

## METACHRONAL CO-ORDINATION OF THE COMB PLATES OF THE CTENOPHORE *PLEUROBRACHIA*

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### INTRODUCTION

The locomotory organs of *Pleurobrachia* are the swimming plates which are carried in eight comb rows of approximately equal length (Fig. 1). Since the effective stroke of the comb plates is directed aborally in normal movement, the animal swims mouth-foremost. The activity of the comb plates is co-ordinated into waves in which the plates beat in sequence from the aboral to the oral end of the comb row; they therefore show antiplectic metachronism.

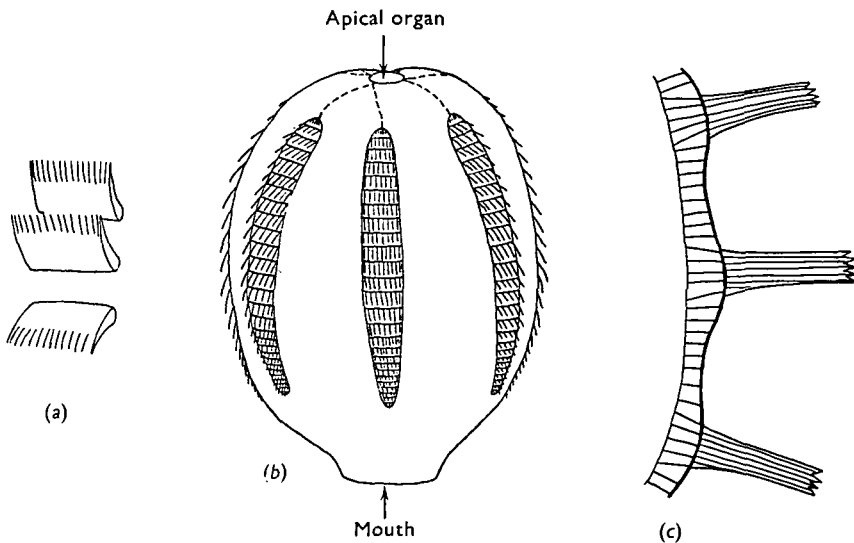


Fig. 1. The arrangement of the comb plates of *Pleurobrachia*. (a) Three comb plates showing their shape and relative position. (b) The comb rows and their relations to the ciliated grooves (dotted) and apical organ. (c) A diagrammatic section along the length of the comb row passing through the bases of three comb plates.

A broad band of cells underlies the comb row and the comb plates occur at more or less equal intervals along the band, except near the ends of the row where the comb plates are closer together. The comb plates are borne on tall columnar (polster) cells, each carrying 15-50 cilia at their outer ends. The polster cells are closely packed together in a ridge which lies transversely to the comb row, and which may bear altogether around 100,000 cilia; all of these cilia move together as a single comb plate which may be 0.7 mm. wide and 0.5 mm. tall in *Pleurobrachia* 6.5 mm. long. At their

inner ends the polster cells make synaptic connexions with fine nerve fibres, believed by Horridge & Mackay (1964) to be part of the ectodermal nerve net which transmits inhibitory stimuli to the comb rows. The ectodermal cells lying between the comb plates and within the comb rows bear cilia in some ctenophores, e.g. *Leucothea*, but not in *Pleurobrachia* (Horridge, 1966). From the aboral end of each comb row a ciliated groove passes towards the aboral pole and joins with another ciliated groove before terminating at one of the balancer cilia in the apical organ. The cells of the ciliated groove are elongated bipolar cells with a dilated central region which bears cilia (Horridge, 1965*b*). Each of the balancer cilia is a cluster of 100–200 cilia forming a conical bundle, and the four balancer cilia support the statolith of the apical organ. The balancers, ciliated grooves and comb plates form the swimming system of the ctenophore; throughout this system the cilia are of an unusual type in which there are links between two opposite peripheral fibrils of the axoneme and the ciliary membrane (the compartmenting lamellae of Afzelius, 1961)—the other cilia of ctenophores lack these links. The cilia of the ciliated grooves are not fused, but in the balancers and comb plates the cilia are partially fused to form large compound structures, being most clearly united in the plane of the compartmenting lamellae. The effective stroke of the beat of the balancer and ciliated groove cilia, as well as of the comb plates, is directed towards the aboral pole of the animal. When a balancer gives a beat, a wave of activity passes along the associated ciliary grooves and comb rows, and each cilium of the system performs a single beat during the passage of the wave.

The mechanism of the co-ordination responsible for this metachronal wave of activity has been the subject of several theories. There is evidence from animals of diverse groups that the propagation of metachronal waves in ciliary tracts is in some cases mediated by viscous-mechanical forces outside the organism and in other cases by some internal conduction process (Sleigh, 1966*b*). Metachronism of the comb plates of the ctenophore *Beroë* was explained by Verworn (1890) as the result of a mechanical process, because he found that if the movement of one or more comb plates is prevented, the wave will not pass the motionless plate. However, in this case the beat and the wave propagation are in opposite directions, the cilia are far apart, and they often show a rest between successive beats, so that actual mechanical contact between adjacent comb plates at a suitable time during the normal beat seems unlikely (Fig. 2*a*). Parker (1905), on the other hand, was led to conclude that in *Mnemiopsis* the co-ordination was due to a cell-to-cell (neuroid) transmission of the ciliary activation, since in his experiments the wave was still transmitted when a comb plate was missing or was prevented from beating by mechanical restraint or local cooling. In *Pleurobrachia* and *Beroë* Child (1933) found that the prevention of movement of a single plate would normally block the passage of the waves, but in some cases an incomplete blockage was observed when some waves passed the stationary plate and some did not. These and other observations led Child to conclude that metachronal co-ordination was the result of the passage of an internal impulse from plate to plate along the row by a path which was not continuous along the row because it involved some activity in the bases of the plates as well as conduction between the bases. This idea is remarkably similar to the step-by-step conduction mechanism proposed for the metachronism of the membranelles of *Stentor* (Sleigh, 1957) when this author was not aware of the theory of metachronism in ctenophores put forward

