THE RELATIONSHIP BETWEEN THE FINE STRUCTURE AND DIRECTION OF BEAT IN GILL CILIA OF A LAMELLIBRANCH MOLLUSC

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ABSTRACT

This paper describes the fine structure and its relationship to the direction of beat in four types of cilia on the gill of the fresh-water mussel *Anodonta cataracta*. The cilia contain nine outer, nine secondary, and two central fibers, such as have been described previously in other material. Each outer fiber is a doublet with one subfiber bearing arms. One particular pair of outer fibers (numbers 5 and 6) are joined together by a bridge. The two central fibers are enclosed by a central sheath; also present in this region is a single, small mid-fiber. The different groups of fibers are connected together by radial links that extend from the outer to the secondary fibers, and from the secondary fibers to the central sheath. The basal body consists of a cylinder of nine triplet fibers. Projecting from it on one side is a dense conical structure called the basal foot. The cylinder of outer fibers continues from the basal body into the cilium, passing through a complex transitional region in which five distinct changes of structure occur at different levels. There are two sets of fibers associated with the basal bodies: a pair of striated rootlets that extends from each basal body down into the cell, and a system of fine tubular fibers that runs parallel to the cell surface. The relationship between fine structure and direction of beat is the same in all four types of cilia examined. The plane of beat is perpendicular to the plane of the central fibers, with the effective stroke toward the bridge between outer fibers 5 and 6, and toward the foot on the basal body.

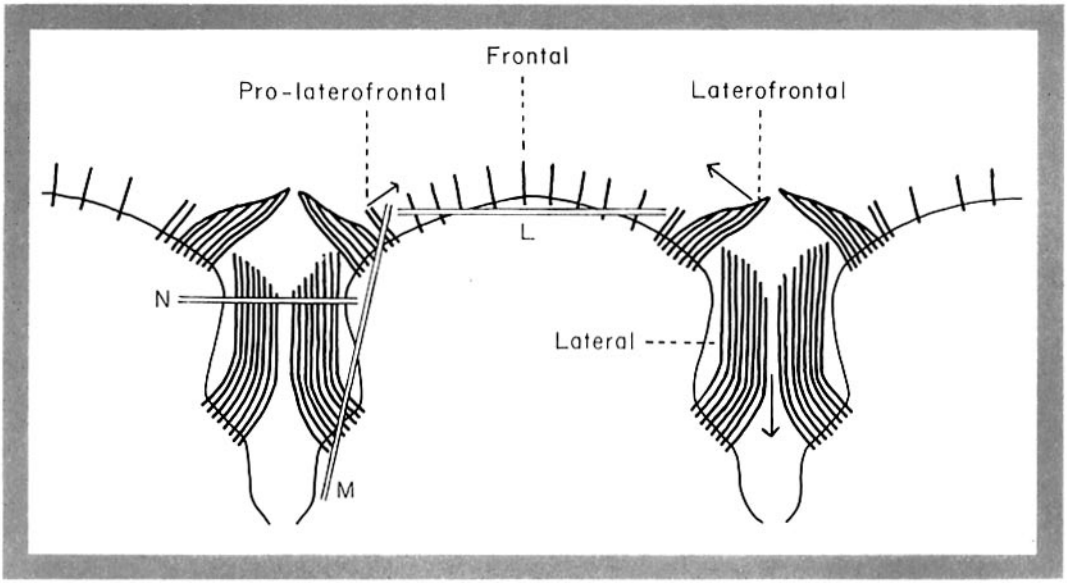
The gills of lamellibranch molluscs possess a highly organized ciliary apparatus that consists of several distinct types of ciliated cells arranged in a regular manner. The cilia on each type of cell beat in a characteristic direction that is related in a simple manner to the general morphology of the gill. This material, therefore, is well suited for correlating the fine structure of the cilia with their direction of beat.

Previous electron microscopic studies on lamellibranch gills have been made by Fawcett and Porter (10) and by Bradfield (7). Since these studies were completed there have been improvements in preparative technique which make it possible to describe the fine structure of the cilia in greater detail; as a result, it is necessary to revise certain of the earlier findings.

The structure of these cilia is similar in general to that of the flagellate flagella described by Gibbons and Grimstone (14). However, there are certain characteristic points of difference, particularly in the transitional region between the basal body and the cilium proper.

MATERIALS AND METHODS

The fresh-water mussel *Anodonta cataracta* was used for most of this study, and all the descriptions refer



Key to Figures

All figures show cilia of *Anodonta cataracta*. All transverse sections are as seen by an observer looking outward along the cilium.

a, arm
bb, basal body
bf, basal foot
bp, basal plate
c, cilium proper
cf, central fiber
cm, ciliary membrane
cs, central sheath
f, frontal cilia
l, lateral cilia
lf, laterofrontal cilia
mv, microvillus of brush border
of, outer fiber
plf, pro-laterofrontal cilia
rl, radial link
sC, distal end of subfiber C
sf, secondary fiber
tf, transitional fiber
tr, transitional region of cilium
tu, tubular fiber associated with basal body

FIGURE 1

Diagrammatic cross-section through surface of gill, showing part of three gill filaments and the four principal types of cilia. The lateral, laterofrontal, and pro-laterofrontal cilia beat in the plane of the diagram, with their effective stroke as indicated by the arrows. The frontal cilia beat perpendicularly to the plane of the diagram. The lines *L*, *M*, *N* indicate the planes of section shown in Figs. 33, 36, and 38. Cell details are omitted.

directly to this species. However, preliminary observations showed the same structures in cilia of the marine mussel *Mytilus edulus* and the long-neck clam *Mya arenarum*.

The movements of cilia on live gills were studied by light microscopy. Sometimes, fine carborundum powder was placed on the gill to show up the ciliary currents.

For electron microscopy, pieces of gill were fixed for about 60 minutes at room temperature in a 2 per cent solution of osmium tetroxide, buffered to pH 7.8, containing 0.004 M magnesium chloride. The following procedure was used in order to preserve the flatness and gross morphological regularity of specimens. Pieces of gill about 4 mm square were carefully cut out and placed flat in the bottom of a small dish. The dish was then flooded with fixative, covered, and left for about 10 minutes. At the end of this time, the specimens were sufficiently hard to be transferred, without damage, to small glass-stoppered

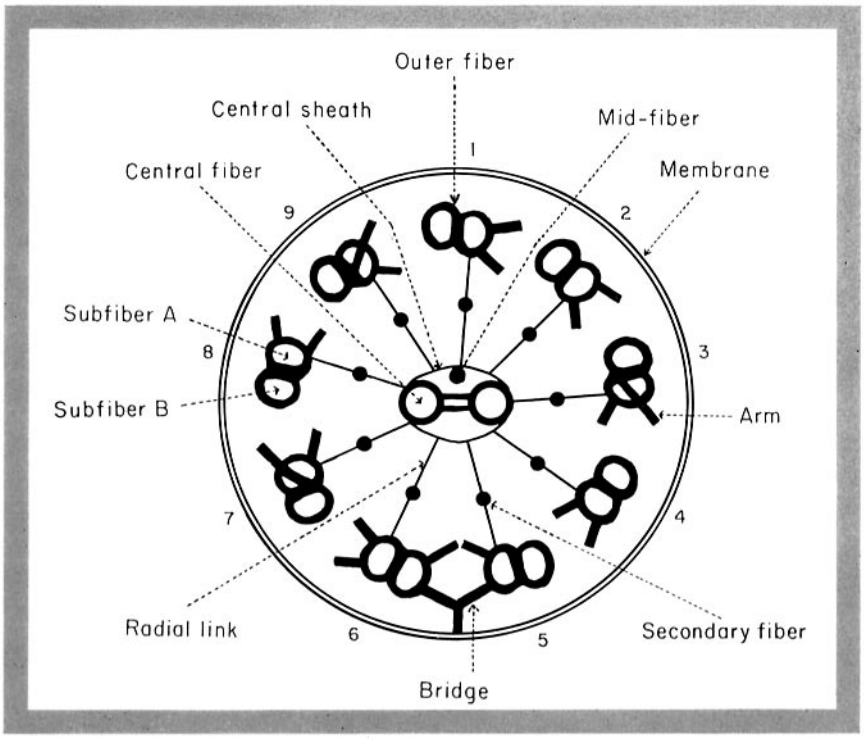


FIGURE 2

Diagrammatic transverse section of a cilium.

bottles of the type normally used for holding specimens. When fixation was complete the specimens were dehydrated through graded solutions of acetone. They were then embedded in Araldite epoxy resin (Ciba A. R. L. Ltd., Duxford, Cambridge, England) essentially according to the instructions of Glauert and Glauert (15).

Sections were cut on a Porter-Blum ultramicrotome fitted with a glass knife and a trough of distilled water. After being flattened with xylene vapors (28) the sections were picked up by applying a carbon-filmed grid to them from above. The majority of sections were stained by floating them, section-side downward, on a fresh saturated solution of uranyl acetate in 50 per cent ethanol for 90 minutes or more, after which they were rinsed thoroughly. (To retard its decomposition this staining solution should be kept in the dark.)

The electron microscope used was an RCA EMU-3D operating at 100 kv with a 50 μ objective aperture and a 125 μ condenser aperture. The microscope was calibrated with a carbon replica-grating obtained from E. F. Fullam Corporation, Schenectady, New York. Measurements were made directly on the photographic plates with a binocular microscope fitted with a calibrated eyepiece.

RESULTS

1. *General Morphology of the Gill*

Many workers have studied lamellibranch gills by light microscopy, and for a detailed description of the gill reference may be made to the papers of Orton (21), Carter (8), and Atkins (4, 5). The following brief account is intended only to introduce the present study of fine structure.

The surface of each gill is formed into a large number of parallel folds, known as the gill filaments. The ciliary apparatus consists of four main types of cilia disposed in a regular manner on the gill filaments, as shown in Fig. 1.

The beat of the long, numerous *lateral cilia* is directed inward, so that they maintain currents of water flowing toward the gill surface. Food particles in these currents are combed out by the plate-like *laterofrontal cilia* and thrown onto the front surface of the gill filament, where the *frontal cilia* convey them to the oral palps. The *pro-laterofrontal cilia* apparently act as a fence along each

side of the front surface to prevent the food particles from falling off.

The movement of individual cilia has been studied in detail by Gray (17). The bending cycle consists of an effective stroke in which the cilium pivots stiffly about its base, followed by a recovery stroke in which it returns limply to its original position. The net effect of this cycle is to impel water in the direction of the effective stroke.

2. *Fine Structure*

So far as it has been possible to tell, the four types of cilia differ only in length and disposition and are identical in fine structure. The following description applies equally to all four types. It is convenient to consider, in order, the cilium, the basal body, the transitional region between cilium and basal body, and the fibers associated with the basal bodies.

a) Cilium: The cilia are typical, long, cylindrical structures composed of a complex bundle of fibers embedded in a matrix and enclosed by a membrane. Fig. 2 illustrates the structures to be seen in transverse section and gives the nomenclature used in the descriptions below.

The two central fibers are approximately circular in cross-section, 240 A in diameter and about 360 A apart (center to center). In transverse sections they have a dense annular outer region about 45 A thick, and a less dense core (Figs. 3, 7 to 11). Favorable areas of longitudinal sections show evidence of helical substructure similar to that already reported in the central fibers of protozoan flagella (14).

In transverse sections there appear to be three distinct structures associated with the pair of central fibers (Figs. 3, 7, 9 to 11): (*a*) The central sheath, which appears as a moderately dense line, about 45 A thick, encircling the two fibers. (*b*) A dense dot, about 50 A in diameter, that lies just inside the central sheath opposite outer fiber 1 (the numbering of the outer fibers is described below). This structure is probably the same as that described in ctenophore cilia by Afzelius (2), and, following his terminology, it will be referred to here as the mid-fiber; however, it must be pointed out that there is as yet no evidence that it represents a continuous longitudinal structure. (*c*) One or, more often, a pair of moderately dense lines that bridge the gap immediately between the two central fibers.

It has not yet been possible to identify these three structures in longitudinal sections. The only feature seen in this region has been a series of slanting lines, regularly spaced about 135 A apart, that run across from one central fiber to the other (Figs. 4 H and 23). These lines most likely represent the central sheath, which is possibly composed of one or two filaments coiled around the pair of central fibers; however, this interpretation is not yet certain.

