THE FINE STRUCTURE OF THE CILIA FROM CTENOPHORE SWIMMING-PLATES

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ABSTRACT

The ctenophore swimming-plate has been examined with the electron microscope. It has been recognized as an association of long cilia in tight hexagonal packing. One of the directions of the hexagonal packing is parallel to the long edge of the swimming-plate and is perpendicular to the direction of the ciliary beat. All the cilia in the swimming-plate are identically oriented. The effective beat in the movement of the swimming-plate is directed towards the aboral pole of the animal, and this is also the side of the unpaired peripheral filament in all the cilia. The direction of the ciliary beat is fixed in relation to the position of the filaments of the cilia. The swimming-plate cilium differs from other types of cilia and flagella in having a filament arrangement that can be described as 9 + 3 as opposed to the conventional 9 + 2 pattern. The central filaments appear in a group of two "tubular" filaments and an associated compact filament. The compact filament might have a supporting function. It has been called "midfilament." Two of the peripheral nine filaments (Fig. 1, Nos. 3 and 8) are joined to the ciliary membrane by means of slender lamellae, which divide the cilium into two unequal compartments. These lamellae have been called "compartmenting lamellae." Some observations of the arrangement of the compartmenting lamellae indicate that they function by cementing the cilia together in lateral rows. The cilia of the rows meet at a short distance from each other, leaving a gap of 30 A only. The meeting points are close to the termini of the compartmenting ridges. An electron-dense substance is sometimes seen bridging the gap. Some irregularities are noted with regard to the arrangement of the compartmenting lamellae particularly at the peripheral rows of cilia. In many cilia in these rows there are small vesicles beneath the ciliary membrane.

In a previous communication the detailed morphology of the sea urchin sperm tail was reported (1). It was pointed out that this flagellum was fundamentally asymmetrical. The arrangement of the nine peripheral filaments around the central pair is in fact quite complicated, as was shown with the diagrammatic text-figure (which in many respects is similar to Fig. 1 in the present communication). As illustrated in Fig. 1 the filaments within the flagellum have an organization that makes it possible to give each filament an index number. The starting point of the indexing is to find the "unpaired filament." This is the filament that is transected by a plane which divides the tail into two equally big parts and is perpendicular to the line through the two inner filaments. This step of the indexing is quite critical, as a distortion with regard to the outer filaments of the central pair by only 20° could lead to an erroneous result. In the cilia to be described in this paper this difficulty does not appear, and one has greater confidence in the validity of the indexing.

The analysis of ctenophore cilia turned out to

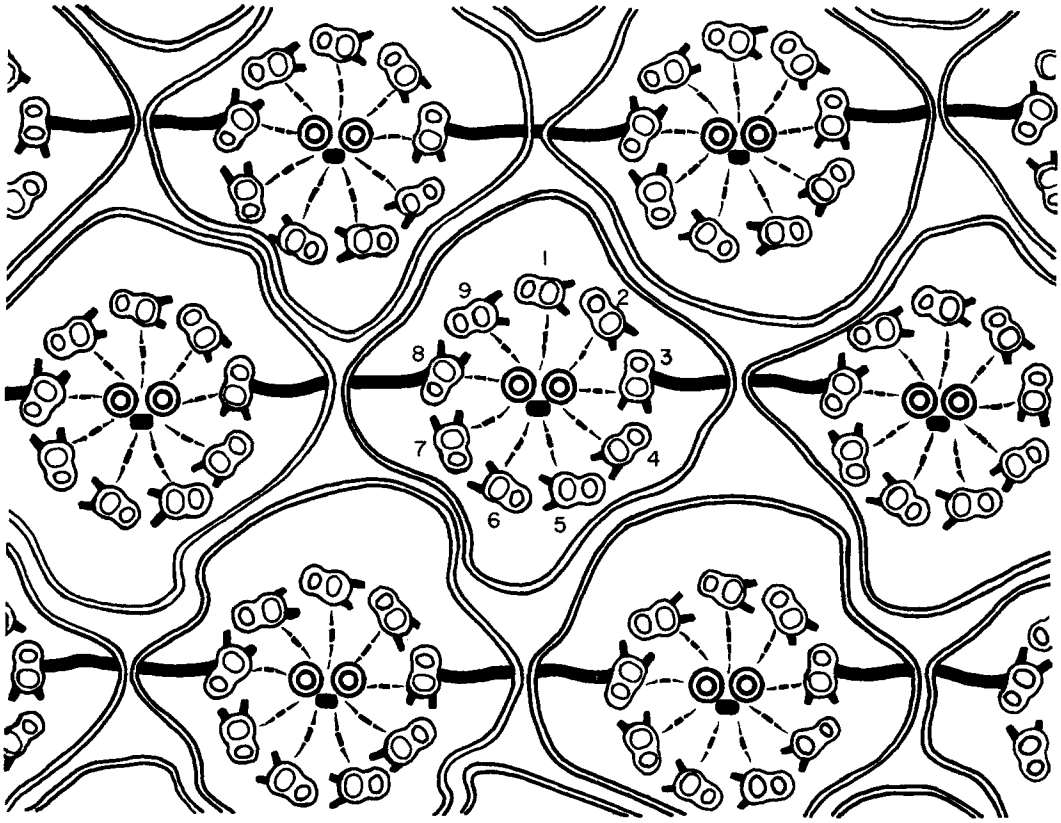


FIGURE 1

A schematic representation of the cilia within a ctenophore swimming-plate. In the central cilium the nine peripheral filaments have been given index numbers. The indexing is performed in conformity with the scheme given in an earlier report (1).

