SOME NEW CYTOLOGICAL OBSERVATIONS IN THE HETEROTRICHOUS CILIATE, *BLEPHARISMA*

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INTRODUCTION

During the course of an experimental, fine structural study of *Blepharisma*, certain aspects of the normal anatomy of the cell came to our attention. Although *Blepharisma* is the subject of two recent reports (9, 10), these features have, to our knowledge, hitherto been neglected in the literature. Furthermore, we feel that the present report may serve to clarify some of the recent work on the closely allied form, *Spirostomum* (7, 15).

MATERIALS AND METHODS

Tube cultures of *Blepharisma intermedium* (8) were concentrated by mild centrifugation and fixed in 5% glutaraldehyde for 1 hr. They were postfixed in Dalton's fixative (2) for an additional hour, dehydrated in an ascending series of alcohols, and embedded in either Maraglas (4) or Epon (11) following overnight impregnation. The polymerization was carried out in flat polyethylene forms, permitting the selection of single, well preserved cells, and precise orientation and plane of sectioning (1). Sections were cut on a Porter-Blum MT-1 or LKB 1 ultramicrotome, stained with uranyl acetate, and examined in an RCA-3F or Siemens 1A electron microscope.

RESULTS

In cross-section (Fig. 1) the surface of the animal presents the familiar scalloped appearance of the heterotrichs (7, 13), with alternating ridges and troughs. At higher magnification (Fig. 2), the cross-sections of the longitudinal kinetodesma (9) are visible in the walls of the ridges. The radially oriented kinetosomes may be seen beneath the floors of the troughs.

Somewhat below the kinetosomes, a dense fibrous material (DFM) delineating the ecto-endoplasmic boundary may be seen (Figs. 1 and 2). It seems to occur in patches and is arranged circumferentially. When seen in longitudinal section (Fig. 3), the disposition of the DFM is apparently similar to that seen in cross-section. The DFM forms a narrow discontinuous band around the organism between the ectoplasm and the endoplasm.

It is only in a grazing longitudinal section through the level of the DFM that the full extent of this material is discernible (Fig. 4). Here, it may be seen as an extensive branching network forming a fenestrated sheath around the endoplasm. Frequently, a rather tight fit around mitochondria or other cytoplasmic inclusions is evident. In these areas, the 40-A fibers (15) making up the DFM seem to condense into a more compact arrangement.

Occasionally, the DFM is seen in close juxtaposition to the fibrils connected to the kinetosomes (Fig. 5). This is particularly apparent at the bases of the adoral zone membranelles (Fig. 6). In these areas, armlike extensions of the DFM penetrate between, and very close to, the fibrils attached to the bottoms of the kinetosomes.

A second cytological feature of *Blepharisma* is apparent as irregular, membrane-bounded spaces. These spaces are of greatest size and extent deep in the cytoplasm (Fig. 1) and they frequently form longitudinal channels directly below the DFM (Fig. 3). Similar, but smaller, spaces are often visible in the ectoplasm (Fig. 3).

In an oblique section near the anterior tip of the animal (Fig. 7), the channels are represented as narrow clefts arising from a larger space deeper in the endoplasm and maintaining a surprising one-to-one relationship with the ridges. Up in the ridges themselves, elements of these clefts, frequently containing small, very dense granules, closely approach invaginations from the surface (Fig. 8).

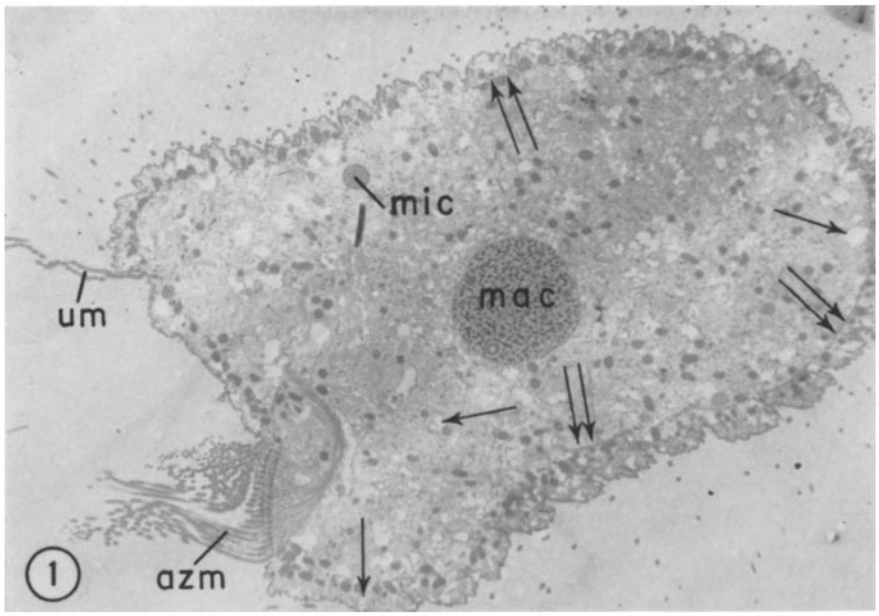
Microtubules, 180 A in diameter, are often associated with the membrane-bounded spaces in the endoplasm (Fig. 9). The small areas and clefts of the membrane-bounded spaces in the ectoplasm have no microtubules directly associated with them.

