

# Novel Bridge of Axon-Like Processes of Epithelial Cells in the Aboral Sense Organ of Ctenophores

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**ABSTRACT** We describe by light and electron microscopy a novel structure in the aboral sense organ (apical organ) of cydippid (*Pleurobrachia*) and lobate (*Mnemiopsis*) ctenophores. An elevated bundle of long, thin, microtubule-filled processes arises from the apical ends of two groups of epithelial cells located on opposite sides of the apical organ along the tentacular plane of the body. This bundle of axon-like processes arches over the epithelial floor like a bridge, with branches at both ends running toward opposing pairs of ciliary balancers that are motile pacemakers for the rows of locomotory ciliary comb plates. The bridge in *Pleurobrachia* is ~40 µm long and 3–4 µm wide and consists of ~60 closely packed processes, 0.2–0.8 µm thick, containing vesicles and numerous microtubules running parallel to their long axes. There are ~30 epithelial cells in each of the two groups giving rise to the bridge and each cell forms a single process, so roughly half of the processes in the bridge must originate from cells on one side and diverge into branches to a pair of balancers on the opposite side of the apical organ. The 150–200 cilia in each balancer arise from morphologically complex cellular projections with asymmetric lateral extensions directed towards a fork of the bridge. Presynaptic triad structures and vesicles are found in this region but clear examples of synaptic contacts between bridge processes and balancer cells have not yet been traced. Cydippid larvae of *Mnemiopsis* have a conspicuous bridge along the tentacular plane of the apical organ. Beroid ctenophores that lack tentacles at all stages do not have a bridge. We discuss the possibility that the bridge is an electrical conduction pathway to balancers that coordinates tentacle-evoked swimming responses of ctenophores, such as global ciliary excitation. *J. Morphol.* 254:99–120, 2002. © 2002 Wiley-Liss, Inc.

**KEY WORDS:** ctenophore apical organ; bridge; axon-like epithelial processes; balancer cilia

Ctenophores are gelatinous marine invertebrates that are voracious predators in zooplankton food chains (Harbison et al., 1978). Ctenophores are the largest organisms to swim by means of cilia and their giant ciliary comb plates and biting macrocilia have served as model systems for investigating the mechanisms and control of ciliary activity (Tamm, 1982).

The aboral sense organ, or apical organ of ctenophores, is the sensory-motor control center of these animals. The principal component of the apical organ is a statocyst. Four groups of motile balancer cilia in the statocyst act as pacemakers to initiate

beating of the eight meridional rows of comb plates that drive swimming behavior of these animals (Horridge, 1965; Tamm, 1982). The nervous system of ctenophores has the appearance of a diffuse nerve net generally lacking concentrations into polarized tracts or ganglia (Tamm, 1982; Hernandez-Nicase, 1991; but see Mackie et al., 1992; Tamm and Tamm, 1995). Several ciliary motor responses are triggered by stimulus-evoked neural input to the ciliated cells (Moss and Tamm, 1986, 1987, 1993). Epithelial conduction, although present in cnidarians (Mackie, 1970; Spencer, 1974; Anderson, 1980), has never been demonstrated in ctenophores.

Here we report a previously undescribed structure in the apical organ of ctenophores that looks like a novel kind of electrical conduction pathway. The structure is an elevated bundle of long, thin, microtubule-filled processes that arise from the apical ends of two groups of epithelial cells located on opposite sides of the statocyst along the tentacular plane. This bundle of axon-like processes arches over the epithelial floor like a bridge and branches at both ends toward opposing pairs of pacemaker balancer cilia. The bridge is thus a new example of bilaterality in the biradiate body plan of ctenophores. We discuss possible functions of the bridge in controlling swimming behavior of ctenophores and its possible significance for evolution of simple nervous systems.

## MATERIALS AND METHODS

### Organisms

*Pleurobrachia pileus* and *Mnemiopsis leidyi* were carefully collected with cteno-dippers from the surface of Great Harbor and Vineyard Sound near Woods Hole, Massachusetts. Ctenophores were maintained in fresh running seawater at MBL. Cydippid larvae of *Mnemiopsis* were obtained by spawning fertile adults, as described previously (Tamm and Tamm, 1981; Nakamura and Tamm, 1985).

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## Light Microscopy

To view the bridge of adult ctenophores, the overlying refractile statolith was first removed from the statocyst with a fine suction pipette operated by a micromanipulator. The apical organs were then dissected, trimmed with a fine iridectomy scissors, and mounted in seawater under a vaseline-supported coverslip on a microscope slide. The bridge on the floor of the apical organ was observed under a Zeiss Universal microscope by DIC optics with a  $40\times/0.75\text{ NA}$  objective. Photographs were taken on Kodak Tech Pan (2415) or Tri-X 35 mm film using an Olympus OM-2N camera with an Olympus T32 flash tube inserted in the light path. Free-swimming cydippid larvae were immobilized on microscope slides by slight compression of a vaseline-edged coverslip (Tamm and Terasaki, 1994).

## TEM

Adult ctenophores were fixed as previously described (Tamm and Tamm, 1988). Briefly, aboral halves were fixed in 2.5% glutaraldehyde, 1% paraformaldehyde, 1% osmium tetroxide, 0.15 M NaCl, 0.01 M CaCl<sub>2</sub>, 0.2 M sodium cacodylate buffer (pH 7.8) for 1 h at 0°C. Pieces were washed in 0.3 M NaCl, 0.2 M sodium cacodylate buffer at 0°C for 1–2 hrs, during which apical organ regions were dissected with iridectomy scissors. Tissue was post-fixed in 1% osmium tetroxide, 0.37 M NaCl, 0.1 M sodium cacodylate buffer (pH 7.8) for 20 min at 0°C, washed in distilled water for 30 min at 0°C, and stored overnight in 1% aqueous uranyl acetate at 4°C. Tissue was dehydrated in an acetone series and flat-embedded in discs of Araldite. Apical organ regions were selected and marked in discs under a light microscope, cut out of the discs with a jewelers saw, trimmed into cubes with Teflon-coated single-edge razor blades (Ted Pella, Redding, CA) under a dissecting microscope, and glued in appropriate orientations on blank stubs for sectioning in three different planes (see Fig. 2). Thin sections were cut on a Reichert OmU2 ultramicrotome with a diamond knife, picked up on Formvar-coated copper grids, stained with uranyl and lead salts, and examined with a Zeiss 10CA electron microscope at 80 kV in the MBL Central Microscope Facility at Woods Hole, MA.

Cydippid larvae were concentrated by gentle centrifugation in 100 ml oil tubes (Nakamura and Tamm, 1985), fixed as above, and processed in glass depression wells without centrifugation. Individual flat-embedded larvae were cut out of discs and mounted on stubs for sectioning the apical organ in appropriate planes.

## RESULTS

### General Structure

Ctenophores have an aboral–oral axis along which the flattened stomodaeum defines the stomodaean or sagittal plane, which is perpendicular to the tentacular plane passing through the pair of feeding tentacles (Fig. 1). The apical organ at the aboral pole has a thickened concave floor of a single layer of flask-shaped epithelial cells, ~100  $\mu\text{m}$  long. The statocyst consists of four sickle-shaped compound cilia, ~50  $\mu\text{m}$  long, with 150–200 cilia per group, called balancers (Horridge, 1965; Tamm, 1982). The bases of the balancers are boomerang- or V-shaped and are arranged as two opposing pairs spaced widely apart along the tentacular plane (Fig. 2; Tamm, 1982). The curved tips of the balancers support a rounded cellular mass 50–75  $\mu\text{m}$  in diameter,

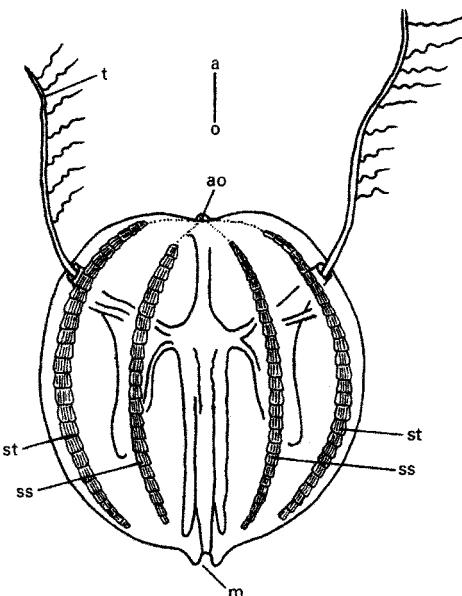


Fig. 1. The ctenophore *Pleurobrachia* viewed from the side along the tentacular plane. a-o, aboral-oral axis; ao, apical organ; m, mouth; t, tentacle; st, ss, subtentacular and subsagittal comb rows. ~1.5 $\times$  lifesize.

the statolith, which is suspended above the epithelial floor (Fig. 2).

Beat frequency of the balancers is controlled by their bending due to the gravitational load of the attached statolith and also by electrical input to the balancer cells themselves (Tamm, 1982; Lowe, 1997). The beat frequency of each balancer is transmitted mechanically to two narrow tracts of small cilia, the ciliated grooves, which run out of the statocyst to activate beating of a pair of ciliary comb rows in that body quadrant (Tamm, 1982). The balancers thus serve as pacemakers to initiate and coordinate geotactic and other swimming responses of ctenophores (Tamm, 1982). Although several motor responses of comb plates are regulated by nerves, metachronal coordination along comb rows is largely, if not entirely, due to hydromechanical coupling between the cilia (Tamm, 1973, 1982).

### Light Microscopy of the Bridge in Adult Ctenophores

DIC views of the aboral surface of dissected apical organs from which the refractile statolith has been removed reveal an elongated structure under the former location of the statolith (Fig. 3). This so-called bridge arches over the epithelial floor, and runs in the tentacular plane. In *Pleurobrachia*, the bridge is ~40  $\mu\text{m}$  long and 3–4  $\mu\text{m}$  wide and has bulbous widenings between the V-shaped bases of opposing pairs of balancers (Fig. 3A). Tracts of short cilia arise from the epithelial floor on either side of

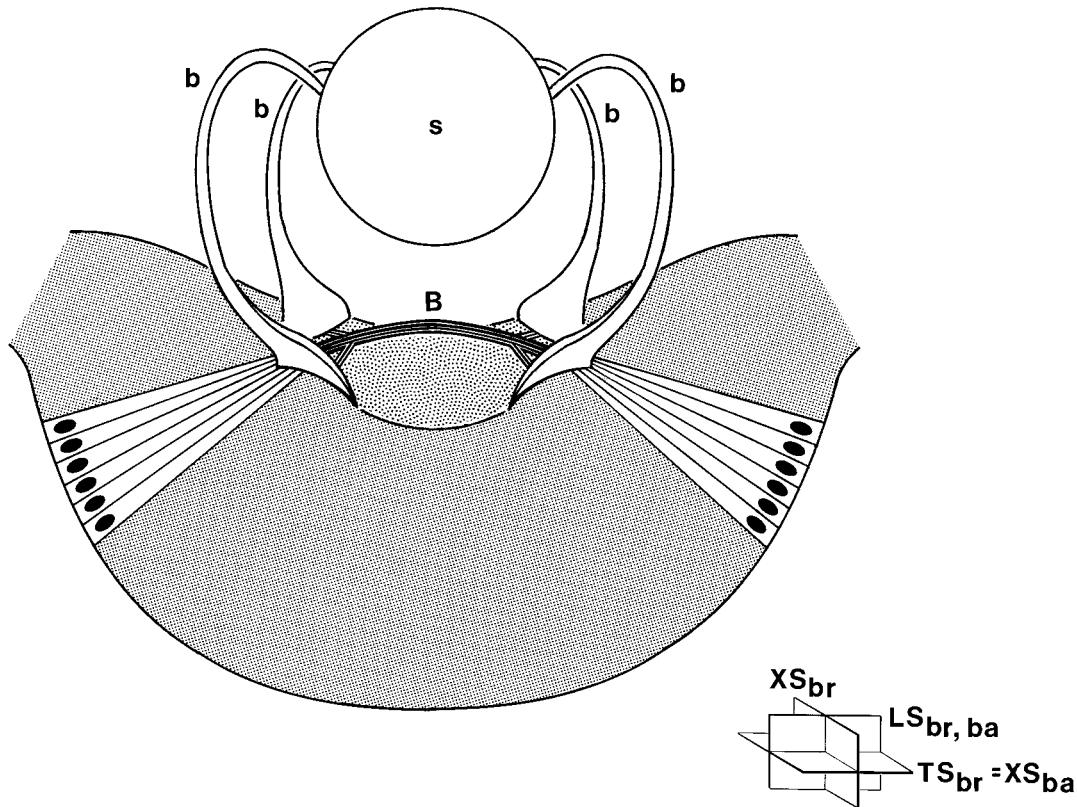


Fig. 2. Diagrammatic side view of the apical organ along the tentacular plane. b, balancer; s, statolith; B, bridge with branches at each end to a pair of balancers. The single-layered epithelial floor is shaded except for the two groups of cells giving rise to the bridge on opposite sides (for clarity, only 6 of the ~30 cells in each group between a balancer pair are shown). Ciliated grooves from balancers are omitted. The three different planes of TEM thin sections are indicated at lower right: XS, transverse section; LS, longitudinal section; TS, tangential section; subscripts indicate appropriate plane through the bridge (br) and/or balancer (ba).

the bridge. The cilia are often inactive when first observed but then start beating in a plane parallel to the bridge like fans before slowing down and stopping again.

