opposite results: waves passed across the region of amputated plates, with all of the stubs (as many as 12) participating in the wave.

#### *Increasing the distance between plates*

The distance between adjacent plates was greatly increased to determine the importance of their relative spacing in metachronal co-ordination (Text-fig. 3*j*). A long region of a comb row, including 20-40 plates with the underlying food canal and some mesogloea, was cut out of an animal. Approximately 1 day after the operation the large wound had completely healed over, mainly by a process of cell migration and elongation. As a consequence of this tissue 'stretching' the plates located at the two edges of the former wound were carried across the intervening gap and became widely separated from each other. In lobate ctenophores the interplate ciliated groove also extended between the widely separated plates.

Increasing the distance between adjacent plates by this method did not block wave transmission in the lobates, *Mnemiopsis* and *Eucharis*. Waves 'jumped' between plates that were separated by up to 10 times the normal spacing. A ciné analysis of the characteristics of metachronal transmission in these preparations, as well as their histology, will be published elsewhere.

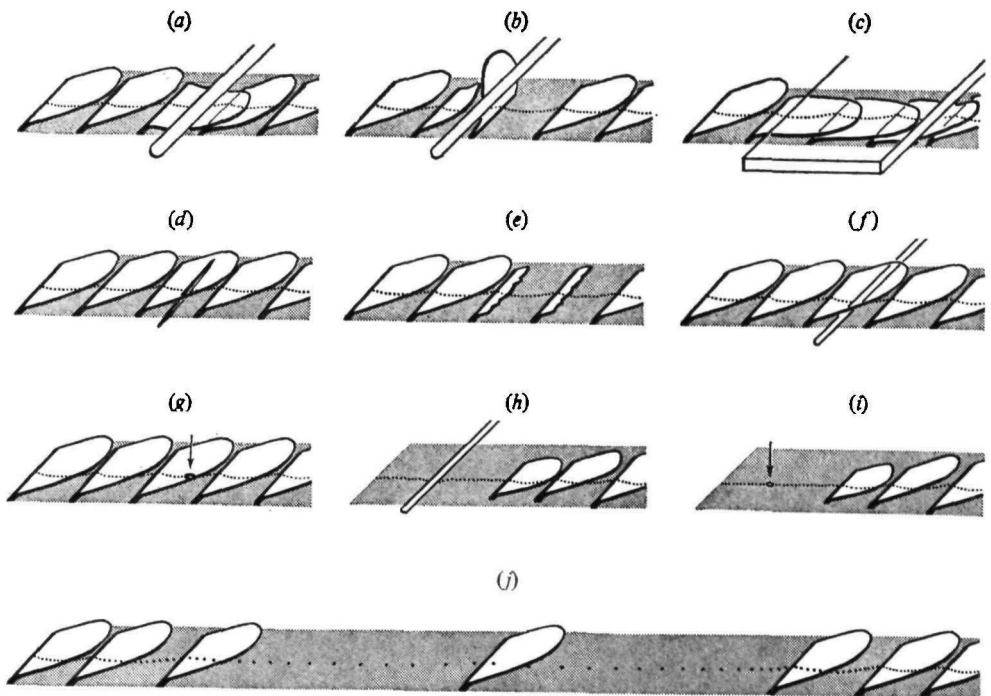


Fig. 3. Different experiments performed on ciliary coordination in comb rows and ciliated grooves. In all cases a lobate ctenophore with an interplate ciliated groove (represented by a dotted line between comb plates) is shown, with the aboral end of the comb row to the left. (a) Pressing down one comb plate with a rod; (b) holding one plate upright with a rod; (c) pressing down several plates with a Lucite strip; (d) cutting across the row between adjacent plates; (e) amputating comb plates near their bases; (f) pressing down cilia of the interplate ciliated groove with a rod; (g) cutting the interplate ciliated groove (arrow); (h) pressing down cilia of the ciliated groove with a rod; (i) cutting the ciliated groove (arrow); (j) increasing the distance between adjacent comb plates.

In identical experiments on *Pleurobrachia* and *Beroe*, waves were not transmitted across widely separated plates. Three to four days after the operation, tiny comb plates appeared between the widely spaced plates in all animals, and these eventually developed into full-sized new plates.

#### *Preventing movement in the interplate ciliated groove*

A fine glass rod was inserted under a comb plate and pressed against the interplate ciliated groove, approximately midway between adjacent plates (Text-fig. 3f). The motion of the overlying comb plate was not impaired in any way.

