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Ultrastructure and organization of the epineural canal and the nerve cord in sea urchins (Echinodermata, Echinoida)

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Summary. The radial nerve cord of *Mespilia globulus* has been examined as an example of echinoid nerve cords. In the radius of echinoids only the ectoneural component of the nerve cord is present which is a derivative of the ectoderm. The nerve cord runs in the interior of the body and is accompanied by the epineural canal. In echinoids, the neuroepithelium makes up the upper and side walls of the epineural canal. Each lateral branch of the nerve cord forms a sort of neural tube. It encloses a branch of the epineural canal which represents an open connection with the sea water. Thus, the epineural canal exhibits numerous openings which probably allow sea water to flow back and forth. This organization is unique in echinoderms. – The neuroepithelium exhibits the organization of an epidermis with well-developed nervous elements. Glial cells are not present. The support cells are the true epithelial cells. Their monociliated cell bodies border the lumen and, by means of cytoplasmic stems that contain a bundle of filaments, they reach up to the basal lamina. The nerve cells and their trunk of nerve fibres fill the spaces between the support cells. – Three types of nerve cells can be distinguished according to their polarity: (1) Primary sensory cells that project a cilium into the epineural canal, the axon hillock region is at the opposite pole. (2) Subluminal cells whose cilium originates in the axon hillock region. (3) Neurones that lie within the trunk of nerve fibres. They are highly stretched in the direction of the nerve cord and are also provided with a cilium. Types 2 and 3 may be homologized with the basal nerve cells of the epidermis. They are possibly multipolar. – The lateral nerve cords make contact with the ampulla and pass the ambulacral plate parallel to the channel that connects the ampulla and the tube foot. The activity of the tube foot-ampulla system is possibly controlled by means of transmitter substances that diffuse through the connective tissue layer between the nerve cord and the myoepithelia of the ampulla and the tube foot respectively.

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A. Introduction

Echinoderms differ radically from other coelomate metazoans by their five-fold symmetry which also includes the nervous system. Recently, several studies aimed for clarification of the neurophysiology of this unique system (for reviews see Cobb 1987, 1990). The present stage of knowledge regarding the histology and ultrastructure of the main nerve cords is a poor basis for these studies and the advanced hypotheses. Especially with respect to the morphology of the echinoid nerve cords little progress has been made since the admirable studies of Hamann (1887) and Cuénot (1891), reviewed by Hyman (1955), except for the scattered ultrastructural findings of Cobb (1970).

The present investigation examines the morphology of the radial nerve cord of *Mespilia globulus* by light and electron microscopy as an example of echinoid nerve cords. Progress in the histological investigation of echinoids was made possible by use of a double-embedding method (Märkel and Röser 1983). Since there is a relationship between the nerve cord and the water vascular system, our study includes the whole complex of radially arranged organ systems. A detailed description of the water vascular system will be presented in a seperate paper.

B. Materials and methods

Materials. Specimens of *Mespilia globulus* (Linné, 1758) were obtained from aquaria supply companies and kept for weeks in artificial sea water. They browsed on the small algae covering the stones and walls of the tank.

Methods. The test of the animal was broken. Small parts of the test, together with the organ complex belonging to the radii, were fixed for about 4 h in 5% glutaraldehyde in 0.05 M cacodylate buffer + 5 mM $CaCl_2$ (pH 7.8) followed by 1% osmium tetroxide for 2 h. $K_3Fe(CN)_6$ was added to the osmium solution to a final concentration of 0.8%. The fixed specimens were embedded in Araldite. The hardened blocks were cut in pieces convenient for the respective purposes by a fretsaw. The more delicate work was

done with emery paper to expose the calcite ossicles. Thereafter, the specimens were decalcified in EDTA and re-embedded in Araldite (Märkel and Röser 1983).

The specimens were cut transversally or horizontally. Series of semithin sections were made with glass knives, stained with crystal violet and mounted with Araldite. The slides were examined with a Zeiss microscope "Axiophot". Series of ultrathin sections were inserted in the series of semithin sections. They were made with a diamond knife and stained with uranyl acetate and lead citrate and examined with a Zeiss electron microscope EM9-S2.

The photographs of cross-cut sections were mounted with the radial water vessel above and the test down. This applies to semithin as well as ultrathin sections without exception.

