STUDIES ON CILIA

III. Further Studies on the Cilium Tip and a "Sliding Filament" Model of Ciliary Motility

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ABSTRACT

This study confirms and extends previous work on the lateral cilia of the fresh-water mussel, *Elliptio complanatus*, in support of a "sliding filament" mechanism of ciliary motility wherein peripheral filaments (microtubules) do not change length during beat (see Satir, 1967). Short sequences of serial sections of tips are examined in control (nonbeating) and activated (metachronal wave) preparations. Several different tip types, functional rather than morphogenetic variants, are demonstrated, but similarly bent cilia have similar tips. The peripheral filaments are composed of two subfibers: a and b. The bent regions of cilia are in the form of circular arcs, and apparent differences in subfiber-b length at the tip are those predicted solely by geometry of the stroke without the necessity of assuming filament contraction. Various subfibers b apparently move with respect to one another during beat, since small systematic variations in relative position can be detected from cilium to cilium. While subfiber-b lengths are uniform throughout, subfiber-a lengths are morphologically different for each filament: 8 and 3 are about 0.8 μ longer than 1, 4 and 5, but each unique length is independent of stroke position or tip type. Subfiber-a does not contract, nor does it move, e.g. slide, with respect to subfiber-b of the same doublet. The central pair of filaments extends to the tip of the cilium where its members fuse. Subunit assembly in ciliary microtubules is evidently precise. This may be of importance in establishing the relationships needed for mechanochemical interactions that produce sliding and beat.

INTRODUCTION

Previous work in a number of laboratories (Satir, 1967) has lent support to a mechanism of ciliary motility in which the peripheral filaments (microtubules) of the axonemal 9 + 2 array do not shorten in length during beating. Because the filaments apparently do move relative to one another as bending proceeds, the alternative hypothesis may be thought of as a "sliding filament" model, analogous in some respects to the sliding filament model of muscle contraction.

Displacement of the filaments with respect to one another has been most directly demonstrated by examination of the tips of cilia fixed in different stroke positions (Satir, 1965). It can be shown that the order in which doublet filaments appear to terminate is different in differing stroke positions, in a manner consistent with the assumption that all doublets morphogenetically are of nearly equal length and with a sliding filament model. However, the doublet filament is a composite of one microtubule and one part microtubule (Phillips, 1966), subfiber-a, bearing dynein arms (the com-

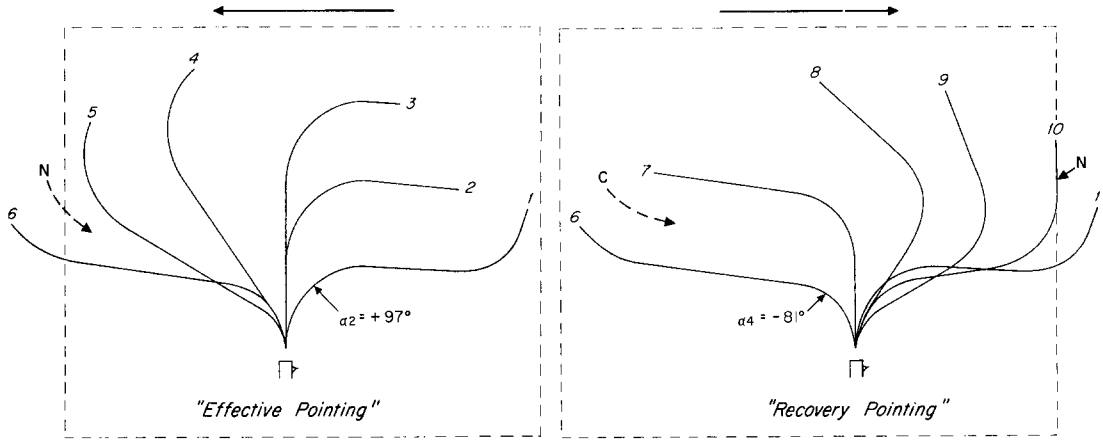

FIGURE 1 Reconstructed stroke of lateral cilia. Arrows indicate direction of motion in recovery stroke (left) and effective stroke (right). In each case, the circular arc at the base of the cilium moves up the shaft as the stroke progresses. The angle subtended by the arc in stroke position *1* is α_2; in stroke position *6* it is α_4, opposite in sense to α_2. Numbers *1–10* correspond to stroke positions of Table I. In stroke positions *1–5*, $\Sigma\alpha > 0$ and the cilia are effective-pointing (E); in positions *6–9*, $\Sigma\alpha < 0$, the cilia are recovery-pointing (R); in position *10* (and also in a stroke position between *5* and *6*), $\Sigma\alpha = 0$ for neutral cilia (*N*). The control position is indicated (*C*), although the control represents a new equilibrium position and is not strictly intermediate between stroke positions *6* and *7*. Redrawn, with the copyright permission of The Rockefeller University Press, from Satir, P. 1967. *J. Gen. Physiol.* 50 (6, p. 2): 241.

plete microtubule), and subfiber-b. At the tip, subfiber-a loses its arms and then continues onward past the termination of subfiber-b as a singlet. The length of the doublets is thus equal to the length of subfiber-b. No information has been available previously concerning the possibility of shortening of subfiber-a.

An essential aspect of the sliding filament model of ciliary motility as it is presently conceived is a close linkage between amount of bend and amount of filament displacement. Brokaw (1965) has elegantly demonstrated that, for certain sperm tails at least, bends occur in the form of circular arcs and that otherwise the shaft is straight. Evidence from electron micrographs has led to a similar conclusion for *Elliptio* lateral cilia (Satir, 1967) and, in this case, stroke form has been reconstructed on the basis of measured maxima of the arcs and straight portions of the ciliary shaft (Fig. 1). If the only considerations for the production of filament displacement at the cilium tip are geometric, then for circular arcs, the equation

$$\Delta l = 2\pi d\, \Sigma\alpha/360 \qquad (1)$$

would be expected to hold, where, for a particular stroke position, Δl is the difference in microns between a longer vs. shorter-appearing doublet (subfiber-b), $\Sigma\alpha$ is the sum in degrees of the angles subtended by circular arcs on the ciliary shaft, and d is the effective distance between the two filaments measured on the axis of the cilium (Fig. 2). Between the bridge (filaments 5–6) vs. filament 1, d is the axonemal diameter and Δl should be maximal (Table I). In this case, the amount of tip displacement expected per degree of bend is approximately 35 A (Satir, 1967).

This paper will attempt to demonstrate, for the cilia studied, that (*a*) subfiber-a does not shorten during beat and (*b*) equation 1 relating tip displacement and bend is experimentally valid within certain limits; that is, all displacement at the tip can be accounted for by the geometrical relations of noncontractile microtubules.

MATERIALS AND METHODS

Material

This report deals exclusively with the lateral cilia of the gill of the freshwater mussel (usually *Elliptio complanatus*). On excision of the gill into water, these cilia come to rest in a characteristic position (Satir, 1961, 1963, 1965) (control preparation) but metachronism may be restored by treatment with 0.04 M