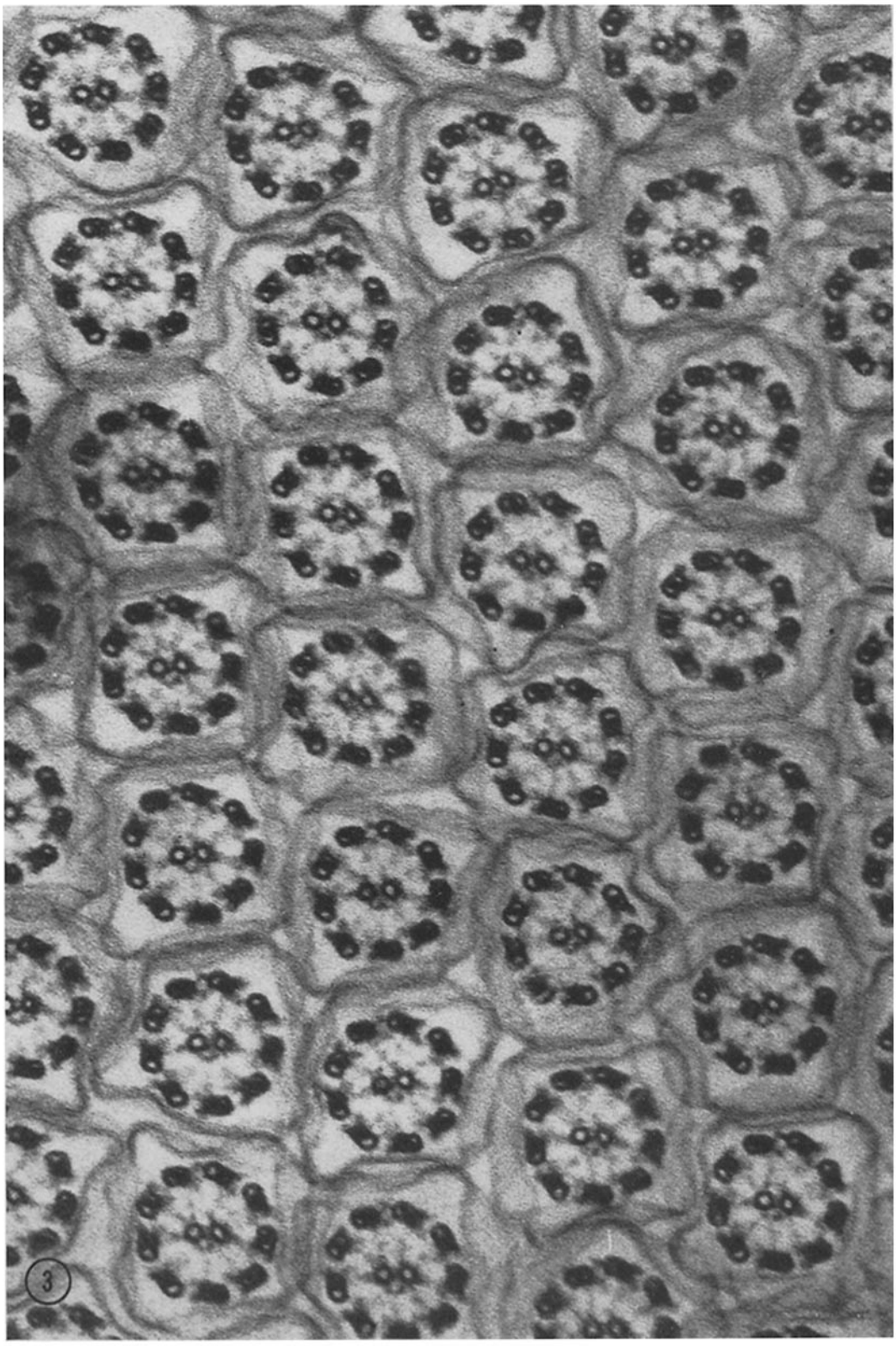
The nine outer fibers each measure about 380 A by 260 A in cross-section (Figs. 8 to 11). They will be considered as doublets composed of two subfibers: subfiber A bears arms and lies closer to the center of the cilium than subfiber B (see below). In cross-section the outer fibers differ somewhat from the typical figure-of-eight form found in many other cilia and flagella. (However, the typical figure-of-eight form does occur near the tip of these mussel cilia, and also in the transition to the basal body.) The subfibers appear to have a dense annular outer region and a less dense core. The central partition between the subfibers is usually thicker than the outer walls and it often appears slanted. Subfiber A frequently appears to have a denser core than subfiber B, and in favorable sections it can be seen that this results from a backward extension of the outermost arm running diametrically across the subfiber (Fig. 11). Occasionally subfiber B also has a dense core.

The longitudinal axes of the two subfibers define the axial plane of the outer fiber. This plane is inclined at about 5° to the tangent to the cilium at the center of the fiber, so that subfiber A lies slightly closer to the center of the cilium than subfiber B.

From subfiber A of each outer fiber there arise short projections, called *arms*. In transverse section two of these are visible on each outer fiber, and their typical disposition is shown in Figs. 3, 4 A, and 9. They are about 50 A thick and 120 A long, and always point in the same direction on all the

FIGURE 3

Transverse sections through lateral cilia. × 110,000.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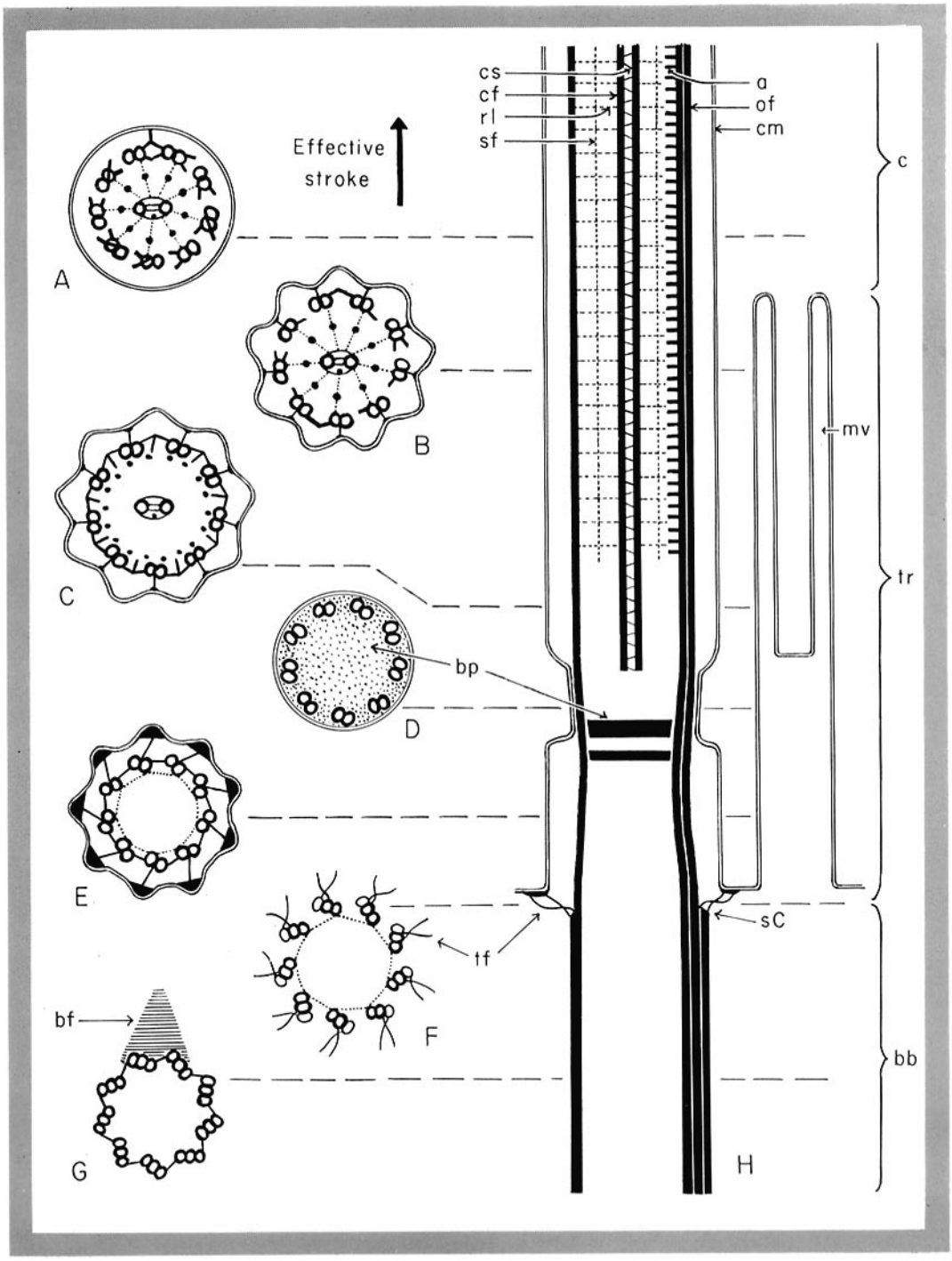


FIGURE 4

Diagrammatic reconstruction of a cilium and basal body. A to G show transverse sections at the levels indicated; they are all oriented with the plane of beat up and down in the figure, with the effective stroke toward the top. H shows a median longitudinal section in the plane of the central fibers; the outer fiber on the right is turned sideways to show it more clearly; the rootlet fibers are omitted; the basal foot does not appear in this plane.

fibers in a given cilium. In longitudinal sections the arms can be identified as approximately rectangular structures spaced about 135 A apart (center to center) (Figs. 4 H and 21).

As has been pointed out previously (12, 14), the arrangement of the arms renders the cilium asymmetrical. The clockwise enantiomorph is defined as that in which, to an observer looking outward along the cilium, the arms point in a clockwise direction. The asymmetry has been examined in gills from four individuals of *Anodonta*, and the cilia have been uniformly of the clockwise form.

One particular pair of outer fibers is characteristically joined together by a *bridge*, which appears to be formed by one B subfiber bearing two arms that meet those on the adjacent A subfiber (Figs. 3, 8 to 11). Sometimes there appears to be a further link extending from the center of the bridge towards the ciliary membrane (Fig. 32); this feature is most clearly seen in laterofrontal cilia, where it perhaps plays a role in holding together the row of cilia. In suitable longitudinal sections the bridge appears as a 225 A periodic variation in density along the outer fiber (Fig. 22); the relationship between this and the 135 A period of the arms is not clear. The bridge always occurs in the same position with respect both to the central fibers and to the direction of effective stroke of the cilium (see below); it therefore forms a suitable fixed point for numbering the outer fibers.