*Mnemiopsis* apical organs have a similar bridge running in the tentacular plane above the epithelial floor (Fig. 3B). The *Mnemiopsis* bridge is longer (~50 µm) and narrower (~2–3 µm) than that of *Pleurobrachia*, perhaps reflecting the larger body size of this genus. More noticeably, both ends of the *Mnemiopsis* bridge fork into two branches, ~10 µm long, which diverge from the main trunk and run toward the bases of the adjacent pair of balancers (Fig. 3B). The so-called fan cilia on either side of the *Mnemiopsis* bridge are grouped into triangular tufts near the middle of the bridge and show activity similar to those in *Pleurobrachia*. We have not studied the fan cilia in *Pleurobrachia* and *Mnemiopsis* further, nor do we know if their activity is related to the bridge.

Similar observations on *Beroë* apical organs without statoliths show no signs of a bridge or arrays of cilia on the epithelial floor. Berooids are the only group of ctenophores that lack tentacles and develop

directly into adults without a tentaculate cydippid larval stage.

#### Electron Microscopy of the Bridge in Adult *Pleurobrachia*

Thin sections were cut through the bridge of *Pleurobrachia* in three different orientations: *longitudinal* (parallel to the tentacular plane), *transverse* (parallel to the sagittal plane), and *tangential* (parallel to the aboral surface) (Fig. 2).

Longitudinal sections show that the bridge consists of closely packed, long, thin extensions of the narrowed apical ends of two groups of epithelial cells located in the tentacular plane at opposite sides of the statocyst floor (Fig. 4). The bridge of cell processes extends over the apical protrusions of intervening cells in the epithelial floor and passes under the statolith.

All of the flask-shaped cells in the floor of the apical organ are connected at their apical ends by a dense web of belt desmosomes (Figs. 4, 5). The cells giving rise to the bridge differ from neighboring epithelial cells by their high density of large mito-

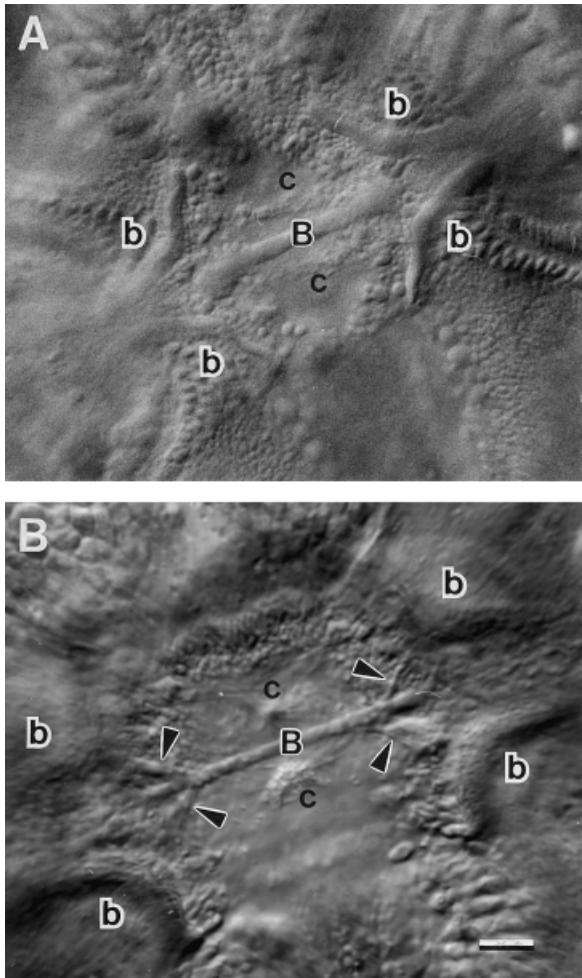


Fig. 3. DIC aboral views of the bridge in dissected apical organs of *Pleurobrachia* (A) and *Mnemiopsis* (B). The statoliths have been removed before imaging. The bridge (B) runs over the epithelial floor in the tentacular plane (diagonally) between the widely spaced pairs of V-shaped bases of the balancers (b). A: The bridge of *Pleurobrachia* is shorter and thicker and widened at its apparent ends. Tracts of cilia (c) occur on either side of the bridge. Brick-like ciliary groups of a pair of ciliated grooves extend from the outer side of each balancer (particularly evident behind the right balancer). B: In *Mnemiopsis* both ends of the bridge send branches (arrowheads) to the bases of the adjacent pair of balancers. Tufts of cilia (c) are present on either side of the bridge. Scale bar = 10  $\mu\text{m}$ .

chondria in the apical cytoplasm (Figs. 4, 5). Adjacent epithelial cells on the inside of the bridge cell groups have a characteristically dense cytoplasm (so-called "dark cells"; Figs. 4, 5) and serve as markers for the boundary of each group of bridge cells (see Fig. 8).

The two origins of the bridge from the widely spaced groups of epithelial cells appear morphologically identical in mirror image, like the two ends of a road bridge. At each origin, the cell extensions begin above the zone of desmosomes and contain numerous microtubules running parallel to their

long axes, similar to microtubule orientation in the apical cytoplasm (Fig. 5). Each bridge cell appears to give rise to a single extension or process. Longitudinal sections through the middle of the bridge show more or less parallel processes, less than 1  $\mu\text{m}$  in width (Fig. 6A). It was not possible to follow a given process across the bridge from one side to the other. The processes appear to extend most of the length of the bridge, since no clear terminations (as opposed to passing out of the plane of section) or end-to-end junctions between processes have been observed in many longitudinal sections through bridges.

Transverse sections through the middle of the bridge show that it consists of ~60 closely packed processes (Fig. 6B). The processes have an irregular shape in cross-section and are 0.2–0.8  $\mu\text{m}$  thick. The processes contain microtubules, here cut transversely, as well as numerous clear vesicles of various sizes and shapes. No neurofilaments or mitochondria were seen in the processes. The processes are not connected laterally within the bridge by adhesions or intercellular junctions.

Abundant nervous elements are found at the wider basal ends of the bridge cells adjoining the mesoglea. The neurites contain numerous clear vesicles, mitochondria, microtubules, and synaptic regions with the characteristic presynaptic triad morphology of ctenophore nerves (i.e., a single layer of vesicles, smooth ER sac, and closely apposed mitochondria; Horridge and Mackay, 1964; Hernandez-Nicaise, 1973). Neurites synapse onto the bases of the bridge cells (Fig. 7), as well as onto other neurites. It is not possible to trace the origins or paths of the neurites in nonserial thin sections. This difficulty might be resolved in the future by antitubulin immunofluorescence studies using 3D confocal reconstruction.

#### Electron Microscopy of Balancer Bases in Adult *Pleurobrachia*

The relationship between the bridge forks and the nearby balancers was investigated by thin sections in two different orientations: transverse sections through the balancer bases (same as tangential sections through the bridge), and longitudinal sections through the balancer bases (same as longitudinal sections through the bridge) (see Fig. 2).

Each of the 150–200 cilia of a balancer arises from the narrow apical projection of a single epithelial cell (Tamm, 1982). There are thus 150–200 cells contributing cilia to a balancer. At the tip of each balancer cell projection is a ciliary basal body, from which a dense striated rootlet extends 8–10  $\mu\text{m}$  to the level of the belt desmosomes. The balancer cells are arranged in the epithelial floor such that cilia in the flared V-shaped base are ordered into ~6 rows in a rhomboidal lattice (Tamm, 1982).

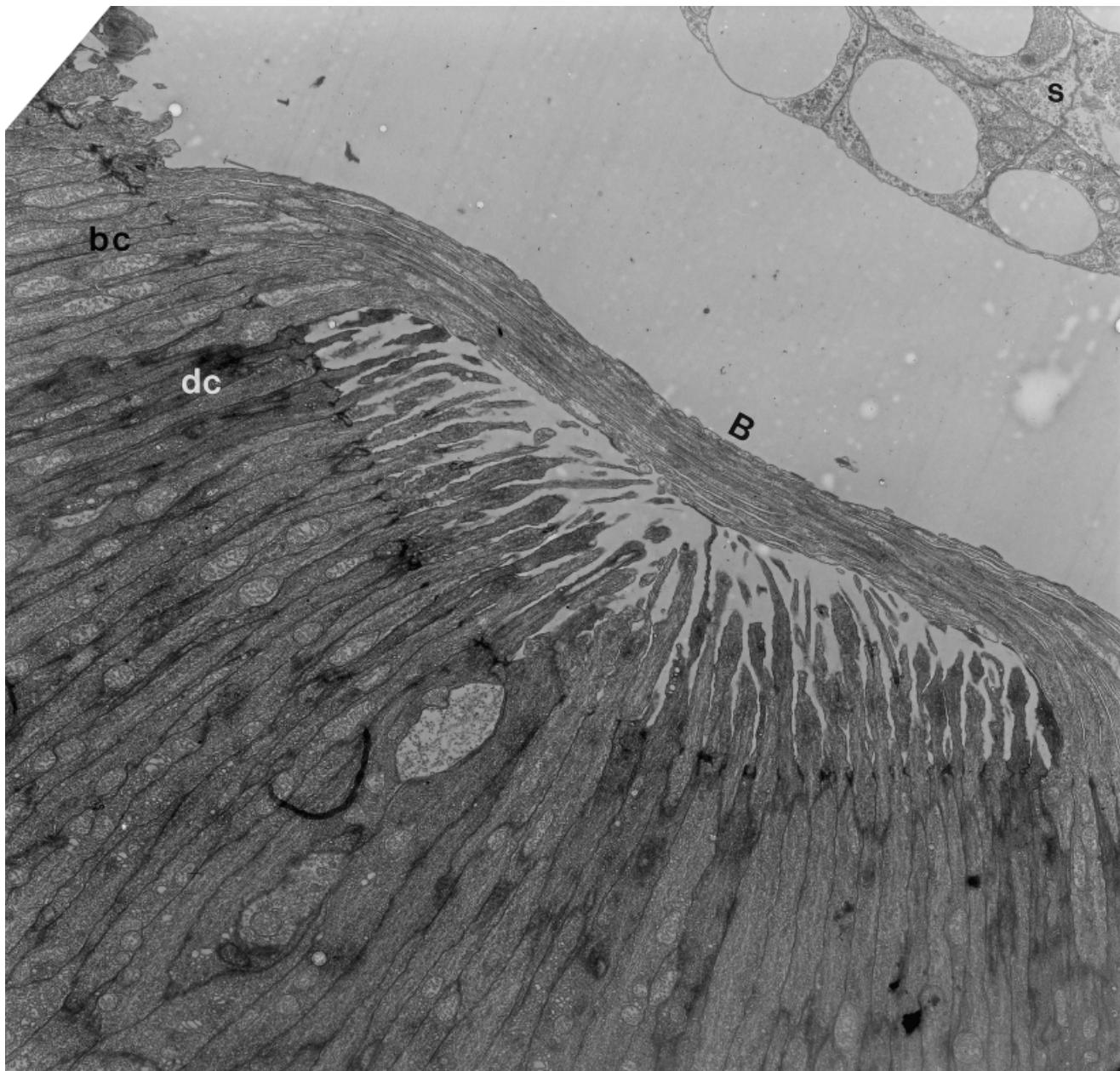


Fig. 4. Longitudinal section in the tentacular plane through the bridge (B). The bridge consists of many thin extensions of the narrowed apical ends of two groups of cells (bc, left group) located at opposite sides of the epithelial floor. Note many large mitochondria in the bridge cells on the left. Cells on the inner border of the bridge cells have a darker cytoplasm and are called dark cells (dc). A dense web of belt desmosomes encircles the epithelial cell apices, from which irregular protrusions underlie the bridge. Part of the cellular statolith (s) is visible at upper right. TEM section through adult *Pleurobrachia* (see Fig. 2 for orientation).  $\times 3,770$ .

