THE STRUCTURE OF CILIA

by

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The structure and mode of action of such fibrous organelles as cilia, flagella and sperm tails present extremely interesting biophysical problems. This paper gives the results of some investigations on the structure of cilia, using the electron microscope to examine them both whole and after thin sectioning. There are numerous publications on the electron microscopy of flagella and sperm tails, but they have usually been observed only after drying on to a collodion supporting film. This technique needs to be supplemented with electron micrographs of sectioned material, for only by this means is a reliable picture of the intact structure obtained.

The introduction of the shadow casting technique[1] so improved the results of electron microscopy that early electron micrographs of flagella were soon superseded. Among recent investigations that should be quoted are those of LEWIN AND MEINHART[2] on the flagella of *Chlamydomonas*, MANTON and co-workers[3,4,5,6,7,8,9,10] on plant cilia and flagella, and GRIGG AND HODGE[11,12] on fowl and human sperm tails. The structure of cilia has not been so extensively studied, but brief observations have been made by SCHMITT *et al*[13] on clam cilia, while ANDERSON[14], JAKUS AND HALL[15], and KRUGER AND WOHLFARTH-BOTTERMANN[16] have examined the cilia of *Paramecium*. These authors examined whole cilia dried on to collodion films. Recently WATSON[17] and CHALLICE[18] have obtained electron micrographs of sections of sperm tails in which their detailed structure can be seen, but published electron micrographs of sections of cilia have not been of high quality. While this paper was being written, a publication by FAWCETT AND PORTER[19] became available, in which sections of cilia showing fine structure are illustrated.

The observations of these authors on the structure of cilia from molluscs, amphibians and mammals, and of MANTON and co-workers[3,4,5,6,7,8,9,10] on plant cilia and flagella are in close accord with those to be presented here, on the structure of cilia from the protozoan, *Paramecium*, and from the gills of an Australian fresh water mussel, *Hyridella australis* (Lam.).

EXPERIMENTAL

Fresh water mussels, of the species *Hyridella australis*, were collected from the River Murray. Cilia were freed from the gills and labial palps by macerating for several days in distilled water or fixative. After this treatment the supernatant liquid contained a high concentration of unwanted cell debris and soluble material, from which a purified suspension of cilia, suitable for electron microscopy, was prepared by differential centrifugation.

An unidentified species of *Paramecium* was grown in an Osterhaut-vegemite culture medium[20], from which, after passing through a coarse filter, the organisms were separated by gentle centrifuga-

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tion. Light centrifuging several times in distilled water served to separate the cells from bacteria and other material, and harder centrifuging, or maceration in distilled water or fixative, freed sufficient numbers of cilia for examination in the electron microscope. The cilia from both sources were examined with and without fixation.

Specimens for the electron microscope were prepared in the usual way by drying a drop of the suspension on to a collodion supporting film, and shadow casting with palladium.

The technique for the preparation of thin sections was substantially that described by PALADE[21]. Pieces of gill from *Hyridella* were fixed for four hours in buffered osmic acid (pH 7.4) and embedded in *n*-butyl methacrylate containing 10–20% methyl methacrylate and 2% benzoyl peroxide for catalyst. *Paramecia* were carried through the same process using gentle centrifugation to effect the change of liquids. A microtome of the thermal expansion type[22] was used for cutting sections, which were then examined in the electron microscope while still embedded.

The electron microscope used for these investigations was a Philips instrument operated at 60 kilovolts. The magnification meter was calibrated against polystyrene latex particles.

RESULTS

Whole cilia

When detached from cells cilia are not seriously damaged as they appear to have a natural cleavage plane[19,23]. Free, unbroken cilia could be distinguished by their slightly bulbous and denser bases.

Fig. 1 shows a group of cilia from the gills of *Hyridella* and Fig. 2 a single cilium from *Paramecium*. Most of the cilia detached from *Paramecium* were 9–12 μ long with diameter about 0.15 μ when intact and about 0.25 μ when more flattened. Those from *Hyridella* were 12–25 μ long with diameter 0.14–0.19 μ, the wide range of lengths probably being associated with the different types of cilia found on the ctenidia and labial palps. The cilia were of uniform diameter except for the base and tip regions, and frequently their fibrillar constitution could be seen (Fig. 1). The tip region of the cilia from *Paramecium* consisted of a tapering zone leading to the narrower tip. In many of the cilia from *Hyridella* the tip region was longer and narrower like a whiplash; some, however, possessed rather a tapering tip, while in others the whiplash terminated in a swollen region. Again these differences might be associated with the different types of cilia.

