J. Cell Sci. **79**, 161–179 (1985)
Printed in Great Britain © *The Company of Biologists Limited 1985*

VISUALIZATION OF CHANGES IN CILIARY TIP CONFIGURATION CAUSED BY SLIDING DISPLACEMENT OF MICROTUBULES IN MACROCILIA OF THE CTENOPHORE *BEROË*

SIDNEY L. TAMM AND SIGNHILD TAMM
Marine Biological Laboratory, Woods Hole, Massachusetts 02543, U.S.A.

SUMMARY

Macrocilia from the lips of the ctenophore *Beroë* consist of multiple rows of ciliary axonemes surrounded by a common membrane, with a giant capping structure at the tip. The cap is formed by extensions of the A and central-pair microtubules, which are bound together by electron-dense material into a pointed projection about 1·5 μm long. The tip undergoes visible changes in configuration during the beat cycle of macrocilia. In the rest position at the end of the effective stroke (+30° total bend angle), there is no displacement between the tips of the axonemes, and the capping structure points straight into the stomach cavity. In the sigmoid arrest position at the end of the recovery stroke (−60° total bend angle), the tip of the macrocilium is hook-shaped and points toward the stomach in the direction of the subsequent effective stroke. This change in tip configuration is caused by sliding displacement of microtubules that are bound together at their distal ends. Electron microscopy and two-dimensional models show that the singlet microtubule cap acts as if it were hinged to the ends of the axonemes and tilted to absorb the microtubule displacement that occurs during the recovery stroke. The straight and hooked shapes of the tip are thought to help the ctenophore ingest prey.

INTRODUCTION

The sliding microtubule model of ciliary motility was initially based on electron-microscopic evidence showing no structural changes in axonemal diameter or microtubule length during ciliary bending (Horridge, 1965; Satir, 1965, 1967, 1968). Satir's thin-section analysis of microtubule termination at the distal ends of molluscan gill cilia showed that the amount of displacement between the tips of microtubules on opposite sides of the axoneme is proportional to the total bend angle and the distance between the microtubules. However, because of the small dimensions of typical cilia and flagella, the displacement of microtubule tips predicted by the sliding model has not been visible in actively beating organelles.

In this report we take advantage of the giant capping structure of macrocilia from the ctenophore *Beroë* to visualize changes in tip configuration of motile cilia caused by sliding displacement of microtubules during ciliary bend formation. Besides providing a direct demonstration of the expected displacement of microtubule tips in active cilia, this feature of macrocilia may also be useful to the ctenophore by facilitating ingestion of prey.

Key words: macrocilia, tip structure, microtubule sliding displacement.

MATERIALS AND METHODS

Beroë cucumis was collected in Sandwich, MA, and also sent from Friday Harbor Laboratory, WA. Methods for electron microscopy and ATP reactivation have been published (Tamm & Tamm, 1984). Films (35 mm, Kodak 2415, Technical Pan) of living macrocilia (Fig. 1A,C–E) were taken through a Zeiss RA microscope with phase-contrast optics, using an Olympus OM-2 camera and an Olympus T32 flash tube inserted in the light path; 16 mm films (Fig. 1B) were taken on Kodak Plus X Reversal film with a Locam (Redlake Labs, Santa Clara, CA) cine-camera.

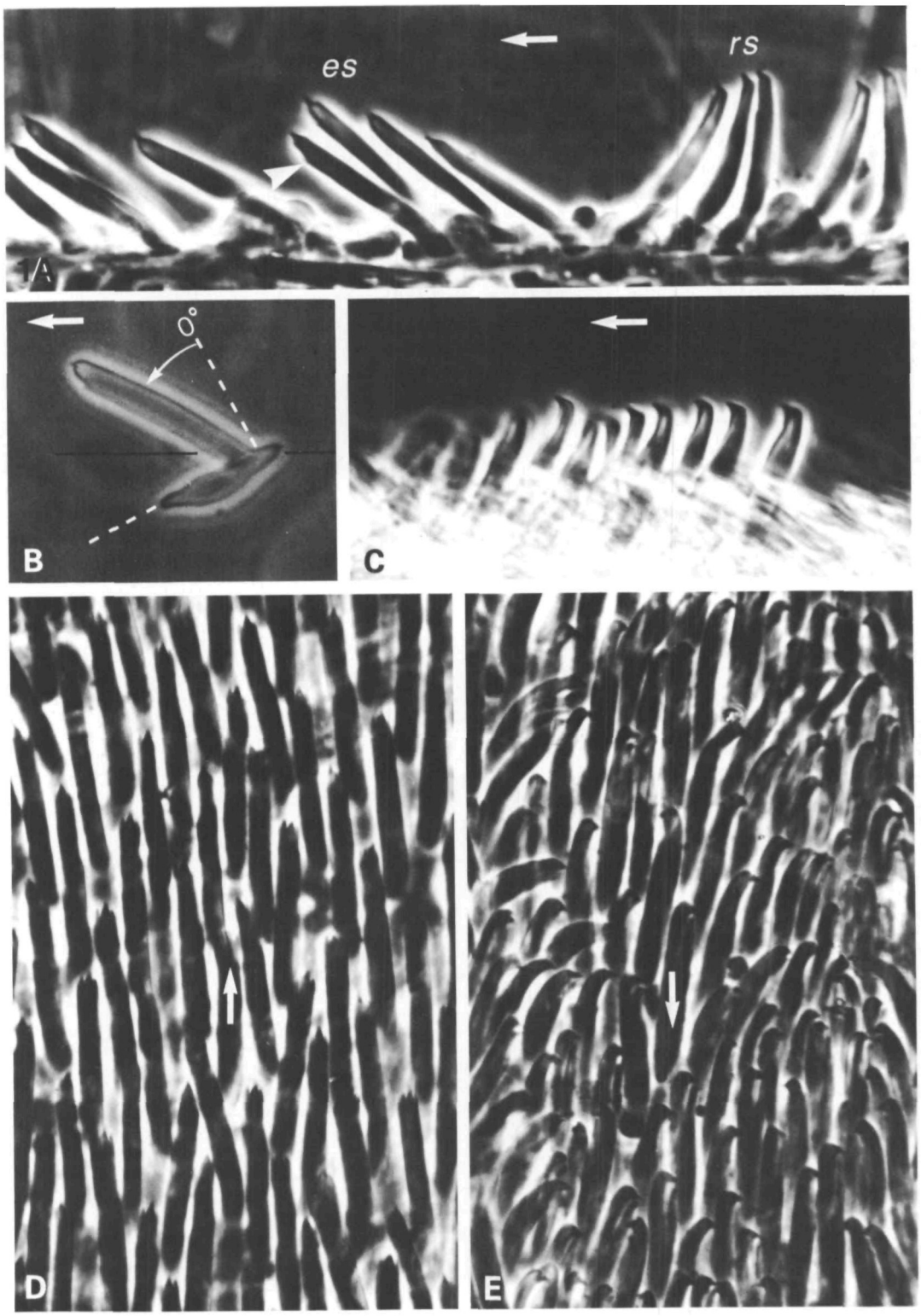
RESULTS

Macrocilia are finger-shaped organelles found in a dense band around the inner margin of the lips of beroid ctenophores. A single macrocilium is 35–40 μm long and ~5 μm in diameter (Figs 1, 2), and consists of a hexagonal array of 200–250 ciliary axonemes enclosed by a common membrane (Fig. 3) (Horridge, 1965; Tamm & Tamm, 1984). The axonemes are connected into ~20 parallel rows by flange-like compartmenting lamellae, which extend from doublet 3 of one axoneme to doublet 8 of the next shaft along the row (Fig. 3) (Tamm & Tamm, 1984).

Macrocilia beat in a planar ciliary pattern with the bend plane perpendicular to the rows of linked axonemes (Figs 1–3) (Tamm & Tamm, 1984). As a result of the close hexagonal packing of the axonemes, the doublet walls of adjacent shafts are superimposed when projected along the plane of bending (see Fig. 10). The effective stroke is directed aborally into the stomach cavity. Reversal of beat direction has never been observed in macrocilia.