Preventing the movement of a small group of groove cilia in this way interrupted wave transmission in *Bolinopsis* and *Eucharis*. The plate immediately aboral to the glass rod was the last one to participate in waves originating from the aboral end of the row. Metachronal waves travelling along the interplate ciliated groove stopped at the glass rod, and were not transmitted to the remaining part of the groove oral to the block.

The block was reversible: upon lifting the glass rod away from the groove, co-ordination was immediately resumed throughout the row.



Control experiments consisted of pressing the glass rod against the tissue between adjacent plates, but not on the interplate ciliated groove. This treatment did not affect wave transmission.

Pressing completely across the region between two comb plates did not block wave transmission in *Beroë*, which lacks the interplate ciliated groove.

#### *Cutting the interplate ciliated groove*

A glass microneedle with a tip diameter slightly larger than the width of the interplate ciliated groove was forced through the groove and then withdrawn, thereby destroying a small region of it (Text-fig. 3g). Care was taken not to damage adjacent comb plates. In *Bolinopsis* and *Eucharis* this operation has the same effect on wave transmission as cutting the tissue between comb plates: waves coming from the aboral end of the row stop at the severed point in the groove.

#### *Preventing movement in the ciliated groove*

A fine glass rod was pressed across the ciliated groove, between the statocyst and the first plate of a row, thereby preventing the movement of a small group of cilia (Text-fig. 3h).

In both cydippids (*Pleurobrachia*) and lobates (*Bolinopsis*) this experiment has the same effect: wave transmission from the statocyst to the appropriate comb row is abolished. If all eight comb rows had been beating continuously, only the row connected to the groove which was pressed upon stopped beating. Upon lifting the rod up from the groove and permitting the underlying cilia to beat once again, the comb row connected to it immediately resumed activity. If the comb rows beat only infrequently before the experiment, preventing movement in a ciliated groove caused the comb row connected to it not to beat when the other member of the pair (connected to the same balancer) was active. Identical rhythms of beating in the two rows were restored when the rod was removed from the ciliated groove.

#### *Cutting the ciliated groove*

The ciliated groove was punctured with a fine glass needle, as described for the above experiment on the interplate ciliated groove (Text-fig. 3i). The same result was obtained with ctenophores belonging to both groups (i.e. *Pleurobrachia* and *Bolinopsis*): destroying a small region of the ciliated groove blocked metachronal transmission along it.

#### *Effect of excess Mg<sup>2+</sup> on co-ordination*

Animals were placed in either isotonic (7.5%) MgCl<sub>2</sub> or a 1:1 mixture of 7.5% MgCl<sub>2</sub> and sea water, in order to anaesthetize nervous functions. Excess Mg<sup>2+</sup> abolished several behavioural responses, including muscular contractions, tentacle withdrawal in cydippids and the temporary inhibition of comb-plate activity when the animal was disturbed. Nevertheless, initiation and transmission of metachronal waves continued under conditions of magnesium anaesthesia.

These observations support the idea that nerves are not involved in co-ordinating the cilia but are responsible for the inhibition of ciliary activity. This suggests that the neurociliary synapses seen in the electron microscope are inhibitory in nature.

*Effect of mechanically stimulating comb plates*

Mechanical stimulation of an inactive comb plate with a glass rod caused it to beat. This result was obtained with every species tested, with or without an interplate ciliated groove (i.e. *Mnemiopsis*, *Bolinopsis*, *Eucharis*, *Pleurobrachia* and *Beroe*).

## DISCUSSION

Ctenophores can be divided into two groups: those species in which the ciliated groove ends at the first comb plate (cydippids, beroids) and those in which the ciliated groove continues between successive plates along the row (lobates). Although this dichotomy was recognized long ago by Chun (1880), its significance for solving the problem of ciliary co-ordination in these animals has been largely ignored by subsequent investigators. The mechanical (Verworn, 1890) and neuroid (Parker, 1905) theories of comb-plate co-ordination were based on similar experiments carried out on ctenophores belonging to different groups. In this paper I have conducted a series of experiments on ciliary co-ordination using ctenophores belonging to both groups. The same experiments performed on animals from different groups gave opposite results, but among various ctenophores within one group the results were consistent (Table 1). Therefore, the mechanism of ciliary co-ordination in lobate ctenophores with an interplate ciliated groove is fundamentally different from that in cydippid and beroid ctenophores which lack an interplate ciliated groove. This finding explains the conflicting results of previous workers, who did not realize this difference and attempted to interpret the results on all ctenophores by one and the same mechanism of co-ordination.