In over a hundred electron micrographs of the cilia from *Hyridella* there were no signs of an enveloping membrane, and some of the cilia from *Paramecium* presented much the same appearance. On the other hand, many of the cilia from *Paramecium*, especially when they had been fixed in osmic acid, were found to be surrounded by a wide, flat, low density structure (Fig. 2). In a number of cases the appearance of folds in this structure indicated clearly that it was a membrane. Although not shown in Fig. 2, this membrane sometimes extended to the tip, and frequently it appeared more swollen in one region, often at a bend in the cilium. In Fig. 3 the base of the cilium has apparently become twisted into a circular shape surrounded by a sac composed of the remnants of the sheath.

The cilia from *Hyridella* had a marked tendency to coil (Fig. 1) and, at the same time, they were often partly frayed. In order to determine whether the coiling was caused in drying, suspensions of cilia in water were examined with a phase contrast microscope. The cilia were found to have permanent configurations resembling those that had been seen in electron micrographs.

Occasional preparations from both *Hyridella* and *Paramecium* showed, without any apparent reason, more disintegration of the cilia than was normally found. The disintegration or fraying of the cilia from *Paramecium* into about eleven fibrils has been

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previously described[15]. In the present investigations a few cases of fraying were observed in which eleven or less fibrils could be counted. These examples were too few for general assertions to be made, but it was clear that two of the fibrils differed somwhat from the remainder by their close adherence and smaller diameter (Fig. 4). The remaining fibrils frequently lay together in a sheet or cylindrical formation (Fig. 4).

Similarly in *Hyridella* only a small proportion of the cilia completely frayed into their component fibrils. However, the large number of specimens examined and the enhancement of disintegration caused by treatment with alkaline 10% potassium chloride yielded a sufficient number of cases for some general observations to be made. A maximum number of eleven fibrils was found (Fig. 5), although quite often there were nine, or sometimes ten, in what appeared to be perfect examples of frayed cilia. As was found for *Paramecium*, two of these fibrils tended to adhere together and were of smaller diameter than the remainder, which tended to lie in sheet formations (Fig. 5). When the cilia had partly disintegrated, they were found to consist of a cylinder of fibrils surrounding a core of one or two fibrils (Fig. 6).

Figs. 6 and 7 show cilia from *Hyridella*, in which some substance appears to stretch between the fibrils. In a few instances, the fibrils of partially disintegrated cilia had regularly spaced ridges on them which suggested a cross striation. As this was observed only occasionally, and as the adhesivelike material already described gave almost the same appearance, the apparent striations may be only another manifestation of this substance between the fibrils. MANTON AND CLARKE[9] have observed somewhat similar effects in some plant flagella. No sign of a substance between the fibrils was found in the cilia from *Paramecium* but fewer specimens were examined.

Although the frayed fibrils of cilia from both *Hyridella* and *Paramecium* often had configurations suggestive of a very loose spiralling in the intact cilium, examination of intact or partially disintegrated cilia also showed the fibrils pursuing a straight and parallel course throughout the length of the cilium. Possibly the twisting of the fibrils could be produced during drying.

Individual fibrils were uniform in diameter which was in the range 350–500 A. Their appearance in cilia from both *Hyridella* and *Paramecium* was somewhat granular, and they showed a marked tendency to segment transversely (Figs. 4, 8 and 9). Some micrographs of more severely damaged cilia (Fig. 8) gave indications that the fibrils were themselves composed of finer sub-fibrils of the order of 50 A in diameter. Occasionally in the cilia from *Hyridella*, the fibrils also showed some signs of longitudinal segmentation into two or three parts (Fig. 9).

The effects of several reagents on the cilia from *Hyridella* were examined but did not yield any conclusive results regarding their composition. No recognizable remnants of cilia were found after digestion with trypsin, while they were unchanged by hyaluronidase digestion. They were completely dissolved by dilute hydrochloric acid and caustic soda, while ammonia and acetic acid produced a "blurring" of their structure as seen in electron micrographs. Slightly alkaline potassium chloride solutions were found to promote fraying and disintegration.