Macrocilia arise from flattened, elongated cells that are inclined about 30° to the epithelial surface and point toward the stomach cavity, overlapping one another like tiles on a roof (Fig. 4) (Horridge, 1965). As a result, the bases of the macrocilia are not parallel to the lip surface but are tilted about 30° in the aboral direction (Figs 1B, 4). The straight unbent position of macrocilia (i.e. 0° bend angle) therefore corresponds to a 30° deviation in the aboral direction from a line vertical to the tissue surface (Fig. 1B). In this report, angular shifts in the effective (aboral) and recovery

Fig. 1. Phase-contrast micrographs of living macrocilia in the two arrest positions: at the end of the effective stroke and at the end of the recovery stroke. Arrows pointing to the left in A–C show effective-stroke (aboral) direction. A. Profile view. Effective-pointing cilia (*es*) have straight pointed phase-dense tips and a notch (arrowhead) on the lower side. Recovery-pointing cilia (*rs*) have tips hooked to the left and a tapered distal end. B. Beating macrocilium of an isolated cell at the end of the effective stroke (from cine-film). The print is rotated to mimic orientation of cells in A. Although the bend angle is actually +30° from the straight position (0° broken white line), the +30° aboral tilt of the shingle-shaped cell (lower broken white line) with respect to the epithelial surface (indicated by black horizontal line) makes the bend angle appear to be +60° from the apparent vertical in the intact tissue (compare with A). Note the straight pointed tip and notch on the lower side of the distal end. C. Profile view of recovery-arrested macrocilia, showing phase-dense tips hooked toward the stomach cavity and tapered appearance of the distal aboral sides. D,E. Views from above the epithelial surface of macrocilia pressed down by a coverslip. Vertical arrows (centre) indicate the effective-stroke (aboral) direction. D. Effective-pointing cilia have straight tridentate tips with a larger central tooth and two shorter lateral ones. E. Recovery-pointing cilia, with a total bend angle of about −120°, have strongly hooked tips that point aborally. A, C–E, ×790; B, ×850.


es
rs
A
0°
B
C
D
E

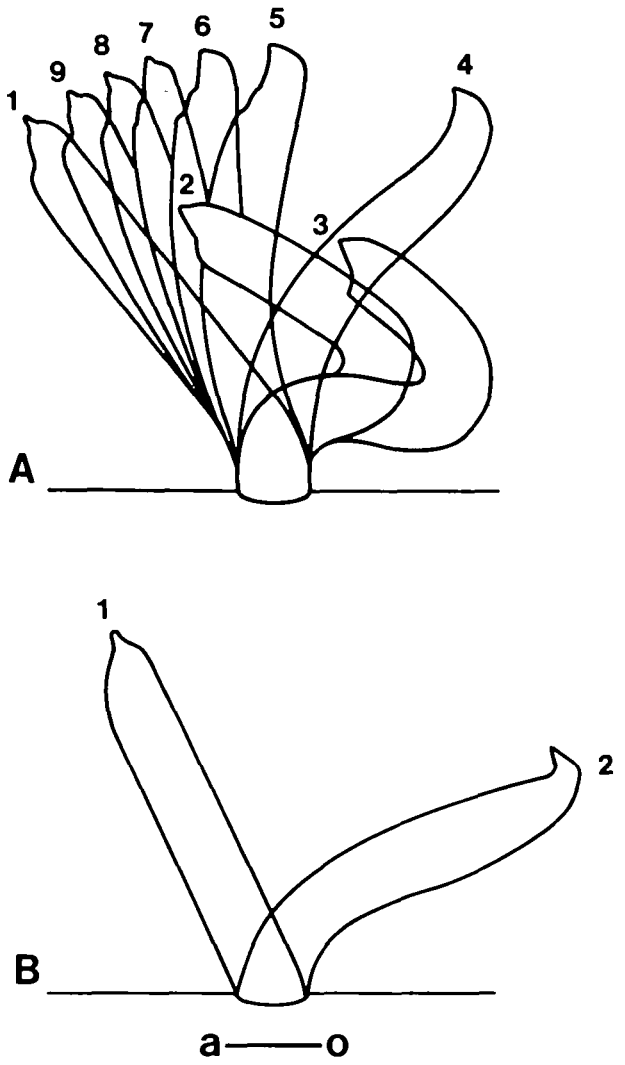


Fig. 2. Tracings from cine-films of continuously beating macrocilia (profile views). Effective-stroke direction is from right to left. Position 1 is at the end of the effective stroke. a–o, aboral–oral axis. A. Successive positions of a macrocilium beating at 2 Hz, showing changes in tip shape during the beat cycle (see the text). Time intervals between positions 1 and 9 are 1/12, 1/25, 1/25, 1/25, 1/25, 1/25, 1/12, 1/12, 1/12 s. B. Positions at the ends of the effective (1) and recovery (2) strokes of an ATP-reactivated macrocilium. Note changes in tip shape at the two extreme beat positions. Except for a brief pause at the end of the recovery stroke, this cilium beat continuously at 4 Hz.

(oral) stroke directions from the straight position are designated positive (+) and negative (−) bend angles, respectively.

Macrocilia typically beat discontinuously with separate effective and recovery strokes, resulting in two different arrest positions: a resting position of indeterminate duration at the end of the effective stroke, and a brief pause of rather constant length

at the end of the recovery stroke (Fig. 1) (Tamm, 1983). This unique type of activity results in a split-cycle pattern of metachronal coordination (Tamm, 1983). Continuous beating without intervening arrests is sometimes observed, and can be induced by high concentrations of Ca and K, or by the Ca ionophore A23187.

The tips of macrocilia undergo characteristic changes in shape during the beat cycle, correlated with the total bend angle of the organelle. In the rest position at the end of the effective stroke, macrocilia are bent toward the stomach cavity with their straight distal portions inclined 60° to the apparent vertical (Figs 1A,B, 2) (Tamm,

