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Electron Microscope Studies on the Ciliary Apparatus of the Gill Cells of *Mya arenaria.** (19359)

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The nature of the ciliary apparatus has long been a subject of discussion. For reviews and literature pertaining to this subject consult Engelmann(1), Saguchi(2), Grave and Schmitt(3) and Worley(4). Morphology of the ciliary apparatus becomes especially significant in the formulation of theories concerning the physiology of ciliary movement (5). Clear pictures have been drawn of certain fixed ciliated cells showing the cilia, basal bodies and intracellular fibrils which often converge, forming a cone-like structure with its apex at one side of the nucleus(1). Because of the fact that the intracellular fibrils cannot be easily seen in the living cell with the light microscope, they have been thought of as artifacts by some (see discussion in 3).

The purpose of this report is to present electron micrographs of cells from the laterofrontal and frontal regions of the gill filaments of *Mya arenaria,* showing cilia, membranes, basal bodies, and intracellular fibrils.

Methods. Material used in this study was fixed in Regaud's solution and sectioned at 0.2 μ according to the method of Beams, *et al.* (6). The electron microscope used was an R. C. A. model EMU-2B, equipped with an unbiased gun. Magnification of the electron micrographs is indicated by the 1 μ scale drawn on Fig. 1.

Results. The cilia of laterofrontal cells (a, Fig. 1 and 3) appear to be compound *i.e.*, consisting of 3 to 4 filaments of approximately 250 Å in diameter. However, extensive fraying of them into the individual fibrils was not observed as has been demonstrated for the cilia of *Paramecium* and filaments of sperm tails (unpublished). At their bases the cilia are spaced only about 500 Å apart. This is close as compared to the distance between the cilia of the frontal cells (Fig. 2). The bodies of the cilia seem to be in many instances in contact with each other. However, no mucous-like substance which might function to hold them together as a unit was observed. This is mentioned in view of the findings of Carter (7) who observed that in the laterofrontal cells of *Mytilus* all the elements of the vibratory apparatus of a cell beat as a unit or "cilium", comparable to the cirri of certain protozoa(8).

Short extracellular filaments (Fig. 3) are thought to be broken cilia. Some evidence for a cuticular border consisting of short filamentous processes is shown at upper left in Fig. 1. However, it cannot be determined for certain whether or not these filaments are continuous all the way across the border. In the frontal cells, definite cuticular processes of about 400 Å in diameter and 8300 Å in length are sharply defined (g, Fig. 2). These are in many ways comparable to those seen in the kidney tubules and certain intestinal cells (9,10). Only the proximal portions of the cilia are shown in Fig. 2; they are more widely spaced than in the laterofrontal cells

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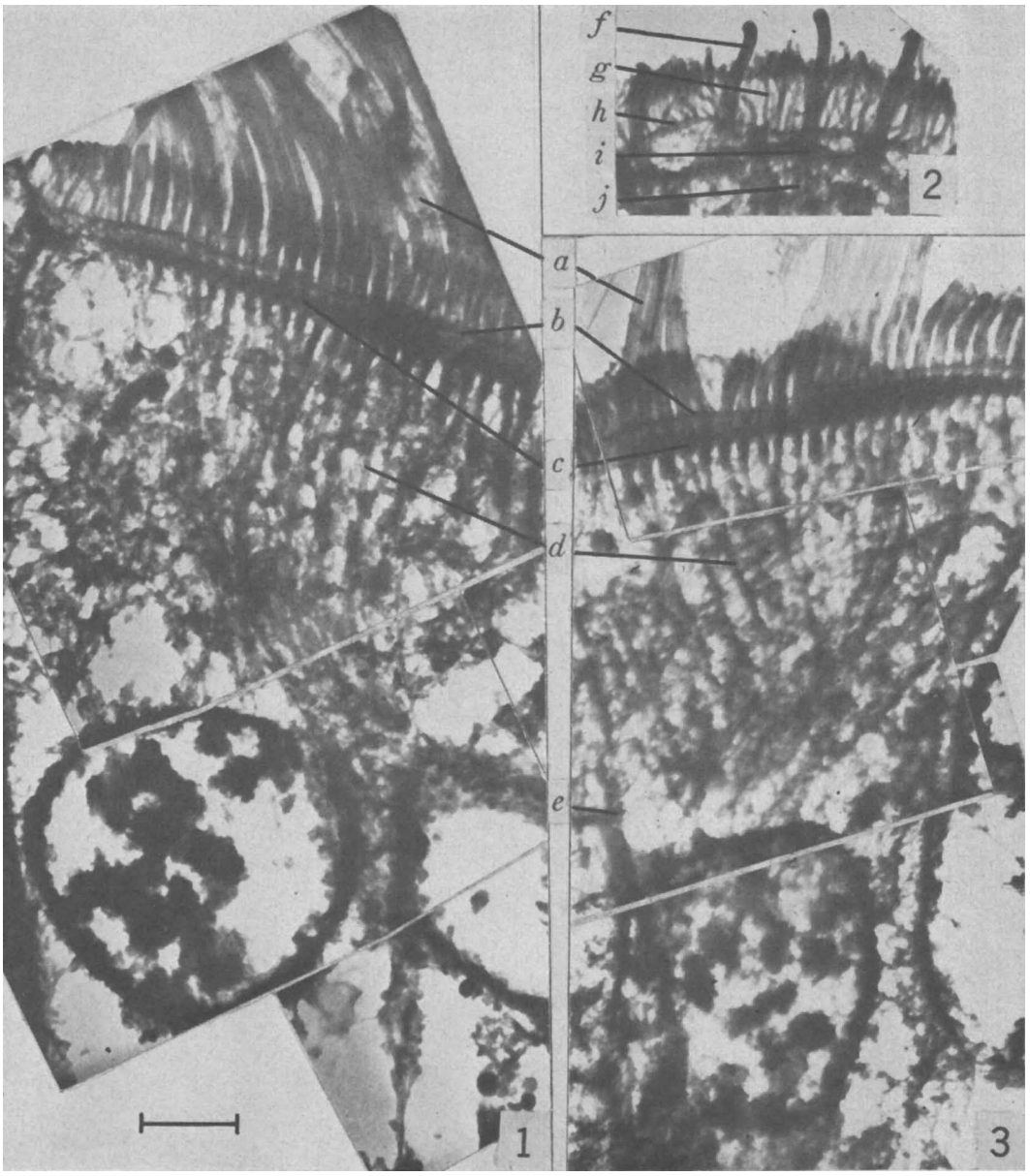