In both groups of ctenophores the initiation and transmission of ciliary waves is not affected by excess magnesium ions at concentrations which completely block all sensory, muscular and cilia-inhibitory functions of the nervous system. This confirms the work of others (Lillie, 1908; Horridge, 1965*b*), and indicates that nerves are not involved in co-ordination of the cilia.

*The mechanism of co-ordination in ctenophores without an interplate ciliated groove*

The experiments on ctenophores lacking an interplate ciliated groove (cydippids and beroids) show that co-ordination of comb plates in this group is by mechanical interaction. Preventing the movement of plates in *Pleurobrachia* and *Beroe* blocked wave transmission. Although this finding suggests that mechanical coupling is responsible for the stimulation of one plate by the preceding one, motion of the comb plates may be necessary for some other reason. For example, a depolarization accompanying the beat could spread by epithelial conduction to trigger the next plate (Sleigh, 1968).

To separate the possible effects of beating *per se* from the mechanical effects of the beat, comb plates were amputated near the base. Waves were not transmitted past an amputated plate, even though the stub beat. Therefore comb-plate activity in itself is not important for co-ordination; rather, it is the movement of a full-sized plate which is essential for triggering the beat of the next one. That the effect of this movement is a short-range one, as would be expected for mechanical stimulation by local water currents, was shown by the interruption of wave transmission between

widely separated plates. The passage of waves across a cut made through the tissue between adjacent plates also shows that there is no direct internal pathway that conducts a co-ordinating 'impulse' in cydippids and beroids.

Recently, Sleigh (1973) obtained additional evidence for mechanical co-ordination in *Beroë*. By sliding a thin celluloid sheet between adjacent plates, water currents caused by the active stroke of one plate were prevented from exerting any mechanical drag on the following plate. The result was that the waves were blocked at the obstruction. Moreover, analysis of high-speed ciné films of plate beating in *Beroë* revealed that 'at the beginning of the movement of one plate the second plate beneath it is drawn up in the basal region where the two plates overlap, and it is several milliseconds later before the second plate begins its full active stroke. . . ' (Sleigh, 1973). Sleigh concluded that the passive upward movement of the base initiated the active process causing the plate to beat.

Other evidence indicates that a depolarization of the comb-plate cells is involved in plate activity, perhaps as an intermediate step between the mechanically induced motion at the base and the triggering of the active stroke. Horridge (1965*a*) recorded intracellular depolarizing potentials correlated with beating in the comb-plate cells of *Cestus*. My preliminary experiments have shown that cathodal stimulation of a comb plate in *Pleurobrachia* and *Bolinopsis* causes the plate to beat; the same result is obtained in excess magnesium ions at concentrations which abolish typical nervous responses.

The above data are consistent with the following model of comb-plate co-ordination in ctenophores which lack an interplate ciliated groove. The active stroke of one plate exerts mechanical drag on the next plate, causing a passive upward movement of its base. This deformation depolarizes the underlying comb-plate cells, triggering the active stroke of this plate, which leads to a mechanical effect on the next one, and so on. It will be important for this hypothesis to confirm Horridge's finding of depolarizations correlated with beating in other ctenophores of this group, such as *Pleurobrachia* and *Beroë*.

#### *The mechanism of co-ordination in ctenophores with an interplate ciliated groove*

The experiments on lobate ctenophores, in contrast to cydippids and beroids, argue against a mechanical explanation of comb-plate co-ordination in this group. Neither preventing the movement of comb plates, nor amputating plates, nor increasing the distance between them, stops wave transmission in these ctenophores which have an interplate ciliated groove. Therefore, mechanical interaction between the comb plates is not required for their co-ordination in lobates.

Preventing ciliary movement in the interplate ciliated groove, or cutting specifically this tract of cilia, does block wave transmission however. Thus, the interplate ciliated groove is the conducting pathway that co-ordinates the comb plates of lobate ctenophores.

The finding that activity of the cilia of the interplate ciliated groove is necessary for wave transmission suggests that these cilia may be co-ordinated by mechanical interaction. Other experiments, however, indicate that this may not be the case. When a long section of a comb row was removed, the wound closed rapidly by tissue spreading, thereby greatly increasing the distance between neighbouring plates. The

ciliated groove between the widely separated plates was correspondingly elongated but it nevertheless conducted waves. Moreover, ciné analysis of these preparations shows that the time interval between successive beats of two widely separated plates is the same as that between the beats of two normally spaced ones, resulting in an apparent increase in wave velocity across the wide space between the plates (Tamm, 1973, unpublished results). Since the spacing of the cilia in the groove between widely separated plates is presumably increased as well, the time interval between excitation of successive cilia in the groove must be independent of their spacing. This argues against mechanical interaction between the groove cilia, and suggests that they may be co-ordinated by an internal mechanism.