The distal ends of the balancer cell projections are connected by brush-like junctions located at the level of the basal body and the start of the striated rootlet (Fig. 10A-D). In transverse sections through these intercellular junctions the apposed membranes are flat and connected by extracellular strands 2-4 nm thick, 25 nm long, and spaced 8-10 nm apart. The strands have dense thickenings that lie in register midway along their length between

the membranes of adjacent projections. A dense cytoplasmic coat underlies the flat junctional membranes. In some specimens the junctions appear stretched along one axis (Fig. 10C), so that strand length and intermembrane distance are increased to 40-50 nm. The thickness of the strands themselves and their spacing remain the same in these "stretched" junctions. Stretching of the junctions may reflect tension or sliding between neighboring

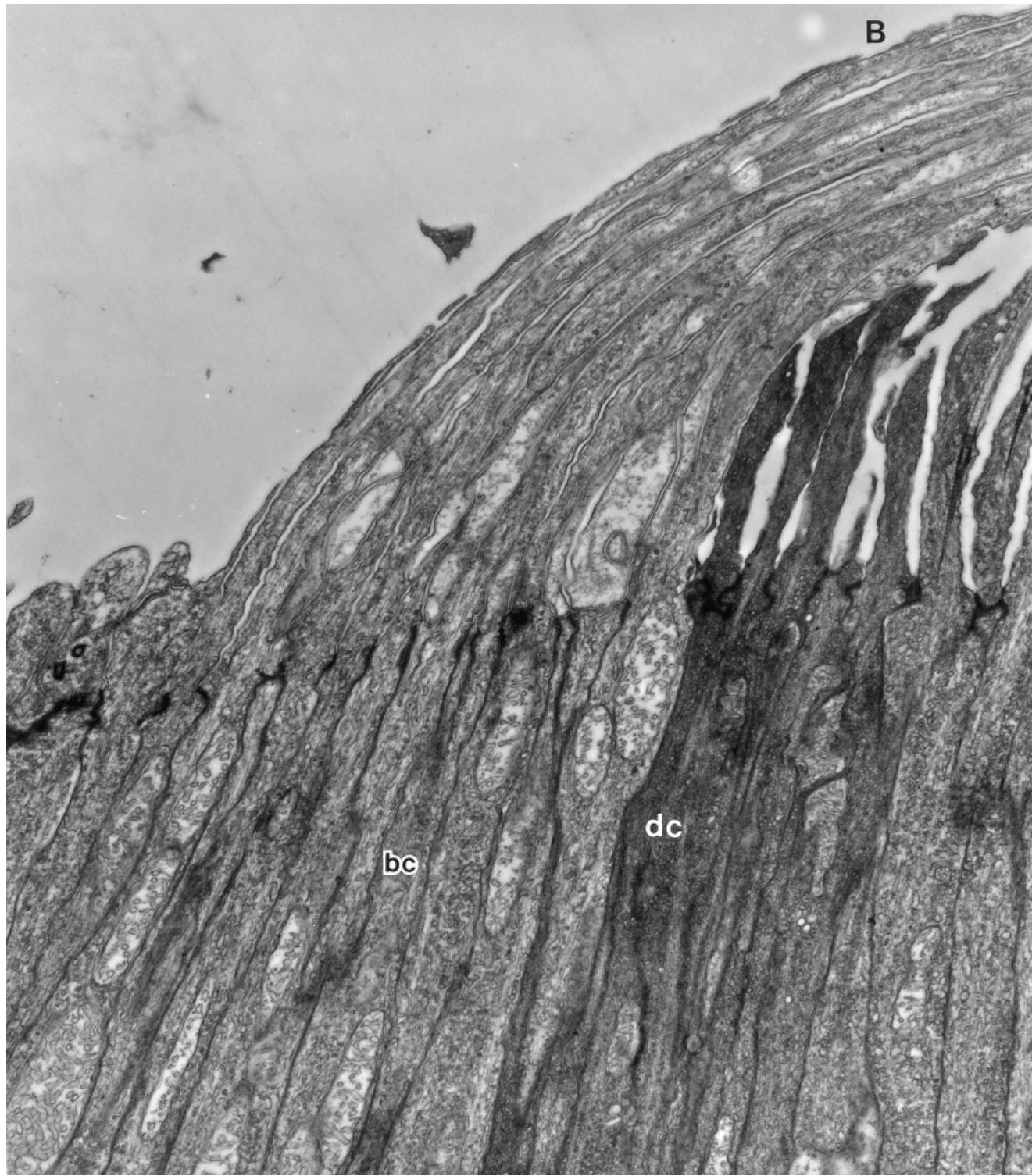
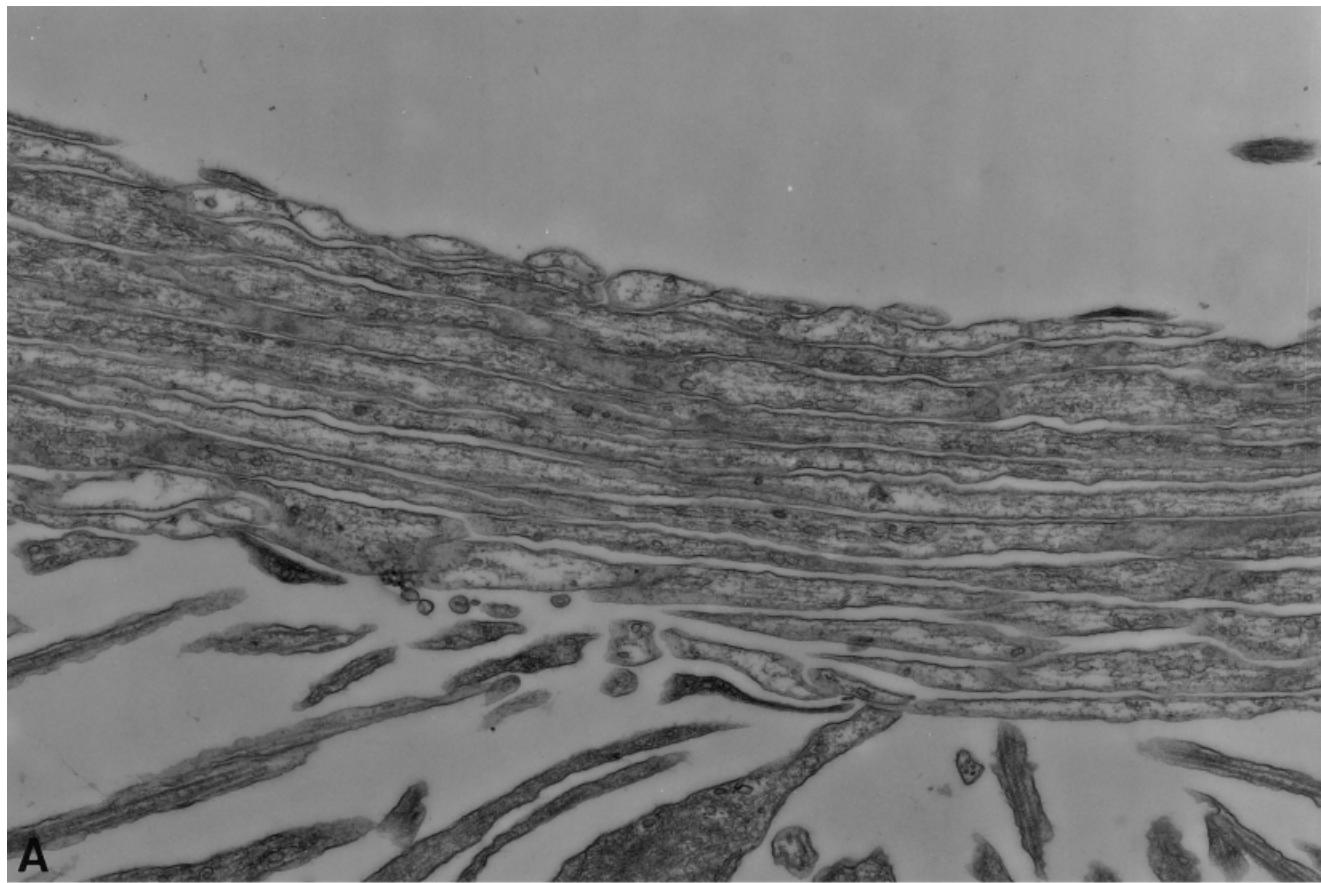
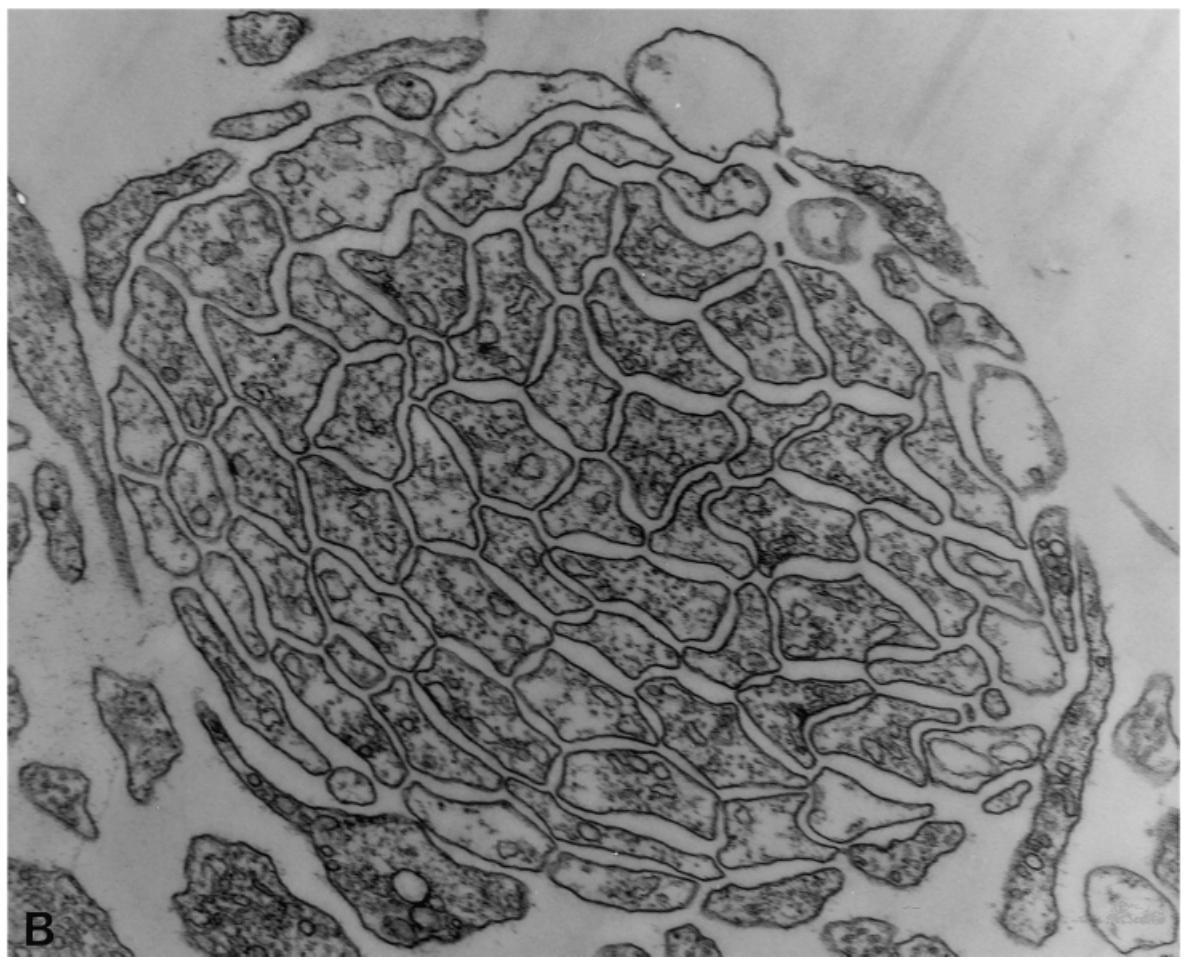


Fig. 5. Higher magnification of an adjacent longitudinal section through the origin of the bridge (B) shown at the left in Figure 4. The apical ends of the bridge cells (bc) contain numerous microtubules running parallel to their long axes and are packed with large mitochondria. Individual extensions of the cells into the bridge begin above the zone of desmosomes and contain similarly oriented parallel microtubules. Note dark cells (dc) with apical protrusions on the inner side (right) of the bridge cell group. TEM section through adult *Pleurobrachia* (see Fig. 2 for orientation).  $\times 9,550$ .



A



B

Figure 6

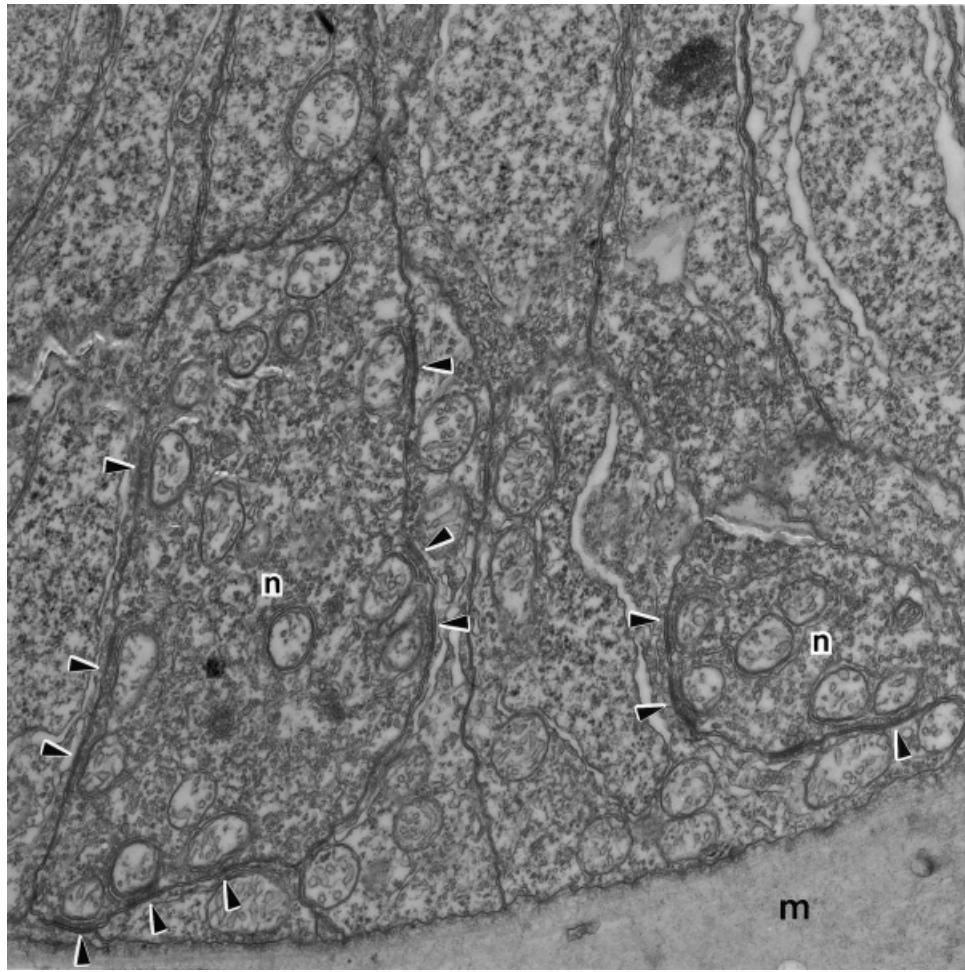


Fig. 7. Synapses (arrowheads on postsynaptic side) of neurites (n) onto the wider basal ends of bridge cells. Note characteristic presynaptic triad morphology at synaptic clefts: a single layer of clear vesicles, a sac of smooth ER, and a closely apposed mitochondrion. TEM section through adult *Pleurobrachia* (see Fig. 2 for orientation).  $\times 12,000$ .

projections due to balancer beating or mechanical deflection by the statolith.

