LOCALIZATION OF ATPase IN ROTIFER CILIA

ALBERT I. LANSING and FRANÇOIS LAMY. From the Department of Anatomy, University of Pittsburgh School of Medicine

Considerable data have been accumulated on the fine structure of cilia, sperm tails, and flagella (4, 5) but the contractile mechanism in these structures has yet to be elucidated. The splitting of phosphate from ATP (8) and the recognition of ATPase activity in myosin (2) have been associated with muscular contractility. A natural sequel to these observations was an attempt to establish a comparable association in vibratile systems (7, 1, 2).

We have found recently (6) that at least in the rotifer there is a peculiar orientation of the peripheral filaments when viewed in true cross-section. When peripheral filaments 9 to 4 are sharply outlined in normal sections, filaments 5 to 8 are disoriented, or the converse may occur. This suggests the possibility that while half of the peripheral filaments is in extension the other half is in contraction. In this study we have attempted to determine whether or not ATPase shows a distribution consistent with the structural bilateral asymmetry of rotifer cilia.

MATERIALS AND METHODS

The rotifer, *Philodina citrina*, was cultured by the method previously described (6). For demonstration of ATPase localization the procedure outlined by Essner, Novikoff, and Massek (3) was employed. Our best results were obtained by fixation of the rotifers in cold 1 per cent OsO_4 for 6 minutes. Fixation for 4 minutes was inadequate for preservation of structural detail, and 10 minutes completely inhibited any ATPase activity. Control animals were incubated with adenylic acid rather than ATP, and in addition rotifers were prepared for electron microscopy after 1 per cent cold OsO_4 fixation without subsequent incubation.

All of the material was embedded in Vestopal and sectioned with the aid of a diamond knife in a Porter-Blum microtome.

RESULTS

Electron micrographs of adenylic acid control specimens were indistinguishable from those fixed in 1 per cent cold OsO_4. There were no apparent regions of elevated electron density in the cilia adjacent structures. The central and peripheral filaments conformed to the established pattern as did the remainder of the cilium in either longitudinal or cross-section.

As illustrated in Fig. 2, cross-sections of experimental material incubated with ATP showed an intense electron density in two highly localized regions: one body is immediately peripheral to filament number 1 and the other is similarly located next to filament number 5. Each of these bodies, roughly elliptical, is approximately one and one half times as long as a pair of filaments and is one half as wide as the diameter of a peripheral filament when these are viewed in cross-section.

The lead deposit which is responsible for the electron density in the ATPase-containing structure is quite granular and hence fine structure is difficult to resolve. It does appear that the ATPase body has an outer rim or membrane with an ill defined pattern of internal vesicles. Electron-opaque strands of material extend from each of the bodies to come into contact with either peripheral filament number 1 or 5. Fig. 1 illustrates the typical fine structure of control osmium-fixed cilia which exhibit no structure that may be associated with the ATPase body.

