J. Cell Sci. 1, 471–474 (1966)
Printed in Great Britain

A SPIRAL SLEEVE IN ROTIFER CILIA TIPS

M. R. DICKSON
John Curtin School of Medical Research, Australian National University, Canberra, Australia

SUMMARY

The fine structure of cilia in the gut of the rotifer, *Philodina roseola*, has been studied. The cilia display the usual structures for the first 9 μ of their length, but for the last 3 μ the shaft consists of plasma membrane clothing an unusual spiral sleeve. The sleeve is made up of 5 parallel fibres twisted in a left-handed helix about the long axis of the cilium.

INTRODUCTION

The fine structure of rotifer coronal cilia has been studied by Lansing & Lamy (1961), and Scholtyseck & Danneel (1962), who reported that they possessed the familiar structures common to all cilia. Cilia are also present in the gut of rotifers, where they stir the contents, and drive them along. This note describes an unusual structure in the tips of the intestinal cilia of the rotifer, *Philodina roseola*.

MATERIALS AND METHODS

Philodina roseola was obtained from moss at the edge of a dry pond near this School. The animals were cultured in filtered lake water and fed on algae and ciliates, which established themselves in the culture dishes. An algal growth medium was added (10% in lake water) to encourage the algae. The medium was changed every 3 days.

Before fixation the animals were narcotized with 10 drops of 2% Xylocaine per 4 ml watch glass. They were then fixed in 5% glutaraldehyde in cacodylate buffer at pH 7·4 for 2 h, washed in 3 changes of buffer for 1 h, and post-fixed in 1% osmium tetroxide in veronal acetate buffer at pH 7·4 for 2–3 h. Washing in 1% aqueous uranyl acetate solution for 1 h preceded dehydration. The specimens were dehydrated in an alcohol sequence, transferred to acetone and embedded in Araldite. Sections were cut on an LKB ultramicrotome using diamond knives. Silver-grey sections were selected and mounted on grids covered with a thin composite carbon/collodion film.

Sections mounted on grids were stained for 20 min in Reynolds's lead citrate solution diluted 1/20 in 0·01 N KOH (Reynolds, 1963). The sections were examined in a Siemens Elmiskop I, operated at 80 kV.

Observations and measurements of cilial movement in living specimens, restrained in a Rousselet compressor, were made with a phase-contrast microscope fitted with an eyepiece micrometer.

RESULTS

At their base the intestinal cilia of *P. roseola* display the structures characteristic of most cilia (Figs. 3A, 5). The ciliary plasma membrane has an unusually thick fuzzy coat, and exhibits many protuberances (Fig. 2).

About 9 μ from the base of the shaft, which may be 12 μ long, a new structural element appears; it is a tapering, cylindrical sleeve which separates the peripheral fibres and the plasma membrane and which runs for at least 3·5 μ up to the end of the shaft. Since cilia with the sleeve may be curved or sharply bent (Figs. 6, 7), it appears that the sleeve is flexible. It consists of a number of dark-staining, parallel fibrils coiled in helices, which have as a common axis the long axis of the shaft of the cilium (shown in diagrammatic form in Fig. 1). The fibrils are about 100 Å thick, and where the

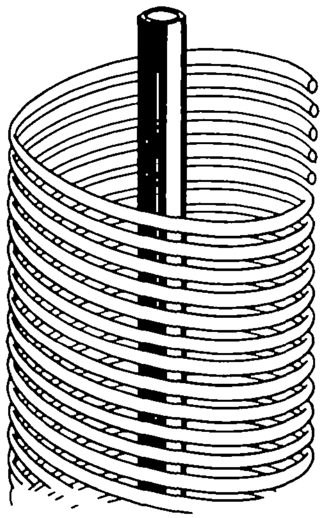


Fig. 1. Diagram of the sleeve showing 5 fibrils spiralling about a single remaining fibre.

packing is regular (Figs. 6, 7) the width of the light bands separating them is about 50 Å. Examination of oblique sections of cilia, in which the direction of the tip of the shaft relative to the section was known, revealed that the helices are left-handed—that is, the spiral rotates in a clockwise fashion as it proceeds to the tip of the cilium. Five fibrils are present near the tip of the cilium (Fig. 1), where the diameter of the tapering shaft is less than the thickness of a section (800 Å), and both sides of the spiral sleeve appear together in a criss-cross pattern from which the number of fibrils may be fooud (Fig. 6).