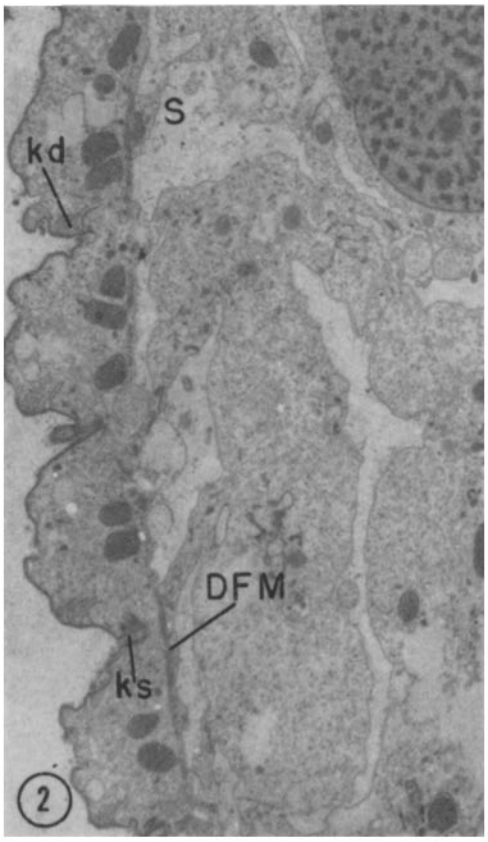
There is reason to believe that the vacuoles and large spaces deep in the endoplasm; the channels, clefts, and large spaces under the DFM near the ectoplasm; and the clefts and small spaces in the ectoplasm proper, all constitute a continuous system of membrane-bounded spaces. The continuity of these spaces is indicated not only by sections showing direct anatomical confluence, but also by the presence of small, very dense granules of variable size throughout all of these spaces (Figs. 3 and 8). These granules, which are not to be confused with the much larger pigment granules found in some species of *Blepharisma* (10), are confined to the membrane-bounded spaces and were not found free in the cytoplasm. In addition to the granules, irregularly shaped dense material was frequently found in the membrane-bounded spaces in the endoplasm (Figs. 3 and 7).

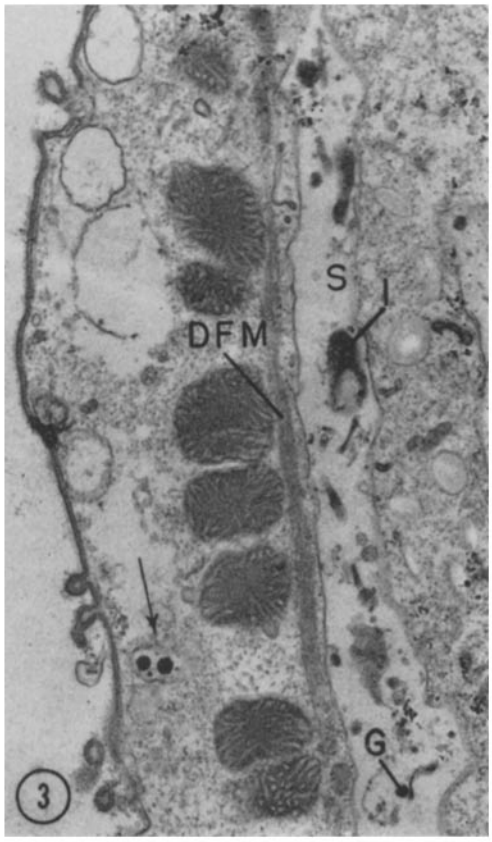
FIGURE 1 Low power cross-section of *Blepharisma*, showing macronucleus (*mac*), micronucleus (*mic*), the undulating membrane (*um*) and an adoral zone membranelle (*azm*). Also visible are the dense fibrous material (double arrows) delineating the ecto-endoplasmic boundary and the membrane-bounded spaces (single arrows). × 1900.