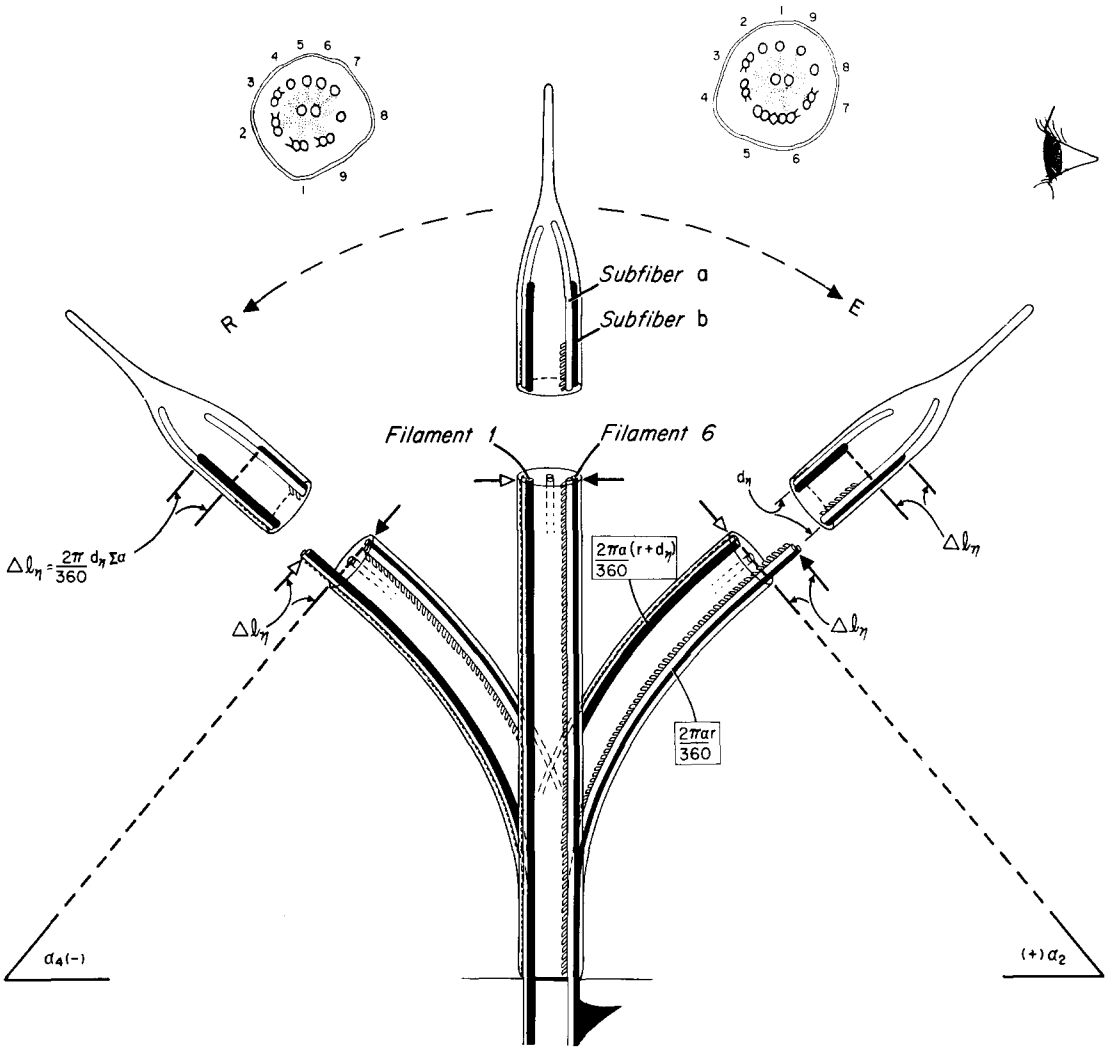

FIGURE 2 Sliding-filament hypothesis of ciliary motility. Behavior of two doublet peripheral filaments (1 and 6) at the tip and base is illustrated when a schematized cilium is bent to either side of a straight position (center). In the neutral position subfibers b of the filaments end together at one level at the tip, i.e., are equally long, while the subfibers a continue onward as naked singlet microtubules to different termination points, i.e., not equally long. The arrows with open and solid arrowheads mark equal shaft lengths from the basal plate (base line) on projections of the two filaments in the plane of the diagram (plane of beat). When the cilium bends, a circular arc arises at the base. The angle subtended by the arc eventually increases to α. At that time, the entire length (to the arrow) of one of the filaments (the outermost) is involved in the arc and, from simple geometry, this length is $\frac{2\pi\alpha(r + d_n)}{360}$ where $(r + d_n)$ is the radius of curvature of the arc. Beyond this point, the filament is straight. Which of the filaments meets this description depends upon the direction of bend, for the length of arc of the innermost filament (nearest cell surface) is only $\frac{2\pi\alpha r}{360}$. The difference in length between the filaments is then Δl_n, given in equation 4. This difference will be apparent at the cilium tip, since the filaments are inextensible. Where filament 1 is apparently shorter, Δl_n (in this case, Δl_6) is positive (E-cilium); where filament No. 1 is apparently longer, Δl_n is negative (R-cilium). A cross-section through the tip at a level where some of the filaments are doublet and some are singlet is shown for both E and R cilia at the top of the diagram. The eye indicates that the view in both cases is from the abfrontal (effective) side of the cell. Axis tilt is neglected.

TABLE I

Predicted Relationships of Stroke Position, Tip Type, and Filament Displacement

Stroke position No.*	Probable tip type	$\Sigma\alpha$	$\Delta l_{5\text{-}6}$
1	9S-E	+16°	···
2	8S, D-E	+97°	+0.35
3	8S, D-E	+97°	+0.35
4	9S-E	+53°	···
5	9S-E	+30°	···
6	9S-R	−33°	···
7	7S, D-R	−81°	−0.28
8	9S-R	−51°	···
9	9S-R	−31°	···
10	9S-N	0	0.00

* From Fig. 1

KCl (KCl-activated preparation). In the activated preparation the form of the metachronal wave may be captured on fixation (Satir, 1963), and various stroke positions are seen. Phase differences between individual cilia are largely preserved (Satir, 1967).

The effective stroke of the lateral cilia is in an abfrontal direction. Cilia whose tips run abfrontally (effective-pointing cilia)[1] can be distinguished in cross-sections from those whose tips run in a frontal direction (recovery-pointing cilia)[1] by the position of the characteristic bridge, by enantiomorphic form, and by other criteria (Satir, 1965). In the control preparation, all cilia are recovery-pointing.

Normally, portions of one gill are kept as controls, while other portions are activated; then both are fixed with osmium tetroxide and embedded in Epon. The material to be illustrated here was sectioned on a Huxley ultramicrotome and viewed in a Siemens Elmiskop I. Precautions were taken to insure that the orientation of the cilia was known at all times (Satir, 1965).

Conventions

NUMBERING THE FILAMENTS: Filaments are numbered by using the bridge as a marker to distinguish filaments 5 and 6, and the enantiomorphic form is used to establish direction (Afzelius, 1959; Satir, 1965). Numbering proceeds in the direction of the arms (see Fig. 2).

TIP PATTERNS: Several distinctive tip patterns are examined in this study. "Tip pattern" refers to the whole three-dimensional reconstruction of the tip, although this pattern is cataloged particularly with reference to one critical cross-sectional level. For example, in one type of tip pattern (the 9S pattern) there will be a cross-sectional level at which subfibers-a of all nine peripheral filaments are present while all subfibers-b have ended. In a second type, at least one filament has ended completely while one or more subfibers-b persist on the remaining filaments. For simplicity, these are usually grouped together as 8S, D pattern for E cilia or 7S, D pattern for R cilia although the number of filaments missing is occassionally different from the indicated abbreviation. A third type of tip examined (9S, D; 8S) is transitional between the 9S and 8S, D types, and may be an artifact of our reconstruction method (see below). This type of tip cannot be scored from single sections. For clarity, the standard convention regarding the central pair of filaments is usually omitted in this notation, but is understood: hence 9S + 2. Some of these patterns have been illustrated by the author (Satir, 1967), and all are shown in detail in Fig. 5.

Δl_n. We adopt the convention of measuring all length differences with respect to filament 1 and

$$\Delta l_n = l_n - l_1 \tag{2}$$

where l_n is the measured length of subfiber-b of filament n past the 9D + 2 configuration; l_1, the corresponding length of subfiber-b of filament 1, and Δl_n, the difference between these quantities. In effective-pointing lateral cilia (Satir, 1965) subfiber-b of filament 1 is shorter-appearing, and Δl_n is usually positive. Subfiber-b of filament 1 is longer-appearing in recovery-pointing laterals and, Δl_n is usually negative. Accordingly, we define

$$\Sigma\alpha = \alpha_2 - \alpha_4 \tag{3}$$

where α_2 is the angle subtended by arc A2 (abfrontal arcs; Satir, 1967) of Fig. 1 and α_4 is subtended by arc A4 (frontal arcs).

d_n. The axis of the ciliary cross-section corresponds in a general way to the plane of beat of the cilium (Fawcett and Porter, 1954), and it might be expected that the effective force bending the cilium would be generated along the axis. Since we postulate that Δl_n is a reflection of this force for each filament, it seems reasonable to assume that d_n is the effective distance between filament 1 and the projection of the filament n on the axis of the cilium. The filaments are assumed to be evenly spaced on a circle for this calculation (Table II), while the diameter of the axoneme ($d_{5\text{-}6}$) is taken to be 0.2 μ (Satir, 1961). Errors in measurement appear to be much greater than the small differences in d_n that may be calculated by other reasonable assumptions. Equation 1 thus becomes

$$\Delta l_n = \frac{2\pi}{360} d_n \Sigma\alpha. \tag{4}$$

[1] Abbreviations: effective-pointing cilia, E; recovery-pointing cilia, R. This convention differs slightly from that adopted in a previous paper (Satir, 1967).

TABLE II

Predicted Maximal and Minimal Values for Δl_n

Filament	d_n*	Predicted Δl_n	
		$\Sigma\alpha = +97°$	$\Sigma\alpha = -81°$
	μ	μ	μ
1	0.00	0.00	0.00
2	0.02	+0.04	−0.03
3	0.08	+0.14	−0.12
4	0.15	+0.25	−0.21
5	0.19	+0.33	−0.27
6	0.19	+0.33	−0.27
7	0.15	+0.25	−0.21
8	0.08	+0.14	−0.12
9	0.02	+0.04	−0.03

* $d_{5\text{-}6}$ = 0.20 μ (axonemal diameter from Satir, 1961)

Serial Section Technique

CONSTRUCTION OF EQUIDISTANT SECTIONS: Short serial sequences (three to five adjacent sections) of regions of one field of lateral cilia containing multiple tips from one preparation have been examined in this study (Fig. 3). Approximately equidistant sections for more than 1 μ length of shaft through one "average cilium" from each field could be obtained by constructing (Fig. 4) a two-dimensional array of sections under the following rules: (*a*) each individual square containing the ciliary cross-section is of equal width and each horizontal row is a real serial sequence; (*b*) spacing is determined so that each vertical column contains identical images of different cilia of the field; (*c*) where intermediate images are obtained between two columns, the columns are separated by a space proportional to the degree of intermediacy; otherwise the columns are touching; (*d*) spacing is identical in all horizontal rows. Comparative section thickness is accurately computed from width of section plus column space if any. A touching row of sections can be easily constructed for long distances as the sum of the array. Such a reconstruction assumes that cilia contributing to the array are identical and that different parts of the shaft are cut. For the controls, this assumption is valid, as will be seen; adjacent ciliary tips in activated preparations are slightly different, but in practice this difference presents little trouble. However, different activated preparations, of course, yield very different reconstructed sequences.