by Child. Both authors pointed out that the idea that the metachronal impulse is renewed or reinforced by the activity of successive cilia should be distinguished from the simpler notion that the impulse passes the length of the row exciting each cilium as it passes.

The beating of a comb plate is associated with an electrical depolarization of the cells bearing the cilia; this has been investigated by Horridge (1965 *a, b*, 1966). Intracellular microelectrodes record a resting potential of about 40 mV. (inside negative), and the depolarization which coincides with the passage of a wave of ciliary beating takes the form of a positive-going 'action potential' of up to about 30 mV. with a steep rising phase and a slow falling phase, the latter taking several seconds in *Cestus*. The ciliary beat occurs at about the time that the action potential starts to rise, and is complete by about the time that the action potential reaches its peak. When ciliary waves follow in rapid succession, the action potentials stand upon the falling phases of previous beats, but they have not been seen to exceed the resting potential. Electrical excitability of the cells remains after treatment with excess  $Mg^{2+}$  which abolishes the nervous inhibition of ciliary activity; there is no evidence of true synaptic connexions between adjacent cells of the comb plate system. Horridge (1966) concludes that 'the transmission between ciliated cells is not nervous but is from cell to cell', and that 'transmission depends on the depolarizing current which accompanies each beat'.

Similar explanations have been proposed for metachronal conduction in the membranelles of *Stentor* and the comb plates of ctenophores, but in *Stentor* a whole train of complete metachronal waves occurs at the surface of a single cell, while in the ctenophore a very large number of cells is involved in each metachronal wave. This paper gives further information about the metachronism of ctenophore comb plates and permits a further comparison of the two systems.

#### METHODS

The waves of activity of the comb plates tend to occur at irregular intervals and move rapidly along the rows, so that the only adequate method of measuring the parameters of ciliary activity is the analysis of cinematograph films. The observations recorded here were made on *Pleurobrachia* which were either allowed to swim freely in a small Perspex box containing sea water or an experimental solution, or were pinned to a cork which was fixed in the box. Short lengths of 16 mm. cine film were taken at 60 frames/sec. using extension tubes and a 75 mm. lens. Analysis of cine films from sequences of single projected frames is a time-consuming process, so that it has not been possible to repeat a number of the experimental treatments enough times for the results to be viewed in more than a qualitative way. The temperature was measured at frequent intervals, and in no experiment was it allowed to alter by more than 1° C.

#### OBSERVATIONS

##### *Activity of the comb plates in normal sea water*

##### (a) *Forward waves*

The frequency of the passage of metachronal waves along the comb rows was very variable; in fact it was unusual for the time interval between successive waves to remain constant for even two or three waves at a time, although occasionally ten to

twenty waves passed with steady frequency. Because of this variation in time interval it was necessary to make an estimate of the 'frequency' for every beat as an indication of the level of excitation of the cilia. The best available measure of this frequency seemed to be the reciprocal of the time interval since the previous beat. Time intervals between beats of 0.5–0.1 sec. (representing frequencies of 2–10 beats/sec.) were usual, but occasionally the cilia beat only once in several seconds or beat as fast as 15 or 16 times/sec.

In the quiescent state the comb plates all lay with their tips directed orally; the rapid effective stroke in the aboral direction was followed by a slower recovery stroke