C. Results

I. Gross anatomy of the radial nerve cord

The radial nerve cord is part of the complex of radially arranged canal-shaped organs (Fig. 1a) which consists of the radial water vessel (*W*), the haemal lacuna (*L*), the haematocoel (*H*) and the epineural canal (*E*). In echinoids, these organs are firmly attached to each other. They are covered with epithelium of the somatocoel (*SO*) which surrounds the whole complex; only towards the test does the epineural canal rest directly on the connective tissue of the skeleton (*SK*). Except for the haemal lacuna, the canals are all clothed with epithelia. The water vessel and the haematocoel are known to be coelomic channels, whereas the epineural canal is of ectodermal origin.

The radial nerve cord is part of the ectodermal epithelium which encloses the epineural canal. The neuroepithelium occupies the interior (*ms*) and the lateral walls (*ss*) of the epineural canal. A flat non-neuronal epithelium (*ep*) closes the canal towards the test. In most specimens the epineural canal is restricted to a horizontal cleft which on both sides delimits the side strips (*ss*) from the main strip (*ms*) of the radial nerve cord (Fig. 1b). For the present investigation a specimen with a widened epineural canal was used.

The radial nerve cord exhibits a bilateral symmetry. The main strip is thinner in the middle and thickened on both sides (Fig. 1a). The thickenings are maintained up to the terminal tentacle which is (in contrast to Hyman 1955) not to be compared with a podium. Moreover, there is a chain of nodose swellings on either side that give rise to the lateral branches of the nerve cord (*ln*). The swellings bulge towards the haematocoel. Horizontal sections (Fig. 1c) through the uppermost level of the nerve cord show a longitudinal gap in the middle and smaller holes on both sides that belong to the haematocoel (*H*). The following section (Fig. 1d) shows areas of neuropile (*arrows*) which lie near the bases of the lateral nerve cords. Finally, at a still deeper level (Fig. 1e), the side strips (*ss*) are cut which appear separated from the main strip (*ms*) by the epineural canal (*E*). The lateral nerve cords arise from the edge between the main and the side strip (Fig. 1b). For details of the lateral nerve cord see below.

II. The fine structure of the radial nerve cord

The radial nerve cord is a specialized neuroepithelium made up of support cells and nerve cells. The support cells may be considered to be the true epithelial cells for they extend without exception from the basal lamina up to the lumen of the epineural canal. Some nerve cells likewise touch the lumen of the canal but they do not touch the basal lamina.

1. The support cells. The support cells (Fig. 2a) consist of the cup-shaped cell body (*su*) that touches the lumen of the epineural canal, a slender stem (*st*) that penetrates the whole nerve cord and a proximal cytoplasmic sheet (*ps*) which is attached to the basal lamina. The proximal sheets of neighbouring cells overlap each other and totally cover the basal lamina to which they adhere by means of extracellular tufts (Fig. 2b, *arrow heads*).

The cell body (Fig. 2c) is monociliated. The cilium (*ci*) is provided with a short rootlet and protrudes into the epineural canal. A shallow ciliary pit is present. The nucleus is U-shaped and the position of the cilium is lateral within the open part of the U. The small Golgi complex is near the base of the cilium. Cytoplasmic protrusions extend irregularly into the epineural canal and spacious vacuoles are often found in the perinuclear cytoplasm (Fig. 2a). They contain residual material that possibly results from phagocytosis. A short adluminal sheet (*as*) spreads out of the perinuclear area. It partially covers the adjacent nerve cells and meets neighbouring cells (support or nerve cells) by apical junctional complexes (*small arrows*). The stem (*st*) arises from the bottom of the cell body. It measures about 0.4 µm in diameter or 1 µm when mitochondria and SER-cisternae are present (Fig. 3c). A conspicious feature of the stem is a bundle of intracellular filaments (*fb*) that reaches from the adluminal cytoplasmic sheet (Fig. 4a) up to the proximal sheet (Fig. 2b). The bundle exhibits a distinct striation with a period of 0.4–0.5 µm (Fig. 3c). The filaments are arranged in a cylinder of about 0.2 µm in diameter which encircles an afibrillar centre (Fig. 3b). The bundle is accompanied by microtubules (mt) which are clearly seen in sections that only touch the stem (Fig. 3d). In horizontally cut sections, the cross-cut stems are difficult to discern due to their small diameters (Fig. 3a, *arrows*). They are 2–10 µm apart.

2. The nerve cells. The spacious interstices between the stems of the support cells are densely populated with nerve fibres (Fig. 2a), most of which run lengthwise in the direction of the radial nerve cord. Scattered nerve cell bodies which are stretched lengthwise lie within the trunk of nerve fibres (Figs. 5b, 6a). The majority of the nerve cell bodies is nearly isodiametric and lies near the lumen of the epineural canal. The nerve cell bodies are arranged in 1–3 layers (Figs. 1b, 4a).