A two-step neuroid mechanism, as proposed by Sleight (1957) to explain metachrony in the membranelles of *Stentor*, would account for these observations on the interplate ciliated groove. Sleight found that the membranelles of *Stentor* are spaced closer together at the beginning of the row, but the time interval between excitation of adjacent membranelles remains constant throughout the row. He postulated an internal transmission mechanism that involves a faster conduction step and a slower ciliary excitation step, so that wave velocity depends on the number of membranelles excited per unit distance, not on their relative distance apart. This type of theory fits the data on the interplate ciliated groove, with one modification: beating must somehow be required for the conduction step, since ciliary groove activity is essential for wave transmission along the groove.

The ciliated groove connecting balancers to comb rows in all ctenophores is the conducting pathway that initiates beating of the first plate in each row. The mechanism of co-ordination in the ciliated grooves is probably the same as that in the interplate ciliated grooves found only in lobate ctenophores, since both ciliated tracts are structurally identical and respond to experimental treatments in the same way.

An important problem is how the ciliated groove stimulates the first comb plate of each row to beat, and, in lobates, how this process is repeated at successive plates along the rows. It seems unlikely that the large comb plates could be stimulated mechanically by water currents created by the movement of the small cilia of the ciliated grooves. Although the most aboral comb plates in a row are not as large as the others, their cilia are still more than  $\times 100$  longer than the cilia of the groove. More convincingly, the interplate ciliated groove of lobates stimulates full-sized plates all along the row. Therefore, excitation at the junction between ciliated groove and comb plate is probably due to an internal mechanism, agreeing with the evidence described above for internal co-ordination within the groove. Since cathodal stimulation of comb plates in *Bolinopsis* causes them to beat, the ciliated groove may excite a plate by depolarizing the comb-plate cells. The characteristic widening of the ciliated groove, and of the interplate ciliated groove, at the aboral side of the plates may act to amplify such an electrical signal.

Likewise, the means by which the beat of a plate is transmitted to the adjacent cilia of the next groove cannot be by mechanical interaction. In lobates waves travel along successive interplate ciliated grooves past plates which are prevented from moving; yet the interplate ciliated groove does not run through the plates. Clearly, an internal conducting pathway must exist between adjacent interplate ciliated grooves. This pathway may or may not be independent of the mechanism that excites the plates to

beat. One possibility is that the long microtubule-filled processes which run orally under the comb-plate cells (Pl. 1A, C) may connect adjacent interplate ciliated grooves, and may provide this conducting pathway. If these processes of the interplate ciliated groove cells are indeed specialized for transmitting electrical signals between successive grooves, they would represent a unique intermediate stage between epithelial and nervous conduction.

If wave transmission in the ciliated groove and interplate ciliated groove is not due to mechanical interaction, as seems likely, ciliary co-ordination in lobates may be entirely a neuroid process. The only step which is unknown in both types of ctenophores is the means by which the balancers initiate waves in the ciliated grooves. The balancers are stimulated mechanically by the statolith, but they may trigger activity at the beginning of the ciliated grooves by either mechanical forces or by an electrical process. This problem is dealt with elsewhere (Tamm, 1973, in preparation).

#### *The reason for two mechanisms of co-ordination*

This work has demonstrated the existence of two different mechanisms of ciliary co-ordination in ctenophores, correlated with the presence or absence of an interplate ciliated groove. A major question arising from this finding is: why are there two different mechanisms?

Chun (1880) believed that the presence or absence of an interplate ciliated groove depended on the distance between plates. He found that the bases of the comb plates were very close together in cydippids, beroids, and cestids, all of which lack an interplate ciliated groove. In lobates and young cestids which have an interplate ciliated groove, however, Chun found that the plates were spaced farther apart. Chun thought that the interplate ciliated groove acted as a nerve to conduct metachronal impulses rapidly between widely spaced plates, whereas in ctenophores with closely spaced plates the impulse could be transmitted directly without the need for a fast pathway.

From my experience, however, it is doubtful whether any parameter of ciliary topology, such as the distance between adjacent plates, or the degree of overlap of adjacent plates, etc., can be correlated with the presence or absence of an interplate ciliated groove, and, thereby, with a particular mechanism of co-ordination. Perhaps, as Samassa (1892) has suggested, one condition is evolutionarily more primitive than the other. In this regard, it is interesting that free-swimming cydippid embryos of *Bolinopsis* have not yet developed an interplate ciliated groove, but co-ordinate the movements of their comb plates nevertheless (S. L. Tamm, unpublished). The mechanism of metachronism in this lobate therefore changes from mechanical to neuroid during development. Chun (1880), however, found just the opposite in *Cestus*: an interplate ciliated groove is present in young cestids, but not in fully grown ones. It is clear that either mechanism of co-ordination can be considered the more primitive one if the embryonic condition is assumed to represent the ancestral state.

