Cell Tiss. Res. 185, 105–113 (1977)

Cell and Tissue Research

Further Observations of Transport within Paddle Cilia

P.N. Dilly*

Department of Structural Biology, St. George's Hospital Medical School, Tooting, London, England

Summary. *Rhabdopleura* has densely ciliated tentacles and cephalic shield. These cilia are of two sorts, ordinary cilia and those bearing paddle-like swellings. These swellings, about 1 μm across, are in the ciliary membrane. The axoneme bundle within the cilium remains intact, keeping the usual spatial arrangements, except that in the swelling its overall diameter is reduced. It is suggested that the mechanism for moving the paddle-like swellings along the length of the cilium is the releasing and reattaching of the side filaments from the axoneme to the ciliary membrane.

Key words. Paddle cilia – Transport – *Rhabdopleura* – Electron microscopy (T.E.M., S.E.M.).

Introduction

Since the initial report of paddle cilia in *Rhabdopleura* (Dilly 1977), further observations with the TEM have correlated the internal morphological changes of these cilia with their external appearance as revealed by the SEM. The range of animals in which these cilia have been observed has also been extended. It is now possible to propose a mechanism by which the enlargement of the shaft is produced and a means of moving such a swelling along the ciliary shaft.

Materials and Methods

Rhabdopleura was obtained by dredging off Stoke Point, Plymouth, England. The specimens were found adhering to the concave surfaces of the separated halves of *glycymeris* shells. The shells were also frequently encrusted with the hard tubes of *Vermiliopsis* and *Dodecacera*. The animals were transported

Send offprint requests to: Professor Dr. P.N. Dilly, Department of Structural Biology, St. George's Hospital Medical School, Cranmer Terrace, Tooting, London SW 17 ORE, England

* *Acknowledgements.* I wish to thank the Director and staff of the Plymouth Marine Biological Association for their help and facilities while collecting the material. Mr. R.F. Moss gave much skilled technical assistance. I wish also to thank Professor W.E. Knight-Jones who identified the specimens of *Vermiliopsis* and *Dodecacera*.

live in cold sea water to the laboratory where they were dissected free from the shells using cornea knives and fixed initially in 4% glutaraldehyde buffered with a cacodylate buffe to pH 7.4, both solutions being made up in sea water. The specimens remained in this fixative for 24 h when they were postfixed for two hours in 1% osmic acid buffered to pH 7.4 using the same buffer and solvents.

Dehydration was undertaken in several ways. Most of the material was dehydrated through graded ethanol solutions into three changes of absolute ethanol. For transmission electron microscopy the specimens were stained in 6% uranyl acetate in absolute ethanol for two hours, washed in absolute ethanol and immersed in epoxypropane for thirty minutes before embedding in araldite. The sections were cut using glass knives on a Porter-Blum microtome, and stained on the grid with lead citrate.

For scanning electron microscopy, the specimens were either taken direct from fixative and placed in liquid freon in liquid nitrogen and transferred in liquid nitrogen to a freeze drying unit, or taken from absolute ethanol into mixtures of absolute ethanol and freon 113 until in pure freon 113, when they were dried in a critical point drying apparatus. Specimen from either preparative method were then mounted on aluminium studs using double-sided sellotape and coated with gold in a sputter coating unit before being examined in a Cambridge IIA scanning electron microscope, using 10K accelerating voltage.

Results

The surface of the tentacles bear rows of cilia most of which have paddle-like enlargements of their shafts. The cilia are in some way sticky, and many small particles and some quite large objects can be found attached to them (Fig. 1–3). Sometimes the larger particles can be seen apparently trapped within a group of cilia. The cilia in such groups are of no specific type and are usually a mixture of normal and spatulate ended ones. The trapped lumps might be prey captured by the tentacular cilia.

Paddle shaped cilia, very similar in morphology to those found in *Rhabdopleura* (Dilly, 1977), have been found in both *Vermiliopsis* and *Dodecacera* (Dilly unpublished observations), and in sponge larvae (Berquist et al., 1977). The TEM confirms that the enlargements of the shaft diameter are in the ciliary membrane alone and that the axoneme itself usually remains intact (Fig. 4, 5, 6, 8) These swellings are not all of the same shape (Fig. 2, 3). They are usually oval with their long axes along the long axis of the axoneme, or disc-like. The enlargements have the same amorphous content as the rest of the ciliary cytoplasm and no unique electron dense contents have been found in them (Fig. 4–9).