SUBFIBER TERMINATION: In most instances it is clear from inspection which subfibers have terminated. However, in critical cases this has been confirmed by placing the appropriate micrographs of serial sections on a light box and matching up the filaments. In addition, terminating subfibers-a often appear less distinct in the section preceding their absence.

SECTION THICKNESS: Interference colors of sections used in this study ranged from silver-gold to gold-purple, that is, from 90 to 150 mμ (Peachey, 1958), but the value of 0.1 μ cilium length per equidistant section has been used consistently in the measurements. A row of sections of one preparation cut consistently at one extreme might introduce a considerable quantitative error into the data, and is a possible cause of some scatter in the results. Measurements on all filaments in such a preparation would be affected equally on a percentage basis, however, and systematic variations would probably be detectable.

ELECTRON MICROGRAPHIC SAMPLING: Micrographs from many gill preparations of the lateral cilia have been accumulated over a period of several years, but, even so, the number of micrographs that illustrate a particular type of tip or stroke position and that may be used for a particular measurement may be relatively small, and the possibility of sampling error cannot be excluded. However, the dense packing of the lateral cilia often does provide multiple opportunities for measurement on a single preparation. In these cases good agreement is found between replicates. Variation from preparation to preparation does not appear to contribute greatly to an extension of the range of measurements.

RESULTS

Control Preparation

The control preparation consists of cilia whose shafts lie in parallel (Satir, 1963, 1965) so that in cross-section a near perfect array of transverse sections through axonemes is readily obtained. Four rows of multi-ciliated cells from one gill filament are involved in producing a particular array, so that, even though individual cilia are nearly identical in stroke form, these cilia originate and terminate at slightly different points. Therefore, within a single array the shaft is cut at various levels (Satir, 1961). This is apparent at the ciliary tip where changes in level of cut are clearly discernible (Fig. 3 *A*). The cilia are bent in similar fashion throughout the preparation to produce the parallel array; the bends occur only at the base and $\alpha = -72°$ (average measurement, 10 cilia; range from -50 to $-89°$; $\pm 7°$ average error; see Fig. 12 of Satir, 1965).

SIMILARLY BENT CILIA HAVE SIMILAR TIPS: Tip type (9S vs. 7S,D) is scored for cilia

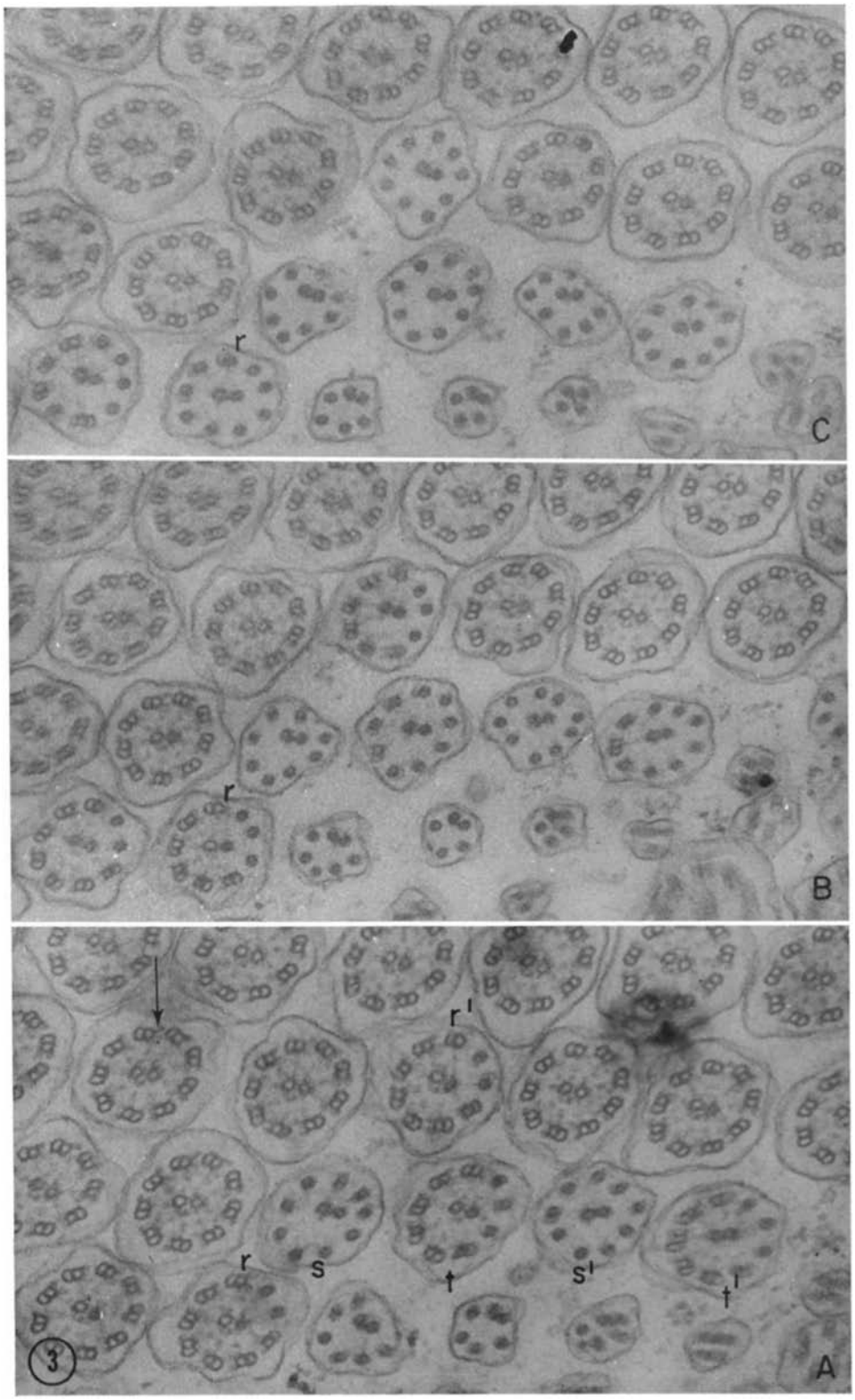

FIGURE 3 Adjacent serial sections of control cilia showing images used in reconstruction of cilium tip. Three pairs of cilia are marked (r and r'; s and s'; t and t'). Members of each pair are sectioned at very similar levels in A and also in B and C. Note 9S + 2 configuration, e.g. s. Arrow indicates bridge. Enantiomorphic form I (Satir, 1965): arms clockwise; cell of origin at bottom of figure (not shown). Outside axonemal diameter, 0.18 μ (Satir, 1961).