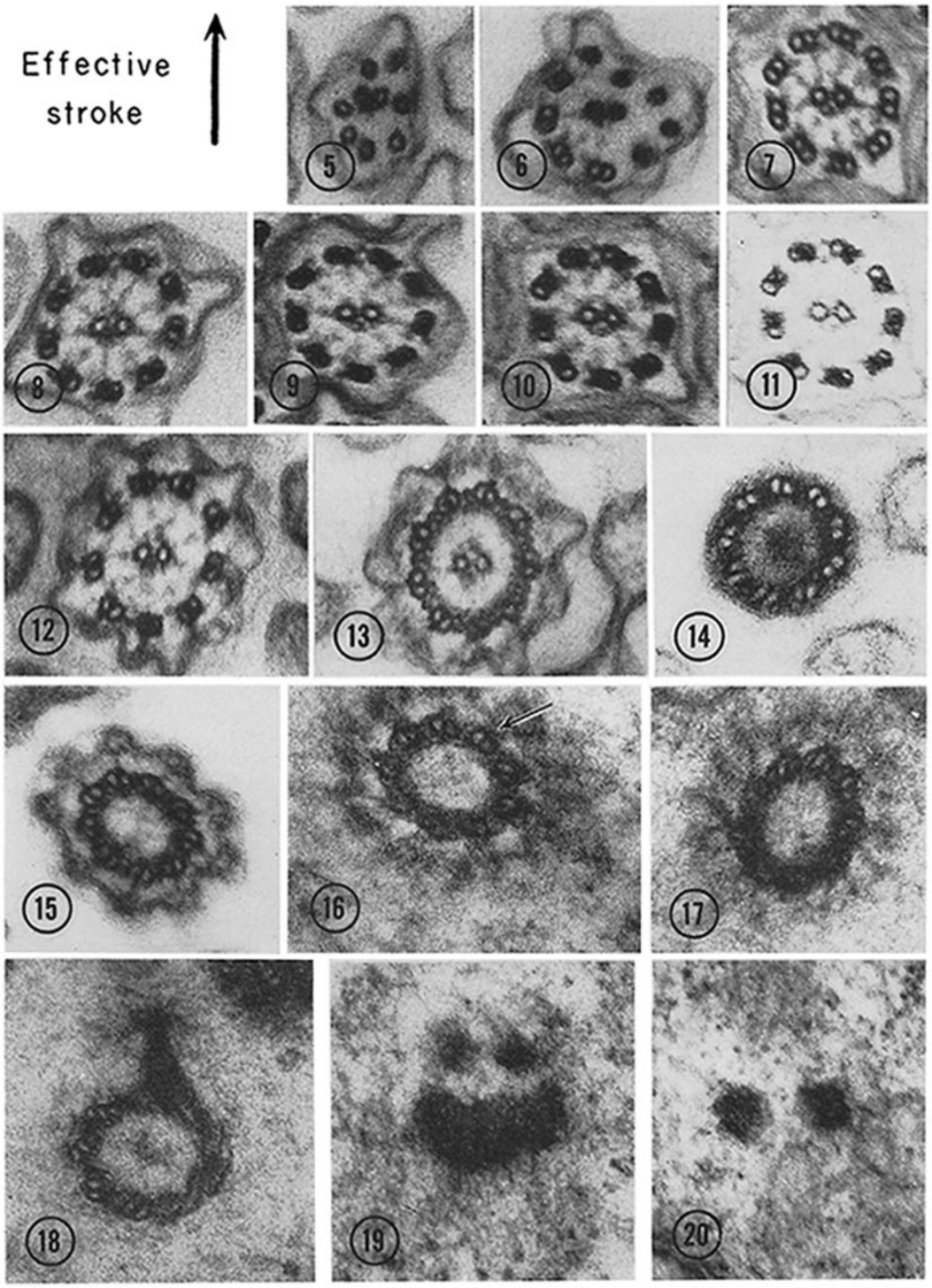
The system used here to number the outer fibers is the same as that proposed by Afzelius (1). Provided that a cilium is not unduly distorted in sectioning, a line drawn through the center of the cilium, perpendicular to the line joining the two central fibers, intersects an outer fiber on one side of the cilium and passes through the bridge between two outer fibers on the other side. Following Afzelius, the intersected outer fiber is called number 1, and the other fibers are numbered in sequence around the cilium in the direction in which the arms point (Fig. 2). In practice, however, this definition is not always easy to apply, because small amounts of distortion often make it difficult to identify fiber 1. A more reliable reference point is provided by the bridge that links fibers 5 and 6.

The region of the cilium between the outer fibers and the central sheath must be considered next. When well preserved this region appears to contain two structures in transverse sections (Figs. 3, 7 to 10). Firstly, there is a set of nine moderately dense dots, one opposite each outer fiber. Secondly, there are lines of lesser density extending radially from each of these dots to the central sheath, and to subfiber A of the nearest outer fiber. The most likely interpretation of these structures is that the dense dots represent cross-sections through a set of longitudinal *secondary fibers*, and that the lines of lesser density represent *radial links* from the secondary fibers to the outer fibers and to the central sheath. In longitudinal sections the radial links appear as a set of evenly spaced lines (about 270 A apart, center to center) extending from the central region to the outer fibers (Fig. 23), and the secondary fibers appear as irregular, nodulous, longitudinal lines of slightly increased density between the central and outer fibers (Figs. 22 and 23). The longitudinal sections are not clear enough to determine whether the secondary fibers are continuous through the whole length of the cilium.

This interpretation is the one that appears most probable on the basis of present evidence, but in certain respects it is, perhaps, still open to question. The secondary fibers do not appear as clearly or as consistently in these cilia as in the protozoan flagella where they were first described (14). In many of the transverse sections some of the dots and lines corresponding to the secondary fibers and the radial links appear to be missing. This apparent variation in structure could be physiological, but it seems more likely that it is an artifact caused by imperfect preservation. (The absence of radial links cannot be ascribed to their being missed by the plane of the section, for the section thickness, 500 to 1000 A, is greater than the spacing between links, 270 A.)

It is clear that all the fibers normally follow a straight course along a cilium. This is implicit in the fact that it is possible to correlate the direction of beat with the pattern of fibers. Furthermore, it is directly apparent in micrographs such as Figs. 3 and 39, where the pattern of fibers is oriented approximately the same way in all cilia, in spite of their being cut at different distances from their bases.

The membrane bounding the cilium is continuous with the general cell membrane. In favorable areas (Fig. 24) it shows the usual triple-layered appearance of a "unit membrane" (26). The membrane shows an irregular and variable profile in transverse sections of cilia; this appearance is presumably an artifact caused by inade-



FIGURES 5 TO 20

Transverse sections at an ordered series of levels through cilia and rootlets. The direction of the effective ciliary stroke is toward the top in all figures. The orientation of the arms is that seen by an observer looking outward along the cilium. Except where otherwise noted, all these figures show frontal cilia. × 110,000.

quate preservation. It is notable, however, that the bundle of fibers (outer, central, and secondary) retains its structure very consistently in spite of these distortions of the membrane.

It is convenient to consider now the structure of the tips of the cilia. Approaching the tip, the first detectable change occurs in the appearance of the outer fibers. These lose their rather irregular profile and take on a much more regular figure-of-eight appearance (Figs. 7 and 39), in which both subfibers have cores of low density, and the central partition between them has the same thickness (45 A) as the outer walls. Somewhat closer to the tip of the cilium, changes occur abruptly. One subfiber of each outer doublet terminates, and so do the arms, the secondary fibers, and the radial links (Fig. 6); these changes all seem to occur at the same level in a given fiber, though at somewhat different levels in adjacent fibers. The outer fibers continue a short distance farther as singlets (Fig. 5), and then end. It is notable that the even spacing and regular arrangement of the outer fibers is maintained only so long as the arms, the secondary fibers, and the radial links are present. This suggests that these components play an important role in maintaining the normal position of the outer fibers.

b) Basal Body: The basal body is a typical, approximately cylindrical structure about 0.35 μ long, formed by nine triplet outer fibers.[1] Its overall diameter is about 0.22 μ at the middle and usually slightly less near each end. The distal end of the basal body is continuous with the cilium, and its proximal end gives rise to the rootlet fibers.

The appearance of a basal body in transverse section is shown in Fig. 18. (Because of the change in diameter along the length of the basal body

[1] Fawcett and Porter (10) used the term *basal body* to include part of what is referred to here as the transitional region of the cilium, but it now seems preferable to restrict use of this term to the region where the outer fibers are triplets.

FIGURE 5

Tip of lateral cilium.

FIGURE 6

Near the tip of lateral cilium.

FIGURE 7

Slightly farther from the tip than Fig. 5.

FIGURE 8

Frontal cilium.

FIGURE 9

Laterofrontal cilium.

FIGURE 10

Lateral cilium.

FIGURE 11

Same as Fig. 10 but printed more lightly in order to show the fine structure of the outer fibers.

FIGURE 12

Beginning of transitional region of cilium.

FIGURE 13

Transitional region just above collar.

FIGURE 14

Transitional region at level of collar. The basal plates appear as moderately dense material within the cylinder of outer fibers.

FIGURE 15

Transitional region below collar.

FIGURE 16

Junction of basal body and transitional region of cilium. In one outer fiber (arrow), it may be seen that subfiber C has lower contrast because it is ending within the plane of the section.

FIGURE 17

Approximately same level as Fig. 16, showing the transitional fibers.

FIGURE 18

Basal body, showing the basal foot. Four of the nine triplet fibers are clearly visible in this particular section (see text).

FIGURE 19

Rootlet material just beneath the proximal end of the basal body.

FIGURE 20

Deeper in cytoplasm than Fig. 19, showing the pair of rootlet fibers that run down into the cell.

the nine triplet fibers are not quite parallel to one another and so it is not possible to see them all clearly in any one micrograph.) Each of the triplet outer fibers measures about 530 A by 260 A over-all. The three subfibers are approximately equal in size, and they all appear to have cores of low density. Both the outer dense wall of the subfibers and the inner partitions between them are about 45 A thick. The axial plane through the centers of the three subfibers is inclined at an angle of about 40° to the tangent through the center of the middle subfiber. In the next section, which describes the transition between basal body and cilium, it will be shown that the innermost subfiber of each triplet in the basal body is continuous with subfiber A of an outer doublet in the cilium, that the middle subfiber is continuous with subfiber B, and that the outermost subfiber (C) terminates at the distal end of the basal body.

Along most of their length, adjacent outer fibers in the basal body are joined together by a series of links between subfibers A and C. In addition to these A-C connections there sometimes appear to be further links that run around the inside of the basal body connecting together the A subfibers of adjacent triplets.

The lumen of the basal body sometimes contains irregularly shaped masses of moderately dense material, but no well defined structures have been observed in this region.

Projecting from one side of the basal body is a dense, approximately conical structure that will be referred to as the *basal foot* (Figs. 18 and 24). It is about 0.1 μ wide at the base and 0.15 μ long, and in favorable sections (Fig. 35) a delicate pattern of cross-striations (over-all periodicity 220 A) may be observed running across it. It gives the basal body a polarity corresponding to that given the cilium by the bridge between outer fibers 5 and 6. As will be shown below, the basal foot always lies in the direction of the effective stroke of the cilium, and is situated on outer fibers 5 and 6.

In the frontal cells one occasionally finds basal bodies lying free in the cytoplasm (up to 1 μ beneath the cell surface), without cilia or rootlet fibers attached (Fig. 29). It is possible that these are newly formed basal bodies which have not yet grown cilia.

c) Transition of Basal Body to Cilium: Before considering the transitional region of the cilium, it is convenient to describe the general surface structure of the gill epithelial cells. Almost the entire free surface of these cells is covered with a well developed brush border about 0.8 μ thick (Figs. 24 and 30). The inner portion of this border consists of a system of narrow anastomosing cytoplasmic ridges (Figs. 33 and 40). The outer portion (about 0.4 μ thick) is formed by numerous finger-like processes, or microvilli, that rise up out of the tops of the ridges. The individual ridges

FIGURE 21

Tangential longitudinal section of a laterofrontal cilium. The arms are visible on the right-hand side of the outer fiber in the center of the figure (region between two arrows). × 110,000.