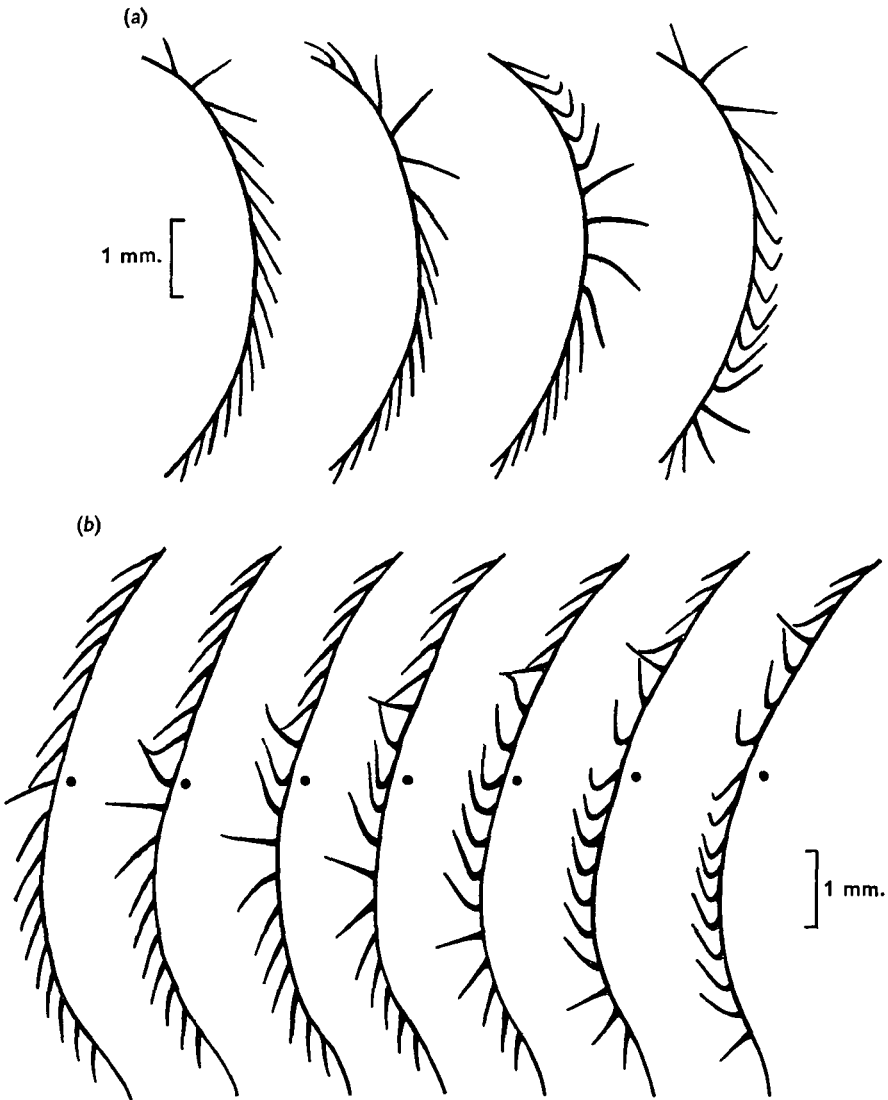


Fig. 2. Movements of the comb plates of *Pleurobrachia*. (a) The passage of a forward wave down the comb row. (b) an abnormally active cilium (indicated by the dot) is acting as a pacemaker for a reverse wave conducted up the row as well as a forward wave conducted down the row.

during which the comb plate returned to its original resting position (Fig. 2*a*). When the frequency of beat was low there was a considerable interval between the end of one beat and the beginning of the next. During rapid beating there was a decrease in the duration of the beat as well as a shortening or disappearance of the interkinetic period. In the intact row the comb plates were normally totally dependent on the activity of the aboral pacemaker (balancer cilium), but occasionally a comb plate showed an increased excitability and acted as a pacemaker for a rhythm which was propagated to the comb plates in the part of the row oral to the excited cilium, and which was faster than the rhythm in the aboral part of the row.

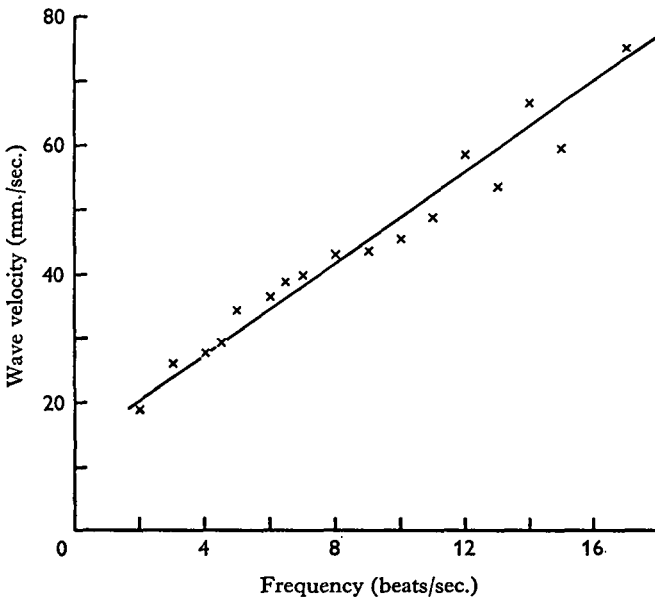


Fig. 3. The relation between wave velocity and frequency of beat. 17° C.

Visual examination suggested that waves of low frequency passed slowly and those of high frequency passed quickly. Figures obtained from the analysis of cine films showed that this was no illusion; there was a very marked and almost linear increase in velocity of the metachronal waves as the frequency of beat increased (Fig. 3). The slope of the line relating wave velocity with frequency varied between about 3 and 6, showing a rough correlation with the size of the animal. There was a tendency for the slope of the line to decrease at the highest frequencies, suggesting an approach to a maximal wave velocity in the region of 60–80 mm./sec. in *Pleurobrachia* of this size. At an average wave velocity of 40 mm./sec each wave would stimulate comb plates at a rate of about 100/sec. Wave velocities below 20 mm./sec. were seldom recorded, even at very low frequencies.