Transverse sections reveal that the apical projections of the balancer cells have an unusual morphology and, most strikingly, a remarkable asymmetry on the two sides of the V-shaped base (Figs. 10A, 11). The convex (inner) side of a balancer base points toward a bridge fork, whereas the concave (outer)

side faces the start of two ciliated grooves running to a pair of comb rows (see Fig. 3). Balancer cell projections in the first and second rows on the inner side of the base have flange-like extensions from the basal body / rootlet axis that are oriented normal to the rows and directed toward a bridge fork (Figs. 10A, 11). Transverse sections at or just below the basal body show that the extensions are filled with an ordered array of flattened membranous sacs or cisternae (Figs. 10A, 11, 12). Two to four cisternae are often stacked side-by-side parallel to the extension, or coiled in closed concentric configurations. The lumen of the sacs is 50–60 nm wide and contains a medial dense layer of particulate material (Figs. 11B,C, 12).

On the outer side of the balancer base only the first row of apical projections bears longitudinal extensions and these face toward the ciliated grooves in the opposite direction to those of the inner side.

Fig. 6 (Overleaf.) Longitudinal (**A**) and transverse (**B**) sections through the middle of the bridge. The parallel processes vary from 0.2–0.8  $\mu\text{m}$  in thickness and contain numerous microtubules running parallel to their long axes, as well as clear vesicles. Although the processes are closely packed, their separation is not uniform and no adhesions or junctions are evident. Approximately 64 profiles of processes can be counted in **B**. Tips of apical protrusions of dark cells underlie the bridge in **A**. TEM sections through adult *Pleurobrachia* (see Fig. 2 for orientation). **A**,  $\times 16,750$ ; **B**,  $\times 34,000$ .

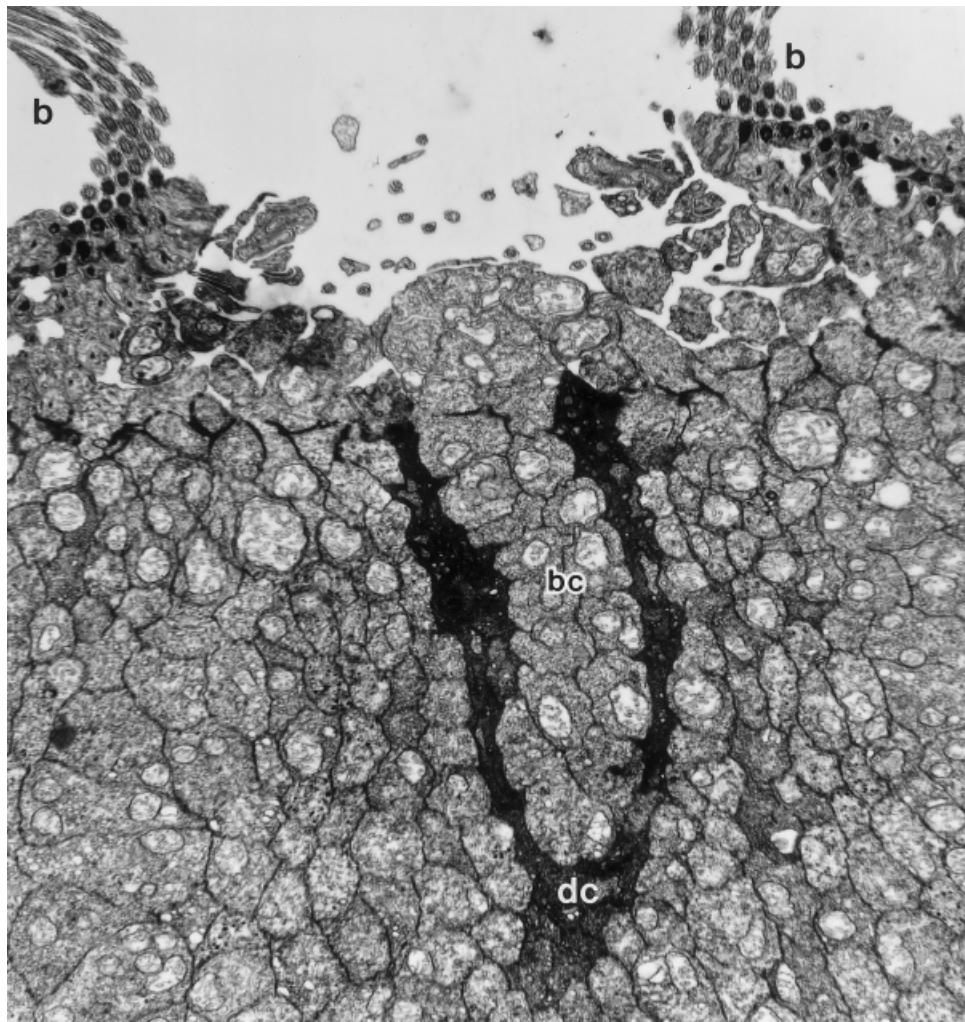


Fig. 8. Tangential section through one side of the epithelial floor, showing the distal ends of one group of bridge cells (bc) surrounded by a wishbone-shaped array of dark cells (dc) between a pair of balancers (b). There are ~24 bridge cells in this group. TEM section through adult *Pleurobrachia* (see Fig. 2 for orientation).  $\times 7,590$ .

Unlike the extensions on the inner side, extensions on the outer side have an irregular shape in cross-section; moreover, their membranous cisternae are not ordered into parallel stacks or coils but appear randomly arranged (Figs. 10A, 13). An additional asymmetry of the balancer base is that the second row of apical projections on the outer side often has denser cytoplasm surrounding the ciliary rootlet (Figs. 9, 13).

Transverse sections through the longitudinal extensions on the inner side of balancer bases reveal complex changes in shape and structure from the basal body downward along the rootlet axis. At both distal and proximal levels the extensions are longer and are widened at the ends facing the bridge fork, becoming thinner and tapered near the basal body / rootlet axis. At the basal body level these flared

regions are filled with clear vesicles of different shapes and sizes lying next to the ends of the membranous cisternae (Fig. 11). At the proximal level near the termination of the rootlets, cisternae are absent in the extensions and the enlarged regions are often filled with vesicles. Presynaptic triad structures, characteristic of ctenophore neurites, are found at this level and may be part of balancer cell extensions (see below) (Figs. 13, 14).

Distally and proximally, the widened ends of the longitudinal extensions on the inner side often protrude above and below their narrow connection with the basal body / rootlet axis. Consequently, the enlarged regions containing vesicles or remnants of membranous cisternae often appear separated and isolated from their origin at the basal body / rootlet (Figs. 11, 13, 14). The distal-to-proximal change

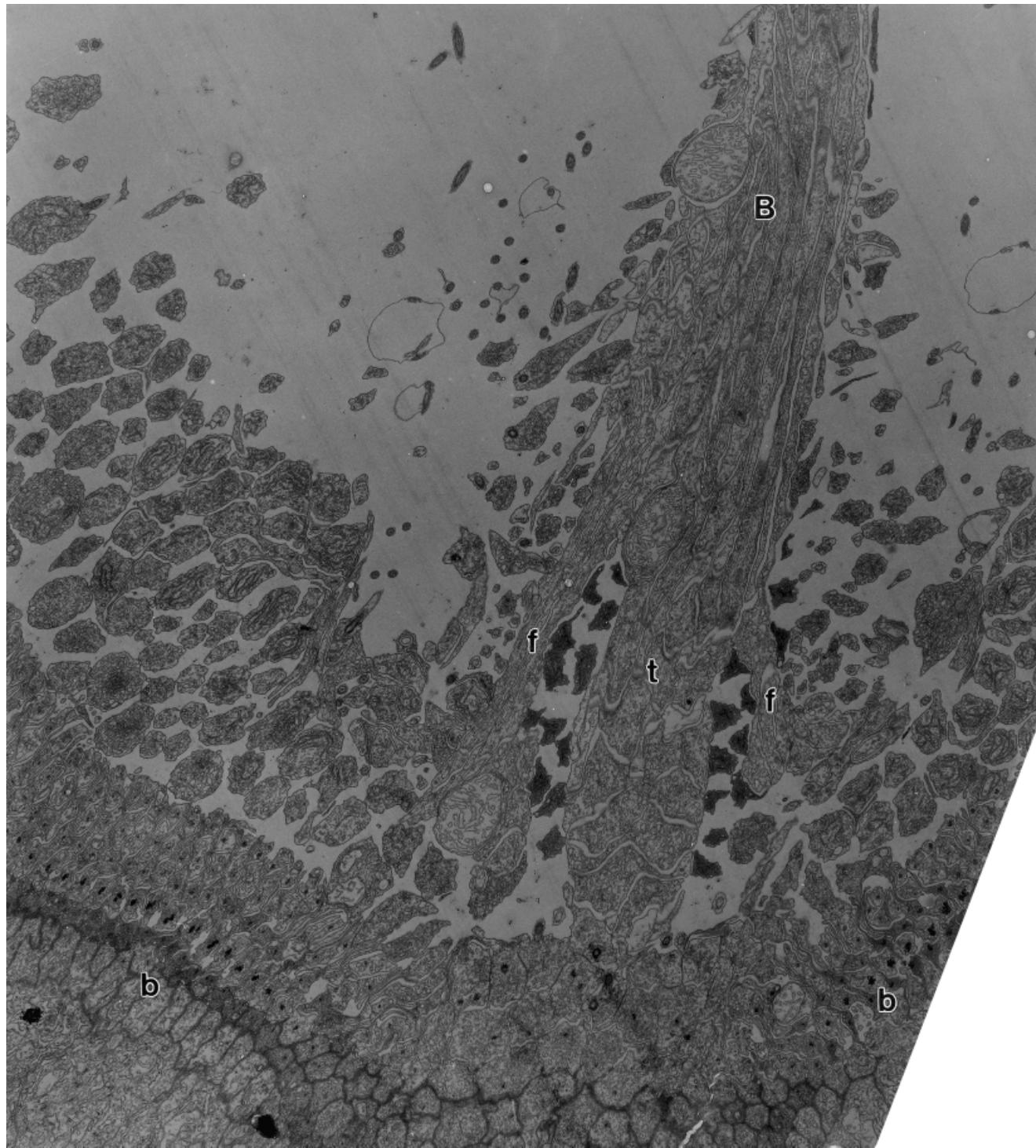


Fig. 9. Tangential section showing two forks (f) at one end of a bridge (B). The medial trunk (t) coming from the bridge cells and the two forks are studded with dense apical protrusions of dark cells bordering the inner side of the bridge cell group (see Fig. 5). The two branches consist of processes that diverge from the main trunk and run toward the bases of the adjacent pair of balancers (b). The section cuts the apical projections of balancer cells below the ciliary basal bodies, at the level of the dense striated rootlets. TEM section through adult *Pleurobrachia* (see Fig. 2 for orientation).  $\times 12,210$ .