Sections of cilia

Sections of cilia from the cytopharynx of *Paramecium* and from the gills of *Hyridella* are illustrated in Figs. 10 and 11 respectively. Such electron micrographs showed very clearly the arrangement of the eleven fibrils, already described, within an enveloping

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membrane. This membrane differed somewhat in the two kinds of cilia. In the sections of *Paramecium* cilia it appeared as a very irregular structure, sometimes with prolongations of 1 μ or more in length, whereas the cilia from *Hyridella* had a less irregular membrane which was thinner, less osmiophilic and hence less conspicuous than that of *Paramecium*.

Fig. 1. A group of cilia detached from the gills of *Hyridella* (× 7,000).

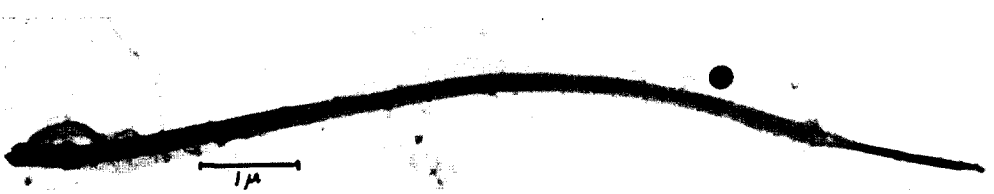

Fig. 2. A single detached cilium from *Paramecium* (× 13,000).

The thickness of the membrane for the latter varied from 100–200 A. The space around the fibrils within the membrane appeared to be occupied by some structureless substance of low density.

Both kinds of cilia showed the same fibrillar structure, namely a circular array of nine fibrils about a central pair. In a small minority of cilia it was not possible to count nine outer fibrils, but this was almost certainly due to disarrangement of the fibrils during processing; sometimes the inner pair of fibrils was not distinctly resolved.

There was no evidence that the fibrils had any kind of spiral organisation. In some longitudinal sections they were seen to follow a straight course over distances of 2–3 μ. Further, in cases where the orderly arrangement of the cilia had been preserved (Fig. 10) there was an obvious preferred orientation of the line joining the two central fibrils which would not be expected in sections cut at random if these fibrils twisted about each other.

Fig. 3. Portion of a partly coiled cilium from *Paramecium* (× 18,000).

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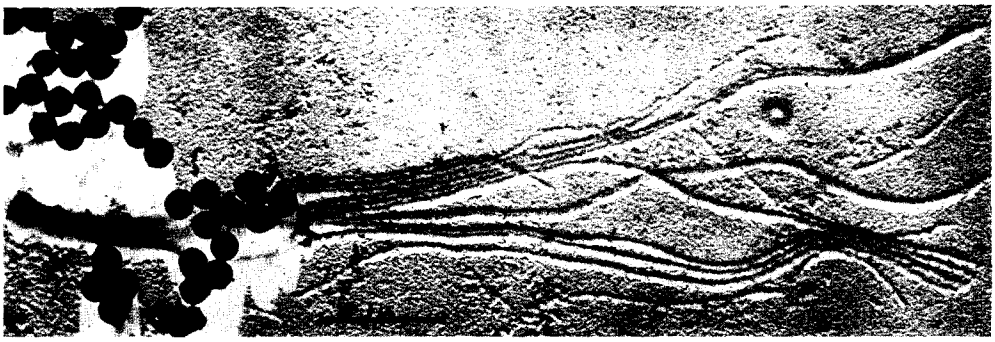

Fig. 4. Disintegration of a cilium from *Paramecium* into eleven fibrils. (× 10,000)

The diameter of the cross sections of cilia from *Hyridella* varied from about 0.12 μ to 0.24 μ while that of the fibrillar cylinder varied from about 0.1 μ to 0.2 μ. The diameter of many of the cilia from *Paramecium* was about 0.16 μ. Some much larger cilia of diameter about 0.25 μ (Fig. 10) were found, and some much smaller of diameter about 0.1 μ. The latter were probably sections through the tips of the cilia. The corresponding diameters of the fibrillar cylinder were 0.12 μ to 0.15 μ for the larger cilia and about 0.06 μ for the smaller ones.

Besides cross sections and oblique sections of cilia, Fig. 11 shows some cilia which are curved with respect to the plane of the section. Prominent features of the more longitudinally cut portions of such sections are the sharp, dense, double lines at the periphery. The space between these lines appears to be of enhanced density while in some places there is a trace of a third line adjacent to the dense pair. Examination of a number of such sections showed that these lines almost certainly corresponded to a section of one fibril. In one place in Fig. 11, two pairs of lines can be seen in the position corresponding to the central fibrils. Some of the fibrils at the ends of such sections, though cut more obliquely, also show a double-stranded character, although some appear to be composed of three strands. The oblique sections of cilia in Fig. 11 also show signs of this double-stranded appearance. Even when curved, the two lines always remain parallel, and the distance across them is about 200 A, the lines themselves being of the order of 50 A wide.