The nerve cells exhibit cytoplasmic differences. Few cells show a homogeneous cytoplasm of medium density with highly developed RER-cisternae. These cells are possibly nerve cells which are not yet differentiated. In

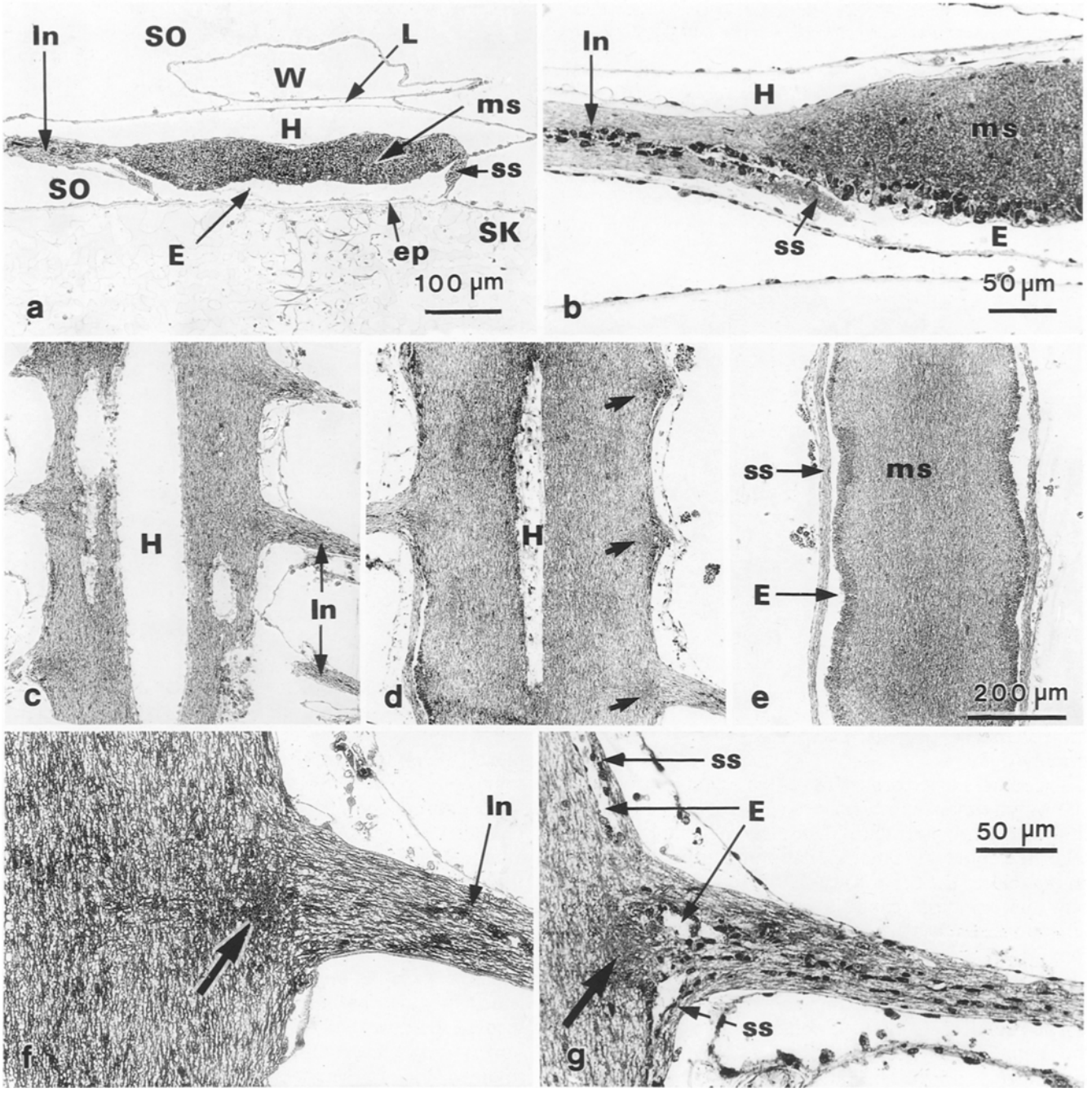

Fig. 1a–g. Semithin sections of the radial nerve cord: **a, b** Cross-sections, **a** shows the radial water vessel (*W*), the haemal lacuna (*L*), the haematocoel (*H*) and the epineural canal (*E*) which is closed towards the skeleton (*SK*) by an epithelium (*ep*). The nerve cord is subdivided into main strip (*ms*) and side strips (*ss*). **b** The origin of the lateral nerve cord (*ln*) enlarged. **c–e** Horizontally cut sections from different levels. **c** Section through the border to the haematocoel. **d** Areas of neuropile (*arrows*) at the bases of the lateral nerve cords. **e** Near the epineural canal the side strips appear separate. **f** Detail of **d**. **g** Section in the level intermediate between **d** and **e**. *SO* somatocoel

most instances (Fig. 4a, b) the cytoplasm of the nerve cells is less electron dense and contains clear and/or dense-core vesicles. Glycogen particles (*gl*) and vesicles which are probably non-neurogranular are likewise present. On the whole, the amount of perinuclear cytoplasm is small.