FIGURE 22

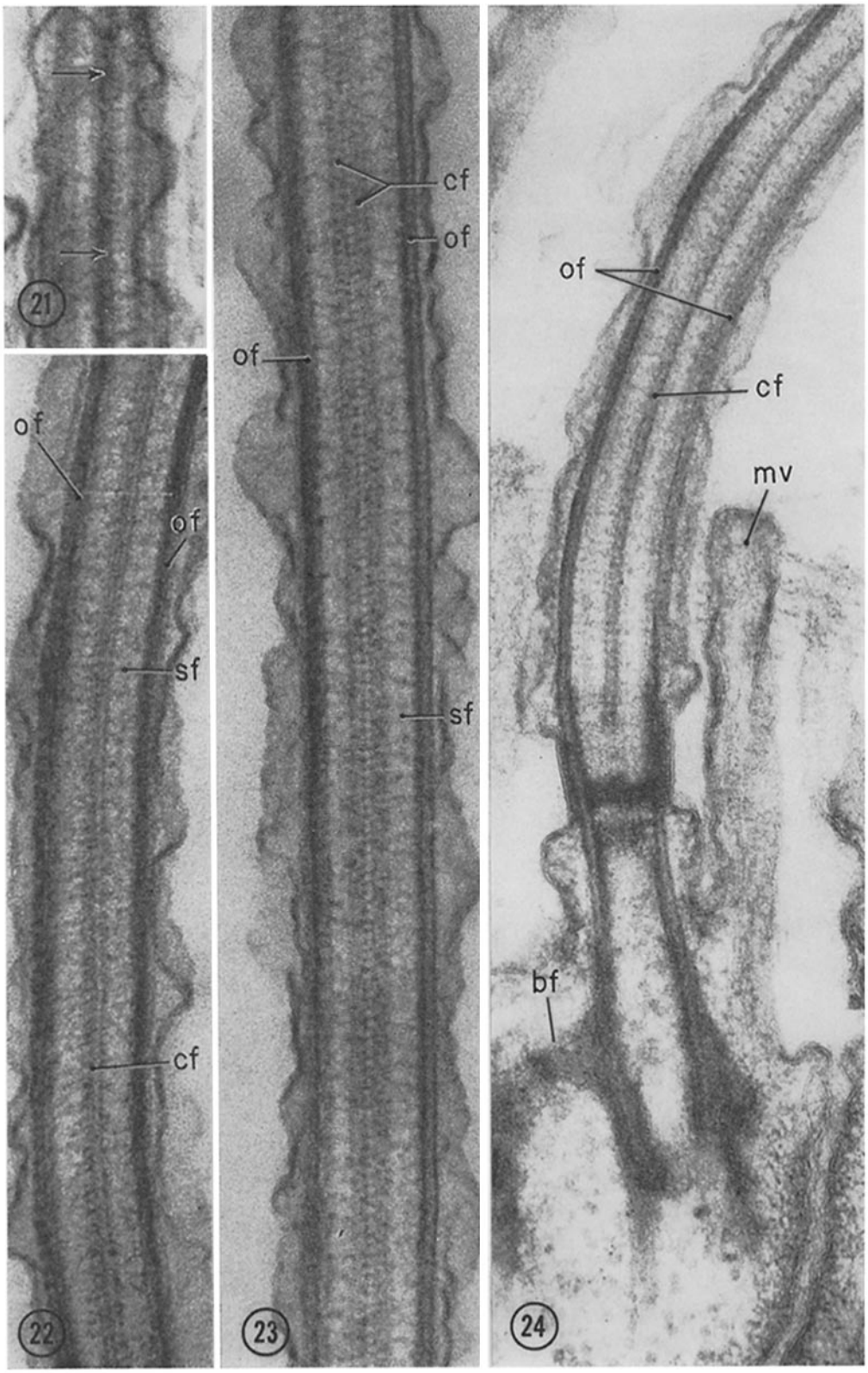
Median longitudinal section of a frontal cilium, perpendicular to the plane of the central fibers. The bridge appears as a periodic variation in density along the outer fiber on left. × 110,000.

FIGURE 23

Median longitudinal section of a frontal cilium, in the plane of the central fibers. × 110,000.

FIGURE 24

Median longitudinal section of lateral cilium and basal body, perpendicular to the plane of the central fibers. The rootlet fibers do not appear in the plane of this section. × 94,000.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and microvilli are enclosed by the cell membrane, and their interior is continuous with the general cytoplasm. Irregular fibrous strands run around and between the microvilli.

The cylinder of nine outer fibers continues out from the distal end of the basal body, through a transitional region in which its structure undergoes several changes, and into the cilium proper (Fig. 24). (The earlier reports of Fawcett and Porter (10) and of Bradfield (7) that the outer fibers of mussel cilia terminate without entering the basal body have not been confirmed.) The transitional region of the cilium is about 0.8 μ long, and corresponds precisely to the region contained within the thickness of the brush border. In describing its structure it is convenient to consider the changes as they occur at successively lower transverse levels passing from the cilium toward the basal body.

The first changes in the ciliary structure occur abruptly at the outer margin of the brush border. At this level (Figs. 4 B and 12), all nine outer doublet fibers become joined by links to the ciliary membrane, which assumes a characteristic nine-pointed profile. The bridge between outer fibers 5 and 6 is replaced by a single link, and a similar link is formed between outer fibers 1 and 2. On the other outer fibers, the arms are still present but the inner arm is now more conspicuous than the outer. The secondary fibers and the central region of the cilium appear substantially unchanged.

Thus modified the cilium continues for about 0.4 μ, and then a further abrupt change occurs (Figs. 4 C and 13). The secondary fibers stop. All nine outer fibers become joined by short links, which sometimes appear T-shaped. A ring of dense dots (probably eighteen in number) appears just inside the circle of outer fibers; it is not yet clear whether this feature is related to the secondary fibers. (In transverse sections which are not sufficiently well oriented, the T-shaped links and the circle of dots appear blurred, so that one receives the impression that the outer fibers are embedded in an amorphous layer of dense material.)

The cilium continues with this structure for about 0.1 μ, and then the membrane constricts to form a tight collar very closely applied to the cylinder of outer fibers (Figs. 4 D, 14, and 24). Near the lower end of this collar, a pair of basal plates run transversely across the lumen of the cilium. The central fibers usually appear to end a short distance above the upper basal plate.

The ciliary membrane re-expands below the collar, and the arrangement of outer doublet fibers is again changed (Figs. 4 E and 15). Their tilt is increased to about 30°. The links between subfibers A and B of adjacent doublets are shorter than they were above the collar, so that the cylinder of outer fibers is smaller in diameter. On the other hand, the links from the outer fibers to the membrane are longer than before, and there is now a large thickening where each link meets the membrane. A further, new set of connections joins together all the A subfibers.

The fibers continue in this way for about 0.2 μ, through to the junction with the basal body, at the end of the transitional region of the cilium.

FIGURE 25

Median longitudinal section of frontal cilium and basal body, perpendicular to the plane of the central fibers. Part of one rootlet fiber is visible. × 62,000.

FIGURE 26

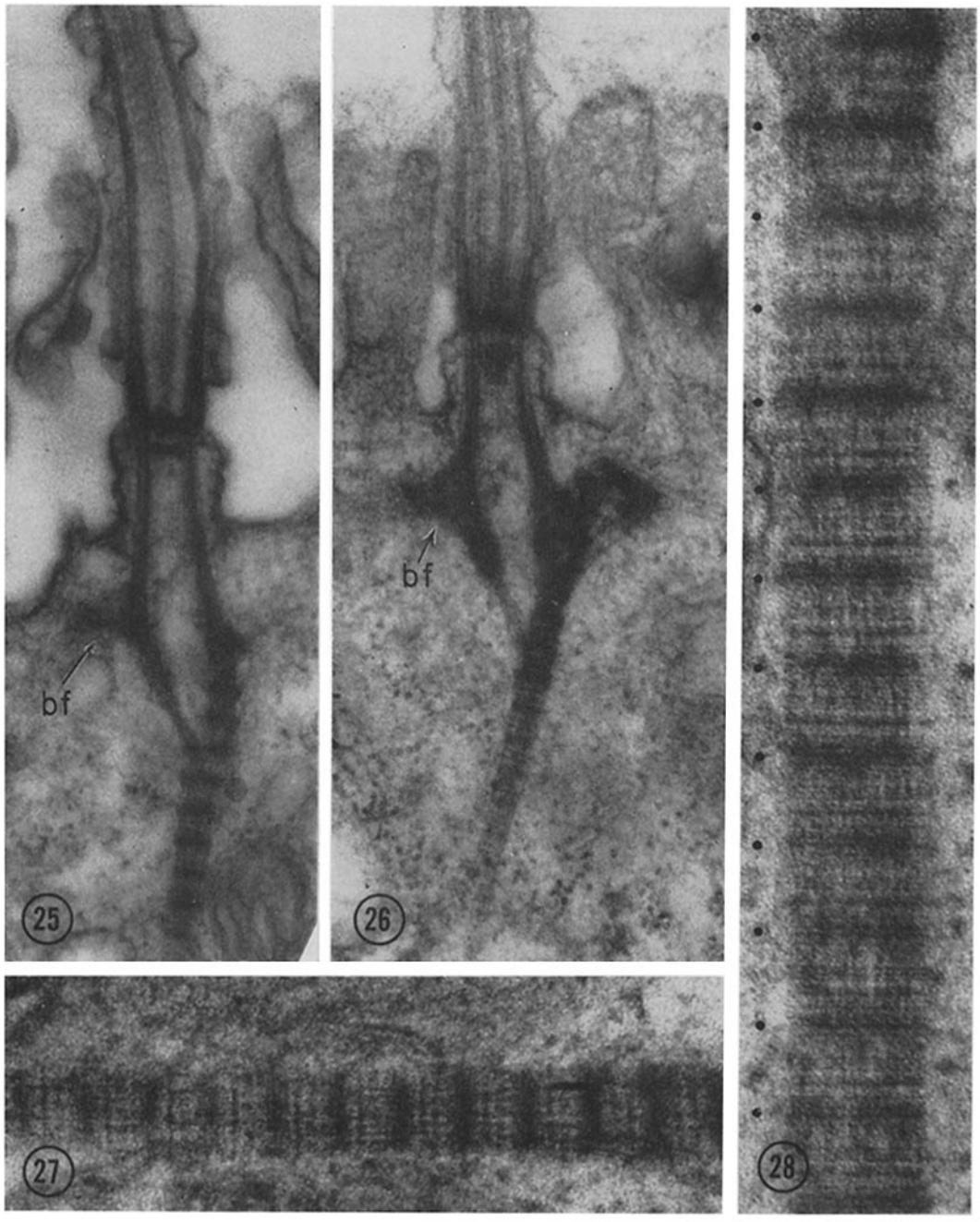
Same as Fig. 25, but this basal body has a large granule on the side opposite the basal foot. × 62,000.

FIGURE 27

Longitudinal section of rootlet fiber showing the fine filaments that compose it. × 125,000.

FIGURE 28

Longitudinal section of rootlet fiber showing the detailed pattern of cross-banding. The dots mark the repeat period. × 175,000.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As already mentioned, the doublet outer fibers become triplets in the basal body. It is the outermost (C) subfiber of each triplet which is acquired on passing from the cilium to the basal body (Figs. 4 F and 16). Subfiber A, which has arms in the cilium, becomes the innermost subfiber in the triplets. From the approximate level at which the C subfibers begin, a set of nine transitional fibers radiates outward toward the cell membrane (Figs. 4 F, 4 H, 16, 17, and 25). In the best micrographs it appears that each transitional fiber is composed of two fine filaments. The ciliary membrane is formed at about the same level as an evagination of the cell membrane.

The complexity of the structural changes that occur in the transitional region is highly remarkable and will be considered further in the Discussion. For the present it suffices to point out that the features occurring at different levels cannot be considered complete artifacts, for they have been observed with great regularity. (See reference 14 for a more detailed discussion of artifacts.)

d) Fibers and Granules Associated with the Balsa Bodies: Two systems of fibers can be identified associated with the basal bodies. The first consists of striated rootlet fibers that extend from the basal bodies down into the cell; the second consists of tubular fibers running approximately parallel to the cell surface.

The rootlet fibers will not be considered here in detail; many features of their fine structure have already been described by Fawcett and Porter (10). In general, a pair of rootlet fibers extends from the proximal end of each basal body downward into the cell and ends freely in the vicinity of the nucleus. The length and exact disposition of the fibers differ in the four types of ciliated cell. When examined in longitudinal sections the fibers show prominent cross-striations of increased density. The pattern of striations is polarized with respect to direction along the fiber, and it repeats about every 750 A (Fig. 28). With the resolution obtained in this study there appear to be twelve striations within each period. In favorable micro-

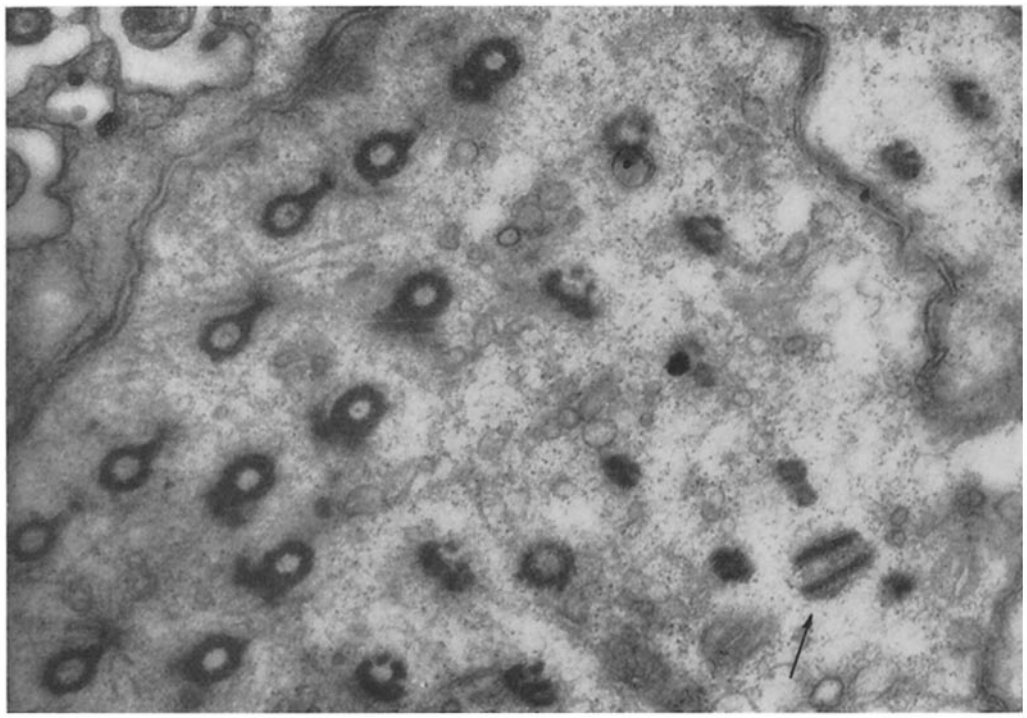


FIGURE 29

Tangential section through surface of a gill filament showing cross-sections of frontal cilia and basal bodies. At arrow is a basal body lying free in the cytoplasm, without cilium or rootlet fiber attached. × 35,000.

graphs (Fig. 27), each individual rootlet fiber appears to be composed of numerous fine filaments, about 45 A thick, all running parallel to the long axis.