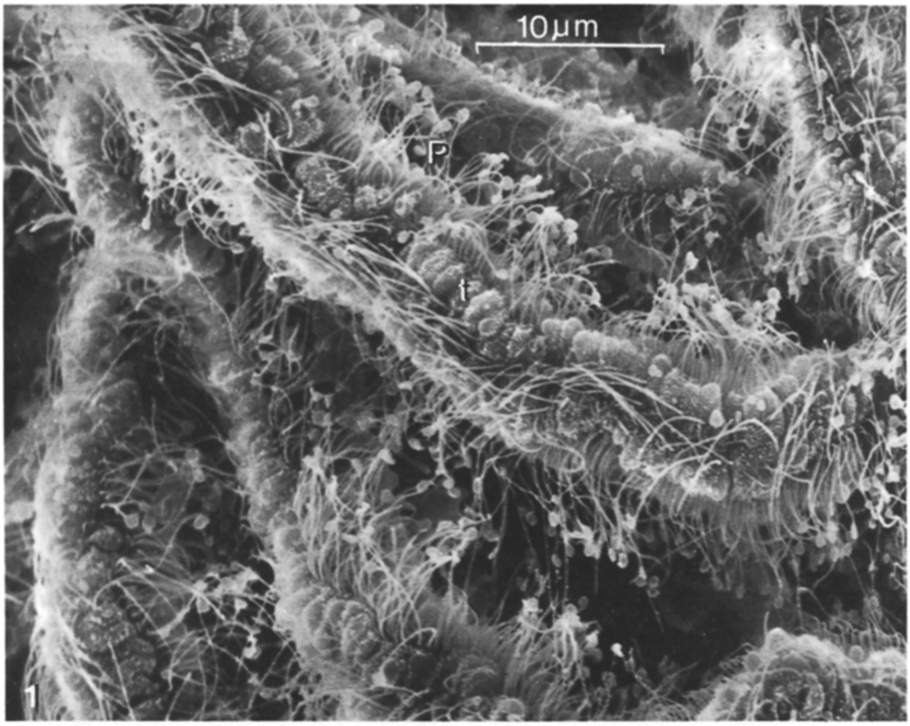
TEM observations have shown that the axoneme filaments are of the usual 9 + 2 pattern (Figs. 7, 9). The central pair of tubules of the axoneme are not regularly orientated but most of their long axes point in the same general direction in the cilia

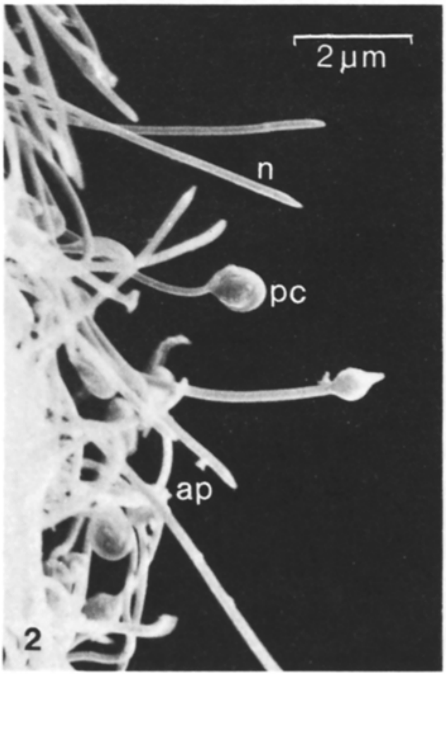
Abbreviations. af, axoneme filaments; *ap,* adherent particle; *c,* normal cilium; *cm,* ciliary membrane; *er,* a swelling made of material apparently enclosed within a kink in the ciliary shaft; *f,*lateral filaments; *fe* flattened enlargement of the ciliary membrane; *k,* kink; *m,* mid-shaft swelling; *mt,* mid-shaft swellings that appear as terminal paddles because the shaft is bent back upon itself; *p,* paddle-shaped enlargements; *pe,* paddle cilia; *se,* spherical enlargement of the ciliary shaft; *t,* tentacle