Where the sleeve begins it touches the plasma membrane (arrowed in Fig. 4), and a little distal to this point tenuous connexions link it for a short distance to the ciliary fibres it encloses (Figs. 3C, D, G). The fibres all end within 1 μ of the beginning of the sleeve, except one, which is continued to within 0·5 μ of the ciliary tip (Fig. 6). The peripheral fibres lose subfibre A (bearing the two rows of arms) where they touch the sleeve, and are represented by single fibres from here to their termination. The fibres neither lose subfibre A nor terminate simultaneously.

The diameter of the tapering sleeve decreases from 130 mμ at its beginning to 50 mμ at its end. As it does so, the angle of the spiral increases, and the thickness of the sleeve wall in transverse sections increases from about 80 Å (Fig. 3B) to about 300 Å (Figs. 3I, J, K).

The movements of the intestinal cilia in the living rotifer may be seen clearly with the phase-contrast microscope. Slow, quasi-sinusoidal waves with a period of about 3 sec pass along the shafts from base to tip, slowly stirring the rectal contents.

DISCUSSION

Apart from the spiral sleeve, the intestinal cilia in *P. roseola* have no unusual features. At their origin they resemble the rotifer coronal cilia (Lansing & Lamy, 1961), their shafts display all the familiar ciliary structures (Fawcett, 1961), and the termination of the fibres is very similar to the termination of the ciliary fibres in certain flagellates (Gibbons & Grimstone, 1960).

The location of the sleeve between the axial complex and the plasma membrane is like that of the fibrous sheath in the principal piece of the mammalian sperm tail (Fawcett, 1961). But this sheath is not a spiral, is associated with the base, not the tip, of the sperm flagellum, and has much thicker walls. Bradfield (1955) thought the fibrous sheath here might function as a resistant wrapping to prevent the flagellum from disintegrating; Challice (1953) considered that it might have elastic properties and act as a spring.

These alternative functions may be proposed for the rotiferan spiral sleeve. But the gut cilia beat slowly, and probably have less need for a protective wrapping than the fast-beating coronal cilia, which have no sleeve. If the sleeve is as flexible as Fig. 6 suggests, it is not likely to function as a spring.

The fact that the cilia beat slowly, with peculiar damped sinusoidal waves passing down them, suggests that the main function of the cilia is to stir gently the contents of the gut. The cilia are about 12 μ long, and the intestine is about 15 μ wide; it may be that their modified tips facilitate the motion of the cilia in this confined space with its rather viscous contents. The sleeve may be a passive weight, or may actively contract; a sliding movement between adjacent turns would produce contraction. The motion of the ciliary tips beyond the ending of the fibres could not be observed since the tips are too fine; there are, therefore, no observations of the way in which the sleeve modifies the motion to enable the dynamical properties of the terminal portion to be judged.

The fuzzy outer coat and protuberances of the ciliary membrane are probably responses to conditions in the gut, and may protect the cilia from digestive enzymes and abrasion. In vertebrates the intestinal microvilli have a similar fuzzy coat (Sjöstrand, 1963).

I wish to thank Dr E. H. Mercer for his invaluable advice and criticism of the manuscript.

REFERENCES

BRADFIELD, J. R. G. (1955). Fibre patterns in animal flagella and cilia. *Symp. Soc. exp. Biol.* 9, 306–334.

CHALLICE, C. E. (1953). Electron microscope studies of spermiogenesis in some rodents. *Jl R. microsc. Soc.* 73, 115–127.