The finding that the comb plates of lobate ctenophores with neuroid co-ordination are as sensitive to mechanical stimulation as those of cydippids and beroids may be relevant here. It is difficult to understand why the comb plates of ctenophores which do not require mechanical interaction between plates should be mechanosensitive, unless one assumes an evolutionary basis. Again, however, we do not know the direc-

tion of evolution. If the primitive condition were mechanical co-ordination, mechanosensitivity of comb plates in present-day neuroid ctenophores may represent a vestigial remnant. On the other hand, mechanical co-ordination might have evolved from a neuroid mechanism, and the presence of mechanosensitivity of comb plates in lobates may foreshadow the change to a mechanical process of metachronism in this group.

## SUMMARY

1. An experimental analysis of ciliary metachronism was performed on ctenophores of two types: those with a continuation of the ciliated groove running between adjacent comb plates (lobates), and those in which the ciliated grooves end at the first plate of each comb row (cydippids and beroids).

2. The results showed that the comb plates of cydippids and beroids are co-ordinated by mechanical forces arising from the movement of the plates themselves. Only along the ciliated grooves and at their junctions with the comb rows is a neuroid conduction process likely in this group of ctenophores.

3. Ctenophores with an interplate ciliated groove, on the other hand, do not depend on mechanical interaction between active plates for co-ordination. Instead, the lobates use neuroid conduction at the junction between the interplate ciliated groove and the comb plate, at the junction between adjacent interplate ciliated grooves, and probably along the ciliated grooves and interplate ciliated grooves as well. Ciliary co-ordination may therefore be entirely neuroid in lobate ctenophores.

4. In both types of ctenophores the comb plates may be triggered to beat by depolarization of the comb-plate cells. Electrical excitation of a plate could be caused by mechanical deformation of its base arising from the motion of the preceding plate (in cydippids and beroids), or by a neuroid process at the interplate ciliated groove-comb plate junction (in lobates).

5. These findings resolve a long-standing controversy over the mechanism of ciliary co-ordination in ctenophores.

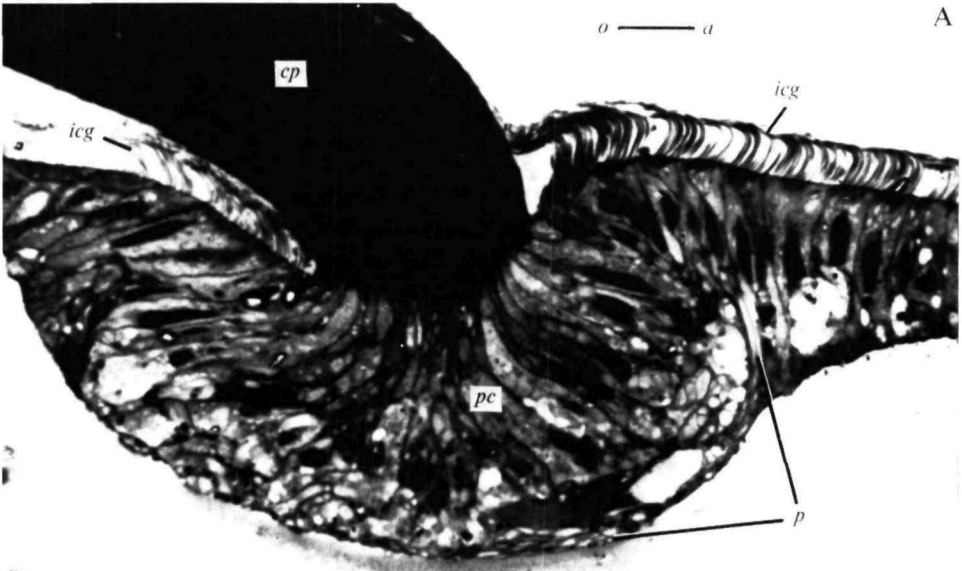
I am grateful to the following people who provided help and facilities at various marine stations: Dr Frank Child, Marine Biological Laboratory, Woods Hole, Mass.; Dr G. A. Horridge, Gatty Marine Laboratory, St Andrews, Scotland; Dr Peter Dohrn and his staff, Stazione Zoologica, Naples, Italy; and Dr Robert Fernald, Friday Harbor Laboratory, Friday Harbor, Washington.