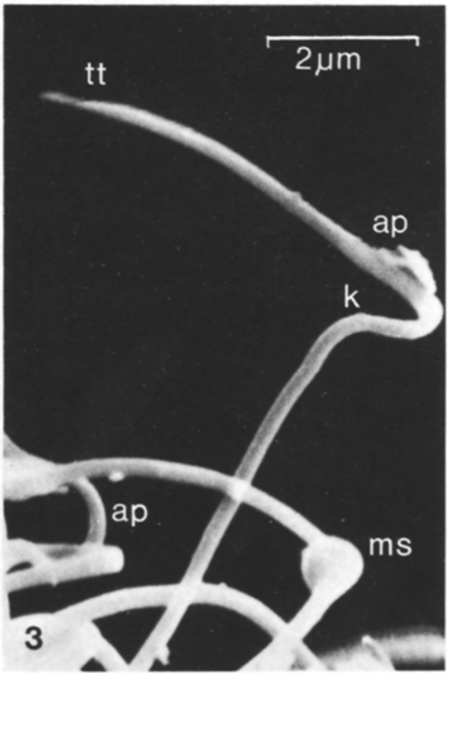
Fig. 1. Cilia on the tentacles of *Rhabdopleura* showing their arrangement in rows with the majority of them bearing paddle-like enlargements

Fig. 2. Another group of cilia from *Rhabdopleura* tentacles with a larger particle enmeshed amongst them showing cilia with and without paddle-like enlargements. The cilia have only short tapering ends

Fig. 3. A mid shaft spherical swelling and a kinked ciliary shaft. There are small particles stuck onto the ciliary shafts, suggesting that the shafts as well as the paddles are sticky