FAWCETT, D. W. (1961). Cilia and flagella. In *The Cell*, vol. 2 (ed. J. Brachet & A. E. Mirsky), p. 217. New York and London: Academic Press.

GIBBONS, I. R. & GRIMSTONE, A. V. (1960). On flagellar structure in certain flagellates. *J. biophys. biochem. Cytol.* 7, 697–717.

LANSING, A. I. & LAMY, F. (1961). Fine structure of the cilia of rotifers. *J. biophys. biochem. Cytol.* 9, 799–812.

REYNOLDS, E. S. (1963). Lead citrate at high pH as an electron-opaque stain. *J. Cell Biol.* 17, 208–212.

SCHOLTYSECK, E. & DANNEEL, R. (1962). Die Feinstruktur des Wimperapparates von *Brachionus urceolaris* (Rotatoria). *Protoplasma* 56, 99–108.

SJÖSTRAND, F. S. (1963). The ultrastructure of the plasma membrane of columnar epithelium cells of the mouse intestine. *J. Ultrastruct. Res.* 8, 517–541.

(*Received* 9 *February* 1966—*Revised* 20 *May* 1966)

Fig. 2. Rotifer intestinal cilium in longitudinal section showing the helical sleeve. Some longitudinal fibres may be seen inside the sleeve. Tapering of the shaft, protuberances of the plasma membrane of the shaft, and the fuzzy outer coat are all evident. Specimen fixed in 5 % glutaraldehyde, 1 % osmium tetroxide and 1 % uranyl acetate, and stained in lead citrate solution for 20 min. × 40000.

Fig. 3. Transverse sections of intestinal cilia at different levels. All micrographs in the series are × 80000, and fixation and staining are as for Fig. 2.

A. Section of shaft below the sleeve showing the usual 9 + 2 pattern of fibres and fine fibrous linkages connecting them.

B. Section of shaft near beginning of sleeve. All the fibres appear normal, and do not appear to be in contact with the sleeve.

C. Further up the shaft. Three of the peripheral fibres have lost subfibre A.

D. All 9 peripheral fibres are now single, and seem to be adhering to the sleeve.

E. Fine fibrous links may be seen joining the single fibres to each other and to the sleeve in this section.

F. Only 8 of the original 11 fibres are present; 4 of these have dense centres (arrowed), probably indicating that they terminate within the thickness of the section. The regular arrangement of the fibres, which is still present in E, has been disturbed.

G–J. With a further reduction in the number of fibres, the shaft diameter decreases, and the transverse width of the wall of the sleeve increases.