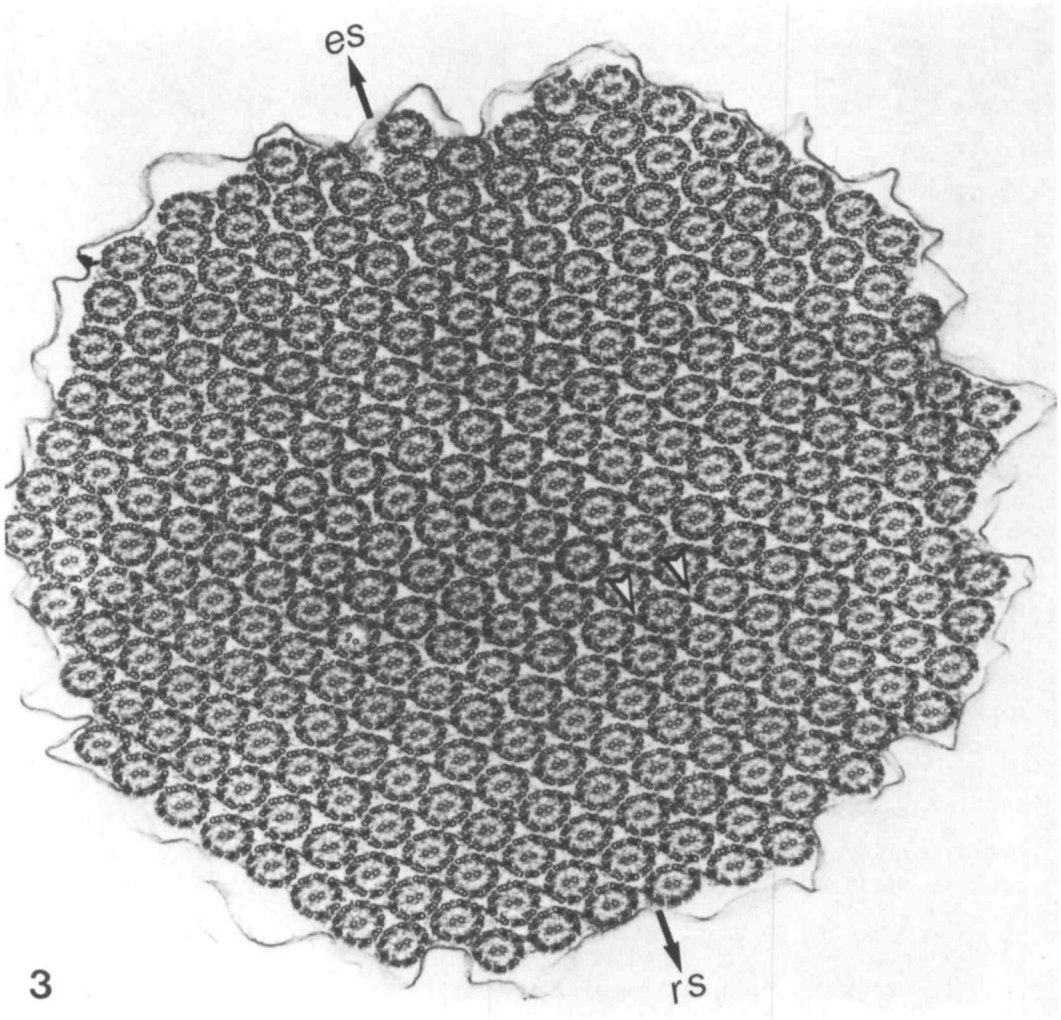


Fig. 3. Transverse section through the shaft of a macrocilium. Several hundred hexagonally packed axonemes are connected by compartmenting lamellae (arrowheads) into approximately 20 parallel rows, which run normal to the plane of bending (arrows: *es*, effective stroke; *rs*, recovery stroke). By sighting along the rows at right angles to the bend plane, it can be seen that doublets on opposite sides of axonemes in adjacent rows lie on the same line (see Fig. 10). ×33400.

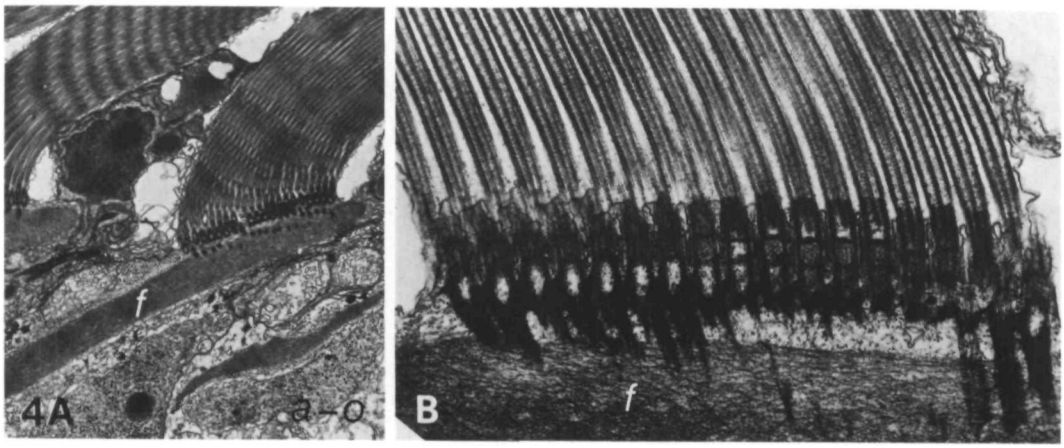


Fig. 4. Longitudinal sections through the bases of macrocilia and underlying epithelial cells, parallel to the bend plane (*a–o*, aboral–axis; effective-stroke direction is to the left). A. Base of the macrocilium is tilted 30° in the aboral direction with respect to the epithelial surface, due to the inclined, overlapping arrangement of the cells. A long bundle of microfilaments (*f*) runs under the basal bodies to the lower end of the elongated cell. B. Higher magnification of the macrociliary base, showing approximately 20 rows of basal bodies and axonemes. Rootlets penetrate the bundle of microfilaments (*f*). At the basal body transition zone, each axoneme is surrounded by a membrane (see Tamm & Tamm, 1984). A, ×4200; B, ×18600.

1983). Because of the 30° tilt of the base, this position actually corresponds to a total bend angle of +30°, as is clearly seen in detached cells where the base is visible (Fig. 1B).

In profile view, the tips of effective-pointing macrocilia consist of a phase-dense pointed cap, almost 2 μm long, which projects in a straight line from the upper convex side of the organelle. A notch or indentation is characteristically present on the lower side of the distal end (Figs 1A,B, 2). Viewed from above the epithelial surface, the tips of effective-pointing cilia appear jagged, with three pointed teeth or caps (Fig. 1D). The middle tooth (i.e. major cap) is longer and broader than the two lateral ones, and corresponds to the pointed cap seen in profile.

At the end of the recovery stroke, macrocilia typically remain frozen for a brief period in a sigmoid posture pointing toward the mouth (Tamm, 1983). A sharp negative bend of 90°–100° is present at the base, and a more gradual curvature of 30°–50° in the opposite direction occurs along the distal region, resulting in a total bend angle of about −60°. In some preparations of excised lips, recovery-arrested macrocilia often have a smaller negative bend at the base (i.e. about 50°–70°), and are relatively straight in the distal region, also resulting in a total bend angle of about −60° (Figs 1C, 2B). The net difference in bend angle between effective and recovery-pointing cilia is thus about 90°.

In profile view the distal ends of recovery-pointing macrocilia are hooked or beak-shaped, pointing toward the stomach cavity, i.e. in the direction of the subsequent effective stroke (Figs 1A,C, 2). The beak itself is phase-dense and resembles the

pointed cap seen in effective-stroke cilia, but tilted aborally with respect to the macrociliary shaft. The distal ends of recovery-pointing cilia are tapered on the aboral side; together with their beaked tips, they resemble a row of penguins facing the aboral pole (Fig. 1C).

Forcing recovery-pointing macrocilia to lie parallel to the epithelial surface by pressure on the coverslip increases the negative basal bend to 120°, so that the total bend angle is −120°. The tips of these cilia are more strongly hooked, and are curved through a full 90° arc with respect to the straight distal region of the organelle (Fig. 1E).