The origin of the rootlet fibers from the basal bodies has been studied only in the frontal cells. They overlap somewhat the proximal portion of the basal body (Figs. 25 and 26), and in transverse sections at this level appear as moderately dense material surrounding the triplet outer fibers (Fig. 42). In slightly lower sections, just below the end of the basal body, there are three components (Fig. 19): a large one situated under outer fibers 9 to 2, and two small ones under fibers 5 and 6. The large component bifurcates to form the two rootlet fibers that run down into the cell (Fig. 20). The two small components join the large one at about the same level as the bifurcation (Figs. 25 and 26). (The rootlet fibers diverge in the plane of the central fibers, so that only one of them, at most, is visible in sections with the orientation of Figs. 24 to 26.)

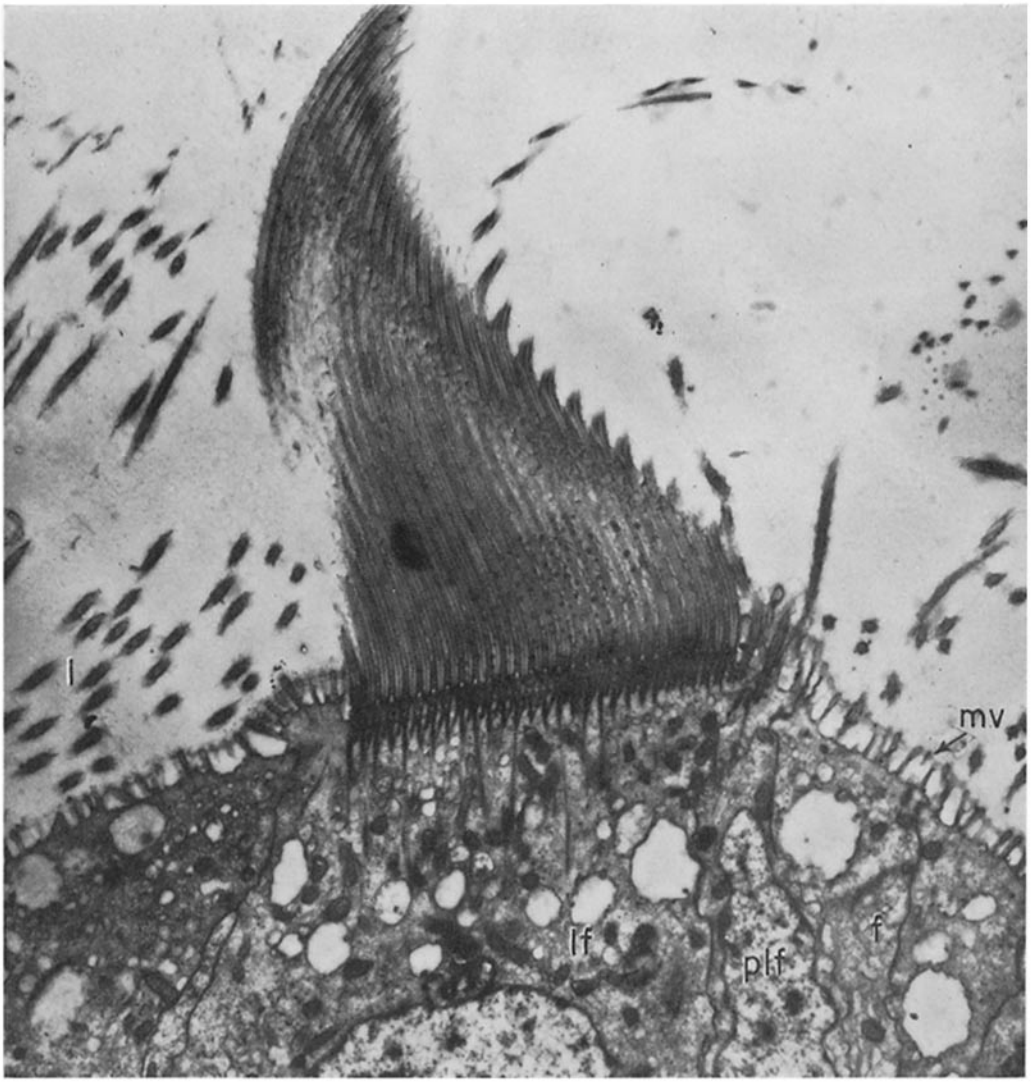


FIGURE 30

Side view of the laterofrontal cilia on a cell. × 8,000.

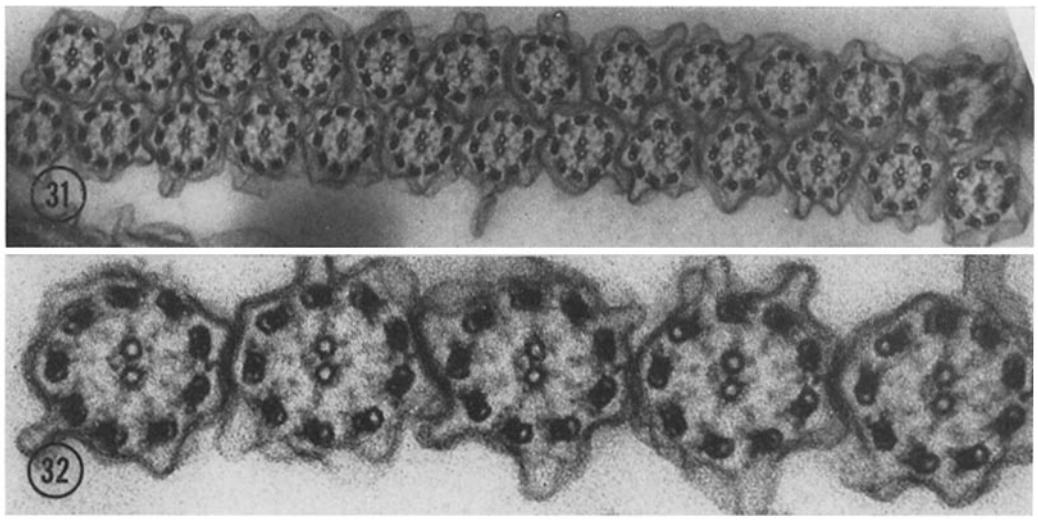


FIGURE 31

Transverse section through the double row of laterofrontal cilia on a cell. × 45,000.

FIGURE 32

Transverse section through part of a row of laterofrontal cilia. × 110,000.

The second system is composed of tubular fibers that run among the basal bodies, approximately parallel to the cell surface (Fig. 35). These fibers appear circular in cross-section (uniformly 230 A in diameter), with a dense annular outer region and a less dense core. Their appearance in longitudinal sections suggests that they consist, at least in part, of a two-strand helix; in this respect they resemble the central fibers of the cilium. Not infrequently they appear to connect with the tip of the basal foot, but no well defined pattern of linkage between adjacent basal bodies has been observed.

Search has been made for tubular fibers in the ectoplasm of the non-ciliated cells between the lateral and laterofrontal cells, but none have been found.

Some of the basal bodies have an irregularly shaped dense granule (Fig. 26) situated near their proximal end, on the side opposite the basal foot. It usually seems connected to the rootlet material. The size of this granule varies in different basal bodies, and in many it is lacking altogether (Fig. 25). In its varied occurrence it is reminiscent of the granules associated with the centriole in mammalian cells (6).

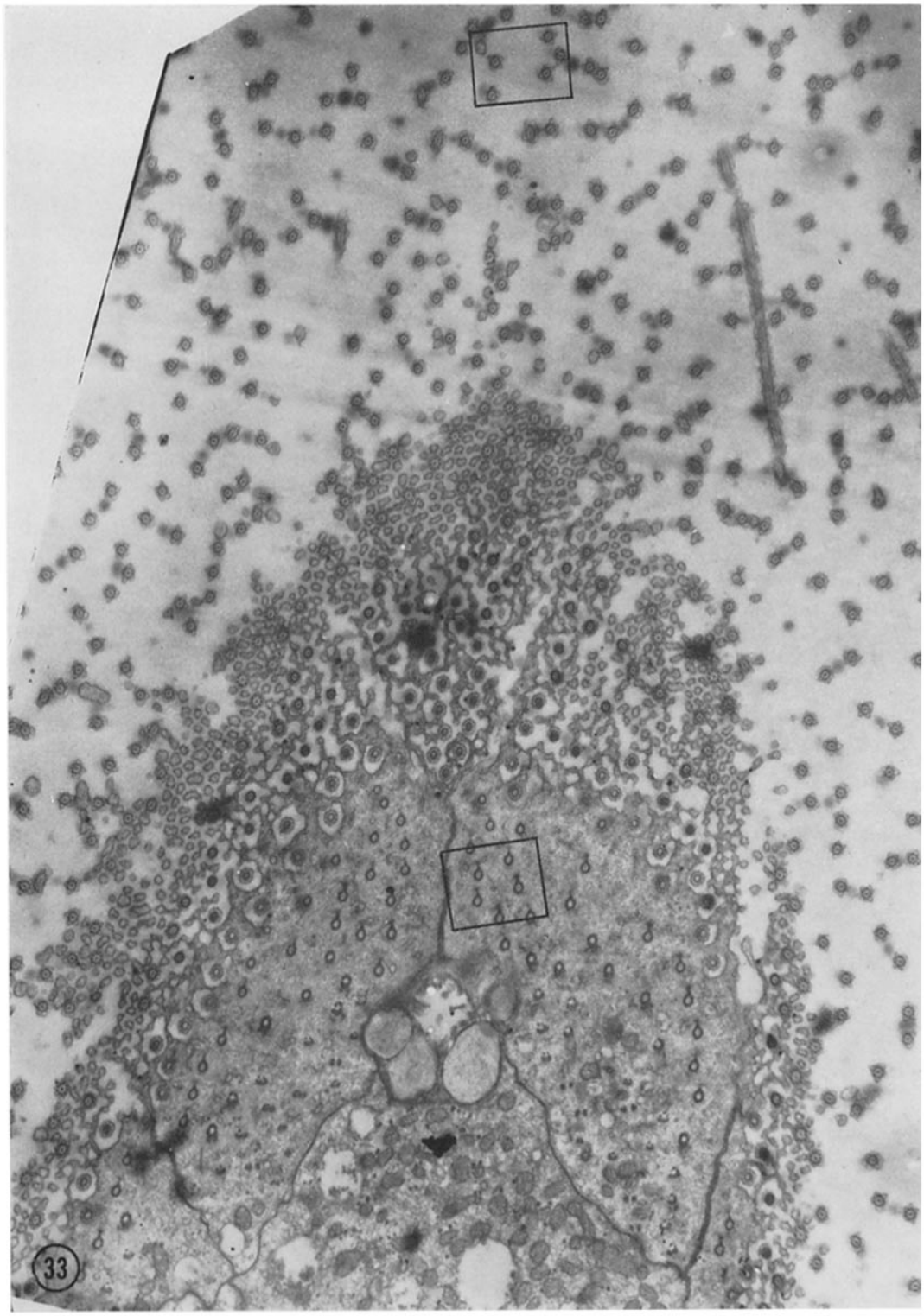
3. *Correlation between Fine Structure and Direction of Beat*

a) Frontal Cilia: The frontal cilia beat along the length of the gill filaments (Fig. 1). On the inner demibranch, the effective stroke of the cilia is toward the free margin of the gill (4).