(b) *Reverse waves*

When a comb plate within the row became a pacemaker, reverse waves were often propagated from it towards the aboral end of the row. The effective stroke of cilia in the reverse waves observed here was directed aborally, as in the normal beat, so that

this reverse metachronism was symplectic. The frequency of reverse waves lay within the normal range, but the propagation of the waves was different from that of the forward waves; over the frequency range from 4 to 16 beats/sec. the wave velocity of reverse waves increased only a little, while the velocity of forward waves of the same comb row increased very considerably (Fig. 4). Perhaps the most convincing demonstration of the difference between forward and reverse waves was seen when a

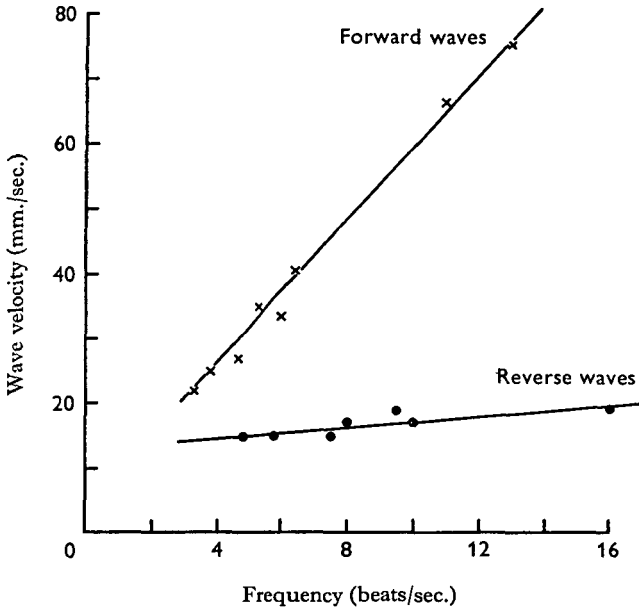


Fig. 4. The relation between wave velocity and frequency of both forward and reverse waves, measured on the same animal.  $17.5^{\circ}\text{C}$ .

comb plate near the middle of the row acted as a pacemaker and waves of identical frequency were propagated aborally as reverse waves and orally as forward waves (Fig. 2*b*); when the frequency of beating was reasonably high, the forward waves travelled much more quickly than the reverse waves. Figure 2*b* also shows that in the reverse wave the beating cilium actually touched the next cilium in the aboral direction before that cilium commenced its beat; it could therefore transmit the excitation mechanically. If such mechanical excitation is involved, the small size of the increase in wave velocity for a large increase in frequency would be expected, since the duration of the first part of the effective stroke up to the time of touching the next cilium is thought to change little over a wide frequency range. Some reverse waves were seen in ouabain solutions which altered the wave velocity of forward waves, but the reverse waves in these solutions moved at the same speed as reverse waves in sea water at the same frequency.

When a reverse wave met a forward wave it was usual for both waves to disappear. In a few cases the forward wave travelled on down the row, re-exciting cilia which had been participating in a reverse wave; these may be cases where the reverse wave had just died out before the forward wave reached it, for many reverse waves failed to propagate themselves as far as the aboral end of the row.

Child (1933) has reported that under conditions of extreme stimulation the reverse waves may involve also a reversal of the effective stroke and a reversal of swimming direction, but this was not observed here.

(c) *Damaged comb rows*

When the continuity of a row was interrupted by accidental damage or by a cut, the two parts of the row behaved independently. A comb plate at the aboral end of the isolated region acted as a pacemaker initiating waves which passed towards the mouth. The characteristics of these waves were normal; measurements made on the complete row before cutting and on both aboral and oral regions of the row after cutting all lay on the same line relating wave velocity with frequency.

*Activity of the comb plates in experimental solutions*

Whole *Pleurobrachia* were allowed to swim in solutions of various substances known to modify the activity of excitable structures by depolarizing cells or by affecting synaptic or other aspects of transmission. The behaviour of the comb plates in the various solutions was analysed by measuring the frequency and wave velocity. Since the frequency was normally rather variable, only cautious general comments on the effects of a solution on the frequency of beat of the pacemaker are usually possible, but by comparison of the relation between the frequency and wave velocity in sea water and in a test solution it should be possible to make more certain assertions about the effects of a treatment on the propagation of metachronal waves, especially where treatments with a similar action produce similar effects.

(a) *Adrenaline*

Adrenaline accelerates the ciliary activity on the tentacles of *Metridium* (ten Cate, Coomans & Walop, 1955), and it was found to increase both the frequency and the wave velocity of membranelles of *Stentor* (Sleigh, 1962).

In a  $5 \times 10^{-7}$  M solution of adrenaline ctenophore comb plates showed a steady continuous beat at a frequency up to 20% higher than the average frequency in sea water, but the activity later returned to a normal level with fluctuations in activity. At concentrations of  $5 \times 10^{-6}$  M and  $5 \times 10^{-5}$  M adrenaline the abnormally regular activity was still observed, but the frequency was often below the average in normal sea water and fluctuations occurred fairly quickly. Measurements relating the wave velocity with frequency in the original sea water, in  $5 \times 10^{-7}$  M,  $5 \times 10^{-6}$  M and  $5 \times 10^{-5}$  M adrenaline solutions and in sea water after these solutions were plotted together on a graph and all lay on the same line.

(b) *Serotonin (5-hydroxytryptamine)*

The ciliary beat and wave velocity of the lateral cilia of lamellibranch gills are accelerated by serotonin (Aiello, 1960); it also accelerates the beat of the velar cilia of nudibranch veligers (Buznikov & Manukhin, 1960). Serotonin is believed to be a neurohumoral transmitter in a variety of invertebrates.

Comb plates beat more rapidly in a  $2.5 \times 10^{-5}$  M solution of serotonin creatinine sulphate. Four to five minutes after addition of the serotonin solution they were beating about 60% faster than the average frequency in sea water during the previous

hour, but by 10 min. later the frequency was dropping back towards the original level, being then only 35 % above the original rate. There was a smaller increase in frequency in  $2.5 \times 10^{-4}$  M serotonin. On a graph showing the relation between wave velocity and frequency for this experiment, the points plotted for sea water initially, for serotonin concentrations of  $2.5 \times 10^{-5}$  M and  $2.5 \times 10^{-4}$  M at various times after their addition and for sea water after removal of serotonin all lay on the same line and gave no indication of any action of serotonin on the conduction process.