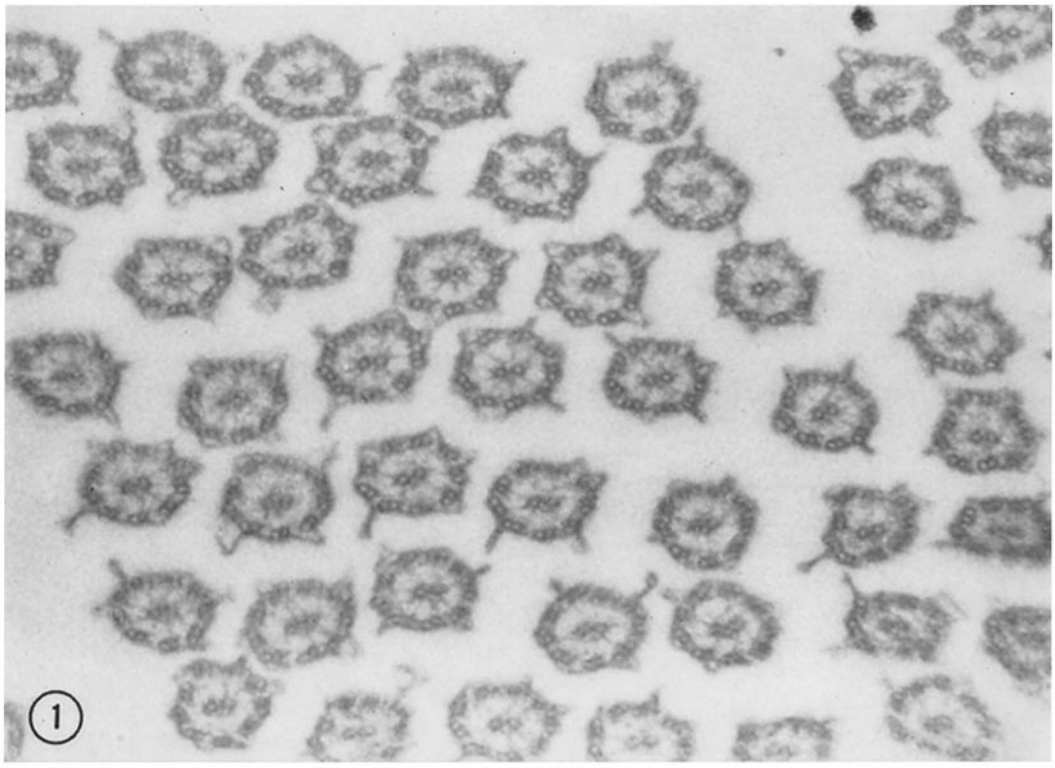
It should be noted that in some sections we have

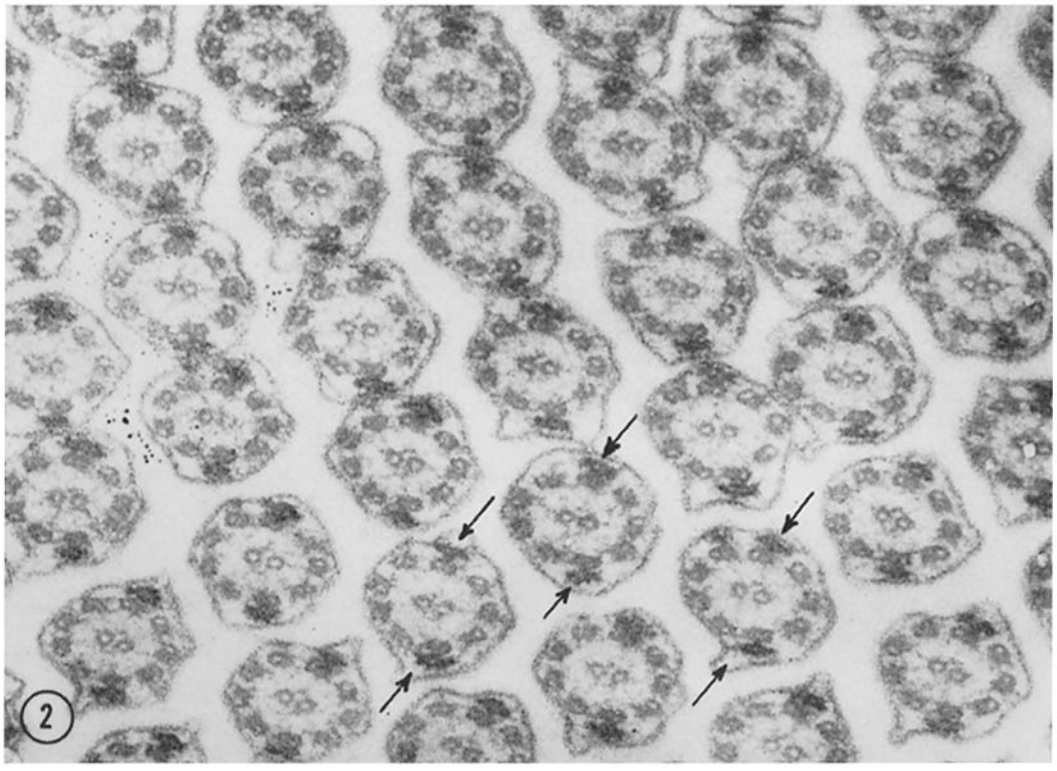
FIGURE 1

Cross-sections of cilia of *Philodina citrina* fixed in 1 per cent osmium tetroxide showing pattern of distribution of peripheral filaments. × 45,000.