Fig. 33 shows an almost tangential section through the front surface of a gill filament, with the frontal cilia and basal bodies cut in cross-section. The plane of beat is up and down in the figure, and the effective stroke toward the top. A group of these cilia is shown at higher magnifica-

FIGURE 33

Tangential section through the front surface of a gill filament (plane *L* in Fig. 1), showing cross-sections of the frontal cilia and basal bodies. The plane of beat of these cilia lies up and down in the figure, with the effective stroke toward the top. × 8,700.


33

tion in Fig. 34. It is apparent that the plane of beat is approximately perpendicular to the plane of the central fibers, and that the effective stroke is toward the bridge between outer fibers 5 and 6. Cross-sections of basal bodies are shown in Fig. 35, where it may be seen that the basal foot lies in the direction of the effective stroke.

b) Lateral Cilia: The plane of beat of the lateral cilia is perpendicular to the length of the gill filament (21), with the effective stroke directed away from the laterofrontal cells (Fig. 1). In the fixed preparations used in this study, the lateral cilia are found uniformly at the end of their recovery stroke. This presumably means that the cilia were stationary in this position when fixative was applied, for it has been shown that fixation with osmium tetroxide will preserve metachronal waves in cilia (7, 22).

Transverse sections through a group of basal bodies of lateral cilia are shown in Figs. 36 and 37. The basal foot lies in the direction of the effective stroke.

Figs. 38 and 39 show an area between two adjacent gill filaments in which there appear cross-sections of cilia derived from the lateral cells of each filament. A line of ciliary tips down the center of the figures marks the boundary between cilia from the two sides. In this micrograph the plane of beat is perpendicular to the boundaries of the filaments, and the effective stroke of the cilia on each side is directed toward the center. It is apparent that the plane of beat is approximately[2] perpendicular to the plane of the central fibers, with the effective stroke toward the bridge between outer fibers 5 and 6. This confirms the earlier report of Fawcett and Porter (10) concerning the plane of beat in lateral cilia of *Mya.*

c) Laterofrontal Cilia: Each laterofrontal cell carries two parallel rows of closely packed cilia (Figs. 30, 31, and 40). The length of the cilia decreases uniformly along each row, with the longest cilia nearest the lateral cells. In well fixed specimens the natural cohesion between cilia of a row is preserved so that they form a flat triangular plate. The direction of beat is along the row, and the effective stroke toward the frontal cells (8).

The plane of beat of the cilia is perpendicular to the plane of the central fibers, and the effective stroke is toward the bridge between outer fibers 5 and 6 (Figs. 40 and 41). The foot on the basal body lies in the direction of the effective stroke (Figs. 40 and 42).

These findings confirm the earlier report of Bradfield on the plane of beat of laterofrontal cilia of *Mytilus edulus* (7). However, Bradfield found that the effective stroke lay toward outer fiber 1, which is the opposite of that reported here. This discrepancy is not due to a difference between species, for the preliminary observations made on *Mytilus* during the present study have shown the same arrangement as in *Anodonta.* The most likely explanation appears to be that distortion of the cilia led Bradfield to misidentify outer fiber 1.

d) Pro-laterofrontal Cilia: The pro-laterofrontal cilia were first described by Atkins (5). They consist of a double row of fairly closely packed cilia disposed along each edge of the front surface of the gill filament. Their plane of beat is perpendicular to the length of the filament, with the effective stroke toward the frontal cells.

Transverse sections through the basal bodies of pro-laterofrontal cilia are shown in Figs. 40 and 42. The basal foot lies in the direction of the effective stroke.

e) Summary: a Ciliary Unit: In all four types of ciliated cell included in this study, the cilia have the same fine structure and the same relationship between fine structure and direction of beat. They differ only in length, and in number, disposition, and orientation. It seems legitimate, therefore, to consider all the cilia of the gill as

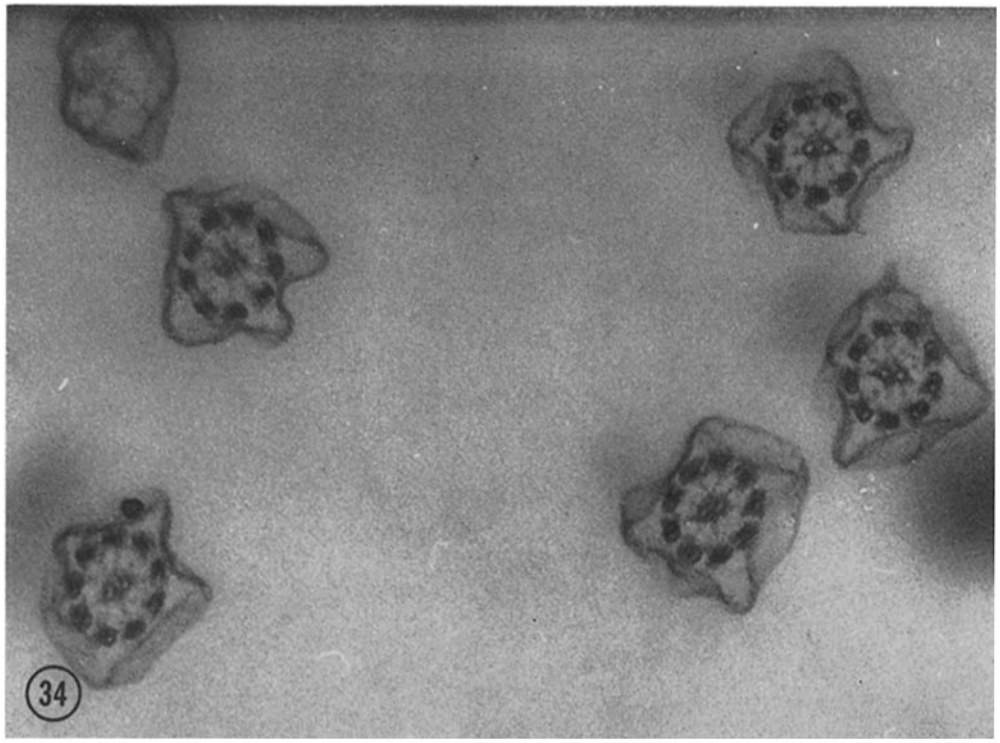
[2] In some instances the plane of beat appears to deviate by up to 15° from the perpendicular to the central fibers. It seems unwise to place emphasis on such small variations until more precise observations on the ciliary beat are available.

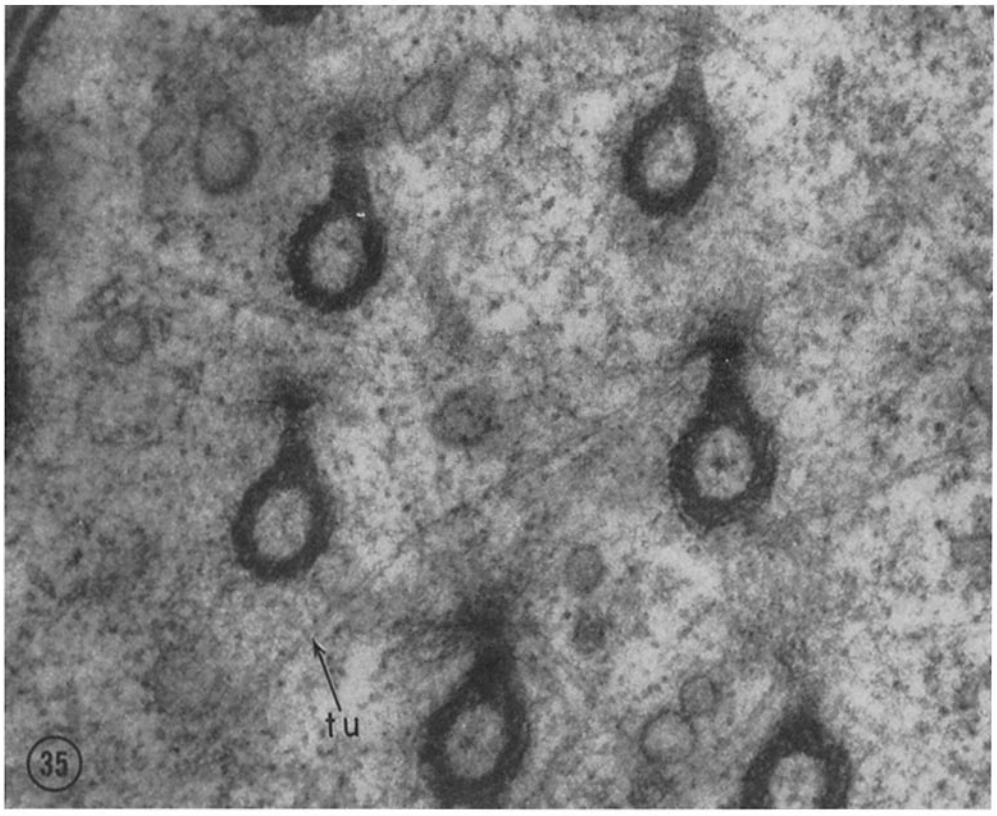
FIGURE 34

Cilia from Fig. 33 (upper squared area) at higher magnification. × 70,000.

FIGURE 35

Basal bodies from Fig. 33 (lower squared area) at higher magnification. × 70,000.


34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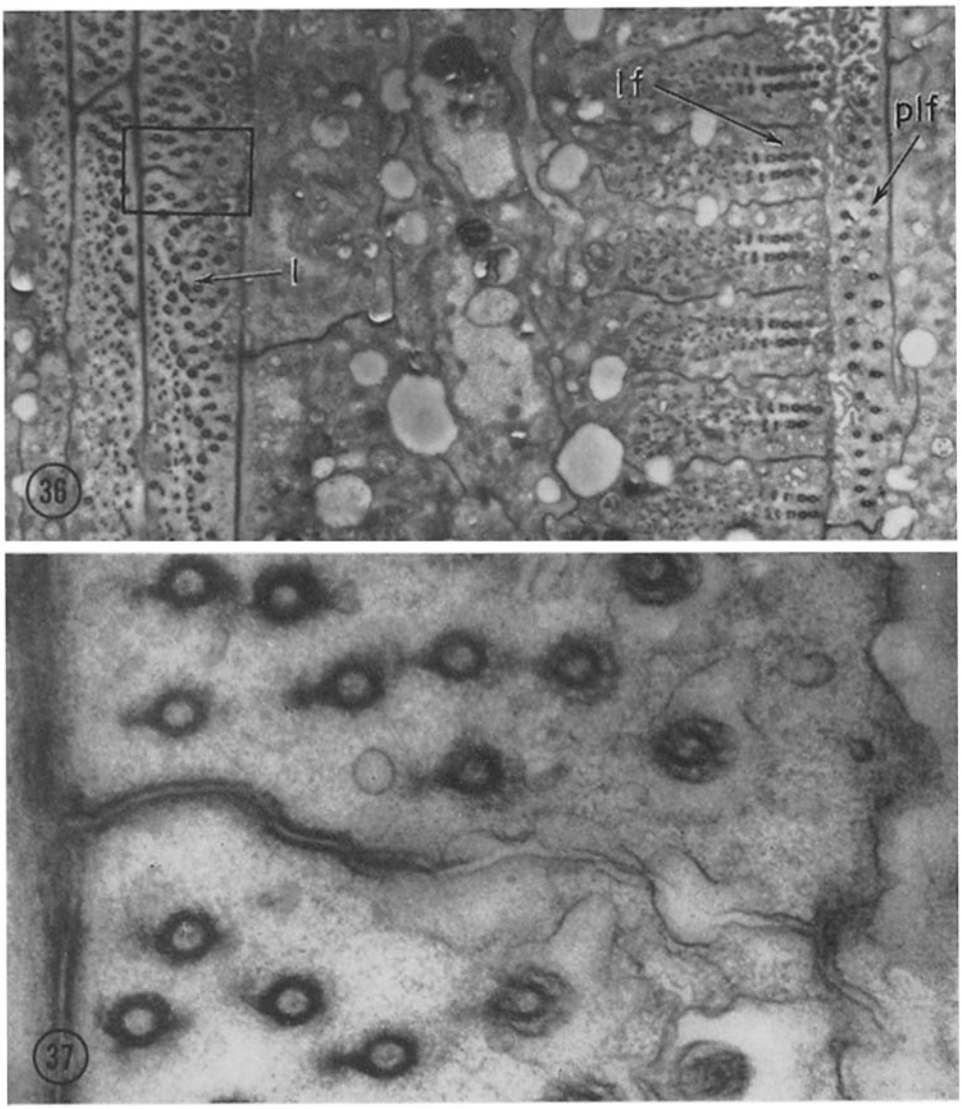


FIGURE 36

Section of gill filament (plane *M* of Fig. 1), showing lateral cilia on left, and laterofrontal and prolaterofrontal cilia on right. × 4,500.