All the cilia in the swimming-plate are identically oriented; in all cilia the unpaired filament 1 points in the same direction, which is also the direction of the effective beat in ciliary motion. The plane perpendicular to the ciliary beat has a unique structure that is a conspicuous feature of these cilia: there are two lamellae in each cilium that connect the cell membrane to filaments 3 and 8 respectively. This is the center axis around which the cilium is bent in its movements. The lamellae have been called "compartmenting lamellae." In the contact regions between the cilia, which are close to the attachments of the compartmenting lamellae, the gap between the ciliary membranes is very narrow—around 30 A. Sometimes a dark substance can be seen bridging this gap; in the picture this has been indicated at the upper central contact region. Adjacent to the two tubular central filaments there is a third filament that has a compact and anisodiametric cross-section. This filament has been called "midfilament."

be profitable also in some other respects. It has been possible to determine the arrangement of the ciliary filaments with reference to the direction of the ciliary beat. The ctenophore is a marine organism that swims through the water by means of its immense cilia. The cilia are collected together in plate-like structures called swimming-plates; there are eight meridional rows of swimming-plates; each row may consist of about twenty swimming-plates and each swimming-plate contains a very large number of cilia. The effective beat in the ciliary oscillatory motion is directed towards the aboral pole of the animal, and the animal will thus swim with the mouth, or the oral pole, forward (12). During the preparatory work, the position of the sectioned cilia was noted with reference to the oral side of the animal. It was also possible to judge the position of the poles

of the animal in fragments of the animal, as the cilia during fixation stopped in a characteristic $\int$-shaped resting position, the swimming-plates having been bent towards the mouth with the distal tips of the plates recurving outwards. Due to the enormous number of cilia contained in one swimming-plate and due to their immense length (approaching 2 millimetres) it is very easy to observe the shape and movement of the cilia in the different phases of their activity.

From the above it is clear that the ctenophore would be the organism of choice for analysis of ciliary movement and ciliary structure. In many of the classical investigations on ciliary motion and ciliary coordination ctenophores were used (7, 12, 14), but there have been very few studies performed with more recent methods. In particular, it is peculiar that these cilia have not previously been subjected to an electron microscopic analysis. The early study by Bradfield (2) will not be considered here as the techniques at that time did not allow him to find any structural differences between cilia from the ctenophore and those from other animals. As far as the author knows, ctenophore swimming-plates have not been subjected to cinematographic analysis.

MATERIALS AND METHODS

The ctenophores, *Mnemiopsis leidyi*, used in this investigation were collected at Woods Hole, Massachusetts. The meridional rows of swimming-plates were fixed either in 40 per cent osmium tetroxide in carbon tetrachloride (1), a fixative giving a high contrast, or were fixed in 2 per cent osmium tetroxide dissolved in sea water. Fixation time was 90 minutes, fixation temperature 0°C. As an embedding medium, a mixture of butyl and methyl methacrylate was used (in the proportions 9 parts:1 part) which was catalyzed by 0.2 per cent benzoyl peroxide. The sections were cut on a Sjöstrand microtome (10) equipped with a glass knife, but otherwise following the technique of Sjöstrand (11). In some cases the specimen block was trimmed to an irregular five-sided pyramid, and the sides of the sections were noted with reference to the poles of the animal on the one hand, and with reference to the fine structure of the cilia on the other. Some of the sections from blocks fixed in the usual osmium in sea water were "stained" with uranyl acetate according to the techniques of Watson (13) and Peachey (8). Identical results have been obtained with the two types of fixation.

A Siemens Elmiskop I electron microscope was used, operated at 80 kv, and with a bore diameter of the objective aperture of 50 μ. The improved grid holder by Elbers was used (4). In order to increase the contrast further the exposures were made on the hard Gevaert litholine 08 plates that were developed in Kodak D72 developer.

OBSERVATIONS

One of the swimming-plates in a full grown *Mnemiopsis* may have a length of 2 millimetres, a width of about 1 millimetre, and a thickness of 20 microns. Within this thickness some 80 to 100 rows of cilia are found, and a calculation of the total number of cilia within one swimming-plate will give a value of 100,000 or more. The number of cilia possessed by the ctenophore is of the order of tens of millions.

Compartmenting Lamellae: The cilia within the swimming-plate are tightly packed in an hexagonal array as seen in cross-sections (Figs. 2 to 7). One of the directions in the hexagonal packing is parallel to the long edges of the swimming-plate. This direction stands out due to a unique anatomical feature—a pair of intraciliary lamellae. The lamellae give the cross-cut swimming-plate a striated appearance that is easily visible even at magnifications as low as 10,000. From micrographs at higher magnifications, it is disclosed that the lamellae in a cilium connect two of the ciliary filaments to the cell membrane. Adopting the terminology proposed in the earlier paper (1), it can be specified that it is filaments 3 and 8 that are connected to the lamellae (Fig. 1). The free filaments are separated by the lamellae into two unequal groups; filaments 9, 1, and 2 are on the one side of the bisected cilium and filaments 4, 5, 6, and 7 belong to the other. As the lamellae divide the cilium into two compartments, the descriptive term "compartmenting lamellae" is proposed. The term does not imply that the cilium has two closed spaces, nor does the term have any special physiological meaning. The significance of the finding is the notion that the filaments of the cilium have fixed positions in relation to the direction of the ciliary beat. This statement can be specified further by mentioning another finding: the position of filament 1 is in the direction of the effective stroke, that is to say, this filament is the one that is closer to the aboral side of the animal.