The neurones that form the adluminal layer of nerve cell bodies differ with respect to their polarity. In the first type (Fig. 5a), the cell body breaks through the adluminal sheets of the support cells and extends a cilium into the epineural canal. There is no ciliary pit, the cilium arising from a protrusion. The Golgi complex is close to the cilium, whereas the axonal process (*thick arrow*) arises from the opposite pole of the cell. Microtubules (*mt*) run from the base of the cilium into the axonal process. The latter runs into the trunk of the nerve cord

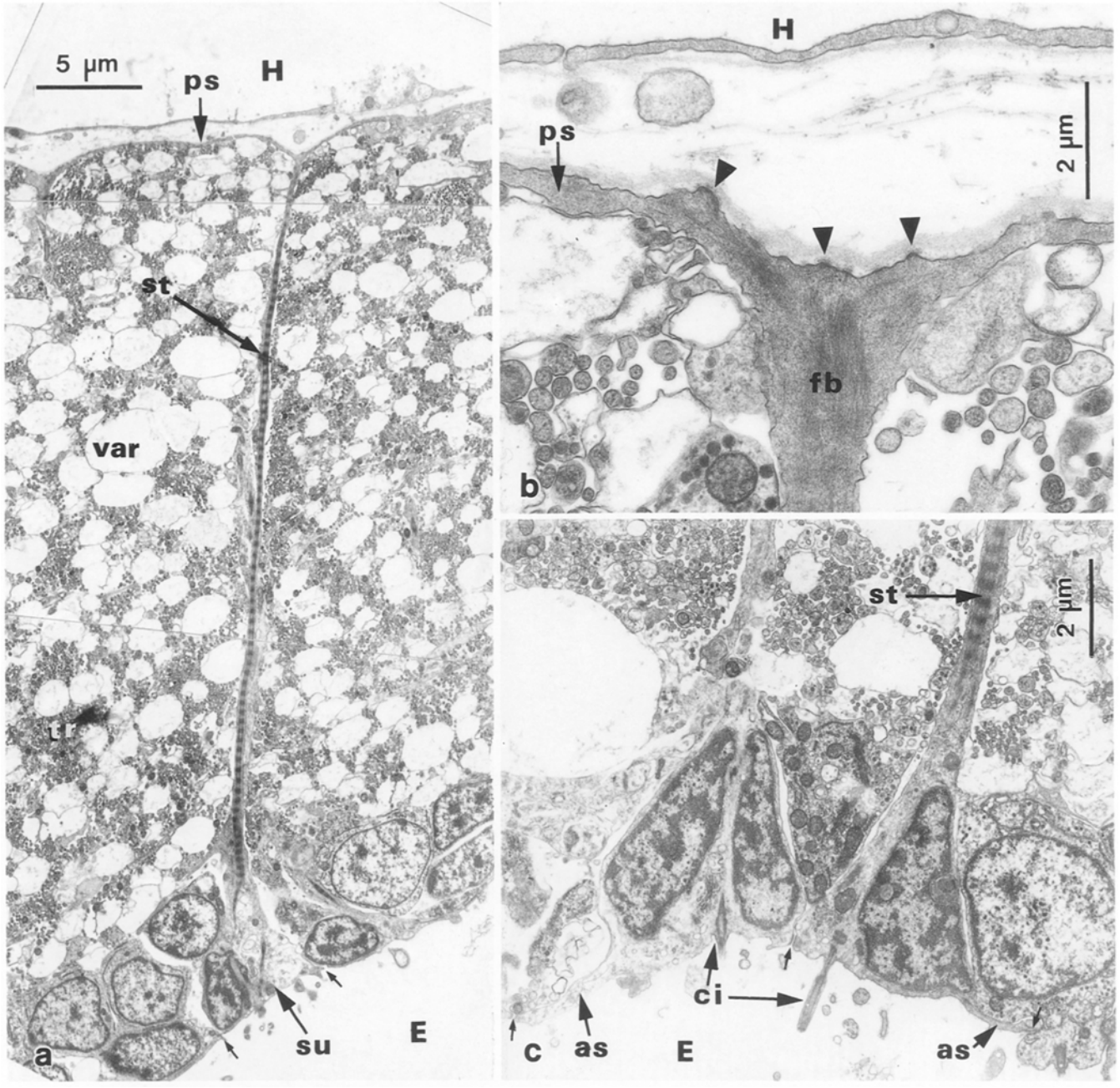

Fig. 2a–c. Support cells within a cross-section through the nerve cord: **a** Low-magnification photograph showing the cell body (*su*), the stem (*st*) and the proximal sheet (*ps*). The nerve fibres of the trunk are arranged in traces (*tr*) and contain varicosities (*var*). **b** Adhesion of the stem to the basal lamina by means of extracellular tufts (*arrow heads*). **c** Two cell bodies with cilia (*ci*). *Small arrows* indicate junctional complexes; *as* adluminal sheet; *fb* bundle of intracellular filaments

immediately. The ramification of the axonal process cannot be discerned. Immediately below the nuclear layer axons are often found that (in contrast to the nerve fibres of the trunk) run transversally (Fig. 4a). They come from a second type of nerve cell body that do not touch the lumen but are covered with the adluminal sheets of support cells. From the cell body labelled *X* the axonal process (*thick arrows*) runs towards the stem of the support cell. It penetrates into the trunk parallel to the stem. In contrast to the neurones with a cilium extending into the epineural canal, the second type of neurone has the Golgi complex and the basal body lying close to the axon hillock region (Fig. 4b, *arrow*). The basal body is probably associated with a cilium. Cilia are often found in the layer of neuronal cell bodies located below the lumen (Fig. 5c, *arrow*) and even in the nerve cells that lie deeply within the nerve cord (Fig. 5b, *arrow*). The cilia lie parallel to the nerve fibres and are easily overlooked.

The nerve fibres (Cobb: axons) within the trunk measure 70–300 nm in diameter. They contain microtubules, dense, dense-core or clear vesicles (diameter about