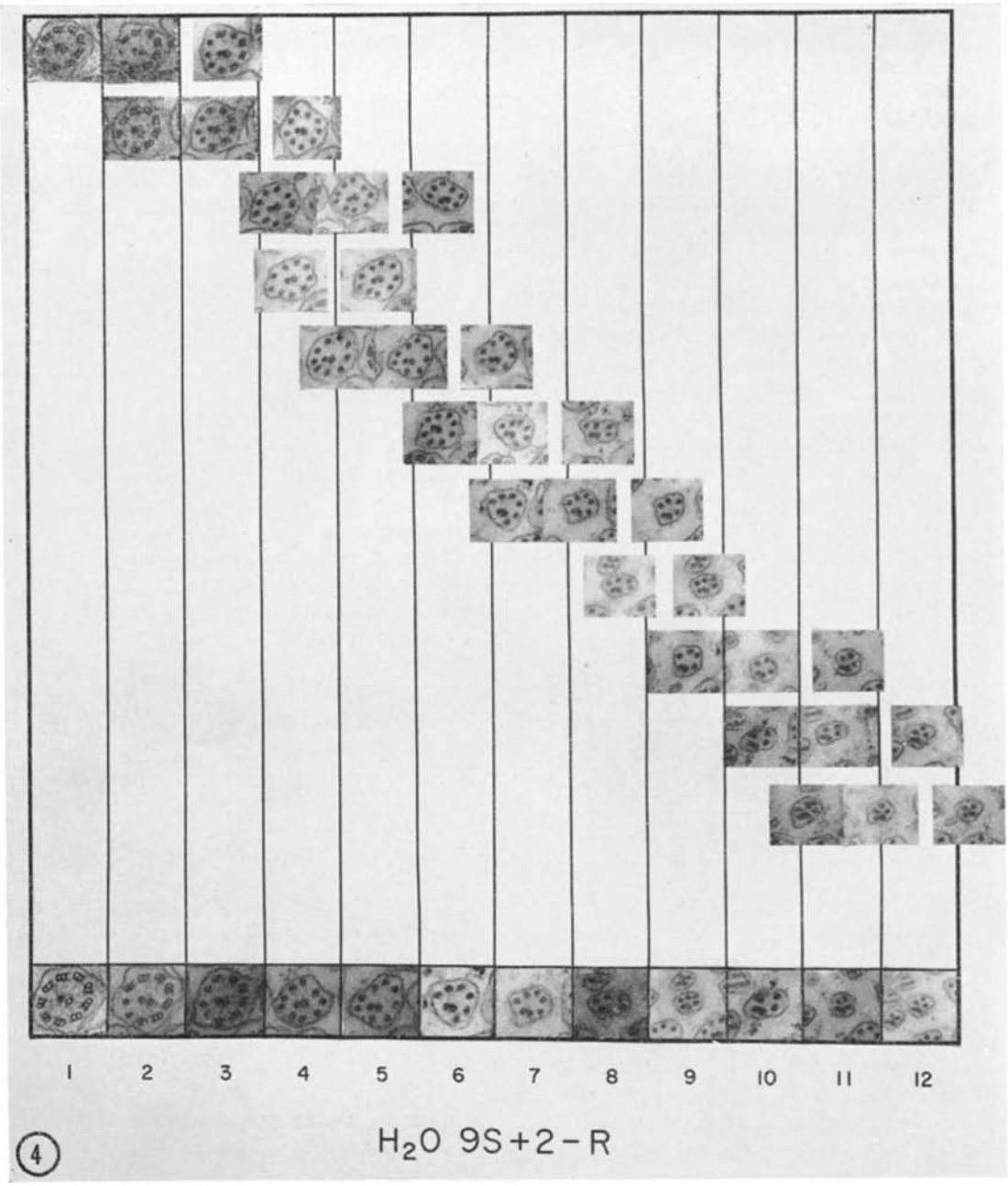

FIGURE 4 Reconstruction of equidistant sections through control tip. Each horizontal row represents a single cilium in Fig. 3 *A*, *B*, and *C*. (For example, row 2 is cilium *r'* of Fig. 3.) Because the change between sections in Fig. 3 *B* and *C* is somewhat greater than that between Figs. 3 *A* and *B* (compare *B* and *C* in row 1 to *A* and *B* in row 2), *B* is probably somewhat thicker than *A*. This is represented on the reconstruction by a displacement of *C* to the right. Columns are arranged to have identical or nearly identical images. Typical images of columns 1–12 appear at the bottom of the figure as representative of equidistant cuts through the ciliary shaft of a single average control cilium. Choices must often be made in the placing of the rows and in the column summations (e.g. column 7 could be 8, 7, 6, or 5S + 2; note, however, that in the summation the shaft goes from 8S + 2 to 4S + 2 in three steps, in agreement with the cilia in rows 6 and 7; duplicates of one row, e.g. *r* for *r'* in row 2, are omitted from the reconstruction but confirm the interpretations as represented in the summation) but variation in termination of individual filaments by more than ±1 section is unlikely. Enantiomorph IV (Satir, 1965): arms clockwise, filament 5 at bottom of cross-section; axonemal diameter as in Fig. 3.

of the control in Table III. If all control cilia had identical bends (all α equal) and if the different tip types were related to the amount of bend as proposed by Satir (1967), then all control cilia should have identical tips, and only one tip type should be present. Over 80% of controls scored apparently have the 9S type tip, which is in reasonable agreement with our expectation. This is not the case for all preparations (Table III).

For any two control cilia, Δl_n should be equal and filaments should end identically. This should be especially apparent in near neighbors of the same preparation. Therefore, if two cilia appear to be cut at the same level in Fig. 3 *A*, they should be exactly or nearly identical in all subsequent cross-sections (Fig. 3 *B*, *C*). Three pairs of tips are marked to illustrate this point in these figures.

SUBFIBER-A VARIES IN LENGTH FOR DIFFERING PERIPHERAL FILAMENTS: Fig. 3 *A–C*

TABLE III

Counts of Differentiable Tip Pattern of Lateral Cilia

Preparation	No. of tips counted	
	9S + 2	7S, D + 2/ 8S, D + 2
Control H_2O-R	14*	2
KCl-R	10	2
KCl-E	20	12

* Usual control configuration

have been used to construct (Fig. 4) 0.1 μ equidistant sections through the control axoneme as described above (Materials and Methods: Serial section technique). The bottom line of Fig. 4 shows the result, a series of 12 sections from the 9D+ 2 pattern to the pattern far along the tip. The central pair of filaments persists to the end of the series. The array of peripheral filaments in these sections is schematically represented in Fig. 5*a*. The variations in peripheral filament array from level to level are caused by subfiber-b of filaments 6–8 apparently ending before the other subfibers and by the extension of the singlet microtubule (subfiber-a) to differing degrees past the termination of subfiber-b. For example, subfiber-a of filaments 3 and 8 extends more than 1.0 μ past the end of subfiber-b, while subfiber-a of filament 5 extends only 0.2 μ. These differences in subfiber-a are relatively small compared to the total shaft length (14 μ), but they are more than four times the apparent differences in subfiber-b. The sequence of termination of the subfiber-a extension is given in Table IV.

Activated Preparation

Construction of a second set of equidistant serial sections is illustrated for an 8S,D effective-pointing cilium from an activated preparation in Fig. 6 and is diagrammed in Fig. 5 *b*, while Fig. 5 *c–e* show other tip types. Electron micrographs of additional sets of serial sections of activated preparations have been illustrated in previous publications (Satir, 1965, 1967). In all cases (not diagrammed in Fig. 5) the central pair of filaments persists throughout the reconstruction. Longitudinal sections (Fig. 7) show that the central pair runs to the very tip of the cilium where the two central microtubules fuse (or a single element executes a hairpin turn?). Longitudinal sections (for example, Fig. 8) also confirm that (*a*) subfibers-b of different filaments are sometimes displaced at the tip and (*b*) differing subfibers a are of differing length, but these sections are more difficult to quantitate, since, at present, particular filaments cannot be identified with any certainty.

At one sectioning level in a fixed metachronal wave the tip pattern from one wavelength corresponds quite closely to that from adjacent wavelengths, and the reconstructions are similar. Apparently, at one section level the tips seen come from only a few closely related stroke positions. This finding is somewhat unexpected, but it is probably due to the conditions imposed by the thinness of the section and the orientation of the tip relative to section plane. However, within short distances in one wavelength both 9S and 8S,D tip patterns can sometimes be found. Therefore, occasional cilia ($\sim$ 10%) do not fit exactly into the serial section reconstruction, but these are easily discernible (see Fig. 6). Differing reconstructions are obtained at differing section levels of one gill filament or at the same sectioning level of different gill filaments in the same section. The most striking differences occur, of course, in E vs. R cilia where subfibers-b of differing filaments appear to end first (Fig. 5 *b* vs. *d*).

THE AXIS OF THE CILIUM IS STABLE DURING BEAT: Wherever reconstruction is reasonably complete (e.g. Fig. 5 *b*, *c*, *e*), subfibers a of filaments 3 and 8 are invariably the longest. Thus, these filaments can be identified unequivo-