(c) *Acetylcholine and eserine*

The nervous inhibition of comb plates appears to act directly on the polster cells, and the nerves involved may be cholinergic. Acetylcholine has been found to have a depressant effect on the cilia of *Metridium* (ten Cate *et al.* 1955), and Aiello (1960) found that while eserine (alone or with acetylcholine) depressed the frequency of beat of *Mytilus* gill cilia, it did not alter the wave velocity.

During normal activity the comb plates of *Pleurobrachia* occasionally stopped beating and frequency tended to fluctuate considerably. The characteristic response to the addition of eserine or acetylcholine was to stabilize the beating activity at a fairly low rate and abolish the intermissions for at least the first few minutes. The effect was found in  $10^{-5}$  M eserine (physostigmine sulphate),  $5 \times 10^{-5}$  M and  $5 \times 10^{-4}$  M acetylcholine chloride and in a mixture containing  $5 \times 10^{-5}$  M acetylcholine and  $3 \times 10^{-5}$  M eserine. The last was most effective; it reduced the rate of beat from an average of about 8 beats/sec. to an average of 3 beats/sec., and on return to sea water the frequency rose to around 9 or 10 beats/sec. In acetylcholine and eserine solutions the wave velocity was depressed to a little below the level recorded at the same frequency in sea water before the experiment; the wave velocity remained below the original level after the return of the animal to sea water, but the differences in level were small and it is doubtful whether any significance should be attached to them.

(d) *Strychnine*

The ciliary activity of frog palate cilia is depressed by strychnine (Ishikawa & Ohzono, 1931), and this drug also suppresses the nervous inhibition of nudibranch velar cilia (Carter, 1926). Strychnine has a variety of effects on other excitable cells; for example, at high concentrations (over 2 g./l.) conduction by frog axons breaks down, at 20 mg./l. and above the amplitude of the action potential of frog muscle is decreased, and at 5 mg./l. and above it causes a decrease in the sensitivity to acetylcholine of the post-synaptic membrane of the frog neuromuscular junction without causing depolarization (Alving, 1961).

The effect of a 100 mg./l. solution of strychnine sulphate was to reduce the activity of comb plates. Within a minute or so the bursts of activity became sporadic and the frequency of waves during the periods of activity progressively declined until after about 20 min. even stimulation failed to provoke more than a very slow beat. The wave velocity was initially within the normal range; later it was very slow, but since the frequency was lower than that recorded in sea water before the addition of strychnine, it is uncertain whether there was a genuine reduction in wave velocity below the normal level as well as a reduction in frequency. After several washes in sea



water the animal resumed activity and at the end of five minutes the frequency and wave velocity were normal.

(e) *Magnesium chloride*

Magnesium salts are used as anaesthetics for marine invertebrates, and the  $Mg^{2+}$  ion is known to interfere with the nervous control of effector organs. The observations of Lillie (1908) indicated that if the Mg:Ca ratio was increased far above the normal (5:1), the rhythmic activity of ctenophore comb plates was increased and inhibition no longer occurred. Horridge (1965*b*) has also used excess  $Mg^{2+}$  to abolish the inhibition of comb plates without preventing metachronal co-ordination. The anaesthetic effect of  $Mg^{2+}$  only becomes marked when the Mg:Ca ratio reaches 20:1 (Mazia, 1940); presumably at this stage  $Mg^{2+}$  has almost completely replaced  $Ca^{2+}$  in the cells.

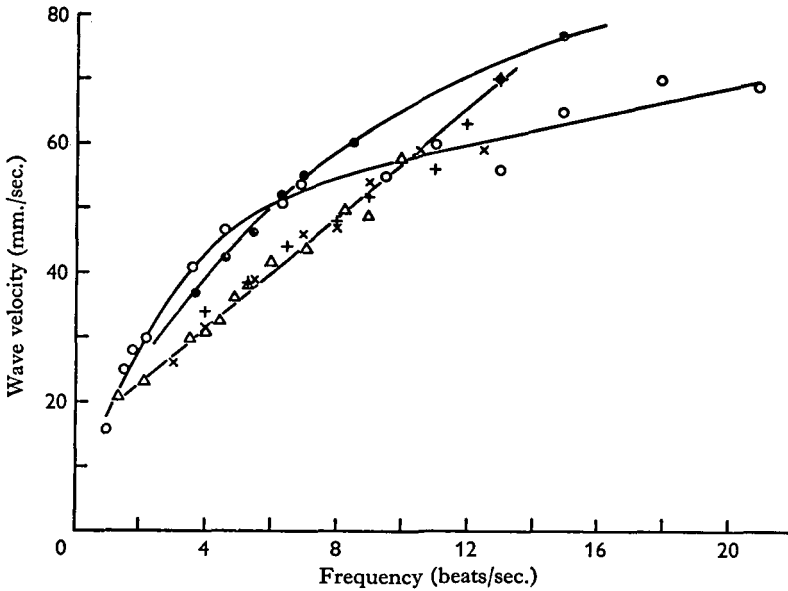


Fig. 5. The effect of increased  $Mg^{2+}$  concentration on the relation between wave velocity and frequency. 16° C.