FIGURE 37

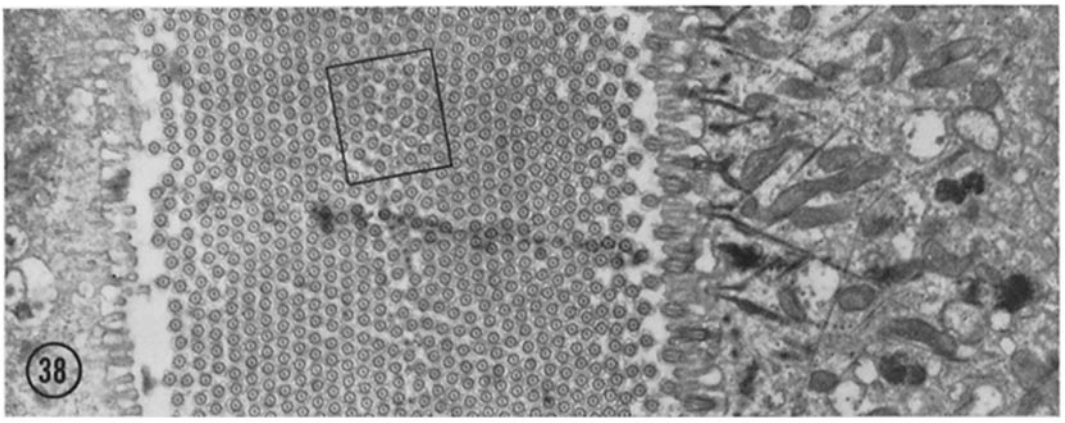
Basal bodies from lateral cells in Fig. 36 (squared area) at higher magnification. × 35,000.

FIGURE 38

Section through the area between two gill filaments (plane *N* of Fig. 1), showing cross-sections of the lateral cilia from both sides. × 8,500.

FIGURE 39

Cilia from Fig. 38 (squared area) at higher magnification. The line of ciliary tips up the center marks the boundary between cilia from the two sides. × 78,000.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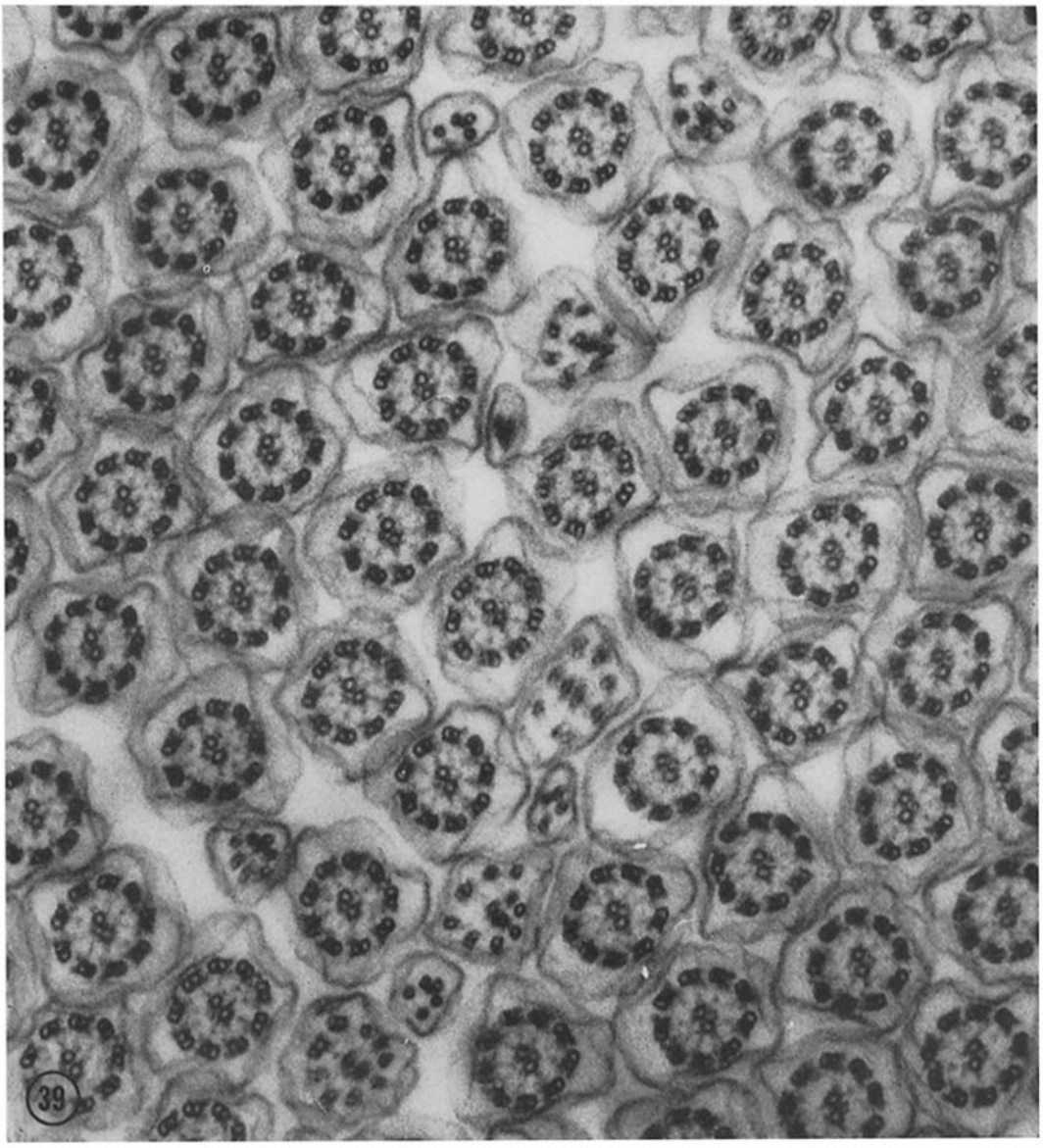
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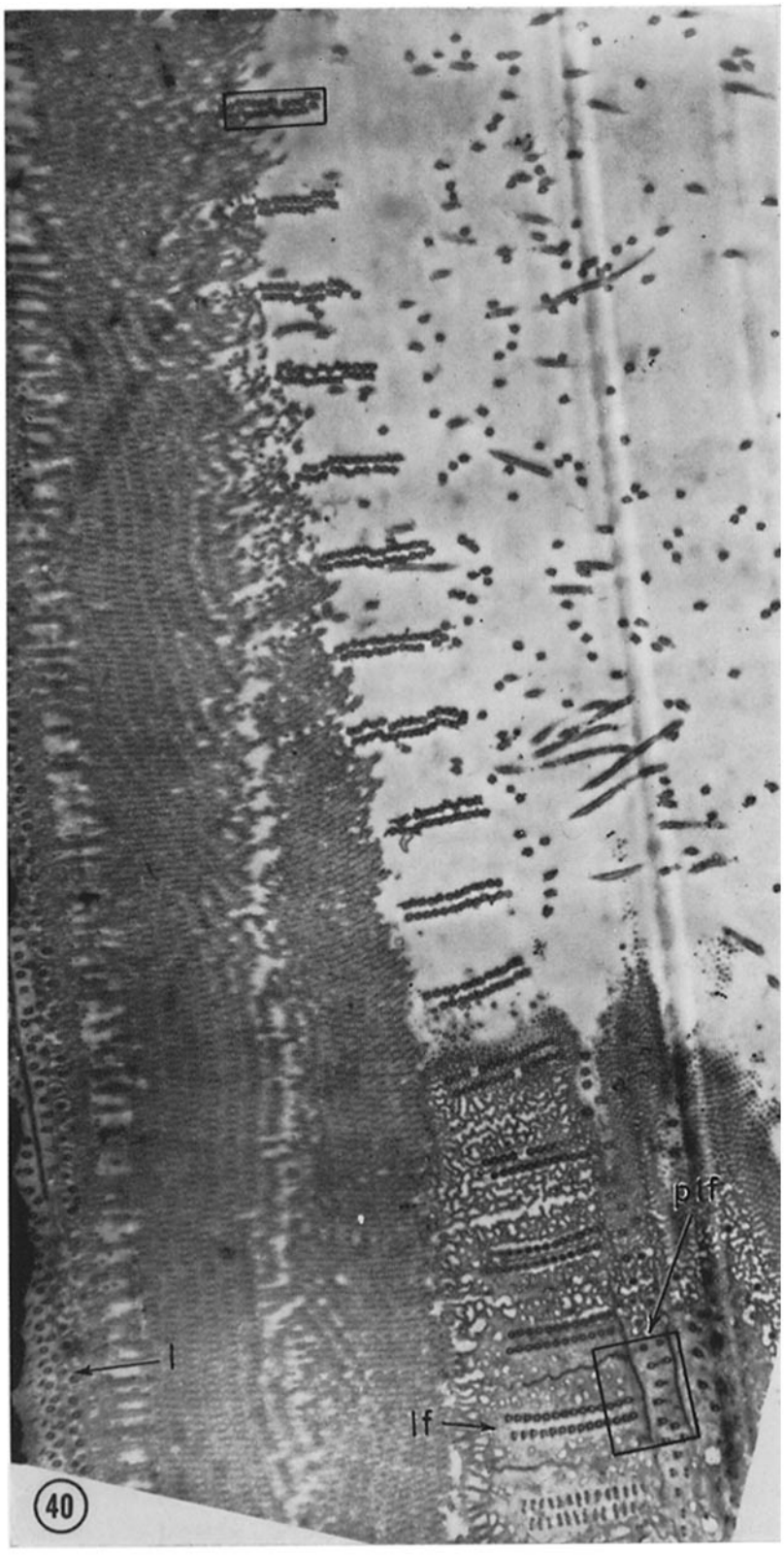