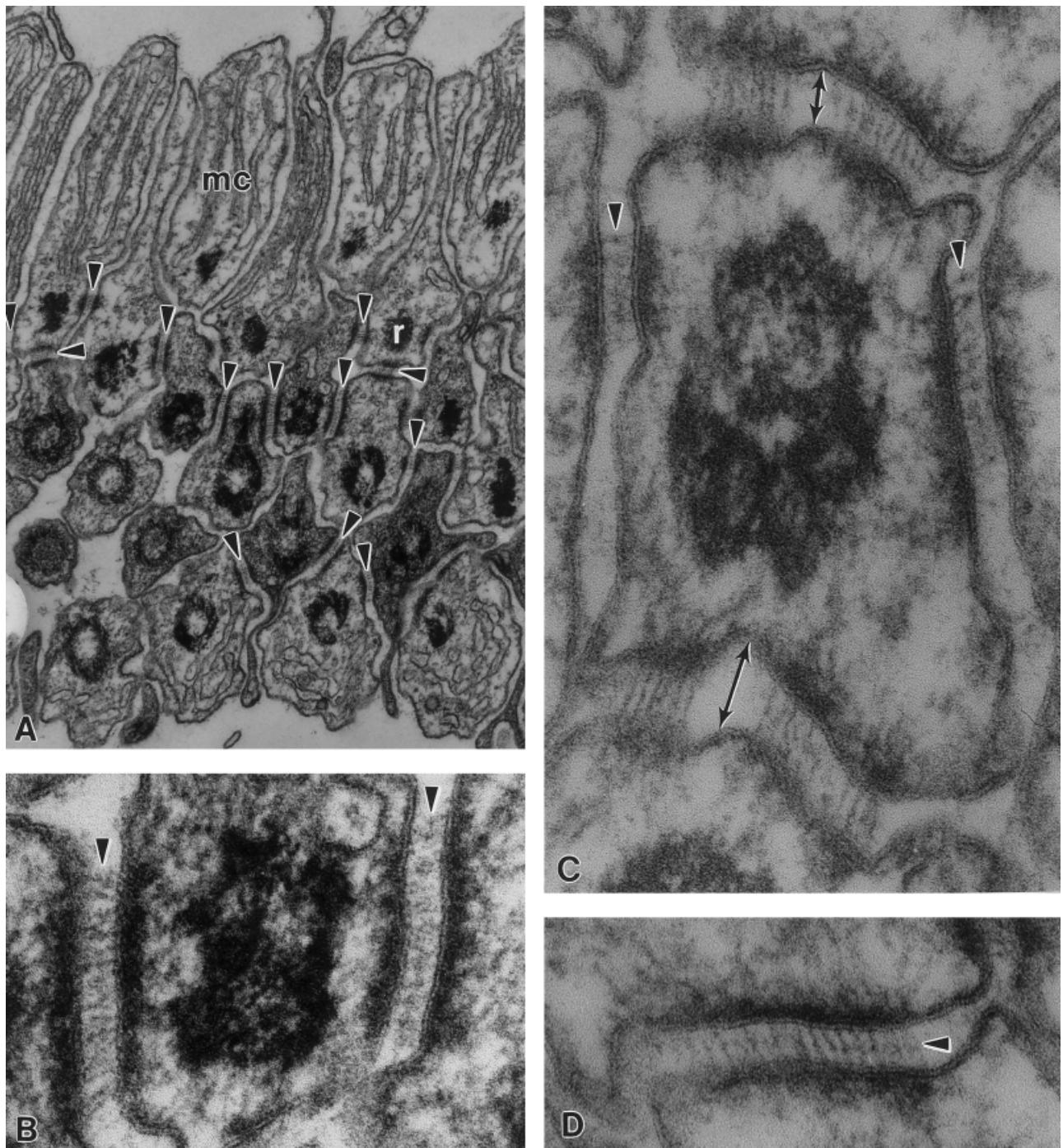


Fig. 10. **A:** Transverse section through six rows of apical projections of cells in the base of a balancer. The projections are cut at different proximal-distal levels, showing a single basal body, dense striated rootlet (**r**), or transitions between. Projections on the inner side of the base (upper) are sectioned more proximally; the first and second rows have narrow orthogonal extensions from the rootlet axis that are directed toward the bridge fork and contain stacks of flattened membranous cisternae (**mc**) oriented parallel to the extensions. On the outer side of the base (lower), only the first row of projections has extensions, which face in the opposite direction and contain an irregular array of membranous cisternae. The second row of projections on the outer side has denser cytoplasm. Intercellular junctions (arrowheads) connect the projections at the basal body-to-rootlet level. **B:** Enlargement of central region in **A**. **B-D:** Apposed junctional membranes are flat, have a dense cytoplasmic coat, and are connected by 2–4 nm thick parallel strands, ~25 nm long and spaced 8–10 nm apart. Dense thickenings midway along the strands lie in register. **C:** Strands between these projections are longer along one axis (doubleheaded arrows), accompanied by increased intermembrane distance. TEM sections through adult *Pleurobrachia* (see Fig. 2 for orientation). **A**,  $\times 40,000$ ; **B,C**,  $\times 200,000$ ; **D**,  $\times 201,740$ .

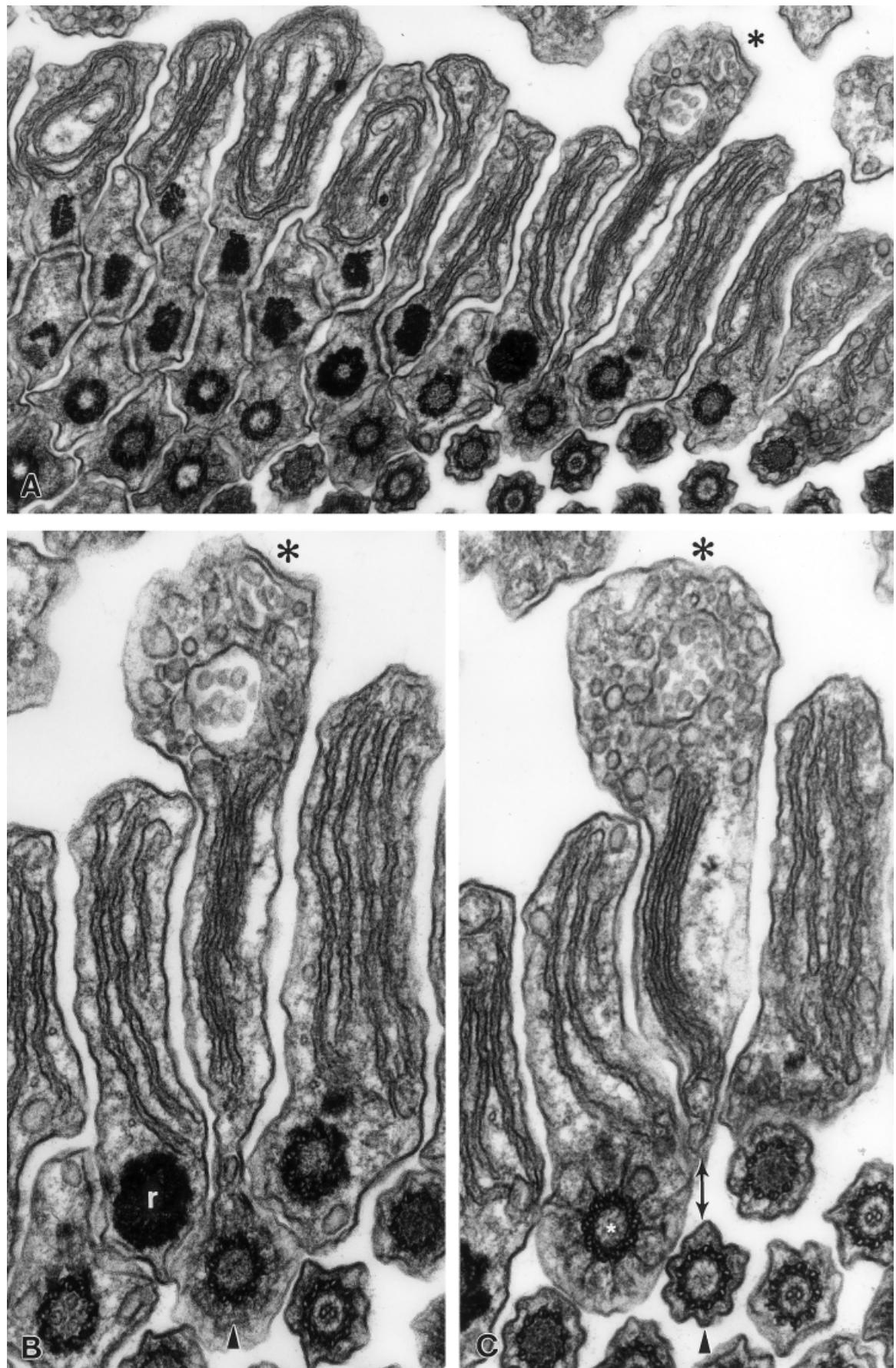


Figure 11

from cisternae near the rootlet axis to vesicles is evident in longitudinal sections through extensions (Fig. 12).

Widenings of the balancer extensions containing membranous cisternae and/or vesicles are commonly directed toward the nearby processes of a bridge fork (Figs. 11, 13). Isolated membrane-bound bodies filled with membranous cisternae and vesicles, which are abundant between a balancer base and bridge fork (Figs. 13, 14), undoubtedly represent the widened ends of balancer extensions protruding out of the plane of section.

Although it seems likely, we cannot be sure that the isolated processes containing presynaptic triads polarized towards a bridge fork (Fig. 13) also belong to balancer cell extensions. This complex architecture prevents clear tracing of connections between balancer extensions and bridge fork processes by nonserial section TEM.

At the deeper level of the belt desmosomes, isolated processes containing presynaptic triads, vesicles, and microtubules, but not membranous cisternae, are found closely interdigitating with proximal regions of the inner row of balancer cell projections (as identified by their rootlet remnants) (Figs. 15, 16). These neurite-like processes lie close to bridge fork processes (identified by their surrounding dark cell protrusions) in the same thin section. Although direct continuity has not been established, it seems likely that the neurite-like endings are terminations of bridge fork processes onto the base of a balancer, since 1) they contain microtubules oriented parallel to those in bridge fork processes (but 90° to microtubules in balancer cell projections), and 2) they lack membranous cisternae typical of balancer cell extensions (Figs. 15, 16).



Fig. 11. A: Transverse section through rows of apical projections in the base of another balancer. The flange-like extensions from projections of the first and second rows on the inner side of the base (upper) contain flattened membranous cisternae stacked side-by-side (right) or coiled concentrically (left). An extension from the second row (asterisk) is very thin near the basal body-cilium transitional region, but is enlarged and filled with vesicles at the other end facing the bridge fork. B: Enlargement of extension marked in A, with its thin connection to the basal body/cilium transitional region (arrowhead). The 50–60 nm wide lumen of the cisternae contains a dense medial layer of particulate material. The widened end of the middle extension contains vesicles of different shapes and sizes and a larger vesicle enclosing smaller ones. C: More distal section through the same extensions. The middle extension no longer appears connected to its basal body / rootlet origin (doubleheaded arrow), which now shows the beginning of the cilium (arrowhead). The enlarged end of the middle extension is wider and contains more vesicles next to the termini of the membranous cisternae. Note that in the left extension, the rootlet (r) in B is now a basal body with radial transitional fibers in C (white star). TEM sections through adult *Pleurobrachia* (see Fig. 2 for orientation). A,  $\times 37,870$ ; B,C,  $\times 67,500$ .

### Light Microscopy of the Bridge in *Mnemiopsis* Larvae

Free-swimming cydippid larvae of *Mnemiopsis* have two tentacles and resemble adult ctenophores of the order Cydippida (i.e., *Pleurobrachia*). The statocyst and comb plates of larvae are disproportionately large relative to their body size ( $\sim 0.5$  mm) (Tamm and Tamm, 1981; Nakamura and Tamm, 1985).

DIC side views of larval apical organs parallel to the tentacular plane show a conspicuous bridge, 8–10  $\mu\text{m}$  long, arching over the epithelial floor between pairs of balancers (Fig. 17). Aboral views in which the statolith is pushed out of the way confirm that the bridge runs in the tentacular plane. However, the larval bridge has not yet developed forks at its ends, as seen in adult *Mnemiopsis* bridges (Fig. 3B).

### Electron Microscopy of the Bridge in *Mnemiopsis* Larvae

Longitudinal sections through the larval apical organ in the tentacular plane show an  $\sim 10 \mu\text{m}$  long curved bridge of thin, microtubule-containing processes extending over the epithelial floor from cells at either side (Fig. 18). The spacing between the processes appears uniform and convoluted, unlike the variable distances between straight processes in the bridge of adult *Pleurobrachia* (Fig. 6). Transverse sections show that the larval bridge consists of 15–20 processes containing microtubules running parallel to their long axes.

Synaptic contacts onto the bases of bridge cells are particularly evident in larvae, due to the smaller size and fewer number of cells in the epithelial floor of their apical organ. In addition, *Mnemiopsis* larvae reveal that bridge cells may synapse onto other cells (Fig. 19), a feature not noticed in adult *Pleurobrachia*. In Figure 19, a bridge cell receives synapses from an adjoining cell and in turn makes synapses onto another cell.

Tangential sections show that each balancer at this stage consists of  $\sim 25$  cilia arranged in five rows in a rectangular base. The apical projections of the balancer cells in *Mnemiopsis* larvae do not form lateral extensions or contain membranous cisternae and vesicles, as seen in adult *Pleurobrachia* (above). Nor does the larval bridge have forked branches between the bases of balancer pairs. In *Mnemiopsis* larvae, therefore, TEMs confirm DIC images of an unforked bridge.