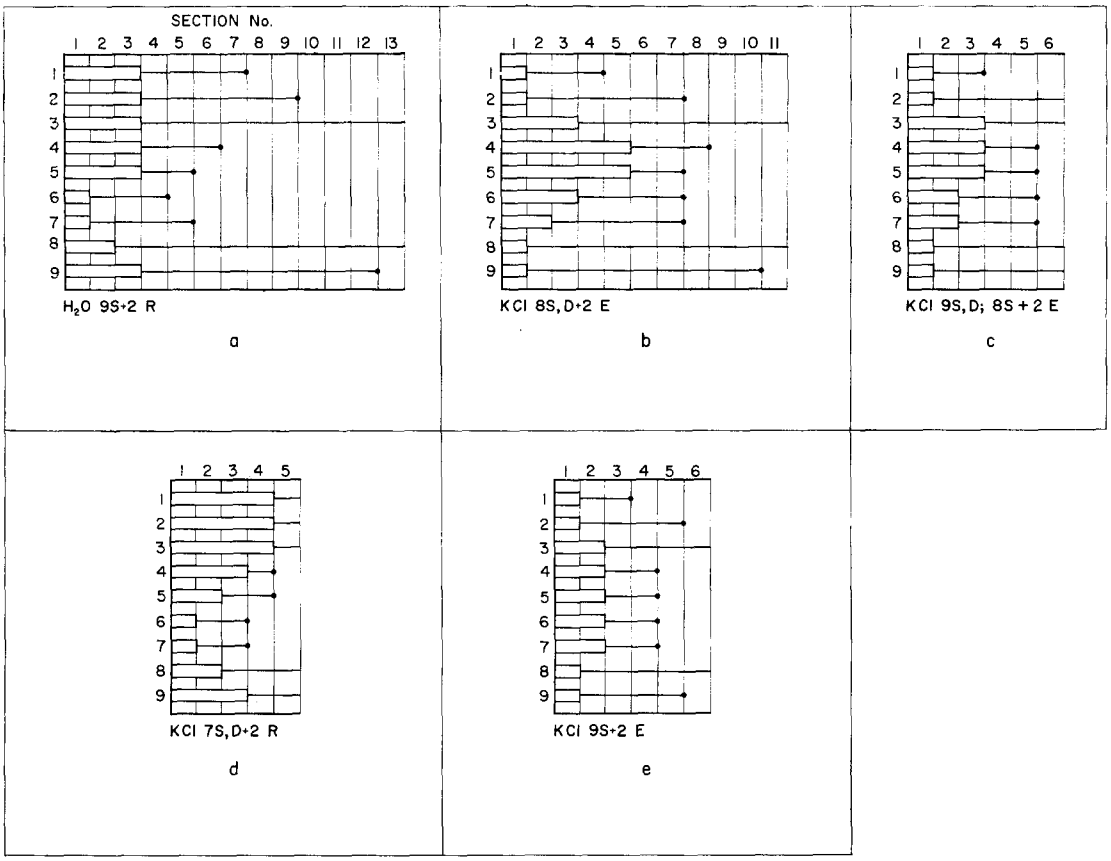

FIGURE 5 Diagrammatic representations of tip reconstruction of several types of cilia. *a*, The cilium of Fig. 4. Open bars represent presence of both subfibers-a and b of indicated filament in summation section; line represents subfiber-a extension (single microtubule); dot shows filament termination. Note that in section 4 all nine filaments are present as singlets, hence: 9S + 2. *b–e*, Active cilia. *b*, The cilium of Fig. 6. Section no. 5 defines tip type. *c*, Transitional type of tip. This is probably an artifact since it is unlikely that filament 1 terminates at precisely the same point as subfiber-b of filaments 4 and 5, but see Fig. 6. *d*, See Fig. 11 *a* from Satir 1967. Probable stroke position 7 of Fig. 1. *e*, A near neutral tip; probable stroke position 5 of Fig. 1.

TABLE IV

Sequence of Termination of Subfiber-a Extension

	Filament*									
H_2O-R (Fig. 5 *a*)	(8	3)	9	2	(7	···	1)	(6	4)	5
KCl-E (Fig. 5 *b*)	(8	3	9)	2	7	6	(1	···	4)	5
Measured length μ‡	>1.05	>.87	.67	.52	.36	.31	.27	···	.23	.19
No. of measurements	2	3	7	9	10	10	10	···	10	10

* Filaments listed by number in order of extension length (long to short). Parentheses enclose extensions whose lengths are indistinguishable in a particular preparation. Subfiber-a of filament 8 appears to be longer than subfiber-a of filament 3 in further reconstruction (not illustrated in Fig. 5).
‡ Averaged.

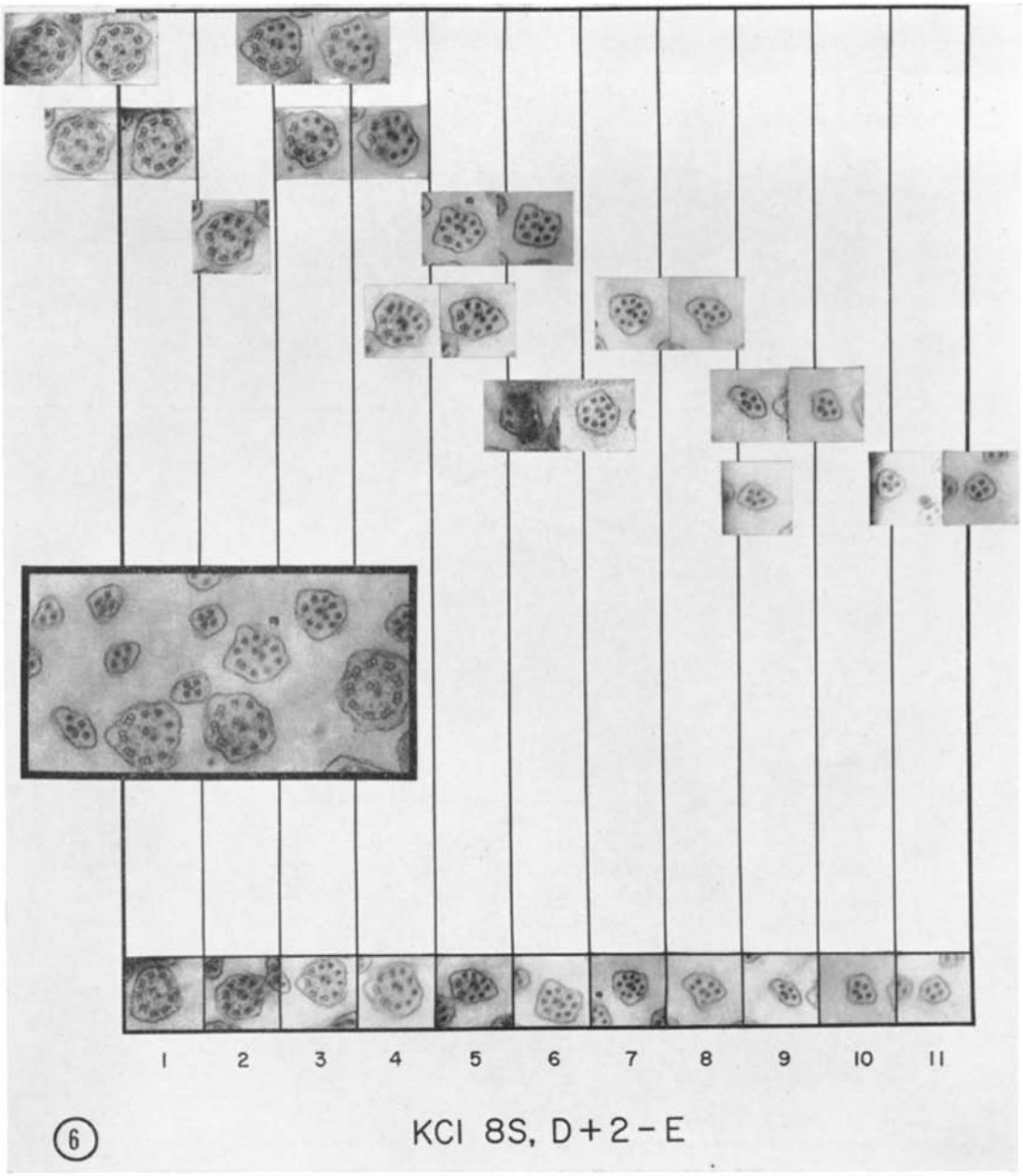

FIGURE 6 Reconstruction of equidistant sections through tip of activated cilium. Insert shows field of tips from which reconstruction is taken (No. 4 of five adjacent serial sections). Note that cilia in rows 3–5 are slightly different, although they are close neighbors (insert) in the fixed metachronal wave: for example, rows 4 and 5 are 8S, D + 2 cilia, but row 3 is a transitional cilium (9S, D; 8S — as in Fig. 5 *c*). This supports the notion that small continuous changes in Δl_n occur during beat, and reflects the phase difference between individual cilia in the fixed metachronal wave. Enantiomorph III (Satir, 1965): arms counterclockwise, filaments 5–6 at bottom of cross-section. Axonemal diameter, 0.2 μ (Satir, 1961).