FIG. 1. Montage of electron micrographs of laterofrontal gill cell of *Mya*. Cilia, a; surface cell membrane, b; basal bodies, c; intracellular fibrils, d; and lateral cell membrane, e; are demonstrated.

FIG. 2. Electron micrograph of the distal portion of a frontal gill cell of *Mya*, showing proximal portions of cilia, f; cuticular border with filamentous processes, g; surface membrane, h; basal bodies, i; and intracellular fibrils j.

FIG. 3. Same as in Fig. 1 except a more oblique section of the cell.

(Fig. 1 and 3), and are too opaque to determine whether or not they are made up of smaller fibrils. At b, (Fig. 1 and 3) and h, (Fig. 2) appears a continuous electron opaque line which probably is the cell membrane. The only other possible interpretation is that it represents a kind of neuroneme similar to that described for *Paramecium* (11). In some areas of the cell this structure appears double (center part of Fig. 1).

The basal bodies are shown at c, (Fig. 1 and 3) and at i, (Fig. 2). They are fused with, and are slightly larger than, the bases of the cilia. In addition, they show a greater electron density than do the cilia. Fused also with the basal bodies and extending basically in the cytoplasm are the intracellular fibrils (d, Fig. 1 and 3; j, Fig. 2). There is a single intracellular fibril attached to each basal body and associated cilium. The intracellular fibrils converge, forming a conelike structure with its apex at one side of the nucleus (Fig. 1). We were not able to determine whether or not the apex of the cone is embedded in any specialized body of the cytoplasm. In certain preparations the side of the cone seems to be in contact with the nuclear membrane (Fig. 1). In Fig. 2 the section is at such an angle that the outline of the cone is not clear. The intracellular fibrils are approximately 1000 Å in diameter. Details of their internal structure could not be determined. Furthermore, we were not able to determine whether or not lateral connections extend between them as reported by Worley(12). In places strands appear to connect the individual fibrils, but this may only represent coagulated protoplasm. At e (Fig. 3) is seen the membrane of the cell.

Discussion. Evidence here presented substantiates the view that a single cilium, basal body and intracellular fibril are morphologically united and conceivably constitute the functional unit of the ciliary apparatus in these cells. The reality of the intracellular fibrils is not questioned. Their sharp definition and consistency in number and appearance argue for their presence in the living cell. Other evidence to support this view is that they are birefringent(13) and have recently been seen in the living cell by use of the phase-contrast microscope(12). A review of the literature on ciliated cells indicates that the basal bodies are probably always present. However, the intracellular fibrils have only been demonstrated in certain of the large ciliated cells of invertebrates. Whether or not they function in the mechanism of regulating cilia beat or are only supporting structures in these cells is unknown.

Summary. The electron microscope demonstrates the morphology of the ciliary apparatus of the laterofrontal and frontal gill filament cells of *Mya* to be composed of cilia which appear to be made up of smaller fibrils, basal bodies which are fused with the basal ends of the cilia and intracellular fibrils which are also fused with the basal bodies at their distal ends. These intracellular fibrils extend basally and converge forming a cone-like structure with its apex ending in a region near the side of the nucleus. Frontal cells of the gills show in addition to the above components, a striated cuticular border composed of short filaments approximately 8300 Å in length and 400 Å in width. The cilia of the laterofrontal gill cells are more closely spaced than are those of the frontal gill cells.

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