In cross sections the fibrils had a core considerably denser than the surrounding medium suggesting either that they were enveloped by a sheath or by material precipitated during fixation. (Fig. 10 and 11) In many cases their appearance also suggested a hollow structure, while occasionally they could be interpreted as double. The overall diameter of the fibrils was estimated to be 300–400 A but precise measurement was difficult. The inner core was about half this diameter.

DISCUSSION

It is clear from the work of a number of authors, and particularly of MANTON and her colleagues[3,4,5,6,7,8,9,10], and of FAWCETT AND PORTER[19], together with the results reported here, that many cilia and flagella (other than bacterial flagella) have essentially the same structural features. The fundamental pattern of structure is one of a cylindrical array of nine fibrils about a central pair. The central pair are narrower and more closely adherent than the peripheral fibrils, but all are parallel throughout the length of the cilium, with no convincing evidence for any spiral structure. Such appearances of a

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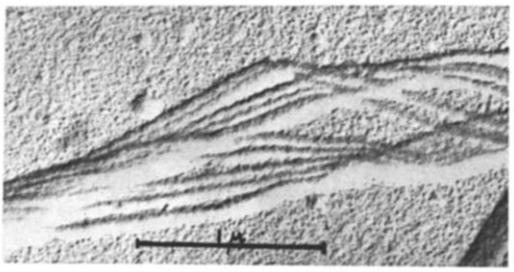

Fig. 5. Portion of a disintegrated cilium from *Hyridella* showing eleven component fibrils (× 25,000).

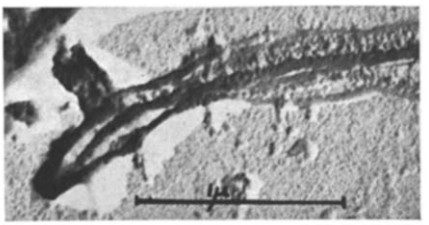

Fig. 6. Portion of a partly disintegrated cilium from *Hyridella* showing its cylindrical structure (× 28,000).

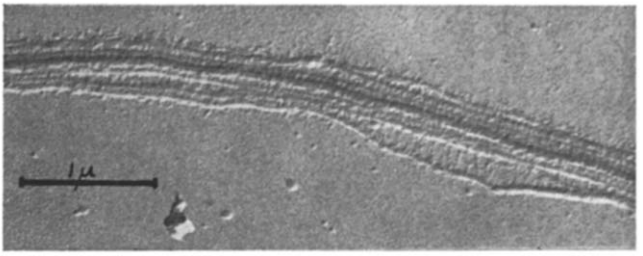

Fig. 7. Portion of a partly disintegrated cilium from *Hyridella* showing the presence of some material stretching between the fibrils (× 18,000).

Fig. 8. Component fibrils from the cilia of *Hyridella* showing disintegration into a number of smaller subfibrils (× 34,000).

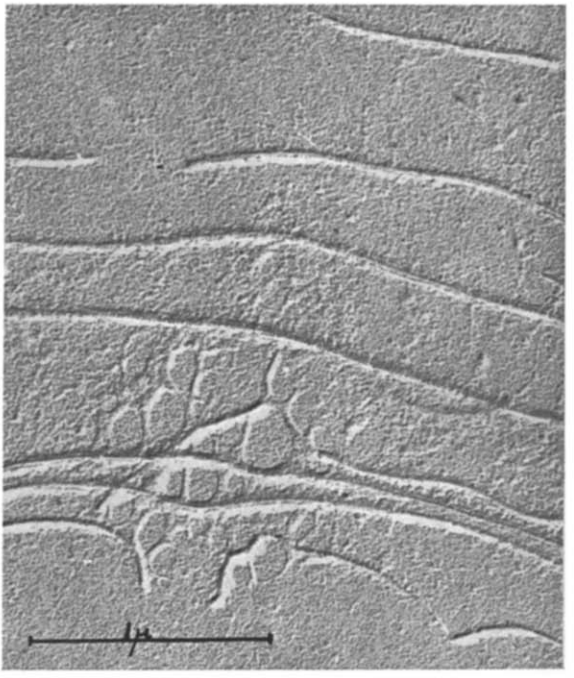

Fig. 9. Component fibrils of a cilium from *Hyridella* showing longitudinal cleavage into two or three parts (× 32,000).