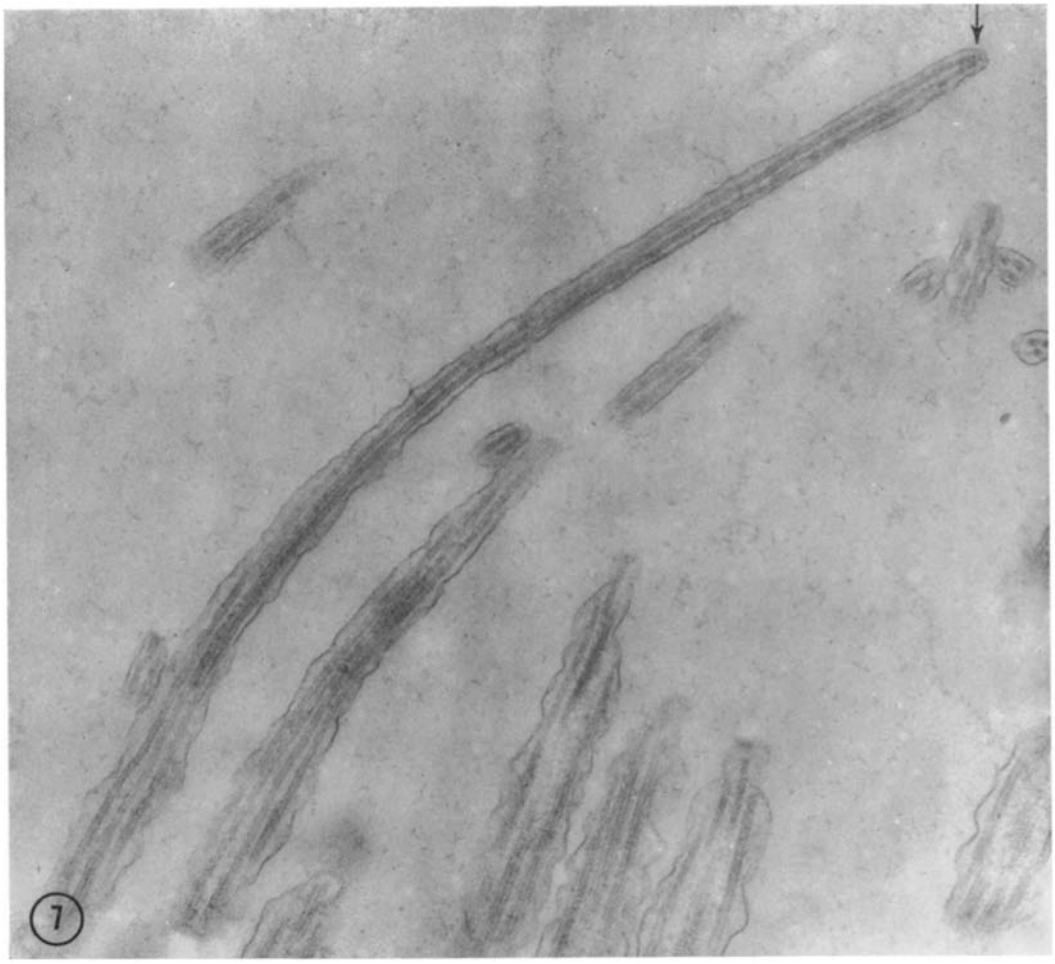

FIGURE 7 Frontal section of lateral cilium tip showing central pair fusion (arrow). × 40,000.

cally, and in turn they allow unequivocal numbering of the axoneme. The original method of numbering (see Methods) is based on the axis of the cilium and assumes stability of the bridge (Satir, 1965) between filaments 5–6. Since the methods agree, the bridge must indeed identify filaments 5–6, in varying beat positions as well as in the control (Fig. 5 *a*). Therefore, another explanation of variation of axis angle (Satir, 1963) must be sought.

TIP DIFFERENCES ARE NOT DUE TO MORPHOGENETIC VARIATION: If intrinsic length differences (morphogenetic variation) were responsible for differences in tip pattern from cilium to cilium as proposed by Afzelius (1959), it might be expected that the proportion of tips of 8S,D type, for example, would remain constant irrespective of tip direction or preparation. (Differences between 8S,D vs. 7S,D cilia are neglected under this assumption.) On the other hand, if the 8S,D pattern were correlated with stroke positions (for example, strokes where $\Sigma\alpha > \pm 70°$), then enrichment of this tip type might be expected in E-pointing cilia (see Fig. 1). Table III shows that many more 8S,D cilia are found in activated E-pointing cilia than in R-cilia or in controls alone.[2] Further, on the assumption that morpho-

[2] The probability from χ^2 analysis that the tip type distributions of KCl-E vs. R cilia (total, KCl + H_2O) are identical is < 0.01, provided that we accept the original data as unbiased. Although micrographs were taken to illustrate ciliary tips without conscious selection of original tip type, bias cannot be excluded entirely.

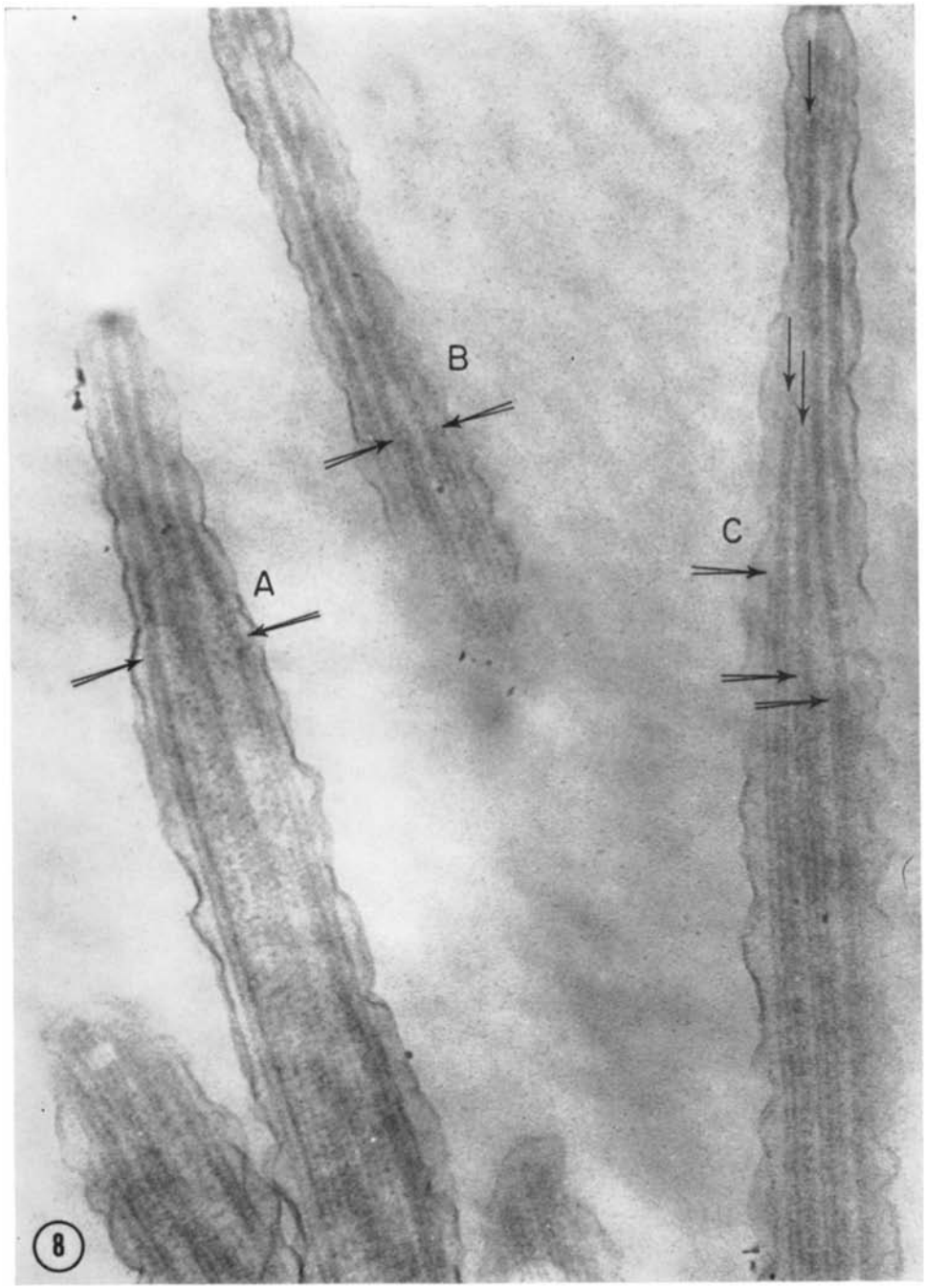

FIGURE 8 Longitudinal section of lateral cilia tips. Double-stemmed arrows indicate probable termination of subfiber-b. Note that in cilia *A* and *B*, subfibers-b terminate together ($\Delta l = 0$), but in cilium *C* they terminate at different levels (greatest difference $\Delta l \sim 0.2\ \mu$). In cilium *C*, arrows mark termination of subfiber-a. From left to right, subfiber-a = 0.3, 0.4, 0.9, and 0.7 μ, respectively. Marked filaments in cilium *B* show relatively long subfibers-a ($\sim$0.8 μ). These filaments are on the opposite side of the cross-section from those in cilium *C*: reading *B* left to right the subfibers are a, b, a, b ... ; in *C* they are b, a, b, a, b, a. From Fig. 9, for an effective-pointing (9S-E) cilium this would be in good agreement with *C*, filaments 6–9; *B*, 3, 2; and *A* = 5 or 4 and 1. Δl_n is depicted for similar tips in Fig. 10 (right). Calibration d = 0.2 μ in *A*). × 85,000.