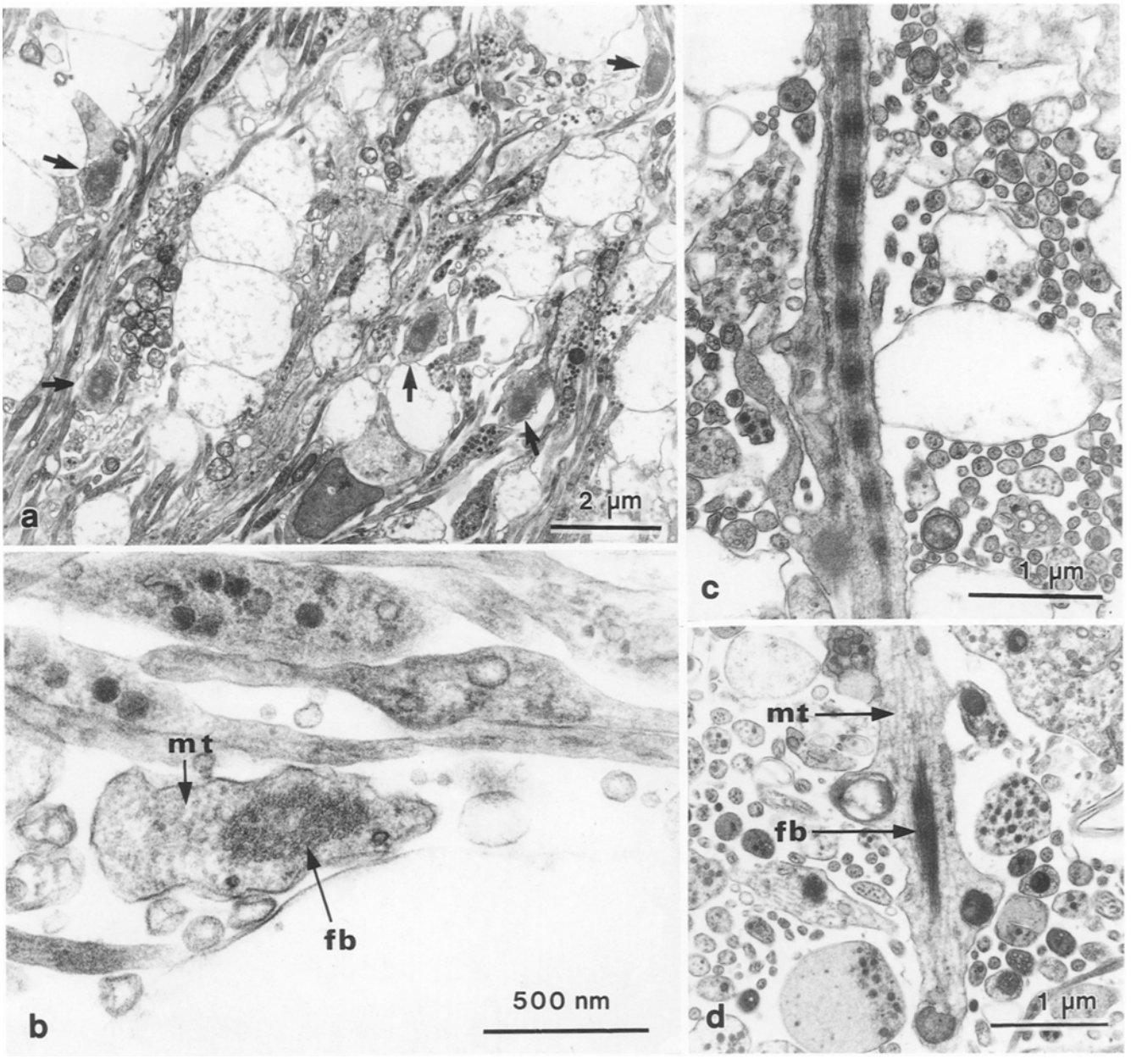

Fig. 3a–d. Stems of support cells: **a** Horizontal section of the nerve cord, *arrows* indicate cross-cut stems. **b** Microtubules (*mt*) and filament bundle (*fb*) within a nearly cross-cut stem. **c** Partial view of a lengthwise cut stem. **d** Partial view of an obliquely cut stem showing microtubules surrounding the filament bundle

70 nm) and mitochondria. The nerve fibres are arranged in tracts (Fig. 2a, *tr*) and exhibit numerous varicosities (*var*) that measure 1.5–3.0 µm in diameter. (Axons that immediately come from the nerve cell bodies do not show varicosities of this sort.) The varicose sections appear empty or contain indistinct precipitates. Occasionally they are completely or partially filled with vesicles and mitochondria are likewise present (Fig. 6c). Vesicle-filled varicosities are especially found in the areas of neuropile (Fig. 6d, e). In these areas the tracts of nerve fibres that run in a radial direction are crossed by traces that run towards the lateral nerve cord. In the neuropile, collaterales (*col*) are found as well as axons which deflect towards the lateral cord (*arrows*). We did not observe well-defined synapses. The structure of the nerve fibres is largely in line with the description given by Cobb (1970) who additionally figured "structures interpreted as synapses" within the area of neuropile.

With respect to the position of the areas of neuropile, however, our findings (Fig. 1f, g) differ fundamentally from Cobb's description. According to Cobb (1970), in asteroids the area of neuropile lies near the bases of the lateral branches, but in sea urchins the areas of neuropile are said to be "consistently localized between successive branches to the podia and the test". Our investigation shows that, with respect to the position of the areas of neuropile, there is no difference between asteroids and echinoids.