10 µm
p
t
1

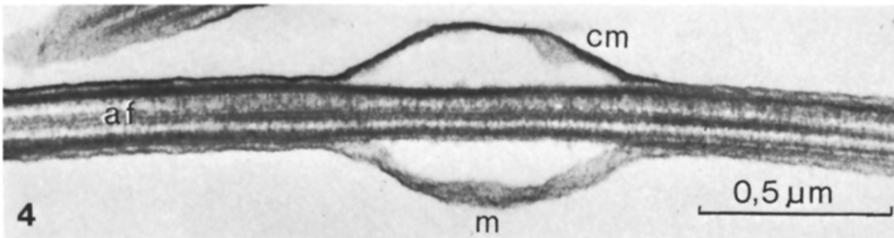

2 µm
n
pc
ap
2

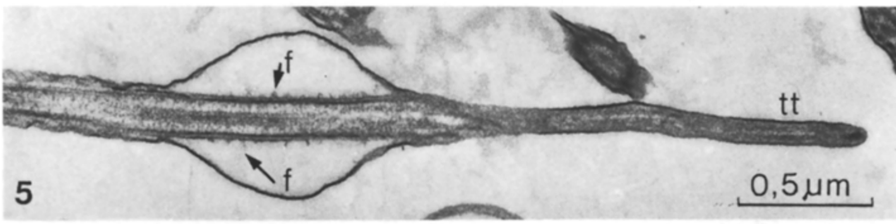

2 µm
tt
ap
k
ap
ms
3

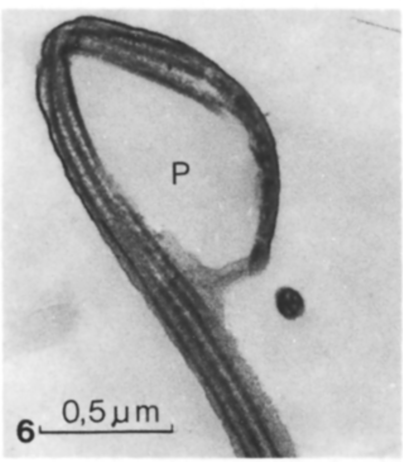
of the tentacles (Fig. 9). The central pair of tubules within the paddle-like swellings are more randomly orientated than those within the normal ciliary profiles. However, it has not yet been possible to determine whether these normal looking cilia in the section would have swellings outside the plane of section, and if so, if the orientation of the central pair of tubules changes within the swelling. The cilia on the tentacles beat with a metachronal wave (Dilly, 1972). Tamarin's (1974) report of randomly orientated central tubules in the cilia of *Mytilus* led him to conclude that the spatulate cilia were able to beat randomly. The tentacular cilia in *Rhabdopleura* beat en masse with a metachronal wave, but this varied orientation of the central tubules in *Rhabdopleura* suggests that these paddle cilia are also capable of random movement. It is probably that as the cilia perform their various functions they beat in different ways. The plastering activity probably involves the random beating mode, whereas feeding and water movements probably involve metachronal waves.

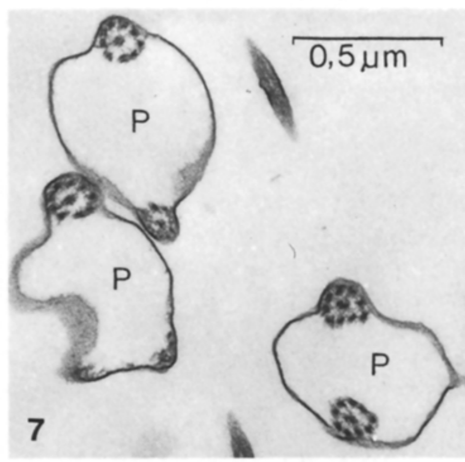
The axoneme filament pattern is maintained throughout the cilium, except in the distal tip of the cilium distal to a terminal enlargement, (Fig. 5). Sections show that the enlargement of the ciliary shaft takes place in the ciliary shaft membrane, and that this enlargement is usually associated with some distortion of the spatial dimensions of the axoneme (Fig. 4, 5, 6, 8). The axoneme fibrils start to come together as soon as they enter the proximal part of the enlargement, suggesting that as they enter an enlargement, they lose something that has previously maintained their separation (Fig. 5). The passage of the fibrils through the enlargement can take place either against one side of the swelling, or through the axis (Figs. 4–12). In those paddles where the shaft can be traced surrounding the swelling in scanning electron micrographs, (Figs. 11, 12), the same structures in TEM sections have the axoneme abutting against the ciliary membrane in the enlargement (Fig. 6–7). Frequently, part of the cylinder of the axoneme is enclosed in the expanded membrane before the membrane diverges from the axoneme and enlarges to become spherical (Fig. 7). Frequently the ciliary shaft bends and changes its direction on either side of a swelling. The bending of the ciliary axoneme takes place within the enlargement. There is usually only one such bend or kink within a single enlargement (Fig. 6). These bends occur frequently but they are only found in those axonemes that remain partially in contact with the ciliary membrane. Axonemes that have become completely separated from the ciliary membrane usually pass directly through the ciliary enlargement. The major structural difference between these two states is that in the former some of the microfilaments still anchor part of the axoneme to the ciliary membrane, and will support one side more than the other against distorting forces, with the subsequent collapse and distortion of the unsupported side (Fig. 6), whereas in the other all the supporting filaments have been broken, and no such asymmetric forces act on the axoneme (Figs. 4, 5, 8).

The TEM confirms that there may be more than one bleb on each ciliary shaft (Fig. 8). In these circumstances the diameter of the shaft between the enlargements does not vary and is the same as that of unmodified cilia, until it traverses the most distal enlargement. However, the axoneme in the proximal swellings decreases in diameter as it enters the swelling and continues to do so until it reaches the middle when it begins to enlarge again and regains its original dimensions as it leaves the swelling to re-enter the non-enlarged part of the shaft (Fig. 4). Distal to the swellings that are near the distal end of the ciliary shaft, the axoneme filaments are collapsed tightly together and the ciliary membrane is tightly applied to them with little or no