## DISCUSSION

We discovered a previously undescribed structure in the aboral sense organ of ctenophores that looks like a new type of conducting pathway for regulating

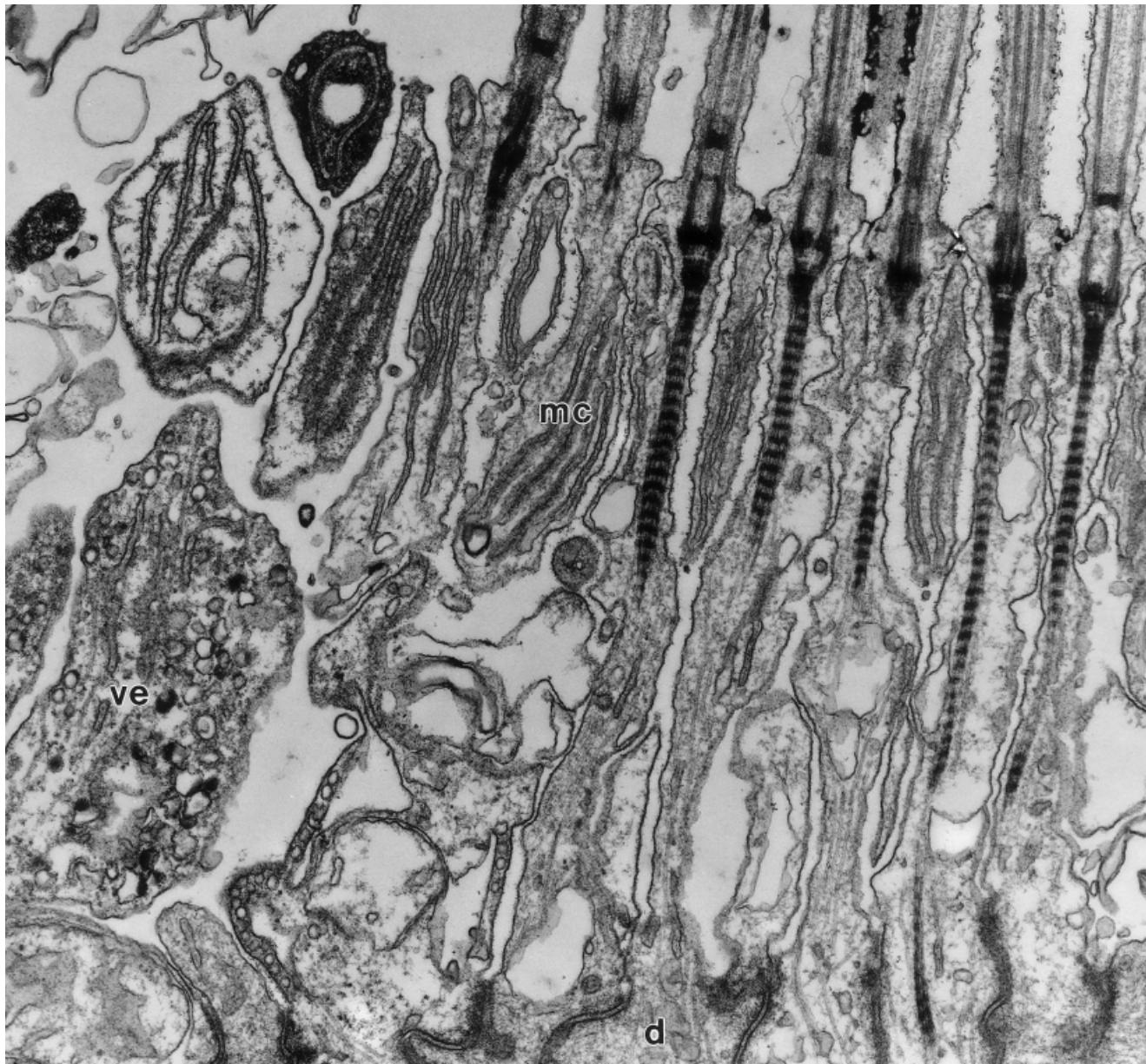


Fig. 12. Longitudinal section of apical projections in the base of a balancer. Basal bodies giving rise to cilia are at the tips of the projections and their striated rootlets extend 8–10  $\mu\text{m}$  proximally to the zone of belt desmosomes (d). Longitudinal extensions of the projections, here cut almost transversely, contain flattened membranous cisternae (mc) distally and numerous vesicles (ve) proximally. TEM section through adult *Pleurobrachia* (see Fig. 2 for orientation).  $\times 31,250$ .

swimming behavior of these animals. The novel structure is a bundle of thin, microtubule-filled processes that arise from the apical ends of two groups of epithelial cells on opposite sides of the apical organ floor along the tentacular plane. The bundle arches over the epithelial floor like a bridge and appears to connect opposing pairs of pacemaker balancer cilia across the sagittal plane.

The reason why the bridge was not described by previous investigators (Chun, 1880; Hertwig, 1880; Heider, 1927; Horridge, 1965; Hernandez-Nicaise,

1991), including ourselves (Tamm, 1982), is probably due to tissue geometry. The refractile statolith completely masks the underlying bridge in views of the apical organ from the aboral surface of the animal. For this reason, we routinely remove the statolith with a suction pipette to obtain light microscopic images of the bridge (Fig. 3). The bridge is also not visible in lateral views of dissected apical organs from adult ctenophores due to the thick walls of the concave epithelial floor and surrounding mesoglea. In cydippid larvae, however, the bridge is

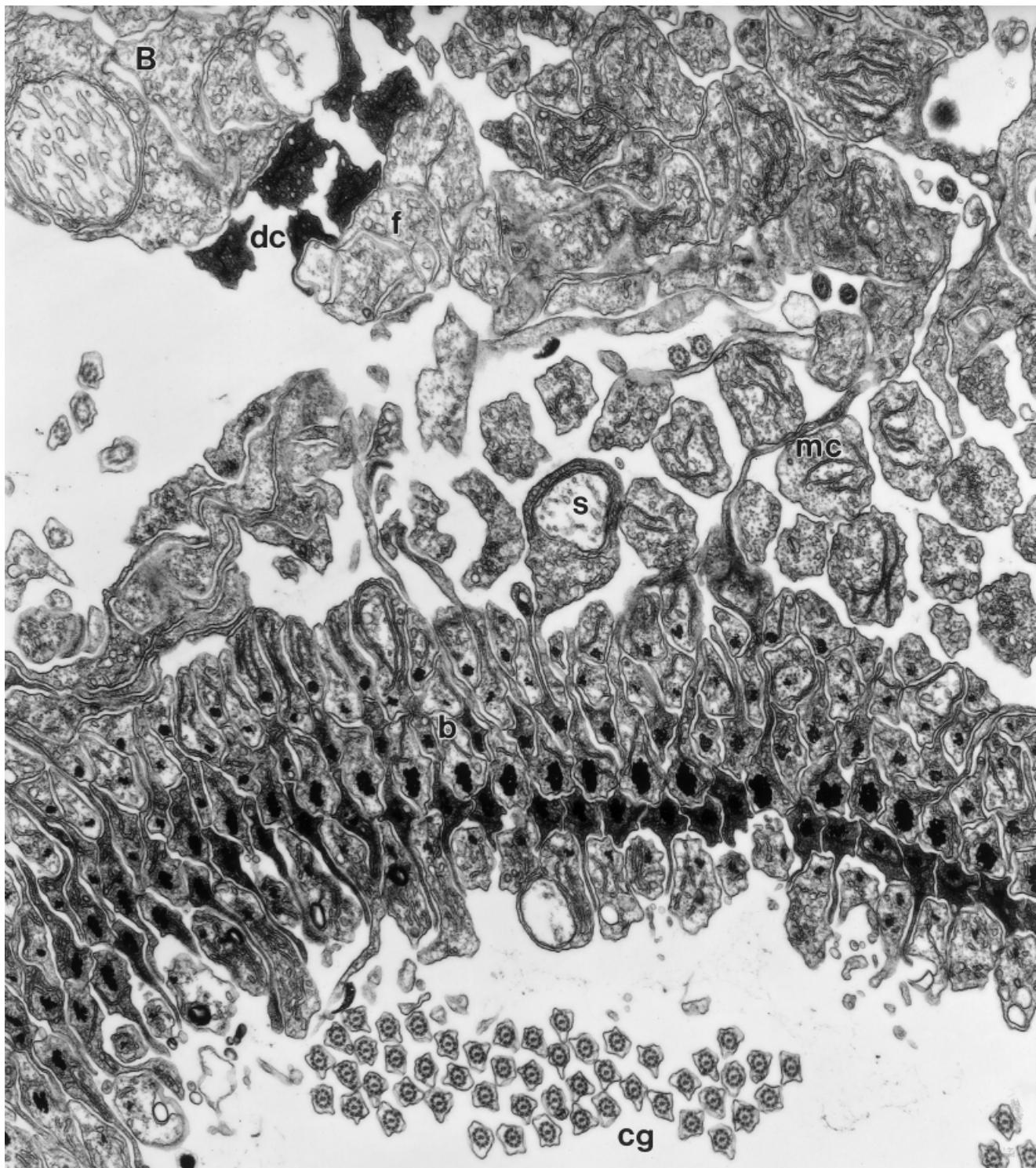


Fig. 13. Transverse section through six rows of apical projections near the center of the V-shaped base of a balancer (b). The section is below (proximal to) the basal bodies and shows the dense striated rootlets at different levels, as indicated by their cross-sectional width. On the inner side of the base (upper), a presynaptic triad structure (s) is present in the widened end of what appears to be an extension from a balancer cell projection in the second row, except that no identifying ciliary rootlet is present in the narrowed end. The presynaptic triad faces the fork (f) of the nearby bridge (B), as identified by surrounding dark cell protrusions (dc). Note numerous membrane-bound bodies containing membranous cisternae (mc) and/or vesicles, evidently connected to extensions of apical projections in more distal or proximal sections. Ciliated groove cilia (cg) lie next to the outer side of the balancer (lower). Note the dense cytoplasm of the second row of apical projections on this side. TEM section through adult *Pleurobrachia* (see Fig. 2 for orientation).  $\times 17,460$ .