K. The last fibre has terminated.

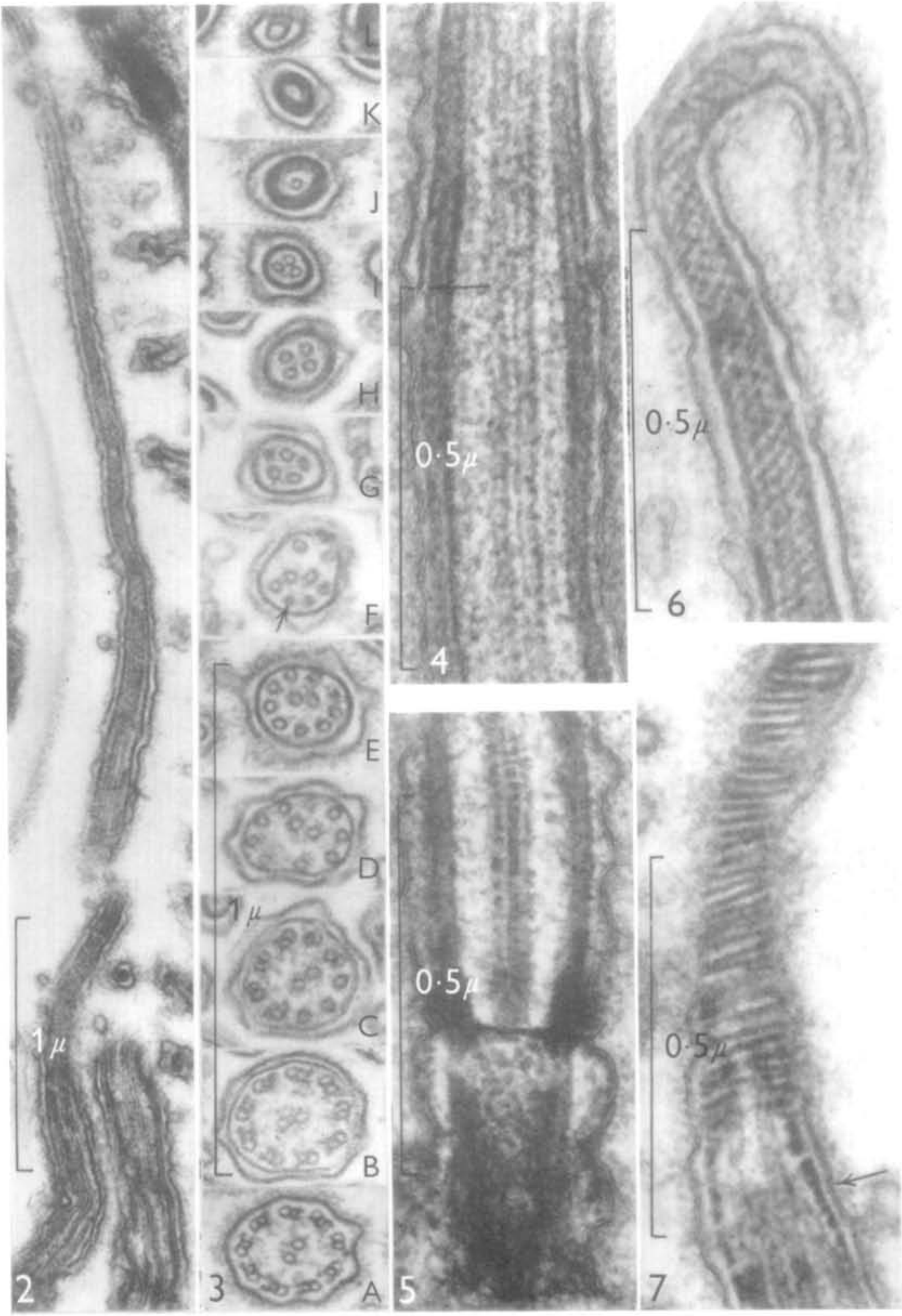
I. Just at the tip of the shaft, the wall of the sleeve once more decreases in width.

Fig. 4. Section of shaft showing point of commencement of sleeve (arrowed). The sleeve first appears in contact with the plasma membrane, then seems to become linked to the adjacent peripheral fibres. Fixation and staining as in Fig. 2. × 120000.

Fig. 5. Basal body and commencement of the shaft. Granules are contained in the basal body interior, and fibres in the basal body wall run through the walls of a dense cup to become the peripheral fibres of the shaft. The axial fibres originate in the floor of the cup. Fibrous links are seen between the peripheral and axial fibres. Fixation and staining as for Fig. 2. × 120000.

Fig. 6. Longitudinal section of cilia tip. The tip is sharply bent, suggesting that the sleeve is quite flexible. The spiral sleeve shows a criss-cross pattern, as both sides of the sleeve lie within the section. Fixation and staining as for Fig. 2. × 120000.

Fig. 7. Near the commencement of the sleeve the angle of the helix is less. Individual fibrils of the helix are occasionally displaced. In some regions the fibrils of the helix seem to be closely linked (arrow); in other places, however, this is not so. Fixation and staining as in Fig. 2. × 120000.


2
1 μ
3
L
K
J
I
H
G
F
E
D
C
B
A
1 μ
4
0·5 μ
5
0·5 μ
6
0·5 μ
7
0·5 μ