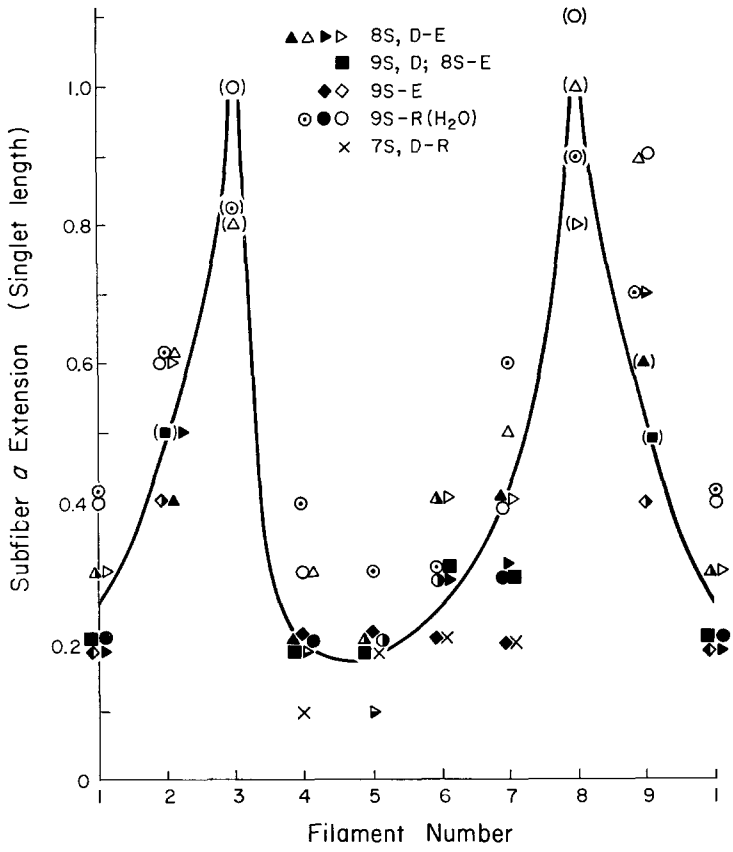

FIGURE 9 Subfiber-a extension vs. filament No. in 11 reconstructed series of different cilia of varying stroke position and tip type. The extension of each filament is measured in microns (to the nearest 0.1 μ). Parentheses indicate that filament had not terminated by the end of the reconstruction, i.e., these values are minimum values. No differences are apparent from preparation to preparation; each subfiber-a extension maintains its unique length throughout. The curve is primarily an aid to visualization of the sequence of singlet lengths, but may have morphogenetic significance.

genetic variation is minimal and subfiber length very precisely determined, all or nearly all tip differences can be explained on the basis of the sliding hypothesis, as shown below.

SUBFIBER-A DOES NOT CONTRACT DURING BEAT: Identically numbered subfibers-a of different cilia with different tip types extend for very similar lengths beyond the subfiber-b of the same doublet. The singlet extensions of filaments 5, 6, 1, and 2 vary by no more than two section thickness total or roughly 0.2 μ (Fig. 5, 9)[3] in every cilium studied, and the measurement for filament 4 is very nearly within this margin. This variation is clearly within the range of technical error. Several controls are shown to indicate reproducibility for identical cilia in identical stroke

[3] In Figs. 9 and 10, "Filament No." (a discontinuous notation) is plotted on the abscissa as a continuous function and a curve is drawn through connecting points. I justify this notation for convenience, because filament No. is directly correlated with distance from filament 1 along the axonemal circumference, which is continuous. This implies that the filaments are simply convenient points on the axonemal circumference to make measurements and that the interfilament matrix moves along with the filaments, which seems reasonable. In Fig. 9, the curve may also have morphogenetic significance in describing the concentration of monomers used to assemble the uniquely different lengths of subfiber-a for each filament (see Discussion).

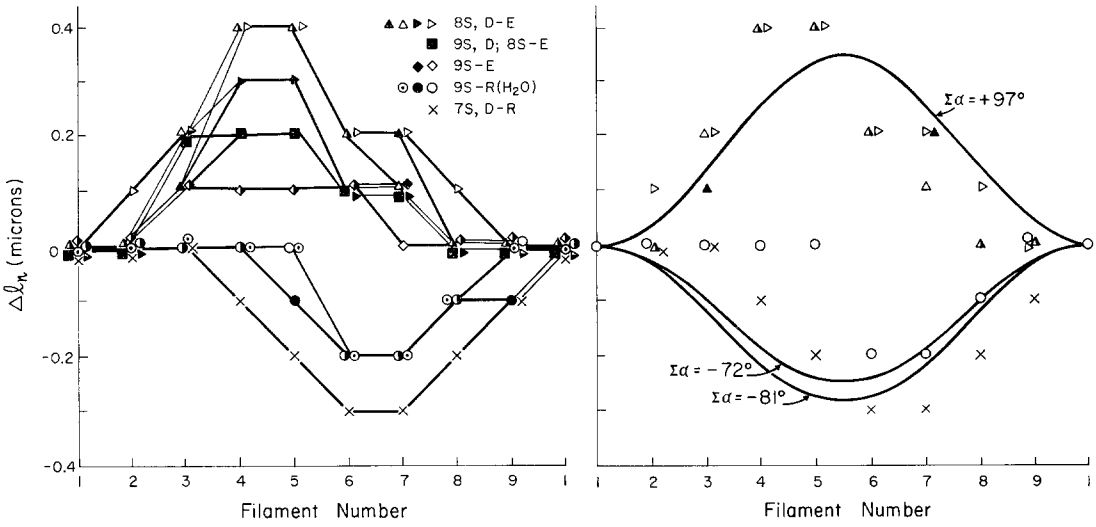

FIGURE 10 Δl_n vs. filament No. Right, measured points from the preparations of Fig. 9. When two cilia of identical tip type give the same measurements, single lines are used to connect points. Curves suggest that Δl_n for one cilium is a continuous function of distance from filament 1. The family of curves suggests the possibility of transformation of 9S to 8S, D tips and back by small shifts of relative position between filaments, e.g. slowly increasing or decreasing Δl_n. Left, comparison of predicted values (curves) of Δl_n from equation 4; (see also Table II) and measured points. The three curves represent cilia in stroke positions *2* or *3* ($\Sigma\alpha = 97°$) and *7* ($\Sigma\alpha = -81°$) from Fig. 1 and the control ($\Sigma\alpha = -72°$). The points are 8S, D-E and 7S, D-R cilia respectively for the stroke positions and a 9S-R control.

positions. The longer filaments 3, 8, 9, and 7 show somewhat greater variation, but this variation is also expected from the method of reconstruction. The order of termination is also qualitatively reasonably constant throughout (Table IV) and does not vary in any consistent way with tip type or stroke position.[4] The least precisely measured lengths correspond to those filaments on the sides of the cilium, roughly perpendicular to the plane of beat. These filaments would also be the most unlikely to cause motion by contraction. Variations in subfiber-a of filament 5, on the other hand, would be expected to be about 0.6 μ if the subfiber contracted in some stroke positions. The small variation found is less than $\pm 1\%$ of the total length of the filament and suggests that extension length for this filament is constant and identical in all cilia of this type. An average (presumably constant) length of subfiber-a extension is given in Table IV for all filaments except 3 and 8. Since the singlet microtubule extensions of filaments 3 and 8 are over 0.8 μ in length, they have not yet been followed to their exact termination. Prior to the singlet extension, subfiber-b measures the length of subfiber-a. Since subfiber-b apparently does not change length during beat (Satir, 1965), neither does subfiber-a to the point where the doublet configuration ends. If the singlet extension is also constant, over its entire length subfiber-a does not contract to within the error of measurement. With respect to subfiber-b of the same doublet, subfiber-a does not move, i.e., slide.

Δl_n VARIES SYSTEMATICALLY WITH CILIUM TYPE (STROKE POSITION): Extra doublet length of each filament measured past an all nine doublet configuration is easily measured from, for example, Fig. 5. Fig. 10 (left) plots Δl_n against "Filament No." for the same preparations as in Fig. 9. The curves drawn are smooth and reflect the possibility of continuous variations of Δl_n. Identical stroke positions (9S-R controls) give nearly identical curves, but there are clear differences between different preparations as to which

[4] The qualitative order of singlet length in 6 and 1 is different in the two cilia of Fig. 5 *a* and *b*, probably because small errors occur in reconstruction when the singlets are very nearly equal length.

doublets appear shortest; in R-pointing cilia these are 6, 7 short; 1, 2, 3 long; in E-pointing cilia they are 8, 9, 1, 2 short; 4, 5 long. This is consistent with the sequences found previously (Satir, 1965). In addition, since filaments may be both longer or shorter-appearing than filament 1, Δl_n may be either positive or negative; for any one type of cilium, Δl_n usually has only one sign and varies systematically in magnitude as predicted.[5] There are also differences in amplitude (maximum to minimum Δl_n) among preparations of the same type; the smaller amplitudes correspond to 9S cilia, the larger to 8S,D (E) or 7S,D (R). Such systematic differences would be expected as sliding at the tip occurred.

APPARENT DIFFERENCES IN SUBFIBER-B LENGTH ARE THOSE PREDICTED BY STROKE FORM: Since $\Sigma\alpha$ is known for the control and can be estimated reasonably well for the 8S,D–E and 7S,D–R type tips because these must correspond closely to the maximum absolute values of α_2 and α_4 respectively, Δl_n can be predicted for these preparations. A comparison between the predicted values (curves) and actual displacements (points) for these preparations is shown in Fig. 10 (right). The fit is reasonable but not exact. Especially, the predicted curves are symmetrical around filaments 5–6, but the actual points are symmetrical around 4–5 for E cilia, 6–7 for R cilia.

DISCUSSION

Validity of Method

The detailed picture of activity of the ciliary microtubules presented here rests upon two basic factors: first, the identification of tip type and stroke position, and second, the serial section reconstruction of the cilium tip. The fixation of the metachronal wave assures that differing stroke positions can be found in one preparation; the methods of correlation of stroke and tip type have been thoroughly discussed previously (Satir, 1965, 1967). The possibility of artifact at the level of microtubule structure cannot be ruled out entirely. For example, these results might be explained if the contractile state of the microtubule was transitory and unable to be captured by osmium tetroxide fixation. This seems unlikely, however; these microtubules are relatively stable structures, and the amount of contraction would be relatively large (0.3–0.6 μ), while fixation is undeniably rapid and certain length differences between microtubules, e.g. those among subfibers a, those between the central vs. peripheral elements, are obviously preserved.