From thick sections cut obliquely through the cilia the lamellar shape of the compartmenting lamellae can be seen, and from cross-sections at different levels of the cilia it is evident that the lamellae extend from the bases of the cilia to their

tips. The thickness of a compartmenting lamella is about 80 A and its extension from the cell membrane to the filament averages 500 A. Rarely, the outer portion of the lamella is branched (Fig. 7, lower arrow).

It might be asked whether the presence of compartmenting lamellae is typical for ctenophore cilia or whether they might be distinctive of fused cilia. As will be shown below the answer is negative for both propositions, although there is evidence that the lamellae do function by cementing the cilia together.

The ctenophore has cilia other than those contained in the swimming-plates. There are, for instance, the ciliated grooves located close to the rows of swimming-plates. The cilia within these grooves have also been examined in this investigation, and it has been found that these cilia have a morphology of conventional type and that they lack compartmenting lamellae. These cilia are free from each other.

The following observations speak in favour of the idea that the compartmenting lamellae are instrumental in ciliary adhesion. The hexagonal packing of the swimming-plate cilia is due to the arrangement in rows of the cilia and to the fact that the cilia have staggered positions in alternate rows. The cilia in a row meet at a short distance from each other, and with meeting points that are close to the termini of the compartmenting lamella. The dimension of the minimum separation between two cilia in a row is of the order of 30 A or less. The ciliary membrane, that should be regarded as a part of the cell membrane, has a thickness of approximately 65 A. (The same values are found after the osmium–carbon tetrachloride fixation method and after ordinary osmium fixation.) The distance between the outer extremity of the compartmenting lamella from filament 3 and that of the neighbouring lamella from filament 8 is therefore of the order of 65 + 30 + 65 A. This dimension is within the order of magnitude over which purely physical forces might be effective in the adhesion of cells (3). When the hexagonal array is distorted and the cilia in a row are at different levels, the compartmenting lamellae will deviate from their natural course but still maintain contact with each other. One gets the impression of an attraction between the neighbouring compartmenting lamellae.

Explanation of Figures

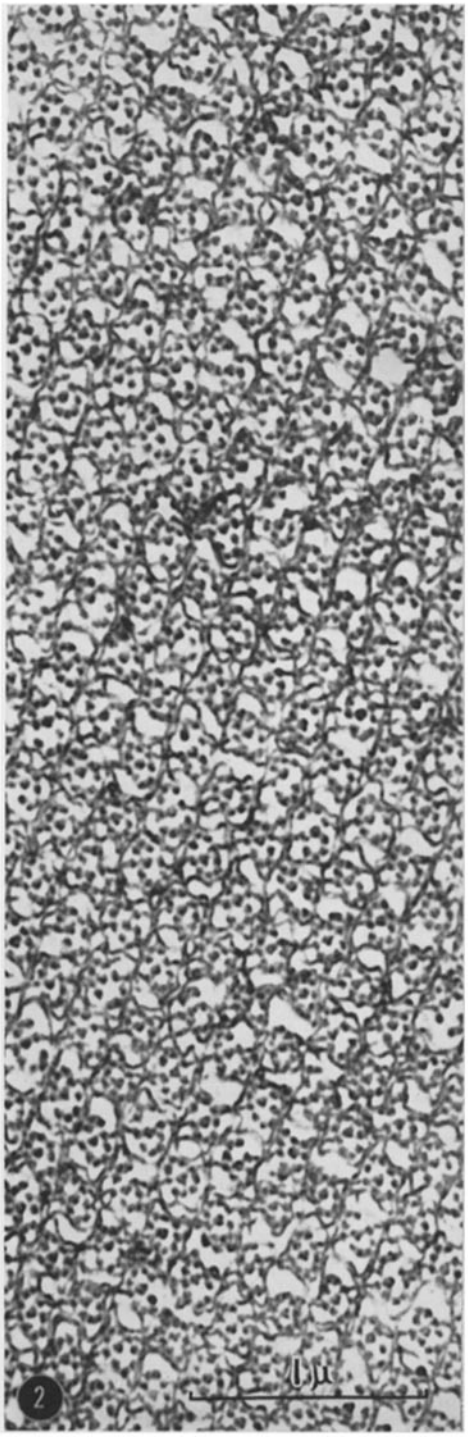
The micrographs represent cross-sections through the swimming-plate cilia from the ctenophore, *Mnemiopsis leidyi*. Fixation is in 2 per cent osmium tetroxide in sea water. In Figs. 3 to 7 and Fig. 9 the sections have been stained with uranyl acetate according to Watson (13). In most of the cross-sections here shown the cilia have been mounted with the unpaired filament 1 towards the top of the plate, and the "arms" (1) pointing in a clockwise direction. In Fig. 7 the unpaired filament is directed towards the upper right part of the figure, and in Fig. 9 this filament is directed to the left. The micrographs 3 to 9 were taken at an initial magnification of 20,000 and enlarged photographically to the indicated magnifications.

FIGURE 2

Survey picture of cross-sectioned swimming-plate cilia. The portion included in this picture has a height that represents one third of the thickness of the swimming-plate and a width that represents $\frac{1}{1000}$ of its width. The uniform hexagonal packing and the fine structure of the cilia are identical throughout the entire swimming-plate. Magnification 31,000.

FIGURE 3

This micrograph represents a very small part of the cross-cut swimming-plate. The eight cilia each have nine peripheral filaments surrounding a group of two tubular filaments + 1 compact midfilament. The arrows point to some of the midfilaments. Two of the peripheral filaments (nos. 3 and 8; compare with Fig. 1 for the indexing) are connected to lamellae, the compartmenting lamellae, that attach to the adjacent ciliary membrane. Magnification 180,000.