Special thanks are due to my wife who spent many hours collecting ctenophores.

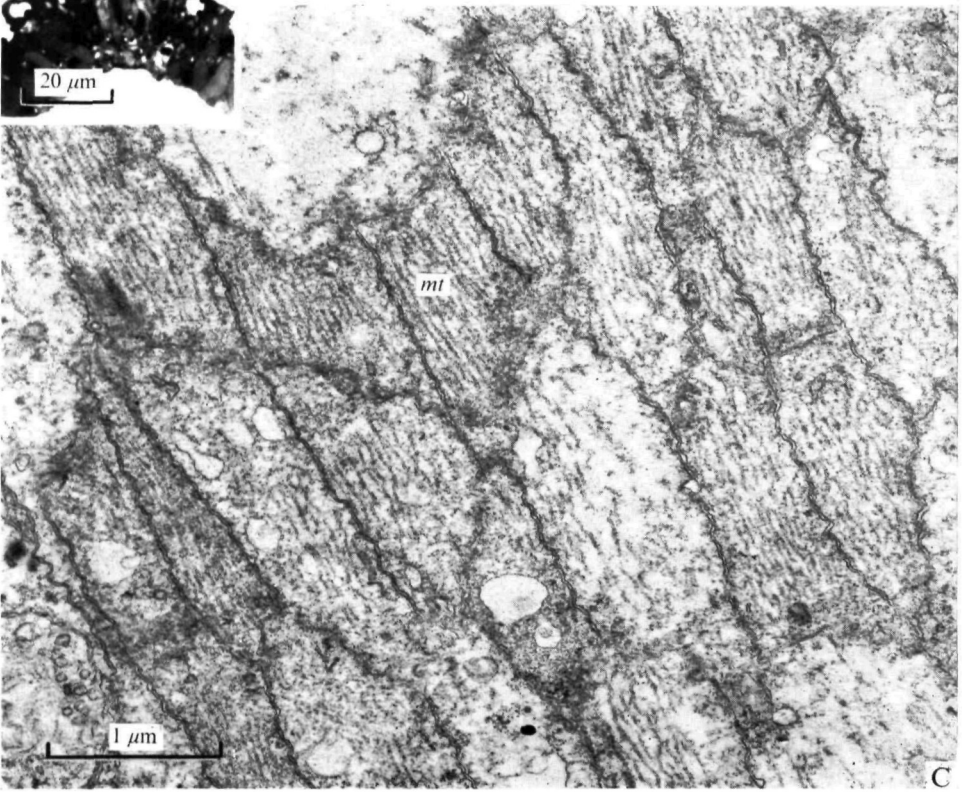
This work has been supported by an NSF Postdoctoral Fellowship, and NSF Research Grants GB-8714 and GB-28821, awarded to the author.