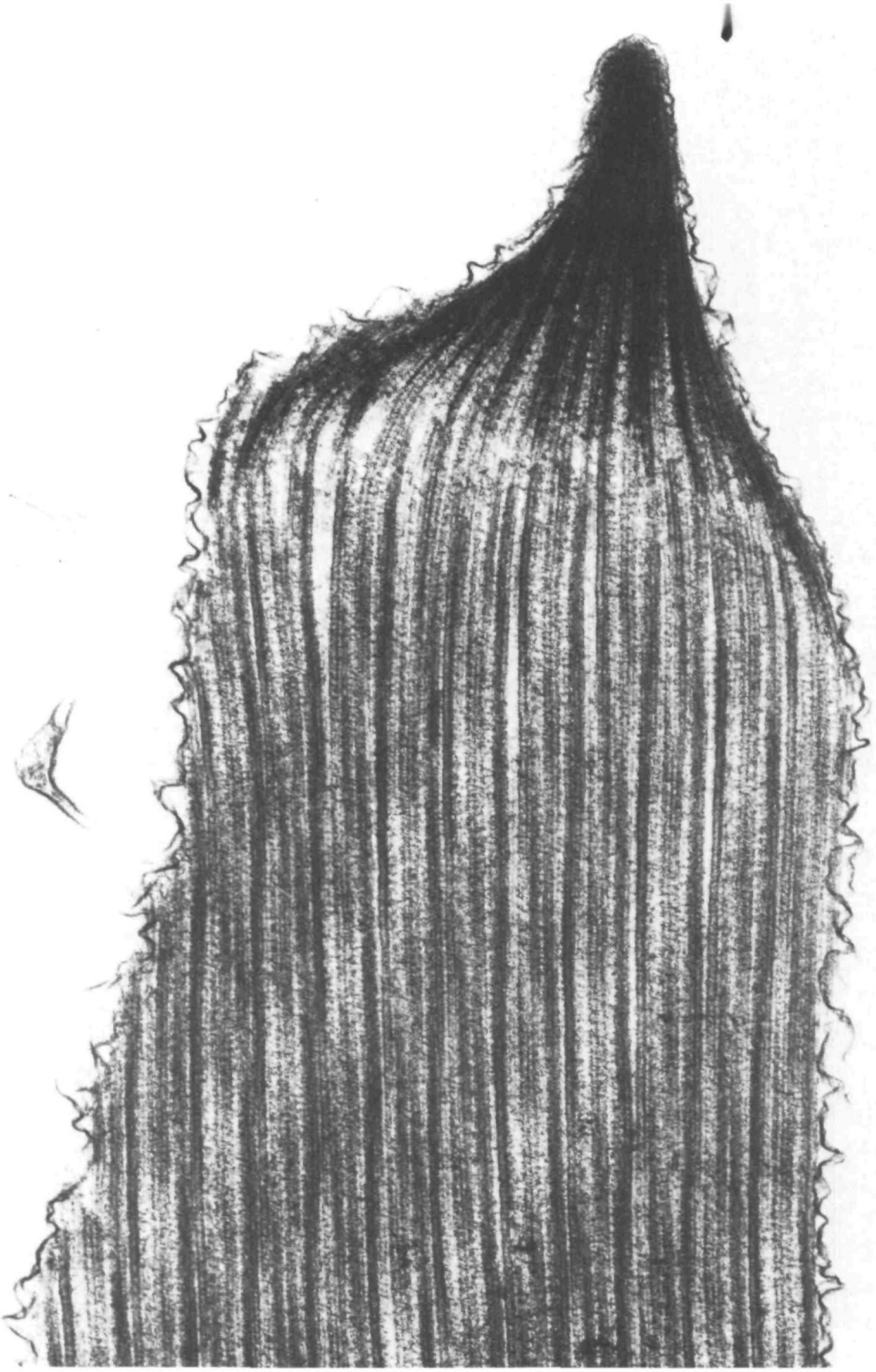
In addition to these static tip configurations of arrested macrocilia, changes in tip shape with bend angle can also be followed during continuous beating (Fig. 2). In the example shown in Fig. 2A, the bend angle at the end of the effective stroke (position 1) was +40°, and the ciliary tip was symmetrically pointed, with a notch on the lower side as described above. During the recovery stroke, a sharp negative bend at the base (−95°) was accompanied by an unusually large bend in the opposite direction (+145°), which propagated distally. As a result, the total bend angle at the end of the recovery stroke (position 3) was +50°, a more positive bend angle than at the end of the effective stroke. The tip was not hooked and the lower side of the cilium was notched, similar to the tip shape of effective-pointing cilia. The positive distal bend disappeared at the beginning of the effective stroke; the total bend angle became negative (−45°) and a distinct beak-like curvature appeared at the tip (position 4). At successive positions in the effective stroke (positions 5–9), during which the total bend angle became more positive, this curvature gradually decreased and the tip became straighter and more conical, with the reappearance of a notch on the lower side.

In another example, a permeabilized macrocilium on a detached cell was reactivated to beat with ATP (Fig. 2B). At the completion of the effective stroke (position 1), the bend angle was +25°, and the tip was symmetrically pointed as in living cilia. At the end of the recovery stroke (position 2), a −70° bend at the base was present without a compensating bend in the opposite direction further distally (see also Fig. 1A). The tip was strongly hooked in the aboral direction.

Electron microscopy of effective-pointing tips

Longitudinal sections (Figs 5, 6) through the tips of macrocilia resting at the end of the effective stroke show that the pointed teeth or caps arise at the termination of the B microtubules of the doublets, and are formed by the continuation and convergence of the A and central-pair microtubules of the axonemes. These singlet microtubules are heavily invested with electron-dense material, which gives the caps a dark, opaque appearance in thin sections and accounts for their phase-density in the light microscope.

Profile views show that the major cap arises from rows of axonemes on the upper, convex side of the bent macrocilium (Fig. 5). Although the axoneme-to-cap transition begins slightly more proximally around the periphery of the macrociliary



5

shaft, axonemes on opposite sides of the cap terminate at approximately the same level, i.e. there is no displacement between the ends of axonemal rows in the cap of effective-pointing cilia. As a result, the A and central-pair microtubules of the cap form a straight pointed projection, about 1·5 μm long. To compensate for the +30° bend angle of effective-pointing cilia, the axonemes on the upper, convex side of the macrocilium must be slightly longer than those on the lower side (see Fig. 11). As the singlet microtubules converge at the tip they become more closely bound together by the electron-dense material, and reduced in number (see below). No connections between the electron-dense material and the surrounding membrane of the macrocilium are evident. The distal ends of the singlet microtubules do not appear to be plugged by electron-dense structures inserted into their lumens, nor do they appear linked to the surrounding membrane, unlike capping structures described in other cilia (Dentler, 1980, 1981, 1984; Dentler & LeCluyse, 1982; LeCluyse & Dentler, 1984).

Longitudinal sections also show that the notch on the lower side of effective-pointing macrocilia is due to the abrupt termination of several rows of axonemes a short distance before the tip, without continuation of their A or central-pair microtubules into the capping structure (Fig. 5).

The tripartite nature of the capping structure is also evident in tangential longitudinal thin sections (Fig. 6). The two shorter lateral caps are structurally identical to the central major cap.

Transverse sections at the level where the cap begins show that transformation of axonemes into singlet microtubules occurs first around the periphery of the macrocilium in a symmetrical manner (Fig. 7), as noted in longitudinal sections. The A and central-pair microtubules of adjacent axonemes are bound together into an irregular network of singlet microtubules by the electron-dense material (Figs 7, 8A).

Further distally, the tip splits into three parts: a major cap on the upper convex side of the macrocilium, and two smaller lateral caps (Fig. 8A). All doublet microtubules have ended at this level and only singlet microtubules remain, linked together by electron-dense material. Pairs of central microtubules are often still recognizable at this level (Fig. 8A).

The distal end of the cap consists of closely packed singlet microtubules embedded in an electron-dense matrix (Fig. 8B). The number of singlet microtubules is less than the total number of doublet and central-pair microtubules in axonemes at the base of the cap. Tapering of the cap is thus due to termination of singlet microtubules as well as to reduction of the spacing between the remaining microtubules.

Fig. 5. Medium longitudinal section parallel to the bend plane through the distal end of an effective-pointing macrocilium. Axonemes on the convex side of the bent cilium (reader's right) terminate at approximately the samel level: there is no tip displacement. As a result, the A and central-pair microtubule extensions, coated with electron-dense material, converge to form a straight pointed (major) cap. Axonemes on the lower aboral side of the macrocilium (left) end abruptly, giving the distal end a notched appearance in profile view (see Fig. 1A,B). ×40300.

Recovery-pointing tips

Longitudinal sections through the beaked tip of recovery-pointing macrocilia show that the beak itself consists of the same electron-dense singlet microtubule cap seen in