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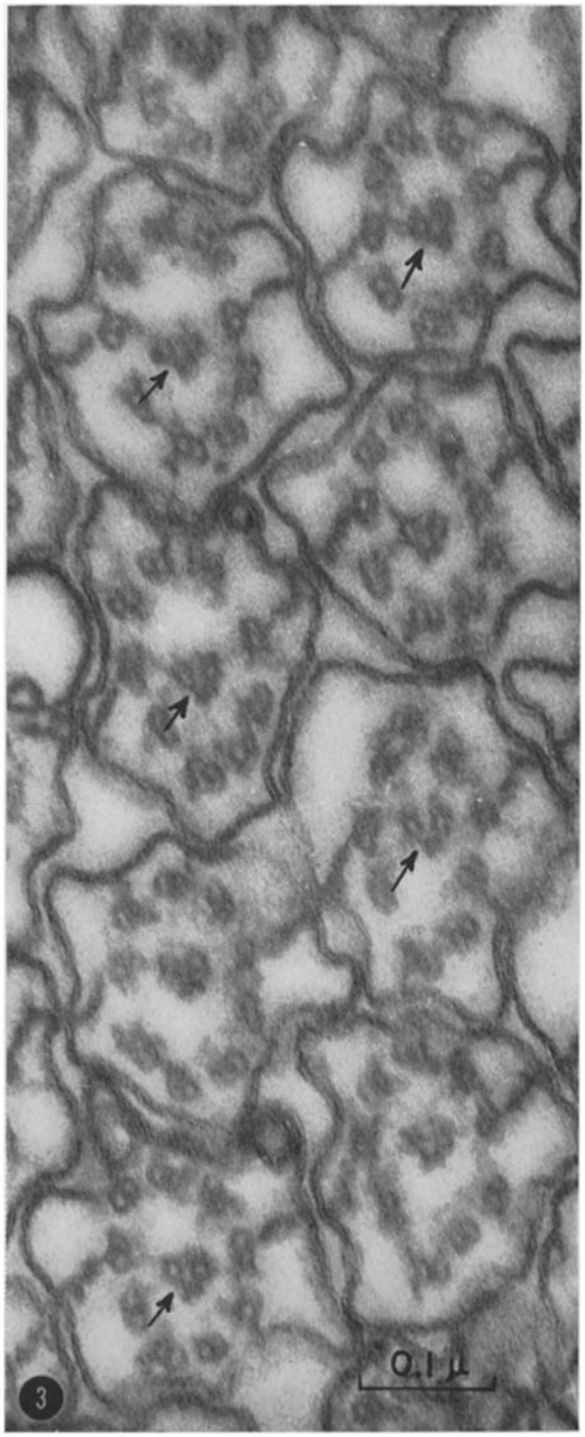

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It is instructive in this connection to study the cilia at the ends of the rows. Some of these cilia do not differ in appearance from the cilia in the interior of the rows. Other cilia have one compartmenting lamella only, and this one is, as usual, directed towards the neighbouring lamella (Fig. 8). This appearance is consistent with the view that the compartmenting lamellae are adaptations with a role in cilium contact.

The cilia in the innermost (Fig. 5) and in the outermost (Fig. 6) rows of the swimming-plate are also of some interest here. (The directions "inner" and "outer" are used here in relation to the body of the animal when the swimming-plate is in its rest position.) It is evident from the two figures and many others of a similar kind that many cilia in these rows have an enlarged cross-sectional area. The ciliary membrane is inflated and the ciliary matrix contains many small vesicles. The hexagonal packing pattern cannot always be maintained and some irregularities can be noted with regard to the arrangement of the compartmenting lamellae. The lamellae might be missing on one or both sides of the cilium (Fig. 5). This is particularly often the case when the cilium is removed from its neighbour. Another deviation from the common pattern is the presence on a third filament of a formation that is similar to the regular compartmenting lamellae. Such an extra lamella is seen in Fig. 6, extending from filament 2 in one of the cross-cut cilia (at the arrow). Rarely, the same feature has been seen in more central cilia, an example of which is shown by the upper left cilium in Fig. 7. These extra lamellae are not met by lamellae from a neighbouring cilium.

It was hoped that a close examination of the contact region might be rewarding when studying the compartmenting lamellae. The ciliary membrane at these regions is identical in appearance to the membrane at other places; it appears as a double membrane (defined as two electron-dense layers separated by a lighter interspace). The gap between the cell membranes is, as stated above, quite narrow, around 30 A or less. Due to this smallness it has been difficult to obtain clear micrographs of the contents within the gap. In some cases, however, it was possible to observe an electron-dense substance bridging the gap. The width of the bridge is similar to that of the compartmenting lamella. Examples of these gap substances, looking like continuations of the compartmenting lamellae, are given in several of the included figures; perhaps those in Fig. 9 are most easily discernible. In other contact regions the gap between the two neighbouring cilia appears quite empty. The two types of contact regions are represented in Fig. 1.