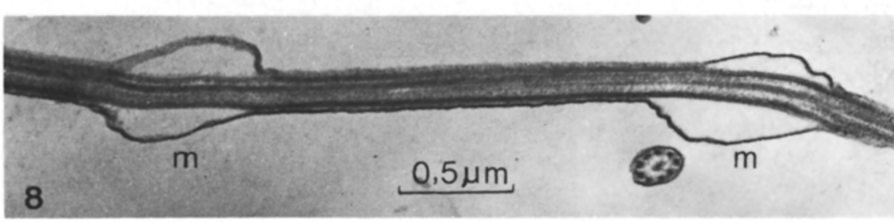


Fig. 4. A mid-shaft swelling showing the axoneme diameter reducing as it traverses the swelling, but being normal in the shaft on either side

Fig. 5. The tapering terminal part of a paddle cilium beyond the enlargement. The diameter of the terminal part is much reduced and filled with collapsed axoneme. Within the swelling, fibrils can be seen on the surface of the axoneme. It was presumably these fibrils that anchored the axoneme to the ciliary membrane

Fig. 6. A juxtaterminal swelling showing the axoneme in contact with one wall and the acute kink caused by the collapse of the unsupported side

Fig. 7. An axoneme partially enclosed within the ciliary membrane. The ridge in the paddle so formed is visible in SEM photographs. The unsupported fibrils are frequently disrupted

Fig. 8. Two swellings along a single ciliary shaft in which the axoneme traverses the swellings without contacting their walls

space between the membrane and the filaments of the axoneme (Fig. 5). It is possible that this reduction in diameter of the ciliary shaft, and the frequent change in direction of the distal fragment might be the result of tension on the ciliary membrane. Such a load could be produced if the extra membrane needed to surround the swelling was dragged back from that enclosing the distal fragment. Why membranes proximal to the enlargement are not collapsed is not understood, but it could be that the pressure of the ciliary cytoplasm differs across the swelling. Confirmation that the axoneme does indeed bend through a circle within the ciliary membrane swellings comes from sections that contain two profiles of the axoneme (Fig. 6, 7). Any tension generated by the bending of the axoneme is usually insufficient to flatten the swelling, but occasional flat disc-like paddles are found (Figs. 11, 12).

Longitudinal sections of the ciliary shaft as it traverses a swelling show that the axoneme within the shaft bends either with a kink or as a gradual curve, but even these curves rarely follow a uniform curve rather they get steeper in one section (Fig. 6). Such bending may suggest that the forces that tend to keep the axoneme rigid are overcome. The rapid bending suggests a local collapse and may be induced by a lack of support to that specific region of the tubules, but occasionally the S.E.M. image suggests a regular bending of the axoneme without kinking (Fig. 12).

All the 9 + 2 filaments of the axoneme traverse the swelling and usually maintain their spatial relationships to one another, although the overall diameter, and the space between the individual units, is reduced (Fig. 9). Sometimes, however, those tubules of the axoneme that have become separated from contact with the ciliary membrane no longer occupy their regular position in the ring of tubules. It appears as if the side fibrils that previously attached them to the ciliary membrane have been ruptured, and they have become distorted out of position (Fig. 7). The filaments still in contact with the ciliary membrane are still anchored, and still maintain their normal position. In those axonemes that are totally separated from the ciliary membrane as they traverse a swelling, the usual spatial pattern of the axoneme is maintained (Fig. 9), but the transverse diameter of the bundle is reduced suggesting that some force which has tended to keep the tubules apart has been removed. This collapse inwards is probably the result of the rupture of the fibrils that attach the axoneme to the ciliary membrane.

The distal tips of the cilium are frequently the most distorted. Here, besides the usual collapse of the ciliary membrane onto the axoneme, there are frequently distorted paddles. These distorted paddles appear to be misshapen because of collapse of their contents. The extent to which they are deformed can vary considerably, and in the extreme cases they are reduced to a few small blebs less than 0.1 μm across. It is possible that the varying degrees of collapse of the paddles represent stages in the discharge of their contents. Some cilia are found in which there is no paddle-like enlargement or remnant of it, but the tapered distal μm or so still remains (Figs. 2, 3, 10, 11). Whether this is the ultimate in collapse of the paddles or if the terminal part of the shaft will regain the diameter of the proximal ciliary shaft is not known.