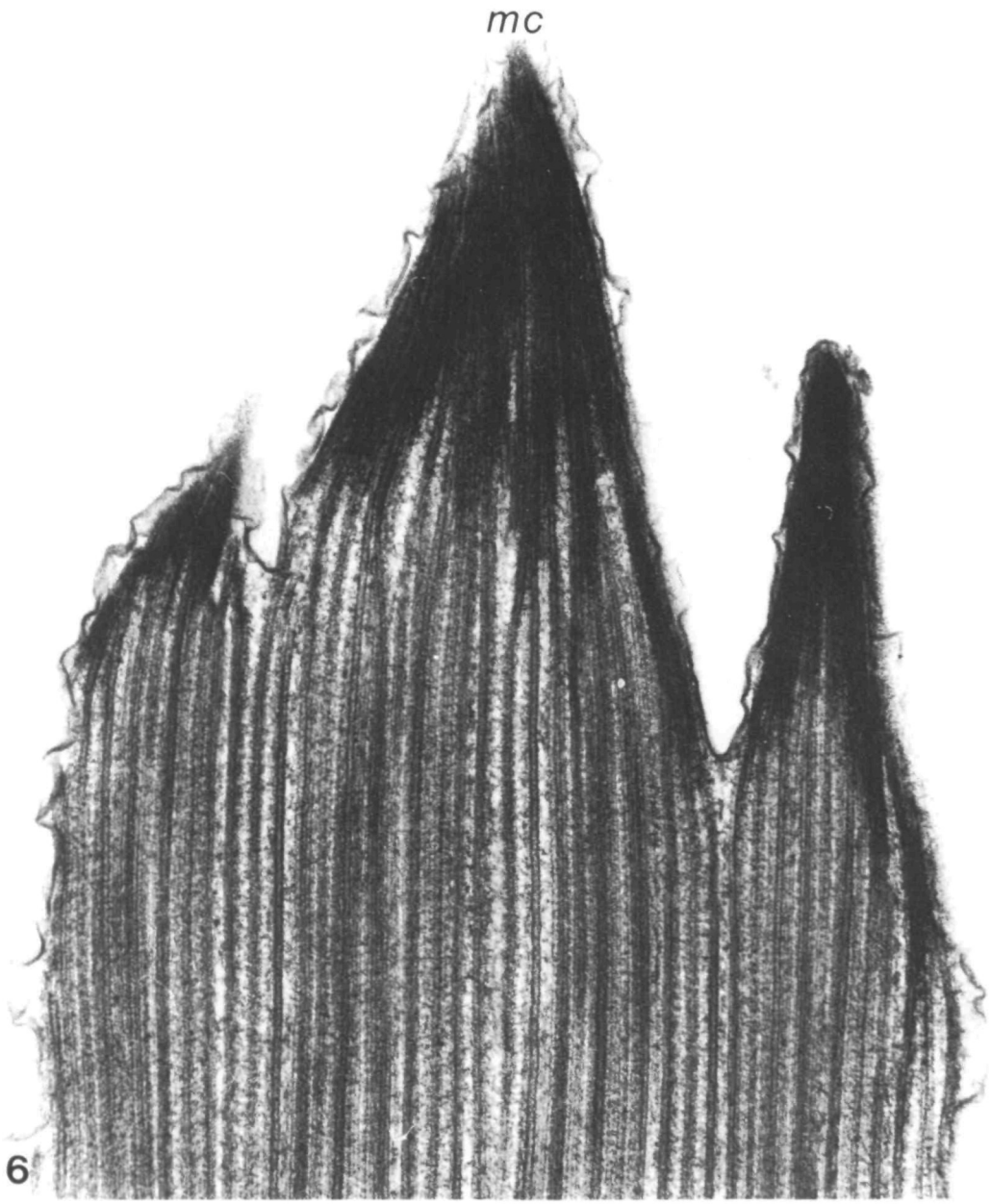


Fig. 6. Tangential longitudinal section normal to the bend plane through the distal end of an effective-pointing macrocilium. The tridentate nature of the capping structure is evident (see Fig. 1D). *mc*, major cap. ×52600.

effective-pointing cilia, but now bent almost 90° in the aboral direction with respect to the shaft of the organelle (Fig. 9). Axonemes just below the tilted cap are curved and bowed outward on the oral side, giving the beaked tip a rounded head as seen by light microscopy (Fig. 1c).

Double or treble hooks are sometimes evident at the distal end of recovery-pointing cilia, depending on the plane of section. In such cases each hook consists of a tilted, electron-dense singlet microtubule cap as described above, reflecting the tripartite nature of the capping structure.

Longitudinal sections also show that rows of axonemes along the aboral side of recovery-pointing cilia end abruptly at various levels along the distal portion of the shaft (Fig. 9). These rows correspond to the shorter non-capped rows that form the

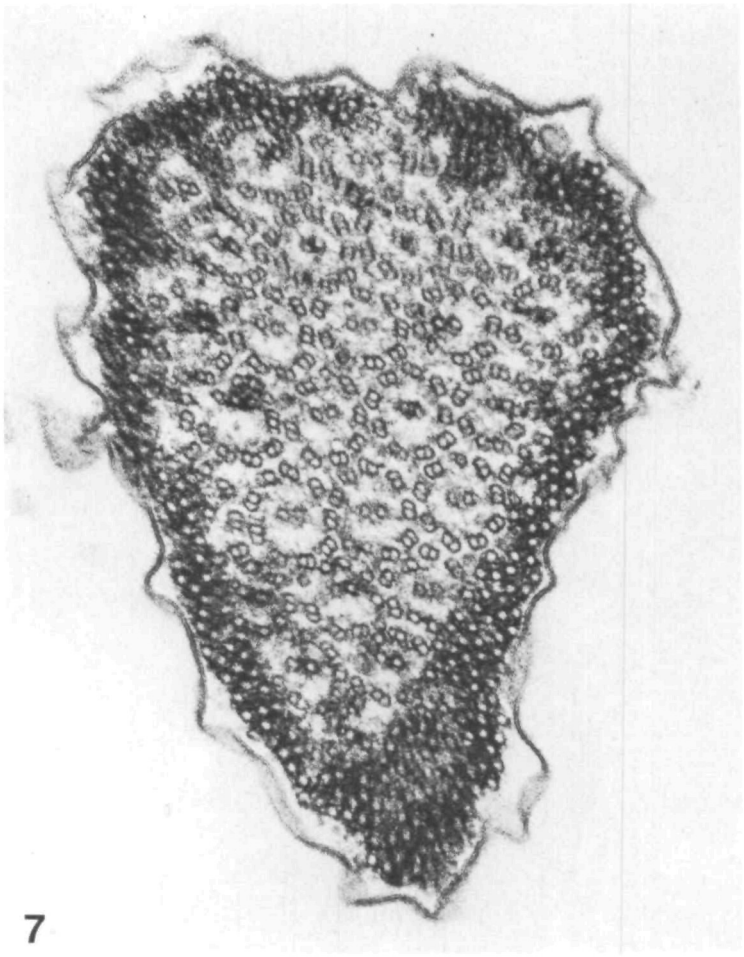
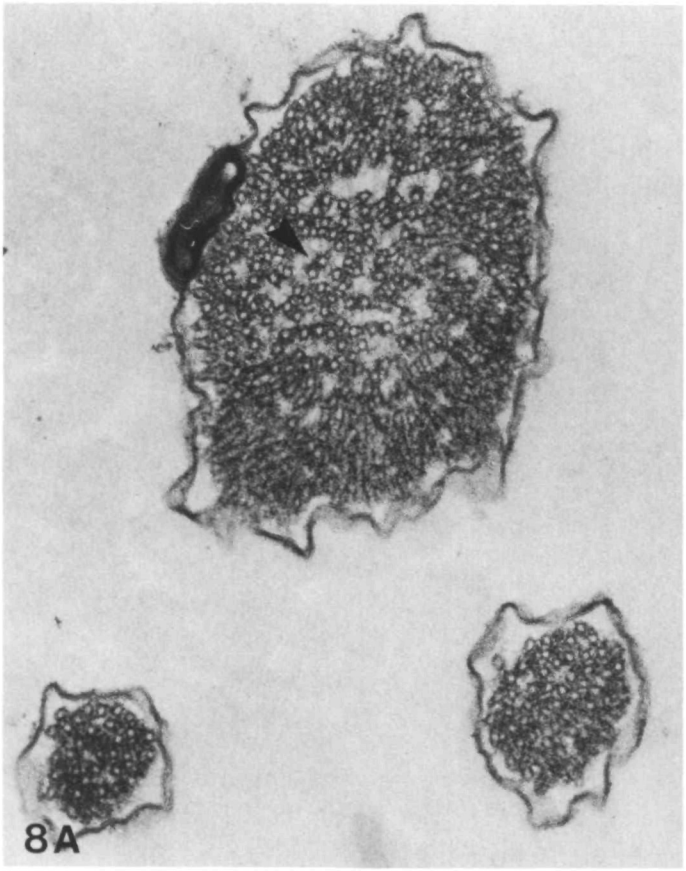
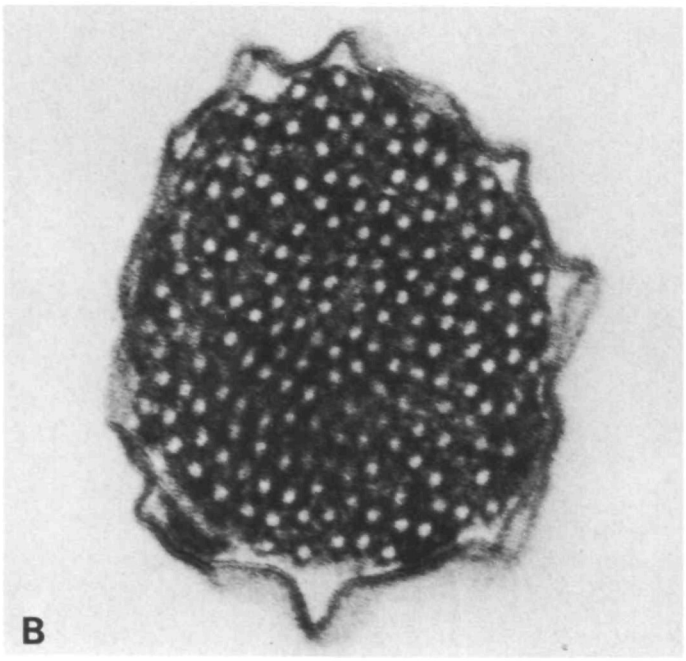