FIGURE 2 Cross-section. The extracellular area is at the left side of the figure. Profiles of the kinetodesma (*kd*) and the kinetosomes (*ks*) are visible in the ectoplasm. The dense fibrous material (*DFM*) and the membrane-bounded spaces (*S*) are also evident. A portion of the macronucleus is visible in the upper right corner. × 6100.

FIGURE 3 Longitudinal section. The extracellular area is at the left side of the figure. The dense fibrous material (*DFM*) is visible below a row of mitochondria. Directly below the DFM is a membrane-bounded space (*S*) in the form of a channel, in which a very dense granule (*G*) as well as irregularly shaped dense material is visible (*I*). In the ectoplasm, near the cell surface, a small membrane-bounded space containing two prominent very dense granules is visible (arrow). × 26,000.







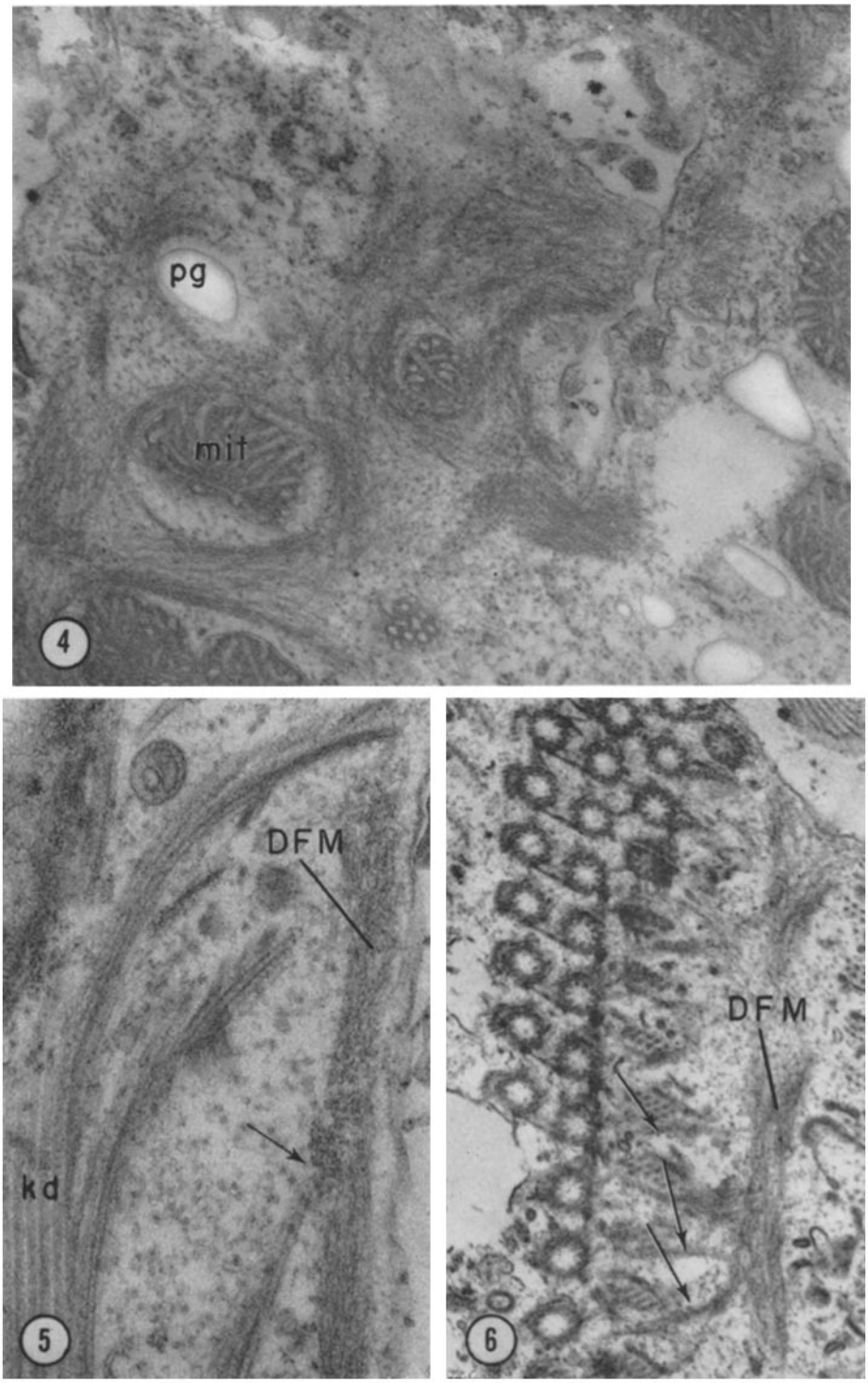


FIGURE 4 Grazing longitudinal section through the level of the DFM. The dense fibrous material appears as a fenestrated sheetlike structure with condensations around organelles such as mitochondria (*mit*) and a paraglycogen body (*pg*). × 52,000.

FIGURE 5 Longitudinal section through the kinetodesma (*kd*). The long curving fibrils lead to the kinetosomes which are out of the plane of this section. A close juxtaposition of DFM and one of the fibrils is visible at the arrow. × 80,000.

FIGURE 6 Longitudinal section through the base of an adoral zone membranelle. Three rows of kinetosomes are visible. The right row is represented in part by cross-sections of fibrils from the bottoms of the kinetosomes. Armlike extensions (arrows) of the DFM are visible in close association with these fibrils. × 34,000.

DISCUSSION

The dense fibrous material seen in *Blepharisma* is evidently identical with that seen in a variety of other ciliates, including both contractile (5, 6, 13–15) and noncontractile (12, 16) forms. In the former, elements of the endoplasmic reticulum have been observed in association with the fibrous material. No such association is apparent in *Blepharisma* or in other essentially noncontractile forms, leading to the speculation that the membranous elements of the endoplasmic reticulum play an essential role in contraction (12, 16).

In *Spirostomum*, a contractile form closely related to *Blepharisma*, fibrous material has been observed at the ecto- endoplasmic boundary where it is associated with what are apparently elements of the endoplasmic reticulum (15). However, the lack of any clear-cut attachment of this material to the pellicle or any other rigid structure presents a serious difficulty for the understanding of how the fibrous material could serve a contractile function. It could, of course, be argued either that the ectoplasm itself is rigid enough to serve as a point of attachment, or that an as yet undiscovered attachment does, indeed, exist.