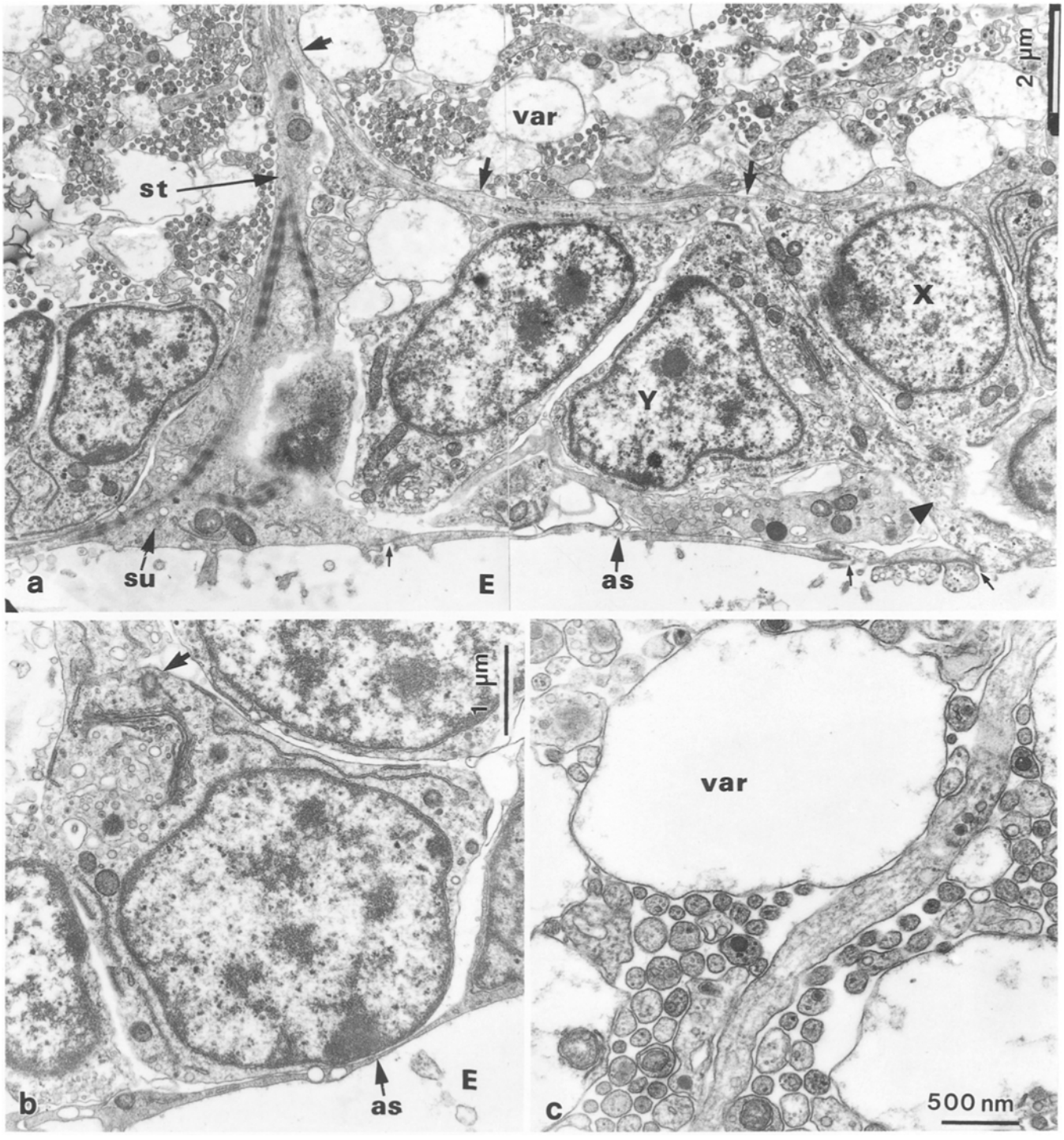

Fig. 4a–c. Cross-cut sections of the radial nerve cord: **a** Some nerve cell bodies (*Y*) touch the lumen (*arrow head*), the cilium is not cut. The cell marked with *X* does not touch the lumen, its axon (*arrows*) runs towards the stem of the support cell. **b** Nerve cell with a basal body (*arrow*) within the axon hillock region. **c** Axonal process that pentrates the trunk of nerve fibres

III. The gross structure of the lateral nerve cord

The radially arranged complex of organs exhibits a segmented organization. Each of its components, including the nerve cord, gives rise to lateral branches on both sides (Fig. 1a, b). The branches maintain their mutual position (Fig. 7a, d). The lateral water vessel (*LW*) leads to the ampulla (*A*) of a tube foot which is entered via a bipartite valve (Fig. 7b, *v*). The lips of the valve are attached to the upper and lower wall of the ampulla (Fig. 7d) and enclose a slit-like pore that represents the ultimate end of the lateral water vessel. The lips of the valve are made up of (densely stained) muscle cells and blistered cushion cells. (A detailed investigation of the valve structure is in preparation).