Fig. 7. Transverse section through the distal end of an effective-pointing macrocilium at the axoneme-to-cap transition level. Axonemes around the periphery have already transformed into singlet (A and central-pair) microtubular extensions, which are surrounded by electron-dense material. ×70800.


8A


B

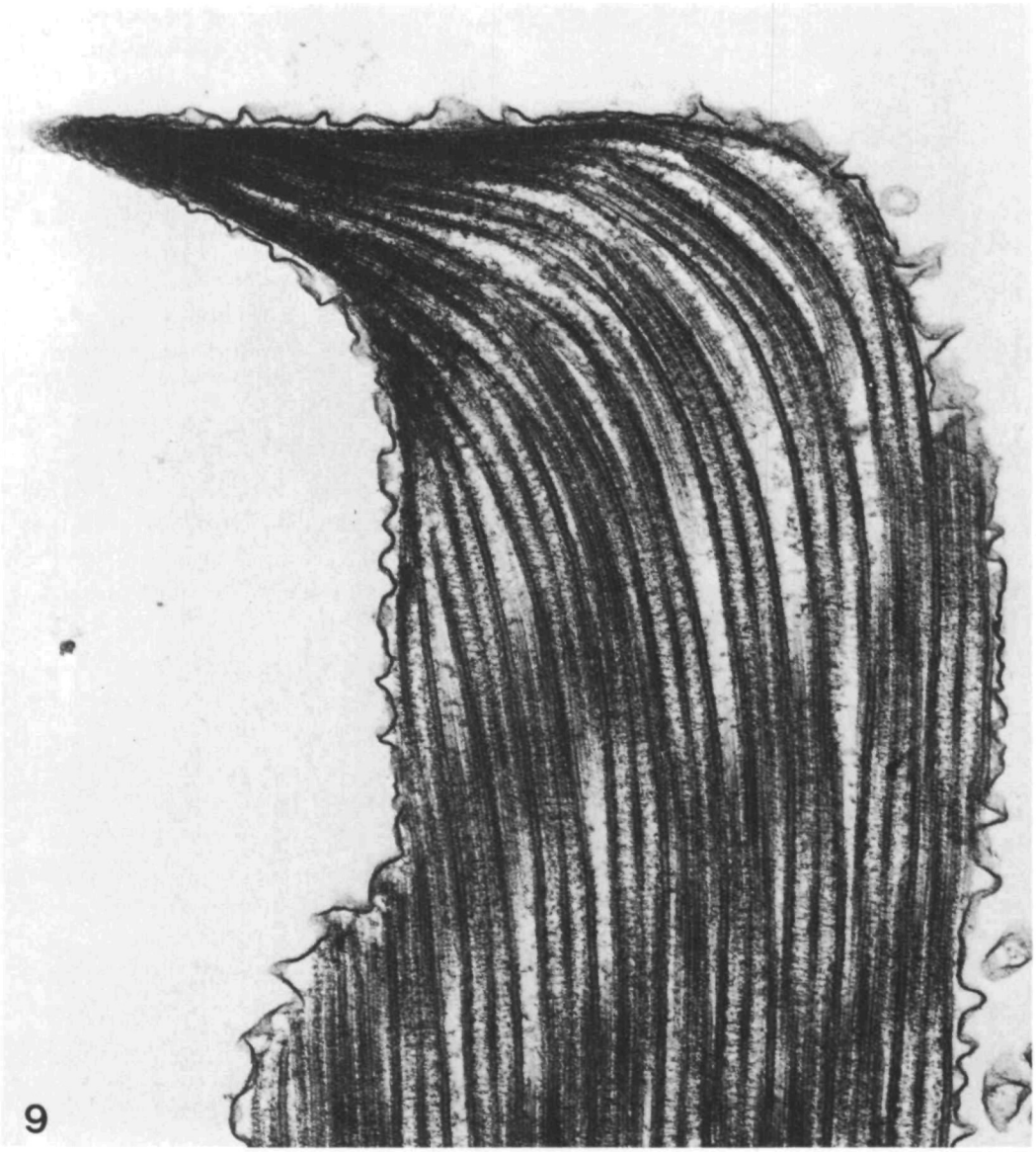


Fig. 9. Longitudinal medial section through the beaked tip of a recovery-pointing macrocilium (see Fig. 1C,E). The electron-dense singlet microtubule cap is tilted almost 90° aborally with respect to the shaft. Axonemes are sharply curved and bowed outward on the oral side (right); on the aboral side (left), axonemes end abruptly, giving the distal end a tapered appearance by light microscopy (see Fig. 1C,E). ×40000.

Fig. 8. Transverse sections through the cap of effective-pointing macrocilia at levels progressively more distal than shown in Fig. 7. A. The capping structure is split into an upper major cap and two smaller lateral caps. Singlet microtubules are more closely packed into an irregular array linked by electron-dense material. Pairs of central microtubules are still recognizable (arrowhead). B. At the distal end of the major cap, single microtubules are reduced in number and embedded in an electron-dense matrix. A, ×71600; B, ×144000.

notch on the aboral side of effective-pointing cilia; in recovery-pointing cilia they give the distal region a tapered appearance as seen by light microscopy (Fig. 1c).

Geometrical projection and model

A two-dimensional projection in the bend plane of doublets of adjacent axonemes at the tip of a recovery-pointing macrocilium illustrates the following points (Fig. 10): (1) the total displacement between the tips of doublets on opposite sides of the macrocilium (Δl_m) results from displacement of microtubules on opposite sides within individual axonemes due to active sliding (Δl_d), as well as displacement between adjacent axonemes due to their passive sliding (Δl_a); (2) $\Delta l_d = \Delta l_a$, because the doublet walls of adjacent axonemes are superimposed in projection along the bend plane; (3) therefore, active intra-axonemal sliding and passive inter-axonemal sliding contribute equally to the resulting tip displacement of the macrocilium (Δl_m); (4) Δl_m is proportional to the number of axonemes in parallel (i.e. diameter of the shaft); (5) this tip displacement is absorbed by tilting of the capped distal ends of the axonemal microtubules; (6) the tilt angle of the cap is independent of the number of axonemes in parallel.

Thus, changes in tip configuration are visible in living macrocilia because of the increased diameter of this organelle and the length of the capping structure that binds the distal ends of the microtubules together.

The observed changes in tip configuration with bend angle are illustrated by a two-dimensional model of a macrocilium, using flexible plastic strips to represent rows of axonemes (Fig. 11). Although this model shows only passive sliding between axonemes and not active intra-axonemal sliding, the resulting angular tilt of the cap is the same (see above). The major cap is represented in profile view by a folded paper strip hinged to the outer rows bearing the cap. The model is based on the structure and geometry of effective-pointing cilia, i.e. the plastic strips under the cap are longer on the convex (oral) side, so as to give zero displacement when their bend angle is +30°. In addition, strips on the aboral side of the shaft are shortened to create a notch, similar to that seen on the concave side of effective-pointing cilia *in vivo* (Fig. 1).