|   | Solution             | $Mg^{2+}$<br>concentration | Ratio<br>Mg/Ca |
|---|----------------------|----------------------------|----------------|
| x | Sea water            | 54 mM                      | ~ 5            |
| ● | Sea water + $MgCl_2$ | 69 mM                      | ~ 7            |
| ○ | Sea water + $MgCl_2$ | 113 mM                     | ~ 12           |
| △ | Sea water + $MgCl_2$ | 238 mM                     | ~ 30           |
| + | Sea water            | 54 mM                      | ~ 5            |

A solution of magnesium chloride isotonic with sea water was mixed with sea water in various proportions to obtain test solutions with increased  $Mg^{2+}$  concentration. The results of a series of observations on the activity of comb plates of *Pleurobrachia* in sea water and mixtures containing isotonic magnesium chloride are shown in Fig. 5. A slight (25%) or moderate (100%) increase in  $Mg^{2+}$  concentration caused an increased wave velocity at lower frequencies; where the concentration was doubled there was a decrease in wave velocity at the higher frequencies. With further increase in

$Mg^{2+}$  concentration these effects disappeared so that the line relating wave velocity with frequency at a concentration  $4\frac{1}{2}$  times the normal is not distinguishable from that for values in sea water before and after the test solutions. The range of frequencies was largest in the solutions where the  $Mg^{2+}$  concentration was doubled, and in this case there was also an increase in the average frequency which was most marked (about 50% above the original level) after 10 min. in the solution. The reason for this curious pattern of changes at different  $Mg^{2+}$  concentrations may be that the activity of the comb plates is only affected when the  $Mg:Ca$  ratio lies within a critical range.

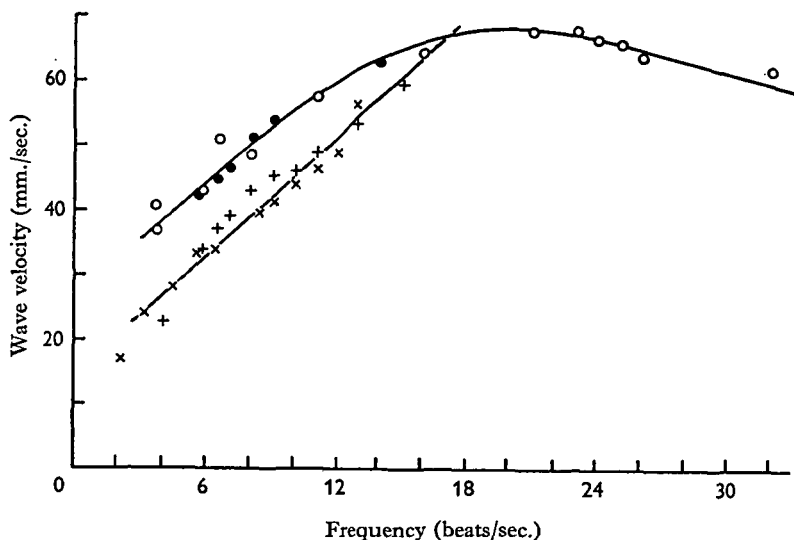


Fig. 6. The effect of tubocurarine on the relation between wave velocity and frequency. 17° C. x, Sea water initially; ●, 10<sup>-5</sup> M tubocurarine; ○, 10<sup>-4</sup> M tubocurarine; +, sea water after washing out tubocurarine.

### (f) Tubocurarine

Curare blocks transmission at some synapses and neuromuscular junctions by competing with acetylcholine for sites on the post-junctional membrane. Its effects on ciliated cells are almost unknown—Carter (1926) reported that in a saturated solution of curare the beat of nudibranch velar cilia was at first abnormally active and remained active for more than 2 hr.; he also found that the nervous control of inhibition of the cilia was not affected. Drugs structurally related to curare are known to act as depolarizing agents.

The effects of solutions of tubocurarine chloride on the frequency and wave velocity of *Pleurobrachia* comb plates are shown in Fig. 6. In a 10<sup>-5</sup> M solution the frequency of beat changed little, but the wave velocity increased to well above the original level, showing a 33% increase at the average frequency of 7.8 beats/sec. The frequency quickly rose to a very high level in a 10<sup>-4</sup> M solution; within 1 min. it had changed from an average of around 7 beats/sec. to an average of 26.7 beats/sec., and 1 min. later it had risen further to an average of 28.7 beats/sec. only to fall back slowly over the next 10 min. to an average of around 13 beats/sec. At the very high frequencies in the 10<sup>-4</sup> M solution the wave velocity fell below the level that would have been expected at that frequency in sea water, but over the normal frequency

range the wave velocity in  $10^{-4}$  M curare was considerably above that in sea water, especially at the lowest frequencies. Both wave velocity and frequency returned to around the original level when the animal was returned to sea water. Similar results were obtained in a repeat experiment.

(g) *Ouabain*

The depolarization of many excitable cells produced by this substance is believed to result from a blockage of the energy supply to the ionic pump moving sodium and potassium across the cell membrane. No report on the effects of ouabain on the activity of ciliated cells is known to have been published.