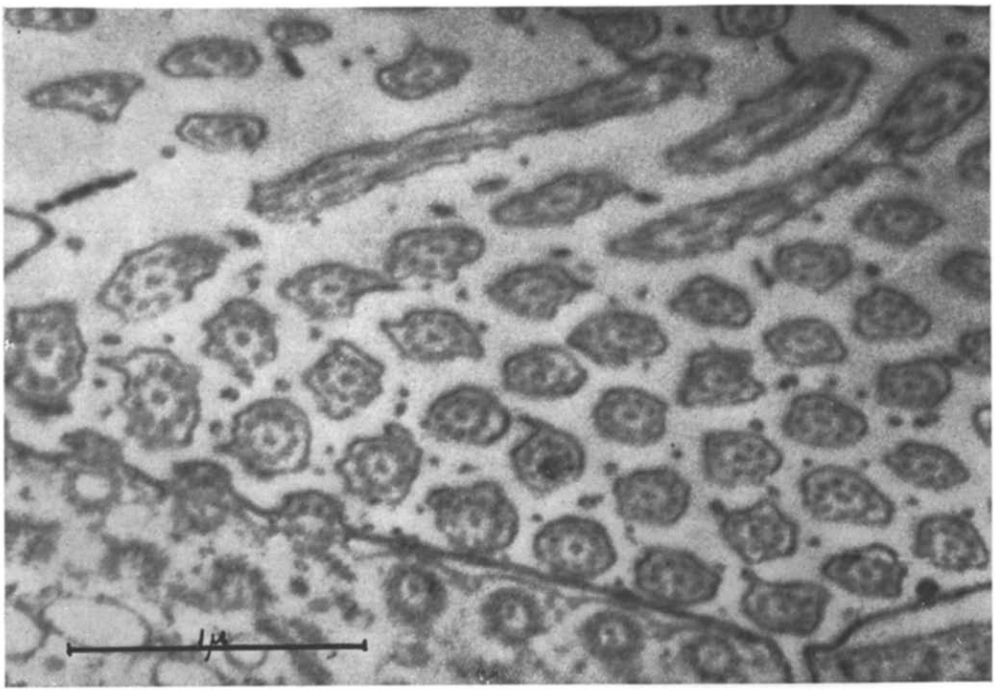

Fig. 10. Sections of cilia from *Paramecium* (× 40,000).