When the model is bent from the effective to the recovery-stroke position, displacement of strips at the tip causes the hinged cap to tilt sharply toward the aboral direction (Fig. 11). The greater length of the strips on the oral side augments this displacement. The shorter uncapped rows on the aboral side of the shaft are drawn further away from the tip, giving the distal region a tapered appearance as seen *in vivo*.

The model thus shows how sliding displacement of parallel microtubules that are attached by a capping structure results in changes in tip configuration similar to those observed in living macrocilia.

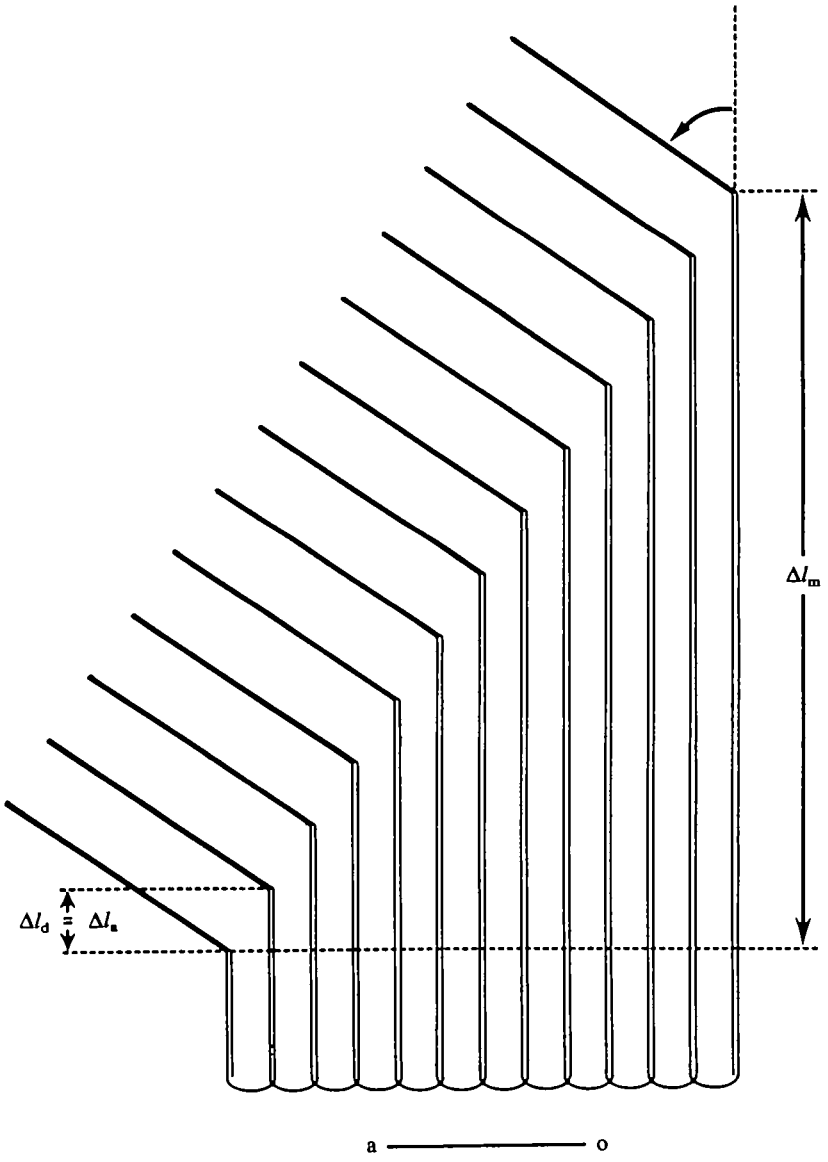


Fig. 10. Diagrammatic projection in the bend plane of doublet walls of adjacent rows of axonemes at the tip of a recovery-pointing macrocilium (i.e. total bend angle = −60°). The rows of cap-bearing axonemes appear to share common doublet walls (open bars) due to their close hexagonal packing (see Fig. 3). The total displacement between tips of doublets on opposite sides of the macrocilium (Δl_m) results from displacement of doublets on opposite sides within individual axonemes (Δl_d) due to active sliding, and displacement between adjacent rows of axonemes (Δl_a) due to passive sliding. All Δl values = 0 in the effective-pointing position (bend angle = +30°), a net difference in bend angle of 90° from the recovery position. As a result of the tip displacements in the recovery position, the cross-linked A microtubule extensions of the doublets (filled bars) are tilted (angular arc) with respect to the shaft. a–o, aboral–oral axis.

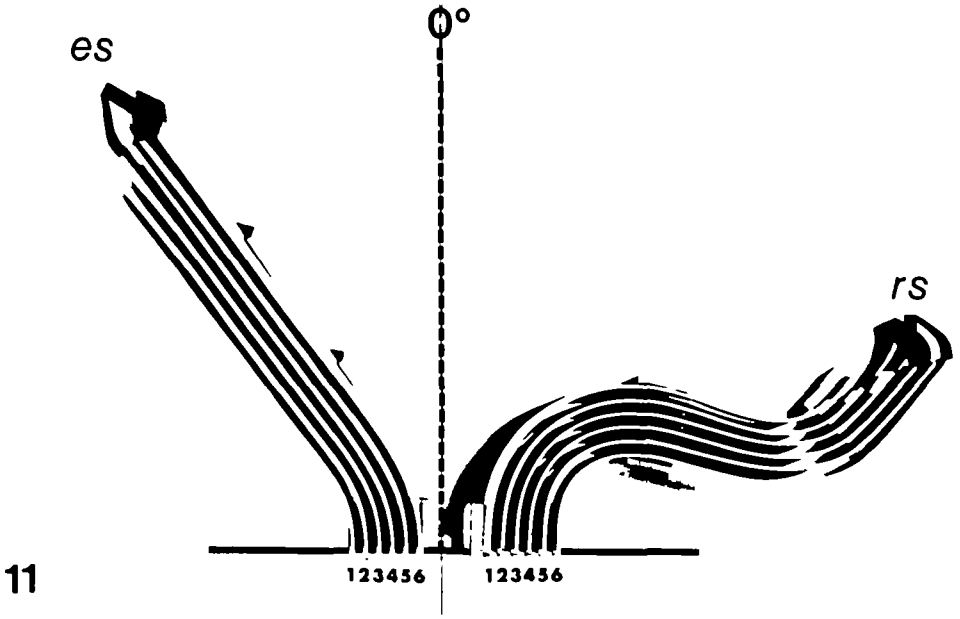


Fig. 11. Profile view of the model macrocilium at the ends of the effective stroke (*es*) and recovery stroke (*rs*). Six white flexible strips represent the 20 rows of axonemes. Strips are held by grooved guides through which they can slide freely when bent from the effective to the recovery-stroke position. A folded black paper strip taped to the ends of strips 3 and 6 represents the major cap. The 0° broken line shows the straight unbent position. Strips 3–6 increase progressively in length, so that in the effective-pointing position (+30°) there is no displacement of their tips, and the pointed cap is straight. Strips 1 and 2 are shorter to represent the notch seen on the lower side of effective-pointing cilia (see Fig. 1). Bending the model into the recovery position involves a net 90° change in bend angle, and results in tip displacement of strips 3–6. This tilts the hinged cap sharply to the left. Strips 1 and 2 are displaced further from the tip, resulting in the tapered distal region seen *in vivo* (Fig. 1).

DISCUSSION

According to the sliding microtubule model of ciliary motility, active sliding between doublet microtubules of constant length results in their relative displacement at the ciliary tip (Satir, 1965, 1967, 1968). The amount of displacement between two microtubules is proportional to the total bend angle and the distance separating them. Because the diameter of a single axoneme is typically 0·2 μm, the maximum displacement of microtubule tips on opposite sides of a cilium bent 90° is about 0·3 μm. Such microtubule tip displacements were initially demonstrated in instantaneously fixed molluscan gill cilia by thin-section electron microscopy, and used to support a sliding-microtubule model of ciliary motion (Satir, 1965, 1967, 1968). However, the displacement of microtubule tips predicted by the sliding model has not been demonstrated previously in actively beating cilia or flagella.