FIGURE 40

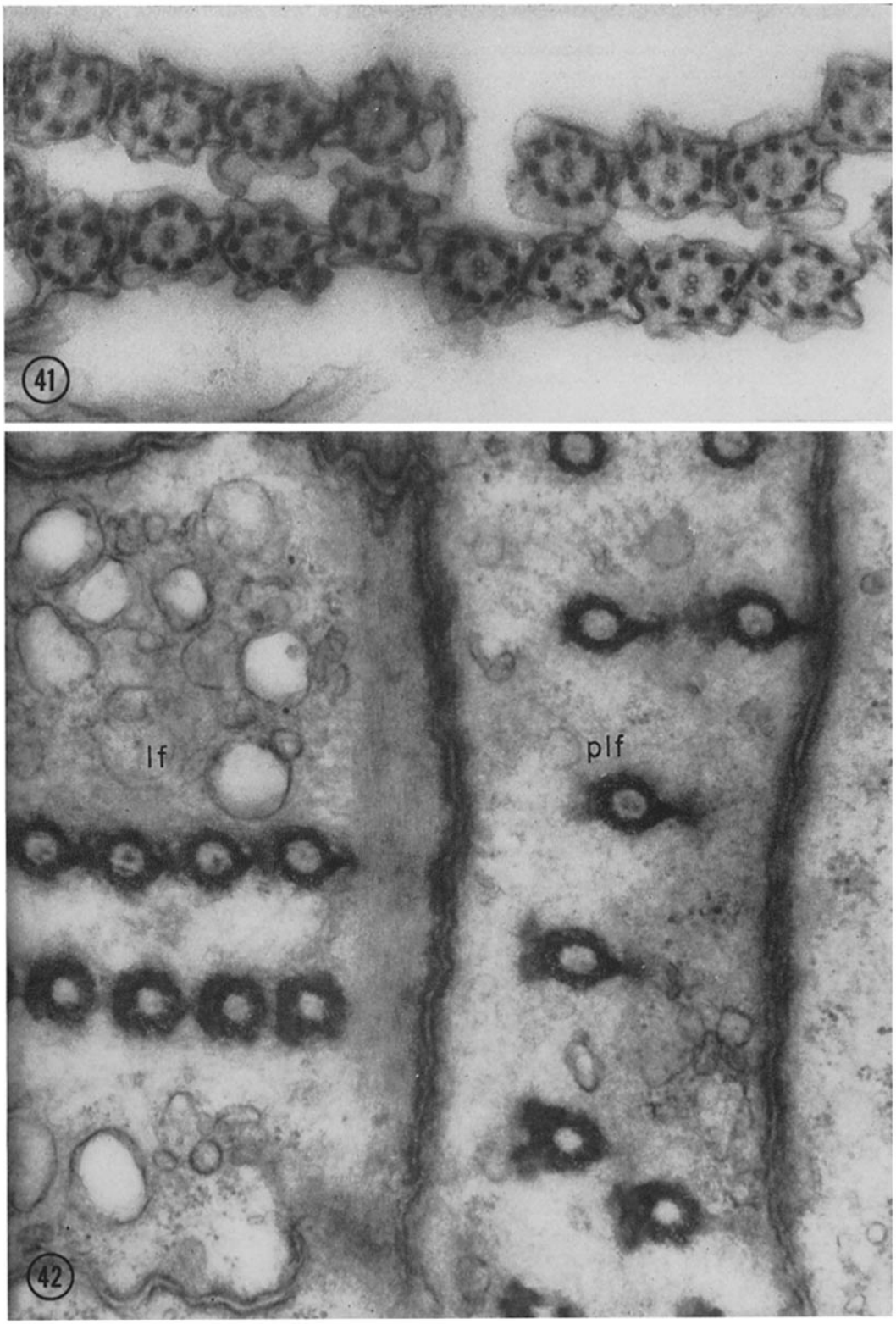
Section of gill filament (plane *M* of Fig. 1) showing lateral, laterofrontal, and prolaterofrontal cilia. × 4,200.

FIGURE 41

Cross-section of laterofrontal cilia from Fig. 40 (upper squared area) at higher magnification. × 62,000.

FIGURE 42

Cross-sections of basal bodies in laterofrontal and pro-laterofrontal cells from Fig. 40 (lower squared area) at higher magnification. × 50,000.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uniform structural units which become disposed according to the type of cell in which they occur.

DISCUSSION

Structure

The more detailed knowledge of the structure of cilia and flagella that is now becoming available gives fresh interest to their comparative study in different organisms and tissues. However, it is necessary to be cautious in interpreting any differences of structure that occur and to allow for the possibility that they may be due to inadequate preservation; it is entirely possible that cilia of some organisms are more difficult to preserve than those of others.

The basic structural uniformity of cilia and flagella is already so well known as to require no detailed comment. All motile cilia and flagella appear to contain nine outer fibers similar to those described here. This uniformity evidently extends also to basal bodies, for the typical arrangement of nine triplet outer fibers has been found in ciliates (11, 20), flagellates (14), mussel, frog, and rat (11). (The same arrangement of triplet fibers occurs also in centrioles (6).) With a uniform arrangement of outer fibers in basal bodies and in cilia, it might be expected that the transition from one structure to the other would show a similar constancy, and this appears to be so. In all cases so far examined (11, 14), subfibers A and B of each triplet continue into the cilium and constitute the outer doublets, while the outermost subfibers (C) terminate, possibly giving rise to the transitional fibers that appear to attach the end of the basal body to the cell surface. Although the fundamental framework of outer fibers appears to be the same in all cilia and flagella, there are many differences of detail.

The *arms* on the outer doublet fibers have been found in many groups of animals (1, 2, 11, 14), and it seems probable that they occur in all inherently motile cilia and flagella. It is interesting to note that arms are present in the sensory cilia of a locust ear (16), where being moved presumably plays a role in function, but that they are absent in the connecting cilia of a rat retina (13, 30), where no movement occurs.

The *bridge* that links outer fibers 5 and 6 in mussel cilia is similar to the one demonstrated in sea urchin sperm tails by Afzelius (1). However, no comparable structure has been reported yet in other cilia and flagella. In the flagella of flagellates, there are only poorly defined connections between adjacent outer fibers, and these occur with equal probability at all positions (14).

In addition to the two central fibers, there are three other components present in the central region of mussel cilia: the central sheath, the mid-fiber, and the dense links directly between the two central fibers. The central sheath occurs widely (11), and is probably always present along with the central fibers. The distribution of the last two components is not yet established. The mid-fiber has been reported in cilia of a ctenophore (2), but seems not to be present in flagella of flagellates (14).

To some extent, the structure of the central region favors the view of Fawcett and Porter (10) that the two central fibers are a modified form of outer doublet fiber. Each central fiber is of approximately the same size as an outer subfiber, the dense links between the central fibers are of about the same length as the arms on the outer fibers, and the mid-fiber might correspond to a modified secondary fiber. Strongly opposed to this view, however, is the fact that the basal body contains triplet fibers corresponding to the outer doublets, but nothing apparently corresponding to the central fibers.

The *secondary fibers* and the *radial links* that join them to the outer fibers and the central sheath were first described in the flagella of flagellates (11, 14). Afzelius described only the radial links ("spokes") in his account of the sea urchin sperm tail (1).

A *basal foot* is present on the side of the basal bodies in ciliated epithelia of frog (10) and rat (25), as well as in mussel. However, there appears to be no comparable structure on the basal bodies of *Paramecium* (13).

The structure of the *transitional region* in mussel cilia, between the basal body and the cilium proper, may be compared with that in flagellate flagella (14). The general features are the same in both cases, but the transition in mussel is more complex than in flagellates. In the former, for example, the transitional region is 0.8 μ long, contains two transverse basal plates, and has five distinct structures at different transverse levels (Figs. 12 to 16), whereas in the latter it is 500 A long, contains one basal plate, and has only two distinct structures at different levels (Figs. 6 and 8 of reference 14). The significance of the additional complexity in mussel is not clear. The

various links and connections associated with the outer fibers at different levels are presumably all related to one another in some way, but the details of this remain wholly obscure.

The general complexity of structure raises obvious problems of morphogenesis. The evidence from light microscopy suggests that cilia are "outgrowths" from previously formed basal bodies. Obviously, such a simple picture fails to account for the variation in structure that occurs at different levels of the transitional region. If a cilium is an "outgrowth" at all, then its structure must be subject to modification by the influence of the cell. In the present case this influence might be mediated by the processes of the brush border.

The similarity between cilia and flagella of different organisms extends also to the dimensions of the component structures. For example, the doublet outer fibers in mussel cilia measure 260 A by 380 A, while in flagellate flagella they measure 250 A by 370 A. The dimensions of the other components are the same to within about 5 or 10 per cent. The longitudinal periodicity associated with the central fibers and with the arms on the outer doublets is 135 A in mussel cilia, and 130 A in flagellate flagella. These minor differences could well be due to experimental error, and no importance should be given them without further study. There is, however, one significant difference concerning the radial links that join the secondary fibers to the outer fibers and the central sheath. In mussel these are spaced 270 A apart, which is twice the separation between adjacent arms on the outer doublets (135 A), whereas in flagellates they are spaced 400 A apart or about three times the separation between arms (130 A) (11). It is probably for this reason that the radial links are less conspicuous in transverse sections of flagellate flagella than in those of mussel cilia.

In striking contrast to the uniformity of cilia is the wide diversity of their rootlets. For example, mussel cilia have two long, striated rootlet fibers running down into the cytoplasm; pharyngeal cilia of frog have a single striated rootlet fiber; in tracheal cilia of rat the "rootlet" consists merely of a short prolongation of the basal body (10). This variation suggests that rootlets are not essential to ciliary function, but play some secondary role, such as providing mechanical support for the cilia. Fawcett (9) has described further examples of rootlet fibers and has discussed their relationship to collagen.

Fine tubular fibers, similar to those described here, occur in association with the basal bodies of many Protozoa, and their distribution has been reviewed by Roth (27).

When the preparation of this manuscript was nearly complete, a paper by Afzelius (2) appeared describing the relationship between structure and direction of beat in cilia of the ctenophore *Mnemiopsis leidyi*. In these cilia the effective stroke lies toward outer fiber 1, which is the opposite of that found here in mussel cilia. This difference is consistent with the hypothesis, presented below, that the direction of the effective stroke of mussel cilia is determined by the presence of an accessory structure, the basal foot, rather than by the special properties of particular outer fibers. The basal body of ctenophore cilia has not yet been described.

Mechanism of Ciliary Movement

As pointed out previously by Bradfield (7) and by Sleigh (29), the bending movements of cilia can be explained as the result of the localized shortening of longitudinal contractile elements. On this hypothesis, the effective stroke is produced by the initial contraction of the proximal region of elements on this side of the cilium; the slower recovery stroke follows as the contractions spread to elements on the other side, and become propagated toward the tip. The hypothesis is not yet fully established, but it is the one that appears most likely at the present time and will be adopted here as a basis for discussion. It defines three problems for consideration: the nature of the contractions, the coordination of contractions to yield a bending movement, and the coordination of cilia to give a metachronal wave.

The nature of the contractile process has been discussed at some length in an earlier paper (14). The evidence available at present favors the view that contraction occurs by actual shortening of limited regions of individual fibers, through some form of molecular rearrangement.

In gill epithelium of mussel, the cilia on any particular cell always beat in the same direction. It has been shown in this paper that there is a uniform relationship between the fine structure and the direction of beat: the plane of beat is perpendicular to the plane of the central fibers, with the effective stroke toward the basal foot and outer fibers 5 and 6. The regular occurrence of the basal foot on the fibers that contract first

suggests that it may be the site of the impulse that initiates a bending cycle. The contractile impulse might then spread to the other fibers, either in one direction around the cilium, or in both. Either possibility could account for the observed bending, but the former seems more probable in view of the asymmetrical fine structure. In this case the plane of beat could be determined by the pair of central fibers, for if they are cross-linked together at frequent intervals and have to bend as a single unit, then the cilium will be resistant to bending in their plane but relatively free to bend in the plane perpendicular to it; a similar suggestion has been made by Fawcett and Porter (10). Such considerations are obviously speculative, but they do receive some further support from preliminary studies showing the same relationship between the basal foot and the direction of beat in cilia on the tentacles of a gastropod mollusc (19) and in tracheal epithelium of rat (13).

In ciliated epithelia the movements of the individual cilia are coordinated into metachronal waves. The direction in which these waves move is usually constant in any particular tissue, but its relationship to the direction of ciliary beat is different in different tissues (18). This indicates that coordination has some fixed structural basis in the cells, presumably lying either in the cell membrane or in some system of ectoplasmic fibers. Coordination by the cell membrane appears unlikely to account for the constant direction of a metachronal wave; moreover, the speed of a metachronal wave (about 0.3 mm/sec (3)) is much less than the speed of an action potential, for example, in a nerve axon (2 to 20 m/sec (24)). It seems more likely that the coordination of mussel cilia is effected by the system of fine tubular fibers associated with the basal bodies, and that passage of the coordinating impulse to a cilium occurs through the connection of these fibers with the basal foot. However, it must be noted that no well defined pattern of connection between basal bodies, corresponding to the direction of the metachronal wave, has yet been observed.

The beating of cilia in ciliates appears to differ in a number of respects from that in ciliated epithelia of higher animals. In *Paramecium*, for example, both the direction of the effective stroke and the direction of the metachronal waves vary according to the direction in which the organism is swimming (23). The same is true in *Opalina* (29). When the fine structure of these cilia is examined it is found that the orientation of the pair of central fibers is not uniform, but varies in an apparently random way from one cilium to the next (13; Plate 3, Fig. 1 of reference 20). It is not at present possible to explain the differences between ciliates and metazoans, but there is no reason to suppose they imply a very substantial difference in the underlying mechanism of ciliary action.

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