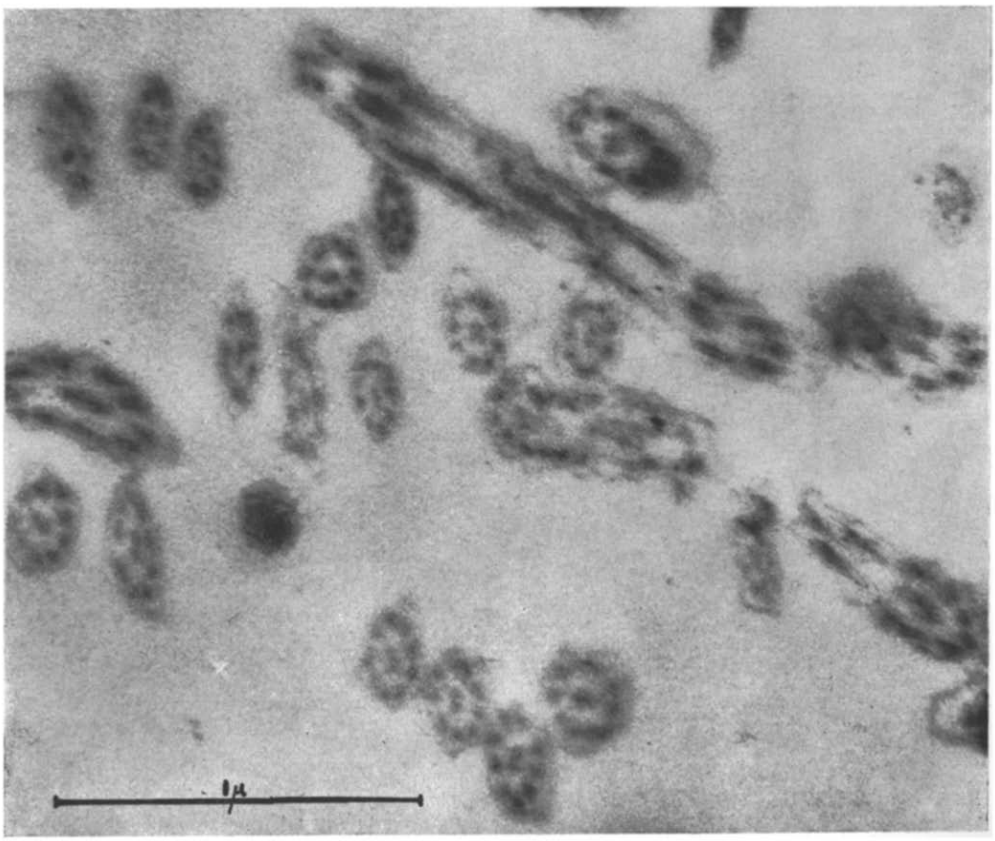

Fig. 11. Sections of cilia from *Hydridella* (× 50,000).

spiral arrangement as occur in micrographs of whole cilia may be discounted as the results of drying. The fibril system is surrounded by a thin membrane, the space within being occupied by a matrix, which, after fixation, appears as a precipitate of low electron optical density.

The main point for discussion concerns the nature of the peripheral fibrils, which are certainly composite in structure. The micrographs of frayed whole cilia usually show the nine fibrils as single threads, but occasionally as in Fig. 9 they are distinctly double or even triple. There is also evidence that extremely fine subfibrils of the order of 50 A in diameter are components of the major fibrils (Fig. 8). FAWCETT AND PORTER[19] have suggested that the appearance of fibrils from whole cilia is due to inadequate cleaning, but this seems unlikely because the fibrils themselves are of uniform diameter (Fig. 5). It is always in more damaged cilia that the composite structure of the fibrils is revealed most clearly. A more likely explanation of these observations is that the fibrils consist of a sheath of different composition surrounding the smaller units.

There is some indication of this sheath in cross sections of fibrils where the appearance of a central osmiophilic core surrounded by a less dense region is often given. None of these cross sections lead to an unequivocal picture of the complex structure of the fibrils, but the impression is often given of a hollow tubular core, or occasionally a double structure is suggested. On the other hand from some of the longitudinal and very oblique sections (Fig. 11) a clearer picture emerges. If, as suggested by FAWCETT AND PORTER[19], the outer fibrils do consist of a pair of tubular structures in contact, longitudinal or oblique sections might be expected to show two or three lines corresponding to the walls of the tubules. This is precisely what is shown in Fig. 11. However, it may be doubted that the tubular appearance is real, for it may be an artifact arising from the fixing or embedding processes. WYCKOFF[24] has obtained sections of collagen showing fibres which appear to be tubes, and in this laboratory it has been found that the embedding process often results in distension of bacteria. Be this as it may, it seems quite certain from such sections that the nine outer fibrils have each a double stranded structure. Whole fibrils show indications of breakdown into very fine units, and both whole and sectioned fibrils appear to be surrounded by a sheath of different composition.

It is a striking fact that cilia, flagella and sperm tails from so many diverse sources should have the same basic fibrillar structure, and it is interesting to speculate on the occurrence of the cylindrical array of nine fibrils. In sections these appear to be rather widely spaced, but it is not clear that this represents the state of affairs in the living cilium where they might be more closely packed. If this were so, the occurrence of nine fibrils might reflect some property of their molecular symmetry. If, on the other hand, the fibrils are really as widely spaced as their sections suggest, it is not clear what preserves the observed structure unless there is some transverse connection between the fibrils or some fairly adhesive material which holds them in place.

ACKNOWLEDGEMENTS

This work was carried out while one of the authors (B.P.P.) held a Commonwealth Scientific and Industrial Research Organization Australian Studentship. The authors wish to thank the staff of the Zoology Department, University of Adelaide, for assistance in collecting mussels and for supplying a culture of *Paramecium.*

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SUMMARY

The structure of cilia from *Paramecium* and from the gills of an Australian bivalve, *Hyridella australis* (Lam.), has been studied by means of electron microscopy. Both whole isolated cilia and sections of cilia have been examined and their fine structure elucidated. This fine structure is similar in both types examined, and appears to be of remarkable uniformity whatever the source of cilia. The fundamental pattern is that of a cylindrical array of nine parallel fibrils and a pair of axial fibrils. Each of the fibrils is itself a complex structure.