In this study we used the unique structural features of ctenophore macrocilia to observe directly the effects of microtubule sliding displacement in motile cilia. The multiple rows of axonemes within a single macrocilium, together with the large

capping structure at the tip, allowed us to follow changes in configuration of the tip resulting from sliding of microtubules during the ciliary beat cycle.

The distal ends of the A and central-pair microtubules are firmly bound together by electron-dense material at the tip of the macrocilium. The rigid attachment of the singlet tubules to one another is shown by the resistance of the cap to distortion by the displacement of doublet microtubules during the recovery stroke (Fig. 9). Instead, displacement caused by intra- and inter-axonemal sliding is accommodated by a sharp curvature of microtubules at the distal end of the axonemes, causing the cap to be tilted toward the stomach cavity. As shown by the model (Fig. 11), the cap acts as if it were hinged to rows on opposite sides of the macrocilium. Displacement of rows is transformed into a proportional angular deviation of the cap from the shaft axis, resulting in the hook-shaped appearance of the tip in recovery-pointing cilia. The extent to which the cap is hooked depends on the degree of tip displacement undergone by axonemal rows during bending (Figs 1, 2).

Electron-microscopic studies of cilia from many different organisms have shown a wide variety of capping structures associated with the distal ends of ciliary microtubules (Dentler, 1980, 1981, 1984; Dentler & LeCluyse, 1982; LeCluyse & Dentler, 1984). The cap of macrocilia, however, is the largest capping structure reported so far. In contrast to other cilia, in which only the distal tips of the microtubules are attached to the cap by electron-dense plugs inserted into their lumens, the A and central-pair microtubules in the cap of macrocilia do not appear to be plugged at their distal ends, but are linked to one another all along their length. As a result, the distal tips of the singlet microtubules in macrocilia do not appear to be connected to the ciliary membrane as they are in other cilia (Dentler, 1980, 1981, 1984; Dentler & LeCluyse, 1982; LeCluyse & Dentler, 1984).

In addition, the electron-dense capping material in macrocilia bridges all the singlet microtubules in an identical manner, so that at the very tip A and central-pair microtubules cannot be distinguished between (Fig. 8B). In contrast, the outer doublet and central-pair microtubules of other cilia are usually capped by morphologically distinct structures (Dentler, 1980, 1981, 1984; Dentler & LeCluyse, 1982; LeCluyse & Dentler, 1984).

Tilting of the capping structure has also been observed in instantaneously fixed bent cilia of vertebrate trachea and palate (Dentler & LeCluyse, 1982; LeCluyse & Dentler, 1984). In contrast to the present work, the amount of displacement of the A-microtubule tips in these cilia was considerably less than that predicted by the sliding-filament model. The reason for this discrepancy is not clearly understood.

Macrocilia do not serve a locomotory function; they are used to spread the lips of *Beroë* over its prey (other gelatinous zooplankton) during feeding, thereby pushing the prey into the stomach cavity. If the food organism is too large to be completely engulfed, the macrocilia act as cutting implements to bite through it 'in the manner of moving teeth' (Swanberg, 1974). Since macrocilia would contact the prey mainly by their distal ends, the capping structure undoubtedly plays an important role in ingestion of prey. In particular, the hooked, claw-like configuration of the tip in macrocilia poised at the end of the recovery stroke seems well-designed for grasping

or tearing through the prey during the subsequent power stroke into the stomach cavity.

The extensive cross-linking of A and central-pair microtubules in the cap would be expected to increase greatly the rigidity and stiffness of the tip, allowing it to transmit effectively the large bending moment of the macrocilium to the prey. Similarly, the A and central microtubules in mucous-transporting cilia are also linked together at the tip, the site of contact with the mucous (Aiello & Sleigh, 1977), perhaps serving to provide greater force with which to penetrate and move the overlying mucous layer (Dentler & LeCluyse, 1982).

The straight, pointed tip of the macrocilium in the resting position at the end of the effective stroke may reflect a neutral, unengaged part of the beat cycle. Although resting macrocilia are not actively involved in transporting or cutting through prey, by pointing into the stomach cavity they may act as a 'non-return surface' to prevent outward movement and escape of struggling prey. A similar function has been ascribed to resting mucous-propelling cilia that point in the direction of mucous flow (Sanderson & Sleigh, 1981). In this respect, the three-toothed cap of effective-pointing macrocilia seems well-adapted for retaining ingested prey.

Thus, the changes in tip shape observed during the beat cycle of macrocilia may be functionally advantageous for ingestion of prey. The displacement of microtubules that develops during sliding is converted into configurational changes of the tip via the capping structure, and this apparently performs useful work for the ctenophore during feeding.

In conclusion, Horridge's (1965) original work on macrocilia provided some of the first supporting data for a sliding-microtubule model of ciliary motion. More recent studies on macrocilia (Tamm, 1983; Tamm & Tamm, 1984) have revealed new insights into the mechanosensitivity of the motile elements in cilia and provided direct experimental support for a switching mechanism regulating active microtubule sliding during the beat cycle (Wais-Steider & Satir, 1979). Together with the present results showing direct visualization of microtubule tip displacement in living cilia, macrocilia truly appear to be 'one of those examples, occasionally presented in the animal kingdom, where a giant structure lends itself to the analysis of general biophysical problems' (Horridge, 1965).

We are very grateful to Claudia Mills for kindly sending *Beroë* from Friday Harbor, WA, and to Dorothy Hahn for skilful secretarial assistance. This work was supported by NIH grant GM 27903 and NSF grant 83-14317.

REFERENCES

AIELLO, E. & SLEIGH, M. (1977). Ciliary function of the frog oro-pharyngeal epithelium. *Cell Tiss. Res.* **178**, 267–278.

DENTLER, W. L. (1980). Structures linking the tips of ciliary and flagellar microtubules to the membrane. *J. Cell Sci.* **42**, 207–220.

DENTLER, W. L. (1981). Microtubule–membrane interactions in cilia and flagella. *Int. Rev. Cytol.* **72**, 1–47.

DENTLER, W. L. (1984). Attachment of the cap to the central microtubules of *Tetrahymena* cilia. *J. Cell Sci.* **66**, 167–173.

DENTLER, W. L. & LECLUYSE, E. L. (1982). Microtubule capping structures at the tip of tracheal cilia: evidence for their firm attachment during ciliary bend formation and the restriction of microtubule sliding. *Cell Motil.* **2**, 549–572.

HORRIDGE, G. A. (1965). Macrocilia with numerous shafts from the lips of the ctenophore *Beroë*. *Proc. Roy. Soc. Lond.* B, **162**, 351–364.

LECLUYSE, E. L. & DENTLER, W. L. (1984). Asymmetrical microtubule capping structures in frog palate cilia. *J. Ultrastruct. Res.* **86**, 75–85.

SANDERSON, M. J. & SLEIGH, M. A. (1981). Ciliary activity of cultured rabbit tracheal epithelium: beat pattern and metachrony. *J. Cell Sci.* **47**, 331–347.

SATIR, P. (1965). Studies on cilia. II. Examination of the distal region of the ciliary shaft and the role of the filaments in motility. *J. Cell Biol.* **26**, 805–834.

SATIR, P. (1967). Morphological aspects of ciliary motility. *J. gen. Physiol.* **50**, 241–258.

SATIR, P. (1968). Studies on cilia. III. Further studies on the cilium tip and a "sliding filament" model of ciliary motility. *J. Cell Biol.* **39**, 77–94.

SWANBERG, N. (1974). The feeding behavior of *Beroë ovata*. *Mar. Biol.* **24**, 69–76.

TAMM, S. L. (1983). Motility and mechanosensitivity of macrocilia in the ctenophore *Beroë*. *Nature, Lond.* **305**, 430–433.

TAMM, S. L. & TAMM, S. (1984). Alternate patterns of doublet microtubule sliding in ATP-disintegrated macrocilia of the ctenophore *Beroë*. *J. Cell Biol.* **99**, 1364–1371.

WAIS-STEIDER, J. & SATIR, P. (1979). Effect of vanadate on gill cilia: switching mechanism in ciliary beat. *J. supramolec. Struct.* **11**, 339–347.

(Received 19 March 1985 – Accepted 1 June 1985)