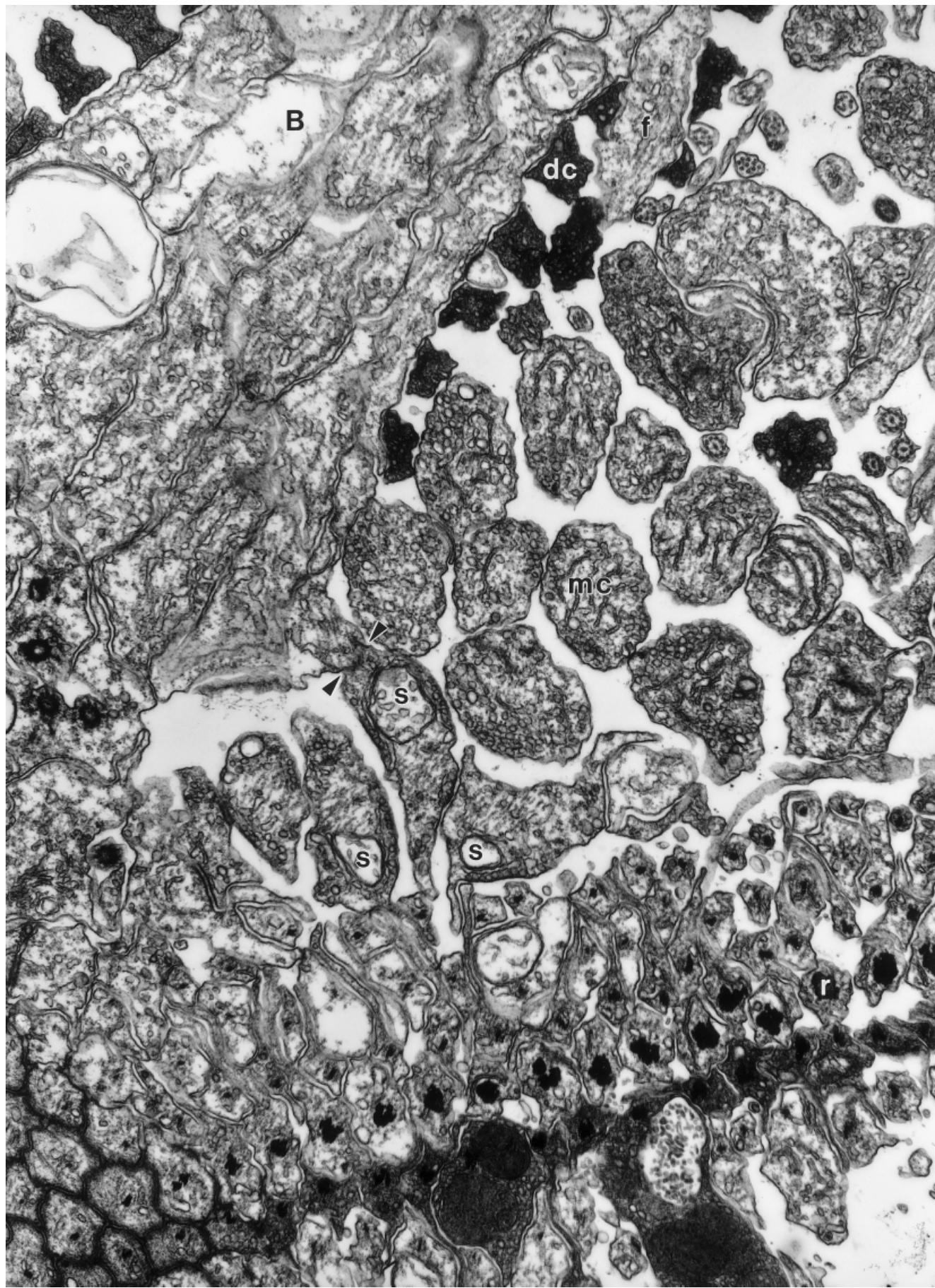


Figure 14

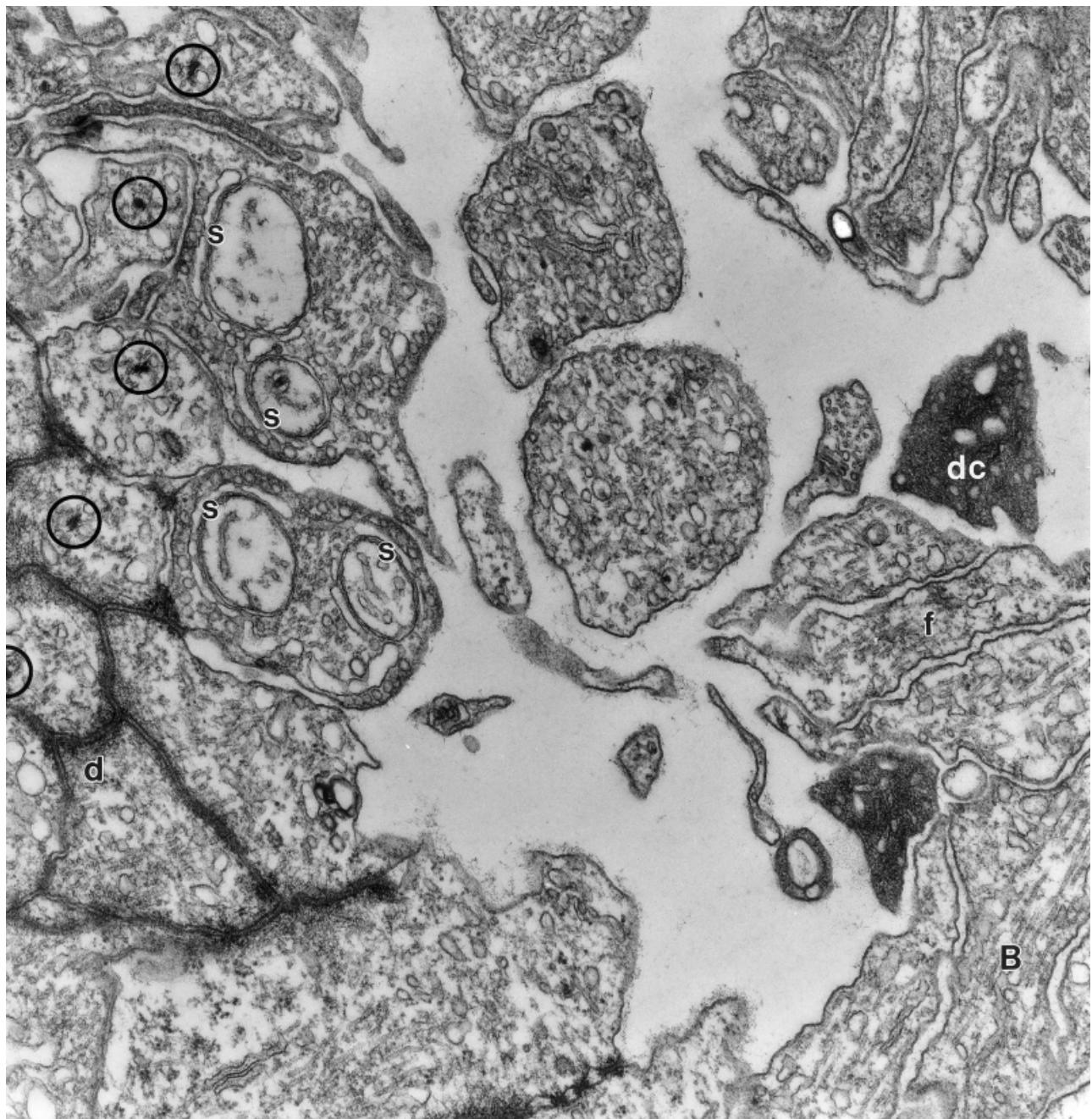


Fig. 15. Higher resolution transverse section deeper through apical projections of a balancer base at the level of termination of striated rootlets (circled) and web of belt desmosomes (d). Processes with presynaptic triads (s) lie close to processes of a fork (f) of the bridge (B) and are closely applied to the apical projections of balancer cells. dc, dark cell protrusions. TEM section through adult *Pleurobrachia* (see Fig. 2 for orientation).  $\times 37,620$ .

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Fig. 14. Transverse section through rows of apical projections with striated rootlets (r) in the base of a balancer (lower right). Membrane-bound bodies containing a variety of presynaptic triads (s), membranous cisternae (mc), vesicles, and/or microtubules are found between the inner side of the balancer base and nearby bridge (B). Note close juxtaposition (or perhaps continuity in adjacent sections) between a process with a presynaptic triad and the bridge (arrowheads). Processes with presynaptic triads and vesicles contain numerous microtubules oriented similarly to those in bridge fork processes. TEM section through adult *Pleurobrachia* (see Fig. 2 for orientation).  $\times 22,150$ .

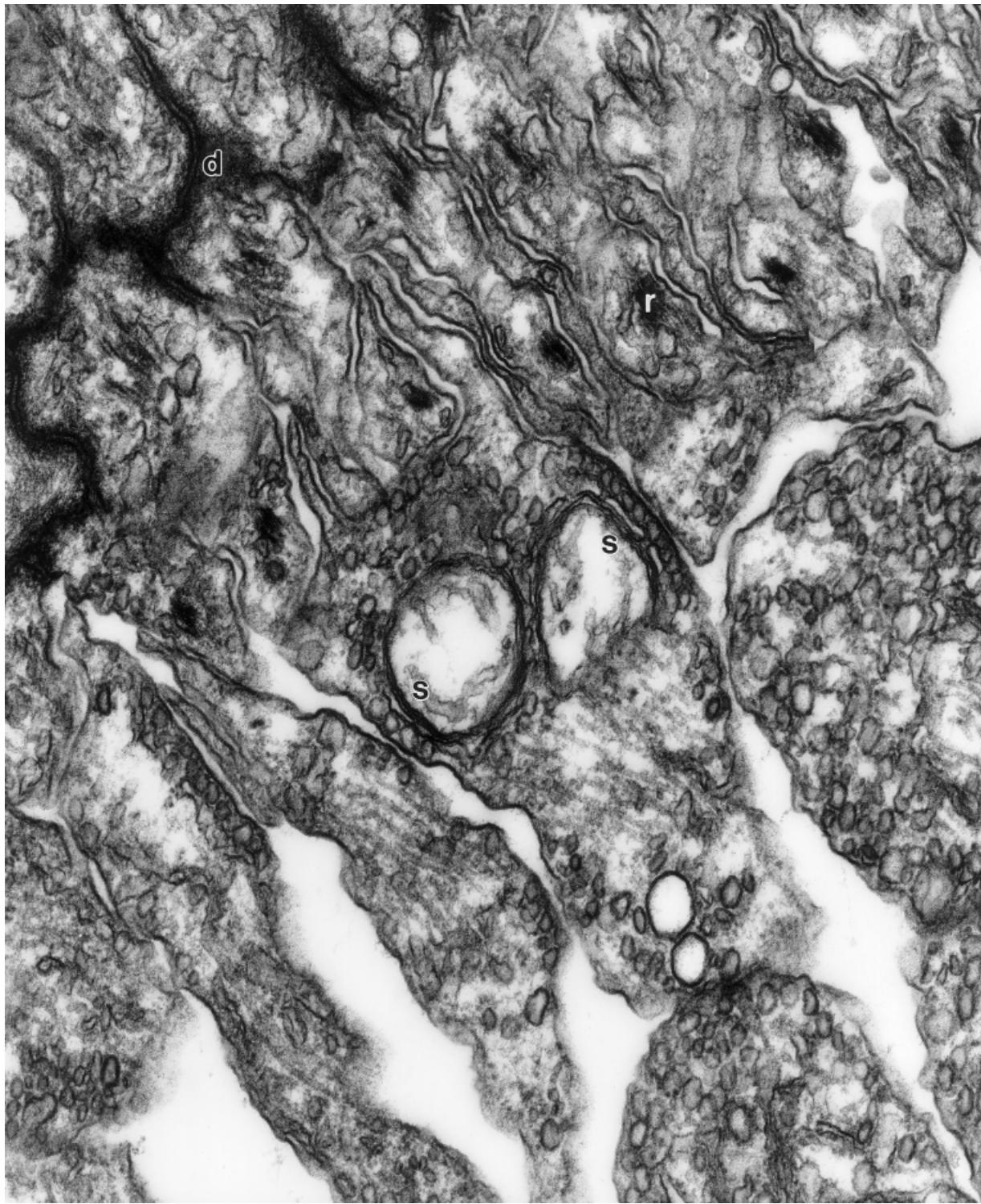


Fig. 16. Section at a similar level showing processes with presynaptic triads (s) and vesicles interdigitating with, and apparently synapsing onto, apical projections of balancer cells with rootlets (r). Microtubules in the processes are oriented similarly to those in bridge fork processes (see Fig. 15). d, belt desmosomes. TEM section through adult *Pleurobrachia* (see Fig. 2 for orientation).  $\times 56,090$ .

readily visible in DIC side views of the apical organ, but only in larvae lying in the tentacular plane (Fig. 17). Earlier investigators apparently did not per-

form careful microscopy of ctenophore larvae immobilized in different orientations on slides. Because of its relatively small size and restriction to the ten-

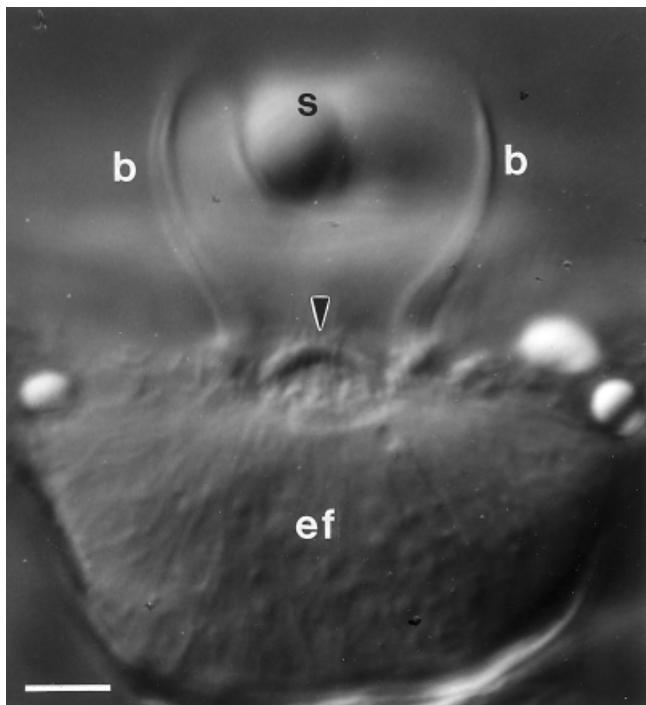


Fig. 17. DIC side view of the apical organ of a *Mnemiopsis* cydippid larva immobilized in the tentacular plane. A conspicuous bridge (arrowhead) arches over the epithelial floor (ef) between the bases of the sickle-shaped balancers (b). Several statolith cells (s) are attached to the tips of the balancers. Scale bar = 5  $\mu\text{m}$ .

tacular plane, the bridge would also be easily overlooked in randomly oriented histological or TEM sections through the apical organ of adult ctenophores. We first noticed the bridge by accident, after removing the statolith to do experiments on mechanical responses of unloaded balancers in *Pleurobrachia*.

The bridge processes are less than a micron thick and contain numerous microtubules running parallel to their long axes, as well as clear vesicles of different sizes. In size and morphology, these extensions resemble axonic/dendritic processes (neurites) in the nerve net of ctenophores (Hernandez-Nicaise, 1973, 1991). However, the bridge processes do not extend from nerve cell bodies, but from epithelial cells. Mitochondria are absent in the processes themselves, but are densely packed in the apical ends of the epithelial cells.

The number of processes in the bridge ( $\sim 60$ ) is about twice the number of epithelial cells ( $\sim 30$ ) in each group giving rise to them. Since each epithelial cell contributes a single process which appears to extend across the bridge, the extensions cannot be cytoplasmically continuous between cells in the two groups. Instead, processes from cells on one side must terminate outside the bridge on the opposite side. We assume that they do so by diverging into

the two branches directed toward a pair of balancers on each side. According to our model of bridge connectivity, the  $\sim 30$  processes from bridge cells on one side branch into two forks of  $\sim 15$  processes each on the opposite side, and these forks contact the bases of the nearby pair of balancers (Fig. 20).

Whether or how bridge forks and balancer cells make contacts with one another has not been clearly resolved by the present TEM study, due to the complex 3D architecture of this region. Presynaptic triad structures and vesicles characteristic of ctenophore nerves and sensory cells (Tamm, 1982; Hernandez-Nicaise, 1991) are certainly associated with both bridge fork processes and balancer cell projections. But unambiguous examples of polarized synapses between the two have not yet been found. It should be noted that ctenophore nerves are isopolar, in that any part of the neuronal membrane can make synapses onto other neurites or target cells (Hernandez-Nicaise, 1973).

At least three types of synaptic polarity between bridge forks and balancer cells are possible: 1) bridge fork processes synapse onto balancer cell projections, 2) balancer cell projections synapse onto bridge fork processes, or 3) both make synaptic contacts with one another. The third possibility seems the most likely from our images so far (Figs. 14–16). Future studies using fluorescent membrane tracers and serial section TEM reconstructions should help clarify the anatomical connections of the bridge.