RÉSUMÉ

La structure des cils de *Paramécie* et des branchies d'un bivalve Australien, *Hyridella australis* (Lam.), a été étudiée au microscope électronique. Des cils entiers isolés et des sections de cils ont été examinés et leur structure fine élucidée. Cette structure fine est semblable chez les deux types examinés, et se montre d'une remarquable uniformité quelque soit l'origine des cils. Le chéma fondamental est celui d'un arrangement cylindrique de neuf fibrilles parallèles et d'une paire de fibrilles axiales. Chaque fibrille possède elle-même une structure complexe.

ZUSAMMENFASSUNG

Es wurde die Struktur der Cilien von *Paramecien* und von den Kiemen einer australischen Muschel, *Hyridella australis* (Lam.), mit dem Elektronenmikroskop untersucht. Es wurden ganze isolierte Cilien und Schnitte von Cilien untersucht und ihre Feinstruktur aufgeklärt. Diese Feinstruktur ist für beide untersuchten Typen ähnlich und scheint von bemerkenswerter Einheitlichkeit zu sein woimmer die Cilien herstammen. Der grundlegende Bauplan ist der einer zylindrischen Anordnung von neun parallelen Fibrillen und einem Paar axialer Fibrillen. Jede der Fibrillen selbst besitzt eine komplexe Struktur.

REFERENCES

1 R. C. WILLIAMS AND R. W. G. WYCKOFF, *J. Applied Physics*, 17 (1946) 23.
2 R. A. LEWIN AND J. O. MEINHART, *Canad. J. Bot.*, 31 (1953) 711.
3 I. MANTON AND B. CLARKE, *J. Exp. Bot.*, 2 (1951) 125.
4 I. MANTON AND B. CLARKE, *Ann. Botany (London)*, 15 (1951) 461.
5 I. MANTON AND B. CLARKE, *J. Exp. Bot.*, 2 (1951) 242.
6 I. MANTON, B. CLARKE AND A. D. GREENWOOD, *J. Exp. Bot.*, 2 (1951) 321.
7 I. MANTON, *Soc. Exp. Biol. Symp.*, 6 (1952) 306.
8 I. MANTON, B. CLARKE, A. D. GREENWOOD AND E. A. FLINT, *J. Exp. Bot.*, 3 (1952) 204.
9 I. MANTON AND B. CLARKE, *J. Exp. Bot.*, 3 (1952) 265.
10 I. MANTON, B. CLARKE AND A. D. GREENWOOD, *J. Exp. Bot.*, 4 (1953) 319.
11 G. W. GRIGG AND A. J. HODGE, *Aust. J. Sci. Res.*, ser. B., 2 (1949) 271.
12 A. J. HODGE, *Aust. J. Sci. Res.*, ser. B., 2 (1949) 368.
13 F. O. SCHMITT, C. E. HALL AND M. A. JAKUS, *Biol. Symp.*, 10 (1943) 261.
14 T. F. ANDERSON, cited by R. WICHTERMAN, *The Biology of Paramecium*, The Blakiston Co. Inc., N.Y., 1953.
15 M. A. JAKUS AND C. E. HALL, *Biol. Bull.*, 91 (1946) 141.
16 F. KRUGER AND K. E. WOHLFARTH-BOTTERMANN, *Mikroskopie*, 7 (1952) 121.
17 M. L. WATSON, *Biochim. Biophys. Acta*, 10 (1953) 1.
18 C. E. CHALLICE, *J. Roy. Microscop. Soc.*, 73 (1953) 115.
19 D. W. FAWCETT AND K. R. PORTER, *J. Morph.*, 94 (1954) 221.
20 B. A. HUMPHREY AND G. F. HUMPHREY, *Br. J. Exp. Biol.*, 25 (1948) 123.
21 G. E. PALADE, *J. Exp. Med.*, 95 (1952) 285.
22 A. J. HODGE, H. E. HUXLEY AND D. SPIRO, *J. Histochem. and Cytochem.*, 2 (1954) 54.
23 L. G. WORLEY, E. FISCHBEIN AND J. E. SHAPIRO, *J. Morph.*, 92 (1953) 545.
24 C. RAGAN, *Connective Tissues*, Trans. Third Conf. Josiah Macy Fn, N.Y., 1952.

Received August 4th, 1954