The reconstruction method, in fact, emphasizes relatively minor real or apparent differences in total length (see below). It is proper to consider whether these differences are significant in terms of function and whether they are inherent in the data or are produced during the reconstruction. Apparent differences in subfiber-b length are of the magnitude expected by the sliding filament hypothesis (Fig. 10) and are largely determined by short sequences of true serial sections, e.g., first horizontal row of Fig. 4 or second row of Fig. 6. This is also true for the most precisely determined lengths of subfiber-a extension, e.g., filaments 1 and 5. For the critical measurements, then, the reconstructed picture is closest to the raw data. Replicate control cilia (identical in stroke position) give very similar results throughout, and the spread in all measurements is readily accounted for by the technique. Comparison between points of two disparate preparations in Figs. 9 and 10 is good to approximately $\pm 0.1\ \mu = \pm 1$ section in the reconstruction. The data for which thorough reconstructions have been made are obviously limited, but many additional images support the information presented as does the previous qualitative study by the author (Satir, 1965).

Implications for Assembly

The results presented are consistent with a picture of ciliary microtubular construction as follows: (*a*) the central element runs to the exact tip of the cilium ($\sim$14 μ); (*b*) each subfiber-a is unique in length; the longest (3 and 8) are roughly 1 μ (7–10%) longer than the shortest (4 and 5); (*c*) all subfibers-b are equal in length. The theoretical curves of Fig. 10 (right) are based on this assumption. With the exception of two points, the maximum difference between a measured value and a predicted one is $\pm 0.15\ \mu$. Disparity of subfiber-b length between filaments might account for this difference, especially when the value for the measured point is consistently higher (or consistently lower) than the predicted value. The maximum difference between subfibers-b could then be about 2–3%.

[5] In R cilia, subfiber-b of 3 and 4 sometimes appears to persist after subfiber-b of 1 has terminated. In these cases, Δl_n would be slightly positive.

For morphogenesis this picture may imply that over-all length of a lateral cilium is governed by growth of the central element (two microtubules or one?), that subfiber-a provides or is the result of an internal numbering system and shapes the tip itself, and that subfiber-b marks the functioning region of the axoneme that contains dynein and spokes. The dynein arms appear to end as much as 0.2 μ before the end of subfiber-b in bent cilia. There must be great precision of site of subunit assembly to produce this over-all result, particularly if, as has been assumed by many workers, there is only one subunit type ("tektin," Mazia, 1968)[6] in the microtubular wall. Among the questions raised by these findings are the following: how is the position of dynein attachment on one side of the microtubule wall of subfiber-a determined; how is the position of subfiber-b relative to dynein determined; when and how are the spokes attached to the filaments during morphogenesis; what is the meaning of the order of termination of dynein, then spoke material and subfiber-b; is this termination suggestive of functional complementarity; why do central elements and subfiber-a persist after such termination; what determines length differences among subfibers a while no differences appear among subfibers b? The curve in Fig. 9 may be taken as an index of the gradient of tektin monomers during growth of subfiber-a extension. Apparently at this stage in ciliary morphogenesis fewer monomers are present for polymerization in the plane of ciliary beat than at the sides. Since monomers presumably are added in growing ciliary buds to the tips of prepolymerized filaments and since, in some instances at least, the cilium is already beating when this occurs, correlations of this sort are not entirely unexpected.

Sliding Filament Model

The results presented are entirely consistent with a "sliding filament" model of ciliary motility in that (*a*) changes in apparent filament length of the cilium tip from preparation to preparation are small and systematic (Fig. 10 left), (*b*) all the apparent filament differences are predictable to within experimental error (Fig. 10 right) on the basis of relative sliding of the filaments to accommodate bending of the shaft and the assumption that (*c*) neither subfiber-a nor subfiber-b of any filament changes length during beat (Fig. 9 and 10). The data are not consistent with hypotheses of microtubular contraction alone accompanying bending. Nor do they imply that microtubular contraction is coupled to microtubular sliding in any manner. Certain variants of the sliding model are also eliminated. For example, subfiber-a does not move relative to subfiber-b of the same doublet.

Sliding filament models of ciliary motility have been discussed at length by Satir (1967) and Brokaw (1968). Additional support for such models from other laboratories is discussed in these papers. The version investigated in this study assumes that sliding at the cilium tip accompanies bend initiation at the cilium base, while bend propagation along the ciliary shaft is not accompanied by sliding.[7] That is: when $\Sigma\alpha$ is constant, no matter where the bend is on the ciliary shaft, Δl_n is a constant; when $\Sigma\alpha$ changes, Δl_n changes. Although Fig. 10 supports this interpretation, it is not conclusive. Furthermore, the differences between observed and expected points in Fig. 10, especially with regard to symmetry, may have explanations other than experimental error or differences in subfiber-b length when $\Sigma\alpha = 0$, and may represent complications in the simple model.

The location in the cilium of the apparatus that contracts to cause coupled bending and sliding remains obscure. Two possibilities have been suggested: (*a*) filament-matrix interaction and (*b*) interactions between adjacent filaments. We may shortly be in a position to distinguish between these. For example, the location of dynein and tektin (the components corresponding to the cross-bridge and actin of muscle?) support the latter possibility. Protozoan axostyles that are arrays of linked microtubules have been shown to undulate (Grimstone and Cleveland, 1965). McIntosh and Porter (1967) have accounted for nuclear volume changes during spermiogenesis in roosters by motion of cross-bridged microtubules relative to one another, without over-all microtubular contraction. Bajer (1967) has proposed to account for chromosome movement in some similar way, although Inoué and Sato (1967) have shown that

[6] The terms "tubulin," "spactin," or "flactin" have also been proposed to designate this protein.

[7] This model has an attractive feature in that it predicts information transfer over distances of tens of microns in ciliary-based systems. If operated so that displacement of filaments at the tip caused bending at the base, such a mechanism might be a feature of energy transduction in certain sensory systems, e.g., the campaniform sensillum.

microtubular polymerization-depolymerization equilibria are also involved in the activity of the mitotic apparatus. In the *Elliptio* lateral cilium, there appears to be a slight helical component to the path of the filament during beat which is reflected in systematic changes in axis angle in activated preparation (Satir, 1963). These changes cannot be accounted for by shifts in the bridge or in the central element relative to the peripherals, as was previously thought, but could be a reflection of interfilament cross-bridge activity of the sort described by McIntosh and Porter. This activity might also generate passive changes in inner area—the matrix component (Satir, 1963) —in analogous fashion to the changes generated in nuclear volume.

In the cilium, however, "cross-bridge" activity if such exists must be recurrent and reversible. Presumably, every doublet moves back and forth during beat (Fig. 5). We do not know whether one of these directions is correlated with bend initiation at the base while the other occurs when the wave reaches the tip. Polarized morphological components such as Huxley (1964) has described in negatively stained preparations of I-band filaments are to be looked for on the ciliary microtubules and in the matrix.

On the other hand, Stubblefield (1967) has recently constructed a workable mechanical model of a cilium where the filaments slide as the model bends because of a regular angular displacement of the axonemal "spokes" connecting center and periphery. He claims to have evidence of such displacement from stereo electron micrographs, but this important point awaits further confirmation. On the basis of our present information, it seems likely that both major axonemal components, filaments and matrix, will ultimately be involved in ciliary beat, either with sliding directly or with propagation.

MECHANO-CHEMICAL COUPLING: Brokaw (1967) has calculated that for sea urchin sperm tails it is likely that on the average one ATP molecule is utilized by one 14S dynein molecule per beat. This is in agreement with the specific activity of dynein as calculated by Gibbons (1966). This appears to place further limitations on the sliding model as it is presently conceived. For the lateral cilium the total displacement of all filaments from a neutral ($\Sigma\alpha = 0$) position is given by the summation of absolute values from the extreme curves for Δl_n in Fig. 10 (right). The average displacement per filament (assuming all filaments move identically) is approximately 0.32 μ or 18 $\times$ 14S dynein step lengths. If action of every 14S dynein along a filament were necessary for one step length (175 A) tip displacement, a hypothesis of filament-matrix interaction would predict 18 ATP utilized per dynein per beat. Brokaw (personal communication) has pointed out that a filament-filament interaction would utilize less ATP to produce equivalent displacement ($2\frac{1}{2}$ times less or 7–8 ATP per dynein per beat). Nevertheless, in either event this discrepancy between measured values and those expected is not easily reconciled unless every 14S dynein along one filament does not normally hydrolyze ATP simultaneously per sliding step, in which case the very neat stoichiometric relationship obtained by Brokaw and Gibbons may be simply coincidental. Regeneration of ATP by phosphate exchange with bound nucleotide of the microtubular protein (Yanagisawa et al., 1968) might also affect the values for *in situ* ATP utilization (Brokaw's values), while tekto-dynein complexes might have a greatly enhanced ATPase activity (Gibbons), but the necessary changes are of about an order of magnitude and are accordingly unlikely.

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