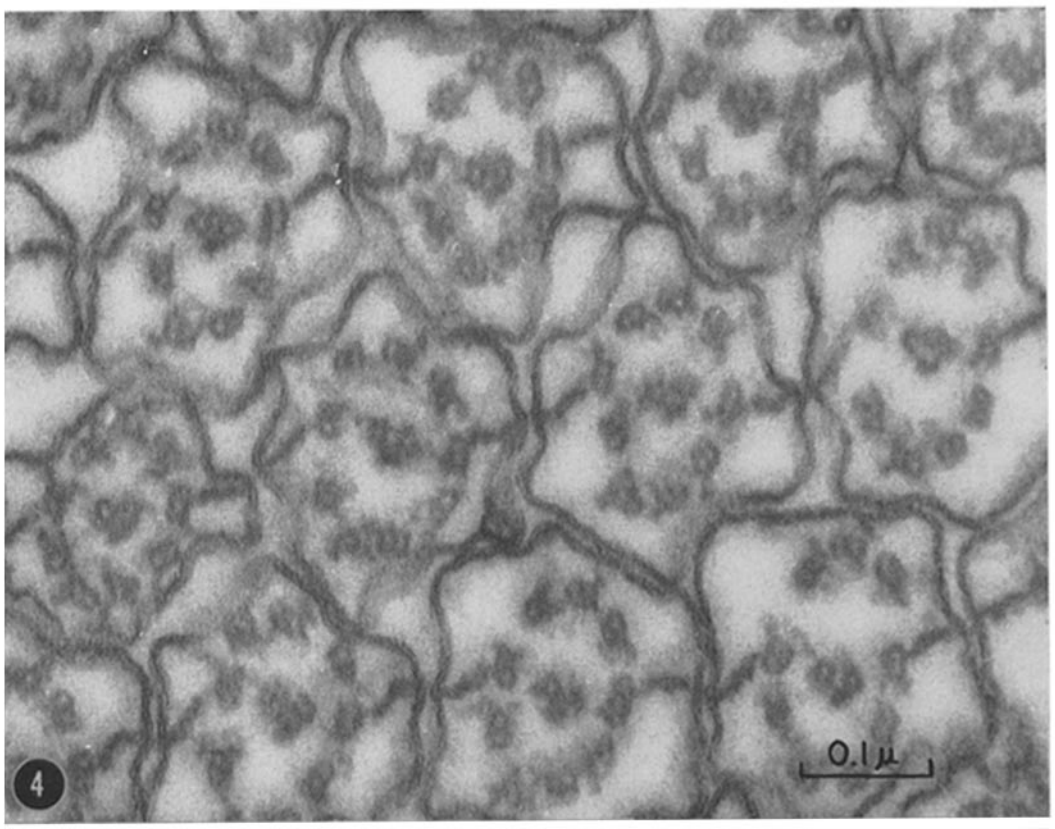
Midfilament: Another unique feature of the swimming-plate cilia is noticed in the arrangement of the ciliary filaments. In contrast to the nearly universal pattern of 2 + 9 filaments in cilia,

FIGURE 4

In the cross-sectioned swimming-plate the individual cilia are closely packed and fit into each other like the pieces of a jig-saw puzzle. The regions where the compartmenting lamellae attach to the ciliary membrane are close to the areas of minimum distance between two cilia. The gap between two cilia here is only 30 A wide. In some of these areas an electron-dense extraciliary substance can be discerned; this might be the case in the three contact areas at the right side. Magnification 175,000.

FIGURE 5

Cross-section through the inner four rows of the swimming-plate. The term "inner" is used with reference to the animal body when the swimming-plate is in its rest position. In the innermost row—the one at the bottom of the figure—there is one cilium that has one compartmenting lamella only (arrow at the right side) and two cilia that are devoid of compartmenting lamellae (arrows to the left side). Another feature that is characteristic of this end row is the presence of many small vesicles within some of the cilia, and a collection of similar vesicles within a covering of several concentric membranes. Magnification 83,000.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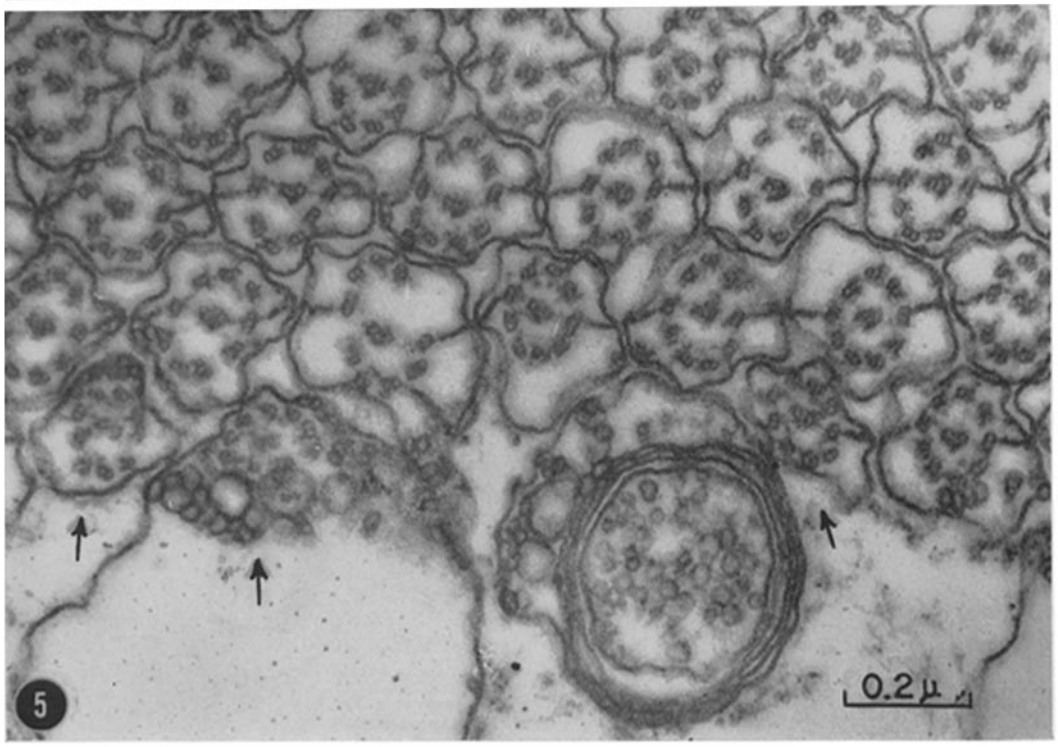
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flagella, and sperm tails, the ctenophore cilia have a pattern that can be characterized as 3 + 9 filaments. The central part of the cilia contains two tubular filaments that are arranged in a plane that is perpendicular to the ciliary beat direction. Moreover, there is a compact filament that is situated close to the other two filaments and, actually, often close to the geometrical centre of the ciliary cross-section (Fig. 3, arrows). The term "midfilament" will be used to designate this filament. In relation to the two other inner filaments, the midfilament is closer to filaments 5 and 6.