Besides these tapered ended cilia there are others with a uniform cylindrical diameter throughout their length (Fig. 2). The alternative explanations of this shape are that the cilium never had a paddle-like enlargement or that either the

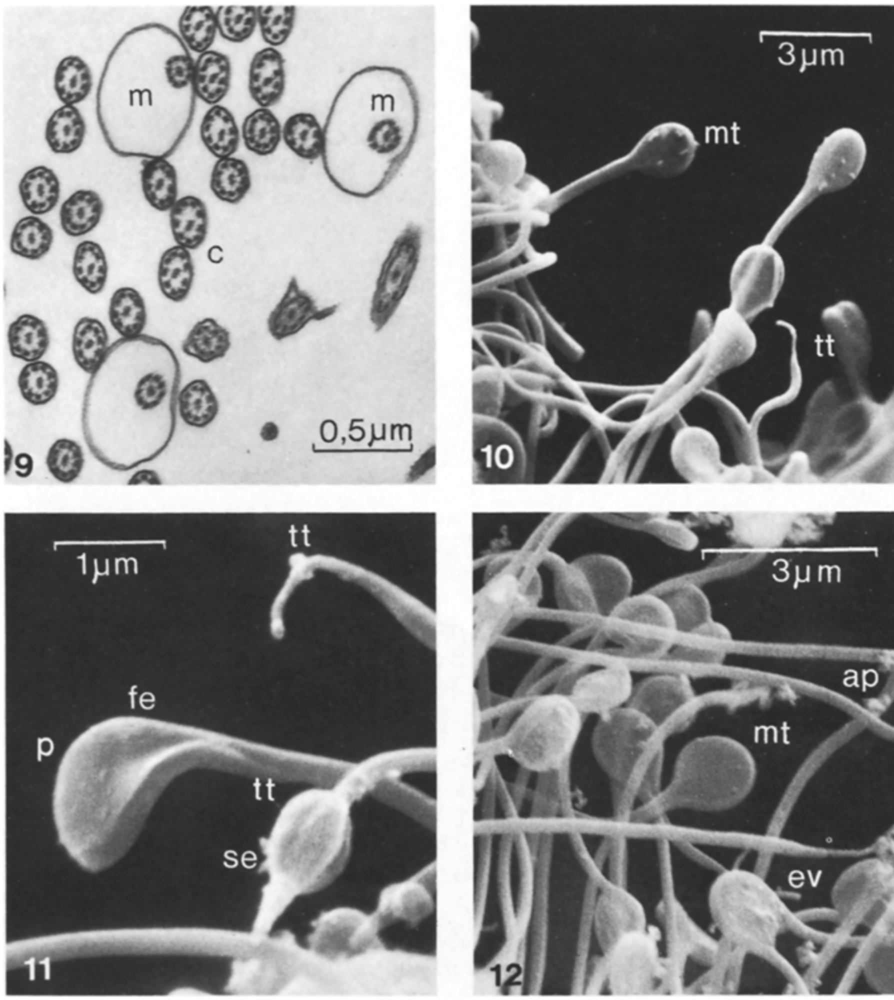


Fig. 9. TEM section of the cilia arising from a tentacle of *Rhabdopleura*. The profiles contain sections of both paddle and apparently normal cilia. Two important features can be seen, one that the enlargements are confined to the ciliary membrane, and secondly that the diameter of the axonemes within the membrane swellings is less than that within the normal profiles

Fig. 10. Terminal paddles and long tapering ends on non paddle bearing cilia. Paddles of this type are frequently supported by a double thickness shaft. Small particles can be seen adhering to the expanded ciliary membrane

Fig. 11. A terminal paddle, a spherical enlargement and a long taper. It is possible to trace the axoneme around most of the paddle perimeter

Fig. 12. A series of flattened paddle-like enlargements around which it is possible to trace the axoneme cylinder and a mid-shaft much less regular enlargement

whole paddle and terminal taper has broken off proximal to the tapering region, or that the taper has been re-expanded. Ocasionally, areas of cilia are seen, especially on the cephalic shield where there is just a terminal tiny button of an enlargement. This button may be the result of the membrane tearing during the breaking off of the distal fragment.