FIGURE 2

Cilia cross-sections after staining for ATPase, showing metal deposits adjacent to peripheral filaments numbers 1 and 5 (arrows). × 84,000.


1


2

observed only one of the two ATPase bodies. We have not yet been able to determine the significance of this pattern nor have we been able to associate the presence of one or two bodies with the stage of ciliary beat at the time of fixation.

In no specimen have we seen ATPase bodies in the region of the base of the cilium. In this region, as reported before, both components of the peripheral filament exhibit a low electron opacity and there is no evidence of a bilateral asymmetry (Fig. 3). These two characteristics distinguish the base of the cilium from the bulk of the free portion of the cilium. Fig. 4 illustrates the absence of ATPase concentration in several levels of section of cilia in the region of the cell surface.

Aside from the recognition of ATPase bodies in the free portion of the cilium we have noted a marked concentration of this enzyme, as evidenced by electron opacity, in the medulla of microvilli between cilia and in the cristae and limiting membranes of mitochondria throughout the rotifer.

DISCUSSION

The basic observation being reported here is the appearance of a formed structure in cross-sections of cilia after ATPase incubation for which there is no precedent in conventionally prepared specimens. The ATPase bodies usually are associated with peripheral filaments number 1 and 5 and are located peripherally to them.

The presence of ATPase in cross-sections of cilia obviously does not constitute direct evidence for identification of a contractile system in cilia, but it does indicate a further parallel between vibratile and contractile systems. It is further interesting to note that the location of the two ATPase bodies in each of the two semicircles, which exhibit asymmetry in so far as the peripheral filaments are concerned, is consistent with our suggestion that a "push-pull" mechanism may be operative in cilia. As we have already suggested, pendular beat of a cilium may be a function of contraction of peripheral filaments in one semicircle and extension of peripheral filaments in the other semicircle.

SUMMARY

Rotifers were prepared for electron microscopy after incubation for ATPase by the method of Essner, Novikoff, and Massek, along with appropriate controls. Cross-sections of cilia showed the presence of distinct formed structures of marked electron opacity in association with peripheral filaments 1 and 5 but absence of them in the region of the base of the cilium. ATPase also appeared to be present in the medulla of microvilli and in the cristae and limiting membranes of mitochondria.

This work has been aided by grants H-2560 (C4) and H-2975 (C3) from the United States Public Health Service.

Received for publication, June 16, 1961.

REFERENCES

1. CHILD, F. M., *Exp. Cell Research*, 1959, **18**, 258.
2. ENGELHARDT, W. A., and LJUBINOVA, M. N., *Nature*, 1939, **144**, 668.
3. ESSNER, E., NOVIKOFF, A. B., and MASSEK, B., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 711.
4. FAWCETT, D. W., AND PORTER, K. R., *J. Morph.*, 1954, **94**, 221.
5. GIBBONS, I. R., and GRIMSTONE, A. V., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 697.
6. LANSING, A. I., and LAMY, F., *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 799.
7. NELSON, L., *Biochem. et Biophys. Acta*, 1954, **14**, 312.
8. WEBER, H H. *Symposia Soc. Exp. Biol.*, 1955, **9**, 271.