The outline of the sectioned midfilament is somewhat irregular and it is not possible to give more than very approximate figures for its dimensions. When cross-cut the midfilament often has a somewhat rectangular outline with side lengths of about 180 A and 100 A. The longer side is parallel to the long side of the cross-cut swimming-plate.

Intraciliary Vesicles: Many cilia in the outermost and the innermost rows of the swimming-plate contain vesicles, often in a great amount. The various diameters recorded can be explained partly by the different sizes of the vesicles and partly by the different levels through which the vesicles have been cut. The vesicles have been noticed in cross-sections (Figs. 5 and 6) as well as in longitudinal sections, in cilia at the distal rows (Figs. 5 and 6), and in cilia in the interior of the swimming-plate.

The wall of the vesicles is about 65 A thick, the same as that of the ciliary membrane. The collection of vesicles seen at the lower part of Fig. 5 is of special interest because it is surrounded by many concentric layers of membrane coverings. It is possible that the vesicles, as well as these concentric membranes, represent a storage phase of the cell membrane material. It is also possible that the vesicles make their appearance during the preparation.

DISCUSSION

The micrographs described above illustrate the unique type of cilia that make up the swimming-plates of the ctenophores. Two new submicroscopic components have been observed in these cilia; they have been called the midfilaments and the compartmenting lamellae. From earlier light microscopical studies (7, 12, 14) two characteristic features have been reported in this material —the relatively enormous length of the cilia and the great number of cilia that are associated in one swimming-plate. The achievements of the ctenophore cilia are also unique, as the ctenophores are the only macroscopic animals that totally depend on ciliary movements for their swimming motion. It is to be expected that the two structures here described can be associated with the unique features in the ciliary function.

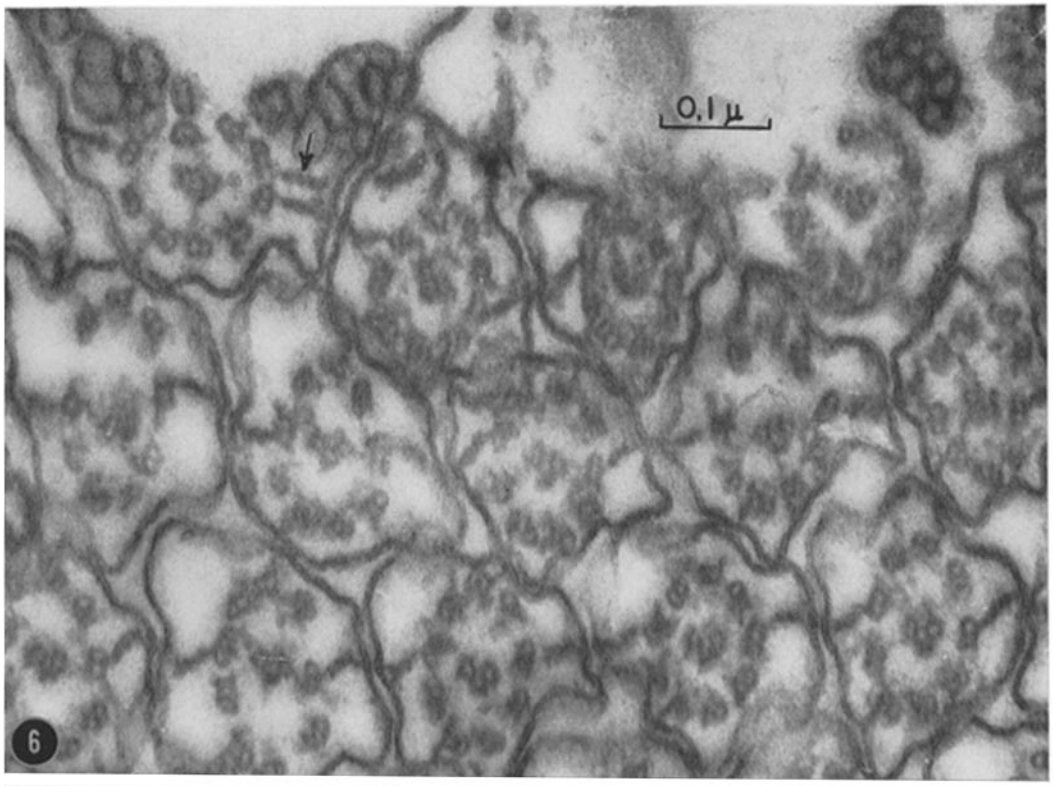
It is often stated in the literature that the swimming-plate cilia are fused, as are the laterofrontal cilia in mussel gills and the cilia in the protozoan cirri and membranelles. The fusion of the cilia implies that the cilia cannot beat separately, nor are they able to exhibit the metachronous rhythm that usually characterizes the cilia in ciliated epithelia. On the other hand, it is very easy to dissociate the individual members, for instance, by fraying the swimming-plate

FIGURE 6

Cross-section through the outer three rows of the swimming-plate. In the outermost row (as in the innermost one) there are many cilia with enclosed small vesicles. The cilium at the upper left corner of the figure is apparently provided with a structure that could be called an extra compartmenting lamella (arrow). This lamella is attached to peripheral filament 2. Magnification 145,000.