Tapering of the tips of ciliary shafts appears to be found in different circumstances both in cilia with juxtaterminal enlargements and in cilia without swellings. The tapers associated with most swellings are much more gradual than those of the terminal parts of the shafts without swellings, and extend for a shorter distance (Figs. 2, 11) although occasionally longer much narrower tapers are found associated with the swellings. The long narrow tapers frequently have terminal blebs and are usually found in the terminal parts of cilia without swellings (Figs. 3, 10, 11). The findings suggests that they are part of the population of cilia that have either lost their terminal swellings and collapsed locally, or because their shafts are frequently curved are part of the population of cilia that transport material outside the ciliary membrane. There are many cilia with the normal regular cylindrical profile.

Discussion

Both microtubules and microfilaments are well known to have important skeletal functions within the cytoplasm both in providing rigidity and tensile strength. Microtubules normally show few acute bends indicating some degree of stiffness, and the axoneme of the cilium is connected to the surrounding ciliary membrane by filamentous extensions of the tubule walls. It is probable that the axoneme is vital in maintaining the shape of the cilium and that distortions of the ciliary membrane must disrupt some of the connections between the axoneme and the surrounding membrane. It may be the breakdown of the mutual support between the membrane and the axoneme that accounts for both the genesis of the swellings and of the occasional acute angulation of the axonemes. A similar hypothesis would explain the contraction of the axoneme diameter. The tension between the filaments joining the axoneme and the ciliary membrane normally hold the axoneme and the ciliary membrane at a specific distance from one another, and once these linkages are broken down the axoneme tubules can contract slightly and come together, making a smaller overall diameter, whereas the membrane is free to move away from the axoneme and expand. If the fibrils joining the axoneme to the ciliary membrane are capable of making and breaking the contact and tensions between the axoneme and the ciliary membrane, then there exists a mechanism for moving the swellings and their contents along the ciliary shaft, those linkages distal to the swelling breaking down and those proximal to the swelling reforming and so squeezing the swelling along the shaft.

The occasional really acute bends in the axoneme filaments may represent a collapse of the axoneme because it has been distorted and undermined by the swelling and has been unable to maintain its rigidity.

In the tapering tips of some cilia the axoneme is completely collapsed.

Once the axoneme has collapsed, the surrounding ciliary membrane becomes very closely applied to its surface, unlike the membrane of the normal ciliary shaft which is usually a few nm away from the outer ring of nine compound tubules. This collapse may represent either a crushing of the microstructures that keep the membrane separate from the axoneme or it may be that the cytoplasm in this terminal part of the shaft has been discharged reducing the turgidity of the tube and allowing the membrane to shrink inwards onto the axoneme surface.

The swellings in the ciliary membrane appear empty in TEM sections and have a similar amorphous appearance to sections of normal parts of the cilium. This, of course, does not mean that they are without content during life, but rather that either the preparation for electron microscopy has removed the content or failed to make it electron dense.

It is possible that these swellings do not represent transport within the cilium but are a structural modification with some other function. The idea that they might act as spatulae plastering secretions onto the tube is attractive, and is supported by my observations of similar cilia in *Vermiliopsis* and *Dodecacera* and the findings of similar cilia in *Mytilus* byssus thread secreting region (Tamarin, 1974), but I have been unable to find similar cilia in *Cephalodiscus* or in *Saccoglossus.*

References

Berquist, P.R., Green, C.R., Sinclair, M.E., Roberts, H.S.: The morphology of cilia in sponge larvae. Tissue and Cell **9** 179–184 (1977)

Dilly, P.N: The structure of the tentacles of *Rhabdopleura compacta* (Hemichordata) with special reference to neurociliary control. Z. Zellforsch. **129** 20–39 (1972)

Dilly, P.N: Material transport within specialised ciliary shafts on *Rhabdopleura* zooids. Cell Tiss. Res. **180,** 367–381 (1977)

Tamarin, A., Lewis, P., Askey, J: Specialised cilia of the byssus attachment plaque forming region in *Mytilus californianus.* J. Morph. **142** 321–328 (1974)

Accepted June 20, 1977