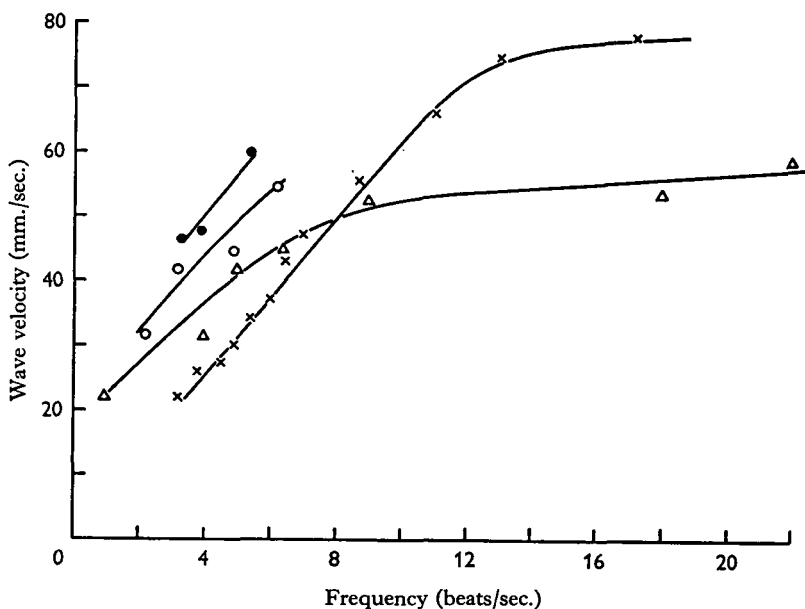


Fig. 7. The effect of ouabain on the relation between wave velocity and frequency. 17° C. x, Sea water; ●,  $10^{-6}$  M ouabain; O,  $10^{-5}$  M ouabain; Δ,  $10^{-4}$  M ouabain.

In two sets of experiments on the effects of ouabain (strophanthin G) on the activity of comb plates of *Pleurobrachia* similar results were obtained (e.g. Fig. 7). At concentrations of  $10^{-6}$  M and  $10^{-5}$  M the frequency of beat was within the normal range, but the average tended to be below that recorded in sea water. The wave velocity in these solutions was increased by up to 100% above the levels found at the same frequency in sea water. During the first few minutes in  $10^{-4}$  M ouabain the frequency was low and the wave velocity was well above the normal level, but after 20–30 min. the frequency had risen from an average level of 6.3 beats/sec. to an average frequency of around 20 beats/sec., and at this time the wave velocity was no higher than at about 9 beats/sec. in sea water. It is possible that a similar delayed acceleration of beat and depression of wave velocity would also have been found at the lower concentrations had the animal been left in these solutions for longer than the 30 min. that was allowed. Animals that had been placed in  $10^{-4}$  M ouabain died within a few hours; normal activity could not be restored by thorough washing in sea water.

## DISCUSSION

Variations in the rate of beat of cilia are not normally accompanied by changes in the wave velocity in the internal co-ordination of the membranelles of *Stentor*, but when the cilia of a group are co-ordinated by viscous-mechanical forces acting through the water around the cilia, it is expected that changes in the rate of beat will be reflected in changes of wave velocity. The almost linear relation between the rate of beat of ctenophore comb plates and the wave velocity suggests that a viscous-mechanical mechanism of metachronism might be involved. However, it is likely that previous authors were correct in assuming that the metachronism of comb plates is internal; in a viscous solution the wave velocity is not changed when the angular velocity of the beat is considerably reduced (Sleigh, 1966*a*), and in several of the treatments mentioned here it has been possible to change the wave velocity at a particular frequency by addition of drugs or ions. By contrast, the velocity of reverse waves, which seem to depend on actual mechanical contact between comb plates for their propagation, is not affected by similar treatment with drugs. If the forward metachronism of the ctenophore is internal, and comparable with that of *Stentor* membranelles, it becomes necessary to attempt an explanation of the difference in behaviour.

The two systems appear to differ functionally in at least one important respect. The membranelles of *Stentor* are rhythmically active unless inhibited, while each comb plate of the ctenophore is inactive unless it receives some activating stimulus (in addition all comb plates may be subject to immediate inhibition by a nervous stimulus which prevents their activation). This difference may be illustrated by the sequence of events following total inhibition of beat in the two systems. The comb plates lie quiet until the pacemaker cilium at the head of the row gives a beat and a wave of activity involving a single beat of each cilium passes down the whole row; successive beats of the pacemaker are each followed by a single beat of the other cilia following closely the rhythm of the pacemaker. The membranelles of *Stentor* may begin to beat as several separate regions each with their own independent rhythm, the regions being separated by inactive cilia; only when all of the membranelles are active is the rhythm of the whole row unified with that of the pacemaker at the beginning of the row. There seems to be almost complete dependence of the comb plate cells of the ctenophore on some conducted impulse for the excitation of the ciliary contraction. This is perhaps a consequence of the multicellular structure in which the integrated activity of a large number of cells is likely to require a more positive act of co-ordination than the integration of the closely situated membranelles of the single cell. The functioning of both systems may be illustrated by the schematic diagram drawn to show the pattern of events suggested for *Stentor* (Sleigh, 1957, fig. 2), where a conducted impulse and an internal build-up of excitation are involved in triggering the contraction of each cilium. The two systems differ in the relative importance of these two components. In the ctenophore the conducted impulse is entirely responsible for excitation unless a group of comb plate cells is for some reason in such an excited state that the comb plate itself becomes a pacemaker and shows the potentiality for internal excitation. The intrinsic rhythm of the membranelles of *Stentor* is often only a little slower than the frequency of beat of the pacemaker, and the conducted impulse is visualized as being a pulse of excitation 'topping-up' the build-up

within the membranelle to trigger the beat, this beat occurring a short time before a spontaneous beat would have been performed in the absence of the conducted impulse.