FIGURE 7

Cross-section from the interior region of the ctenophore swimming-plate. In this picture there are two uncommon variations of the arrangement of the compartmenting lamellae. The arrow at the lower left points to a compartmenting lamella that is branched, and the arrow at the upper left to an extra compartmenting lamella that is attached to peripheral filament 2. Magnification 115,000.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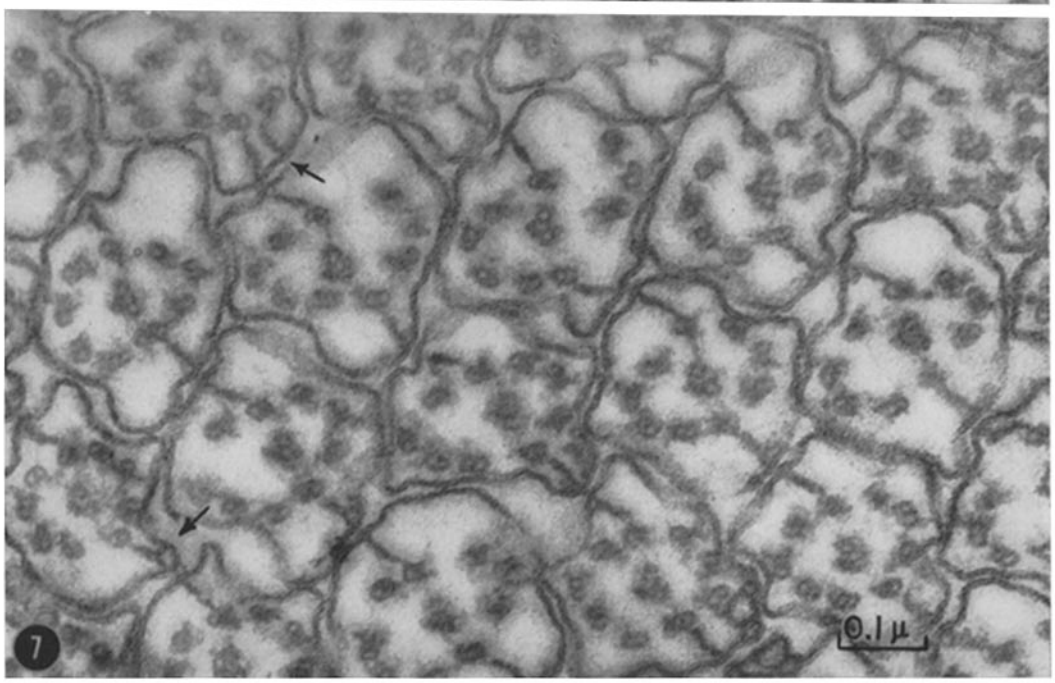
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with a needle. The expression "fused cilia" must not be taken too literally.

The morphological explanation of the phenomenon of ciliary fusion is not known. As described above, under Observations, it is likely that the compartmenting lamellae of the swimming-plate cilia are instrumental in this function. The observed facts are at least not inconsistent with such an interpretation. If the compartmenting lamellae indeed have this function it can be concluded that the cilia are fused in the lateral direction only; there is no connection of a similar kind binding together the different rows. As a consequence, there should be a greater flexibility between the rows, and such a property would certainly be advantageous in the movements of the swimming-plates. It is hard to understand how the cilia could beat if they were cemented to each other in the plane of the beat. Compartmenting lamellae have not been found in other fused cilia such as the protozoan cirri (9) or the laterofrontal cilia of mussel gills (Afzelius, unpublished data). In these cases there are only a few cilia in lateral rows.

The very close approximation of the swimming-plate cilia, particularly in the contact region near the compartmenting lamellae, might well lead to a type of adhesion of the cilia. In other words, the tight packing would not only be a visible sign of ciliary fusion, but might also furnish an explanation of how the fusion is established. According to the analysis by Curtis (3), purely physical forces might suffice in "cementing" together cells at these dimensions. It is also quite possible that chemical substances are present in the contact regions, and that these substances—cementing substances—bind the cilia together. The electron-dense material seen in the interciliary gaps would then represent these cementing substances.

One of the interesting aspects of the swimming-plate cilia is the fact that their separation at places is only 30 A, a value that is only a fifth of the dimension frequently found in intercellular spaces of epithelia.

The presence of compartmenting lamellae is helpful in establishing the fact that the filaments have a fixed and constant arrangement within the cilium. From this fact it is concluded that the direction of the ciliary beat is fixed in relation to the position of the filaments. Similar conclusions have been reached by other authors, Fawcett and Porter (5), Bradfield (2) and Manton (6), who have analysed the fine structure of other types of cilia and flagella.

The compartmenting lamellae cannot be considered to be analogous to the lamellae connecting the coarse dense fibers and the fibrous sheath in mammalian sperm tails, as that represents a fusion between two types of structures that do not exist in ctenophore cilia.