The most intriguing question about the bridge is, why does this novel structure exist? What does it do, and what is its function? The resemblance of the bridge processes to neurites and their probable synapses onto balancer cells suggests that the bridge may serve as an electrical conduction pathway that regulates certain swimming responses of ctenophores by coordinating beating of pacemaker balancer cilia across the sagittal plane. For example, the bridge cells are innervated (Fig. 7), so neural input to the bases of one group of bridge cells might elicit depolarizing potentials that spread distally and trigger regenerative potentials in the apical processes of the cells. Excitable bridge processes might then conduct the action potentials across the bridge to the pair of balancers on the opposite sagittal side via synapses of bridge branches onto the bases of balancer cells. Depolarization of balancer cells would be expected to cause an increase in beat frequency of balancer cilia, leading to a specific locomotory response. We showed previously that beating of a balancer can be excited not only by its deflection in a specific direction by the statolith (or artificial mechanical stimulus), but also by chemical depolarization of isolated balancer cells (Lowe, 1997), or by extracellular electrical stimulation of the entire apical organ (Tamm, 1982). In all cases, transmembrane calcium influx is required for excitation of balancer beating (Lowe, 1997), indicating that me-

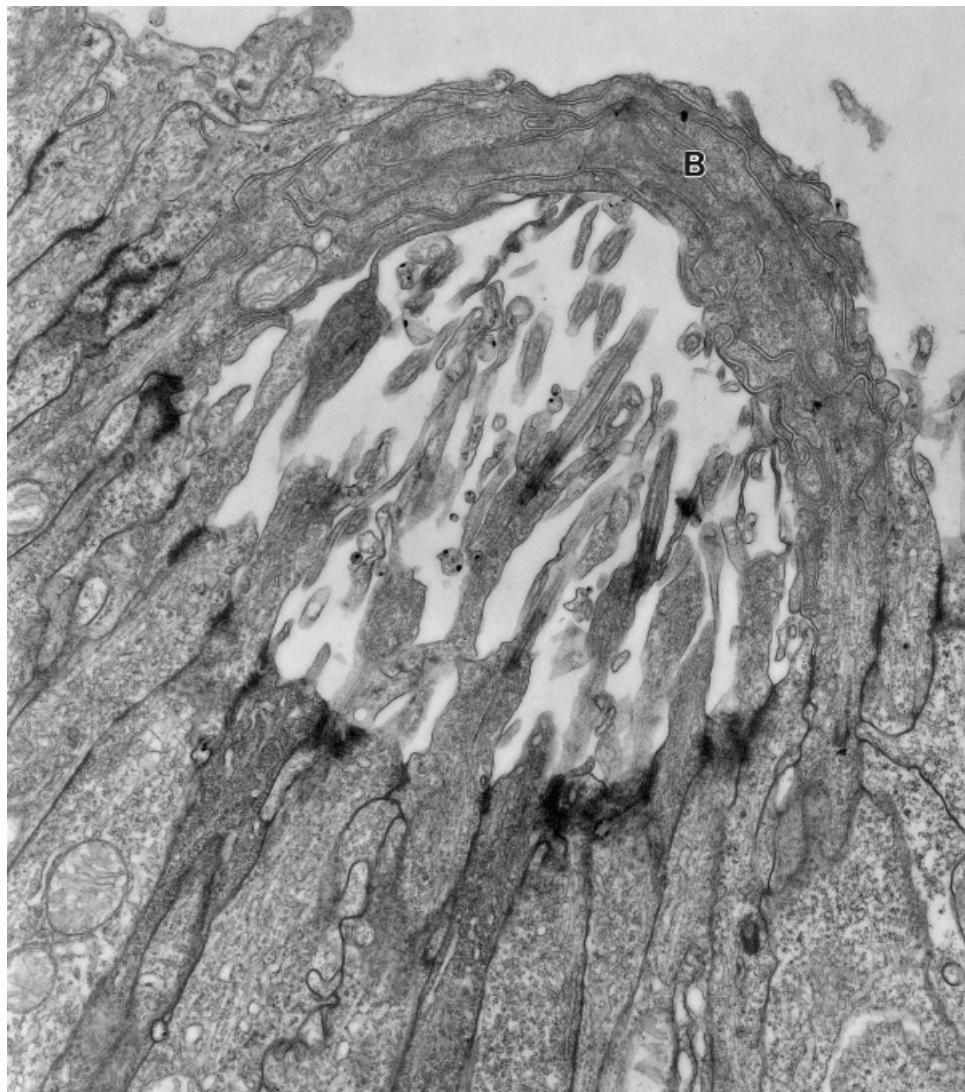


Fig. 18. Longitudinal section in the tentacular plane through the apical organ of a *Mnemiopsis* larva. The bridge (B) of thin microtubule-filled processes arches over the epithelial floor. Note the convoluted, uniform spacing between the processes. The narrowed ends of the epithelial cells are joined by a web of belt desmosomes. Irregular apical protrusions, some bearing single cilia, extend under the bridge.  $\times 15,360$ .

chanically activated and/or voltage-gated calcium channels regulate beat frequency of balancers (see Tamm, 1994). Comb plate cilia also respond to calcium, but by beating in the reverse direction at high frequency (Moss and Tamm, 1986, 1987; Tamm and Terasaki, 1994).

Since the bridge runs in the tentacular plane, it is appropriately oriented to transmit stimulus-evoked signals from a tentacle on one side to the balancer pair on the opposite sagittal hemisphere. A behavioral response that clearly requires such a bilateral conducting pathway across the sagittal plane is global ciliary excitation elicited by tentacle stimulation (Tamm, 1982). Mechanical stimulation of (or prey capture by) either outstretched tentacle of a “fishing” adult *Pleurobrachia* causes a burst of rapid

beating of all eight comb rows, resulting in fast forward swimming for several seconds (Tamm, 1982; Tamm and Moss, 1985). This global ciliary excitation in response to tentacle stimulation also occurs in cydippid larvae of *Mnemiopsis* and other ctenophores. Beroid ctenophores lack tentacles, even as larvae, and do not have a bridge, consistent with the view that bridge function is related to tentacular responses. Previous extirpation experiments on the apical organ of *Pleurobrachia* showed that an intact apical organ is necessary for the spread of tentacle-evoked ciliary excitation across the sagittal plane to the four comb rows on the unstimulated side (Tamm, 1982). Discovery of the bridge, therefore, provides an ideal morphological candidate for transmission of the signal for global ciliary excitation.

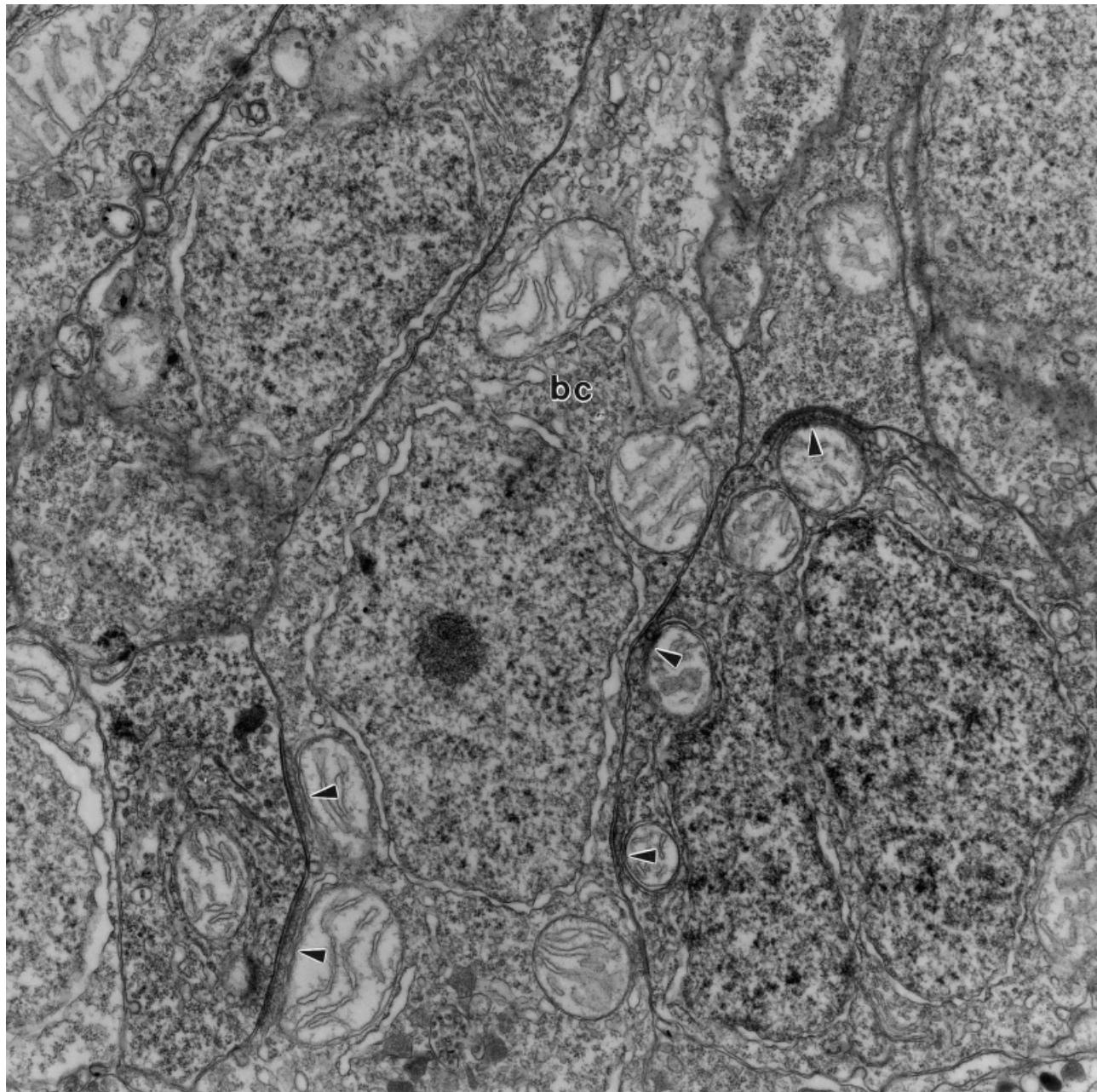


Fig. 19. The basal end of a bridge cell (bc) in a *Mnemiopsis* larva receives synapses from an unidentified adjacent cell (arrowheads on presynaptic side, right) and itself makes synapses onto another cell (arrowheads, left).  $\times 22,480$ .

Whether or not the bridge is indeed an electrical conduction pathway to balancers that mediates global ciliary excitation can be tested by microsurgically cutting the bridge and by electrically stimulating and recording from the bridge itself. The possible function of presumed synapses between bridge forks and balancer cells in the opposite direction, i.e., from balancer cell extensions to bridge forks, is less obvious, since it is not at all clear what excitation of bridge cells might effect.

If future experiments show that bridge processes are electrically excitable and conduct action potentials, this would be the first case of epithelial cells with axons. Propagation of action potentials in the membranes of nonnervous, nonmuscular cells, termed neuroid, nonnervous or epithelial conduction (Mackie, 1970; Spencer, 1974; Anderson, 1980), occurs in a wide variety of protists, plants, and animals, including amphibian tadpoles and mammalian pancreatic islets. Transmission of signals occurs

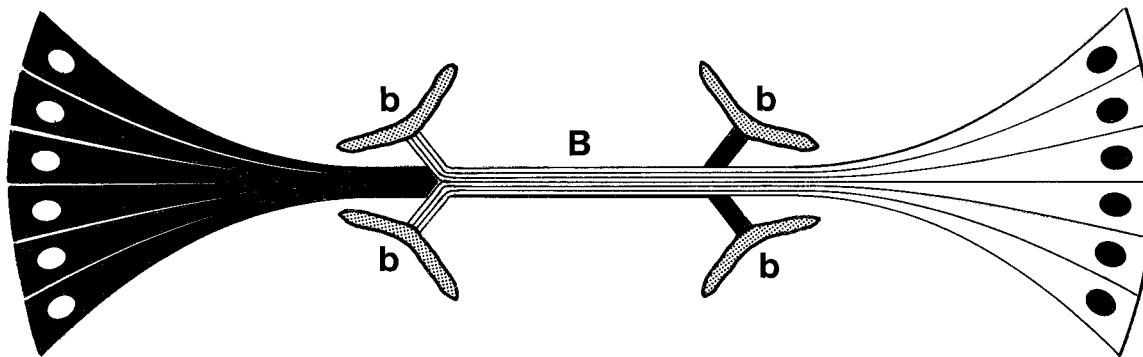


Fig. 20. Model of bridge-balancer connections in the ctenophore apical organ. The tentacular plane is horizontal and normal to the page. The two groups of epithelial cells giving rise to the bridge (B) are depicted in black and white on opposite sides of the epithelial floor (for clarity, only six cells per group are shown instead of ~30). Processes from cells on opposite sides are drawn segregated into two layers in the main trunk of the bridge (white over black), instead of mixed, as is probably the case. In this simplified model, six processes from six bridge cells on one side split into two forks of three processes each on the opposite side to contact the bases of a nearby pair of balancers (b).

through electrically coupled (by gap junctions), electrically excitable cells, typically in all directions. The ctenophore bridge of directionally polarized epithelial cell extensions, if axon-like in function as well as structure, would be an intriguing intermediate between diffuse-conducting epithelial sheets and specific tracts of neurons—perhaps “the long-sought-for missing link between excitable epithelia and true nerves” (Mackie, 1989). Future studies of the bridge may thus advance understanding of the diversity of electrical conducting pathways in animals and evolution of the first nervous systems.

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