## REFERENCES

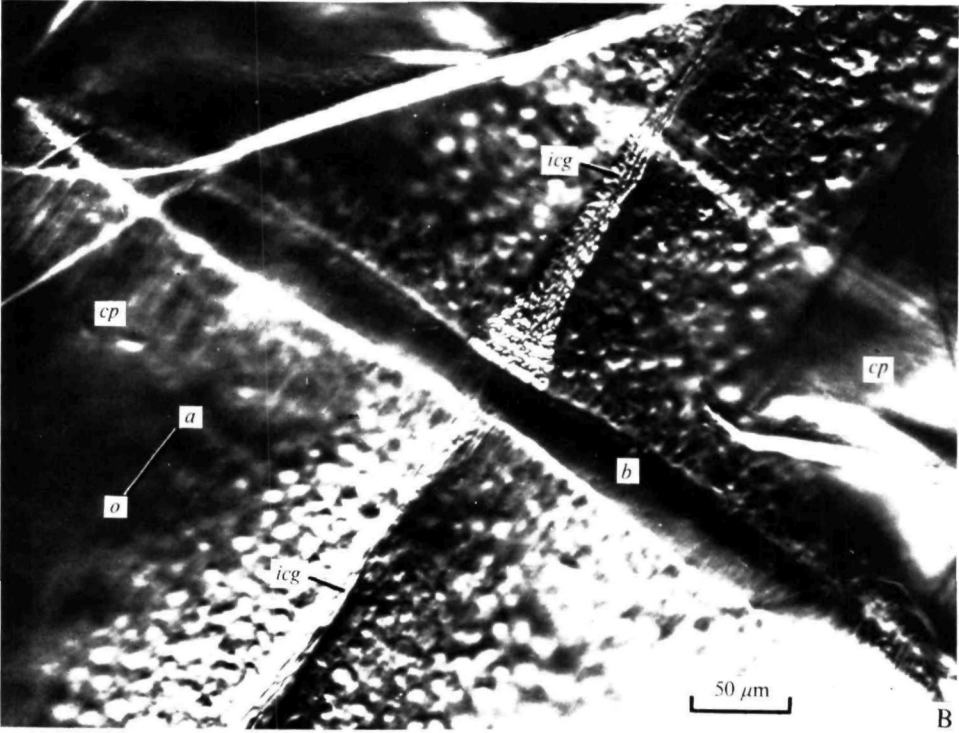
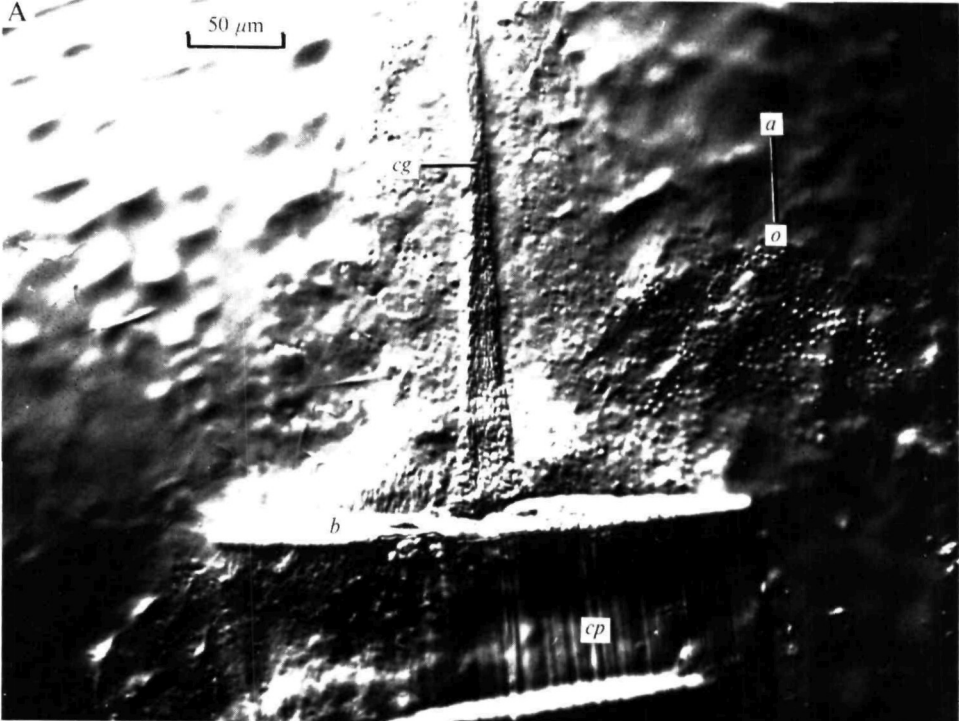
- AFZELIUS, B. A. (1961). The fine structure of the cilia from ctenophore swimming plates. *J. biophys. biochem. Cytol.* **9**, 383-94.
- CHILD, C. M. (1933). The swimming plate rows of the ctenophore, *Pleurobrachia*, as gradients: with comparative data on other forms. *J. comp. Neurol.* **57**, 199-252.
- CHUN, C. (1880). *Die Ctenophoren des Golfes von Neapel und der angrenzenden Meeres-Abschnitte*. Leipzig: Engelmann.
- HEIDER, K. (1927). Vom Nervensystem der Ctenophoren. *Z. Morph. Oekol. Tiere* **9**, 638-78.
- HERTWIG, R. (1880). Ueber den Bau der Ctenophoren. *Jena Z. Naturw.* **14**, 313-457.