The variation in speed of transmission with the duration of the interval since the previous beat of the comb plate is a peculiar feature which requires explanation. There are no grounds for believing that any chemical transmitter is involved in the metachronal co-ordination, since agents active at a variety of types of nerve-effector junctions were without effect on the wave velocity. Those treatments which did change the wave velocity probably act on the membrane potential of the cell, and can be interpreted as supporting the view of Horridge that a depolarizing current of the comb plate cells is the basis of the transmission of excitation from one comb plate to the next. The ciliated cells undergo depolarization at the time that the cilia perform a beat, and the shorter the interval since the previous beat the greater the depolarization and presumably the lower the effective threshold for excitation, since there is faster conduction of the excitation during rapid beating (up to a maximal value of about 80 mm./sec. in these small *Pleurobrachia*). This relation between threshold and depolarization may explain the acceleration of conduction by substances which are expected to act by depolarizing the cells. Each step in the chain of excitations within the comb row takes a time of about 10 msec. when the comb plates are beating at an average frequency. The slowest beats recorded were associated with a step interval of 20 msec. and at the fastest frequencies the wave velocity shows a maximal value corresponding to a step interval of about 5 msec. When the animal is left for some minutes in  $10^{-4}$  M ouabain (which presumably causes considerable depolarization) the minimum step interval is longer than 5 msec. The times taken for each step in the chain appear to be of the right order of size for one to propose that the excitation of each comb plate of the row is a single step, i.e. that the electrical change involved in the depolarization is conducted electrotonically through the intervening cells to the next comb plate and that the active participation of the intervening cells is not involved. One reason why this is believed to be likely is that in the intracellular recordings of Horridge (1965*a*) the action potential rose slowly, taking perhaps 100 msec. for the rising phase in *Cestus*, and a significant part of this time is likely to be required to cause a change in membrane potential which is large enough to produce a threshold depolarization in the cells of the next comb plate; there is not time for a succession of membrane depolarizations of the intervening cells. It is conceivable that the more rapid beats are also associated with a more rapid rising phase of the action potential. The fact that there is a maximum permissible step interval (about 20 msec.) is probably also significant, for the rate of rise of potential must exceed the rate of decay if the threshold is to be reached. It is interesting that the step interval between the excitations of the membranelles of *Stentor* is also about 5 msec.

If the transmission is of this type, it is not easy to see why the conduction system is polarized so that a wave is transmitted rapidly by electrical means in the forward direction, while in the reverse direction only slow mechanically transmitted waves occur. It is conceivable that the polarization of the electrical conduction is a feature of the shape of the cells intervening between the comb plates. It must be remembered that Child has reported the occurrence of reverse waves with reversed beat of the comb plates; it is possible that these are electrically transmitted, but it seems likely that some slightly different message is required to trigger a beat in the reverse direction.

A number of the treatments described influence the rate of beat, and are presumably acting on the state of excitation of the pacemaker. It is suggested that these treatments are affecting the  $\text{Ca}^{2+}$  concentration of the cells, either by competition ( $\text{Mg}^{2+}$ ) or by causing an immediate (curare) or delayed (ouabain) depolarization of the cells. Adrenaline and acetylcholine may also be acting on ionic concentrations, as found with smooth muscle (Daniel, 1964), and the depressant effect of acetylcholine may indicate that the inhibitory system involving the nerve net has a cholinergic mechanism. The action of strychnine may involve a reduction in size of the action potential parallel to that found in frog muscles.

#### SUMMARY

1. The activity of comb plates of *Pleurobrachia* was analysed from cine films.
2. The interval between successive beats of a comb plate varied from several seconds to about 50 msec. in normal sea water; this variation in frequency was accompanied by a variation wave velocity. There was an almost linear relationship between wave velocity and frequency, ranging from a minimal wave velocity of about 20 mm./sec. at low frequencies to a maximal wave velocity of about 80 mm./sec. at the highest frequencies.
3. The wave velocity was accelerated at low frequencies by increased  $\text{Mg}^{2+}$  concentrations, by ouabain and by curare, and the same substances decreased the wave velocity at high frequencies.
4. The frequency of beat was accelerated by certain concentrations of adrenaline, serotonin,  $\text{Mg}^{2+}$ , ouabain and curare. Decreases of frequency were found in acetylcholine (and eserine) and strychnine. These substances act on the excitability of the pacemaker.
5. It is concluded that metachronal transmission is by a flow of electric current from one comb plate to the next. Depolarization, either by a recent excitation of the comb plate or by agents like ouabain causes increased excitability and a more rapid transmission. The minimum phase difference between adjacent comb plates was about 5 msec.
6. The comb plates are almost entirely dependent on a conducted impulse for their excitation; occasionally a spontaneous excitation occurs. The membranelles of *Stentor* depend more on excitation within the ciliary base and less on the conducted impulse. The two systems are believed to share common features, but differ in the relative importance of these features.
7. Reverse waves involving the beating of comb plates in the normal direction were observed. The wave velocity of these waves increased only slightly over a wide frequency range. Reverse waves of this type are believed to be transmitted by mechanical contact between the comb plates.

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