One such possible attachment in *Blepharisma* is suggested in the admittedly equivocal micrographs indicating a close relationship between the DFM and the fibrils attached to the kinetosomes. This suggestion is strengthened somewhat by the rather distinct anatomical relationship between the DFM and the fibrils attached to the kinetosomes in the bases of the adoral zone membranelles (Fig. 6), in which each set of fibrils arising from a single kinetosome is encompassed by extensions of the DFM. Because of the close taxonomic relationship between the two forms, it seems reasonable to suggest that a similar connection may obtain in *Spirostomum*.

The significance of a ramifying, extensive system involving certain of the membrane-bounded spaces of the cell is unclear. While occasional structures similar to partly digested bacteria have been seen within the system, the classification of any of these spaces as food vacuoles is uncertain.

In this connection, we would like to report that we have recently made several preliminary observations of material incubated to demonstrate the presence of acid phosphatase activity. Reaction product was visible both within and just below the ectoplasm, as well as in deeper endoplasmic spaces, but preservation adequate for precise localization of this enzyme has not yet been achieved.

The significance of the microtubules associated with the membrane-bounded spaces in the endoplasm is not understood. Since many of these spaces are in the anterior half of the animal, it is unlikely that they are directly connected to the contractile vacuole, which has been reported to be associated with microtubules (3).

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FIGURE 7 Oblique section of an area near the anterior end of the animal. Clefts (arrows), arising from membrane-bounded spaces in the endoplasm which contain irregularly shaped dense material, are seen traversing the endoplasm and leading into the ectoplasm. × 11,000.

FIGURE 8 Higher magnification of the same section as in Fig. 7. Ectoplasmic elements of the membrane-bounded spaces are seen to approach surface invaginations (arrows). × 42,000.

FIGURE 9 Cross-section. Microtubules (*MT*) are seen in close association with a membrane-bounded space (*S*) near the anterior end of the animal.