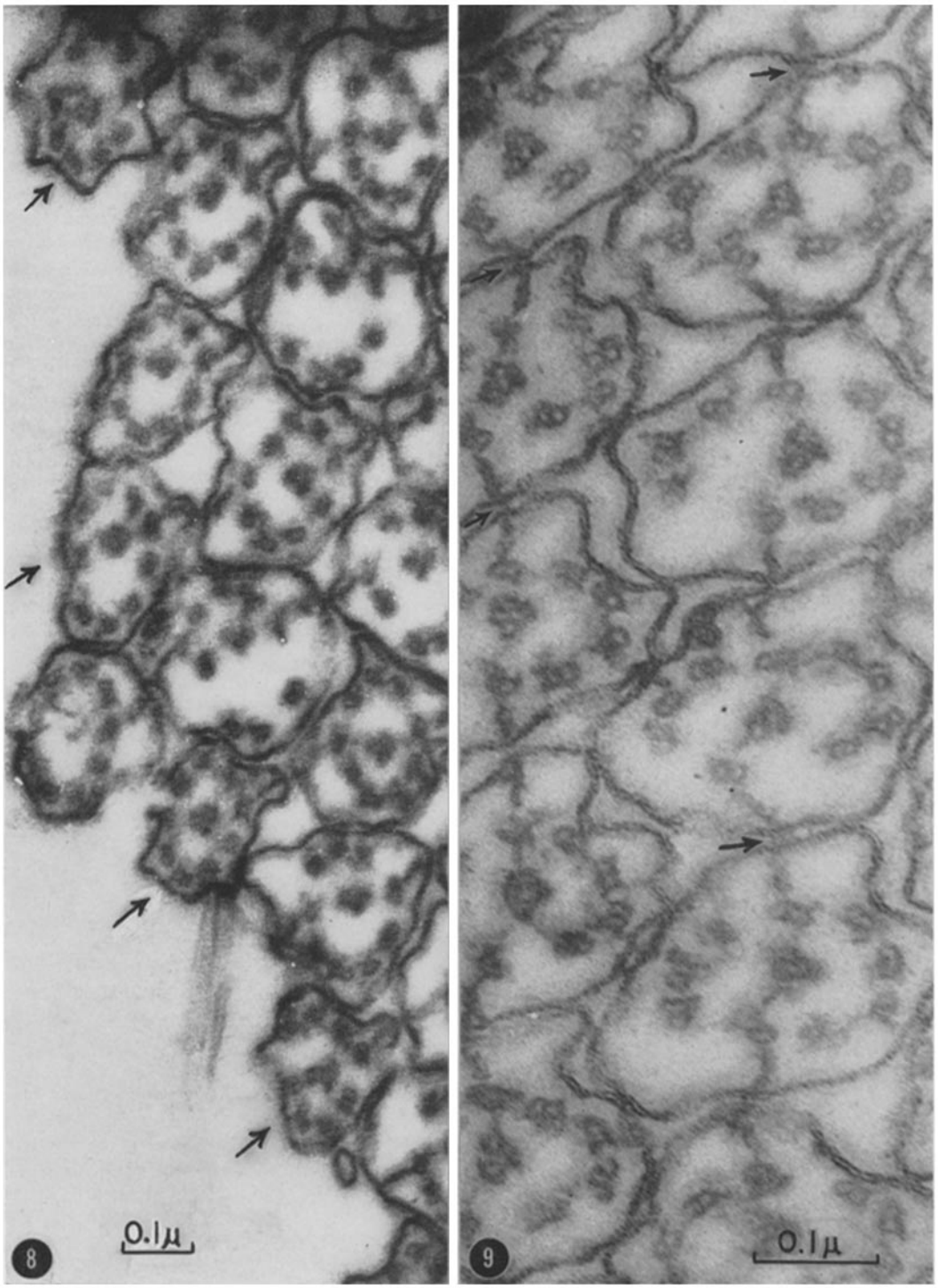
It is not easy to understand the function of the midfilament. Its central location seems to make a supporting role more likely than a contractile or a kinetic one. There is no direct evidence for this point of view, but its anisodiametral cross-section

FIGURE 8

This picture shows the lateral ends of the ciliary rows. Whereas the cilia within the rows are provided with two compartmenting lamellae, many of the terminal cilia have a lamella only at the side turned to the neighbouring cilium. Such cilia are marked with an arrow. Magnification 105,000.

FIGURE 9

The two rows of cilia shown in this picture are oriented to make the compartmenting lamellae run parallel to the long edge of the figure. The membrane surrounding the cilium appears as a double membrane with a thickness of 65 A. Its appearance is identical in regions where the compartmenting lamellae end and in other areas, but the contact regions, adjacent to the lamellae, are remarkable in having an extraciliary substance filling the gap. The arrows point at such interspaces with dense substances. The distance between two cilia is only about 30 A at these contact regions, when measured from the outer boundaries of the ciliary double membranes. Magnification 185,000.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is consistent with this idea. The shorter diameter is in the direction of the beat, and the longer diameter is in a direction that will not be bent in ciliary motion. A midfilament has not been seen in any other type of cilia.

The immense length of the swimming-plate cilia raises many questions that will not be dealt with here. It would, for instance, be of considerable interest to find the source of energy for ciliary movements, and another important task is to find out how the chemical or chemicals representing the energy source are made available to the energy consuming portions of the cilium.

Addendum: Since this manuscript was submitted there has appeared a study on flagellar fine structure which is of considerable interest in this connection (Gibbons, I. R., and Grimstone, A. V., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 697). The descriptions there given of the linkages between the flagellar membrane and the "anchorage granules" might be compared to the "cementing substances" presented in this study.

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