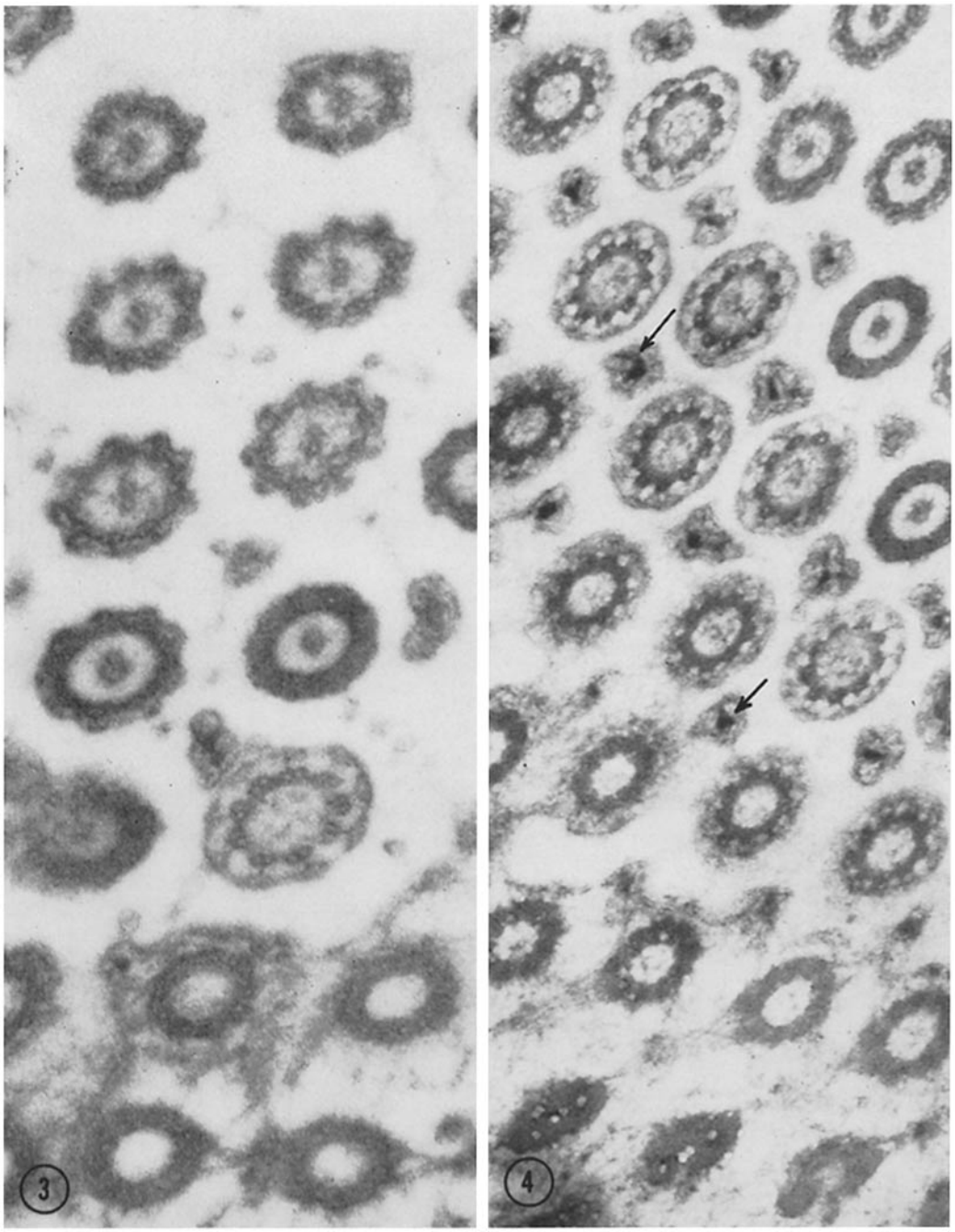


FIGURE 3

Cross-sections of cilia in the region of the cell membrane. Osmium tetroxide fixation (1 per cent). × 70,000.

FIGURE 4

Cilia cross-sections in region of cell membrane after ATPase staining, showing lack of reaction within the cilia. Lead deposits are present in medulla of microvilli (arrows). × 70,000.