A



C





- HORRIDGE, G. A. (1965*a*). Intracellular action potentials associated with the beating of the cilia in ctenophore comb plate cells. *Nature, Lond.* **205**, 602.
- HORRIDGE, G. A. (1965*b*). Relations between nerves and cilia in ctenophores. *Am. Zool.* **5**, 357-75.
- HORRIDGE, G. A. (1966). Pathways of co-ordination in ctenophores. *Symp. Zool. Soc. Lond.* **16**, 247-66.
- HORRIDGE, G. A. & MACKAY, B. (1964). Neurociliary synapses in *Pleurobrachia* (Ctenophora). *Q. Jl microsc. Sci.* **105**, 163-74.
- HORRIDGE, G. A. (1971). Primitive examples of gravity receptors and their evolution. In *Gravity and the Organism* (ed. S. A. Gordon and M. J. Cohen), pp. 203-21. University of Chicago Press.
- KINOSHITA, H. & MURAKAMI, A. (1967). Control of ciliary motion. *Physiol. Rev.* **47**, 53-82.
- KNIGHT-JONES, E. W. (1954). Relations between metachronism and the direction of ciliary beat in metazoa. *Q. Jl microsc. Sci.* **95**, 503-21.
- LILLIE, R. S. (1908). The relation of ions to contractile processes. II. The role of calcium salts in the mechanical inhibition of the ctenophore comb plate. *Am. J. Physiol.* **21**, 200-20.
- MACKIE, G. O. (1970). Neuroid conduction and the evolution of conducting tissues. *Q. Rev. Biol.* **45**, 319-32.
- PARKER, G. H. (1905). The movements of the swimming-plates in ctenophores, with reference to the theories of ciliary metachronism. *J. exp. Zool.* **2**, 407-23.
- SAMASSA, P. (1892). Zur Histologie der Ctenophoren. *Arch. mikr. Anat.* **40**, 157-243.
- SLEIGH, M. A. (1957). Further observations on co-ordination and the determination of frequency in the peristomial cilia of *Stentor*. *J. exp. Biol.* **34**, 106-15.
- SLEIGH, M. A. (1963). Movements and co-ordination of the ciliary comb plates of the ctenophores *Beroë* and *Pleurobrachia*. *Nature, Lond.* **199**, 620-1.
- SLEIGH, M. A. (1966). Some aspects of the comparative physiology of cilia. *Am. Rev. resp. Dis.* **93** (Suppl.), 16-31.
- SLEIGH, M. A. (1968). Metachronal co-ordination of the comb plates of the ctenophore *Pleurobrachia*. *J. exp. Biol.* **48**, 111-25.
- SLEIGH, M. A. (1969). Co-ordination of the rhythm of beat in some ciliary systems. *Int. Rev. Cytol.* **25**, 31-54.
- SLEIGH, M. A. (1973). Features of ciliary movement of the ctenophores *Beroë*, *Pleurobrachia*, and *Cestus*. (In the Press.)
- VERWORN, M. (1890). Studien zur Physiologie der Flimmerbewegung. *Pflüger's Arch. ges. Physiol.* **48**, 149-80.

## EXPLANATION OF PLATES

## PLATE 1

Structural features of the comb plates and interplate ciliated groove of *Eucharis*.

(A) Median longitudinal thick section through a comb row at a comb plate (*cp*). The cilia of the interplate ciliated groove (*icg*) stop at the aboral side of the plate and begin again on the oral side; at rest they point toward the oral end of the body, as do the comb plates. Long processes (*p*) extend orally under the polster cells (*pc*) from cells of the interplate ciliated groove at its widening on the aboral side of the comb plate. *m*, Mesoglea; *a-o*, aboral-oral direction.

(B) Transverse thick section through an interplate ciliated groove, approximately midway between plates. The groove consists of ciliated epithelial cells, and is several cells wide.

(C) Thin section through processes of interplate ciliated groove cells, as shown at lower magnification in (A). The processes are filled with parallel arrays of microtubules (*mt*), and run together in a bundle.

(A) and (B) Light micrographs of material fixed in glutaraldehyde, post-fixed in osmium tetroxide, embedded in Araldite, thick-sectioned (0.5  $\mu$ m), and stained with 1% alkaline toluidine blue. (C) Electron micrograph of material fixed and embedded as in (A) and (B), thin-sectioned (silver-gold), and stained with lead citrate and uranyl acetate.

## PLATE 2

Comparison of the ciliated groove and interplate ciliated groove in living animals.

(A) Junction of the ciliated groove (*cg*) with the first comb plate (*cp*) of a comb row in *Pleurobrachia*. The ciliated groove widens at its attachment to the base (*b*) of the plate. In this species the ciliated groove does not continue past the first plate of each row.

(B) Junction of the interplate ciliated groove (*icg*) with a comb plate (*cp*) in *Bolinopsis*. The interplate ciliated groove also widens at the aboral side of the base (*b*) of the plate, but continues on the oral side, running the entire length of the comb row. The cilia of the interplate ciliated groove, like those of the ciliated groove, do not pass through the comb plate. *a-o*, Aboral-oral direction.

Nomarski interference-contrast optics and electronic flash ( $\frac{1}{2000}$  sec. 60 wS).