Up to the valve the lateral water vessel is accompa-

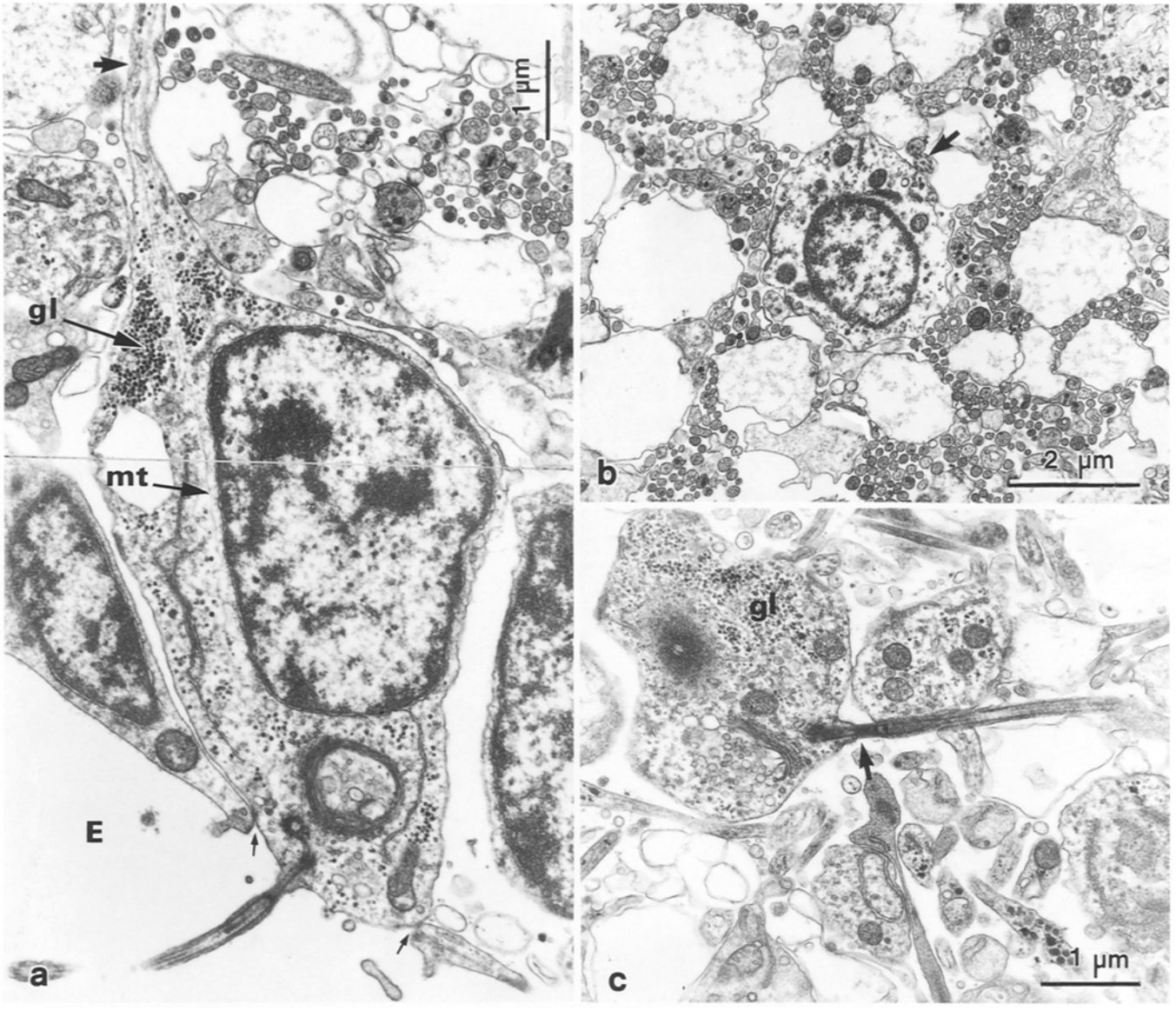

Fig. 5. a Nerve cell whose cilium protrudes into the epineural canal whereas the axonal process (*arrow*) arises from the opposite pole. **b** Cell and its cilium (*arrow*) within the cross-cut trunk of nerve fibres (cf. Fig. 6a). **c** Cilium (*arrow*) of a nerve cell that does not touch the epineural canal (horizontal section). *gl* glycogen particles

nied by a branch of the haematocoel and a branch of the haemal lacuna in between (Fig. 7a, d). The haematocoel terminates at the level of the valve (Fig. 7b) and the haemal lacuna is enclosed between the ampulla and the lateral nerve cord. The latter forms a median groove that harbours the haemal lacuna. On both sides the nerve cord touches the wall of the ampulla. The first contact is just in the attachment area of the valve muscles (Fig. 7c, d, *vm*) although some connective tissue is sandwiched in between. Next to that, the lateral nerve cord runs along the bottom side of the ampulla up to the adradial pore of the tube foot-ampulla system (cf. Hyman 1955), and passes the test through this pore (Fig. 7e). Reaching the surface of the test, the lateral nerve cord terminates with a ganglion-like area of neuropile which gives rise to the podial nerve and makes contact with the basal nerve plexus. The ganglion-like area encloses the opening of the lateral branch of the epineural canal.

IV. The fine structure of the lateral nerve cord

The lateral branch of the nerve cord arises from the edge between the main and the side strip of the radial cord (Figs. 1a, 8a). It is pierced in full length by a branch of the epineural canal (*BE*) which has the shape of a transversal cleft and divides the lateral cord into an upper (*uh*) and a lower half (*lh*, Fig. 7). The upper half originates from the distinct area of neuropile located in the main strip of the radial cord (Fig. 1f, g, *arrows*). The lower half branches off the uppermost part of the side strip (Figs. 1b, g, 8a, *ss*) which deflects laterally. In this manner the branch of the epineural canal is en-

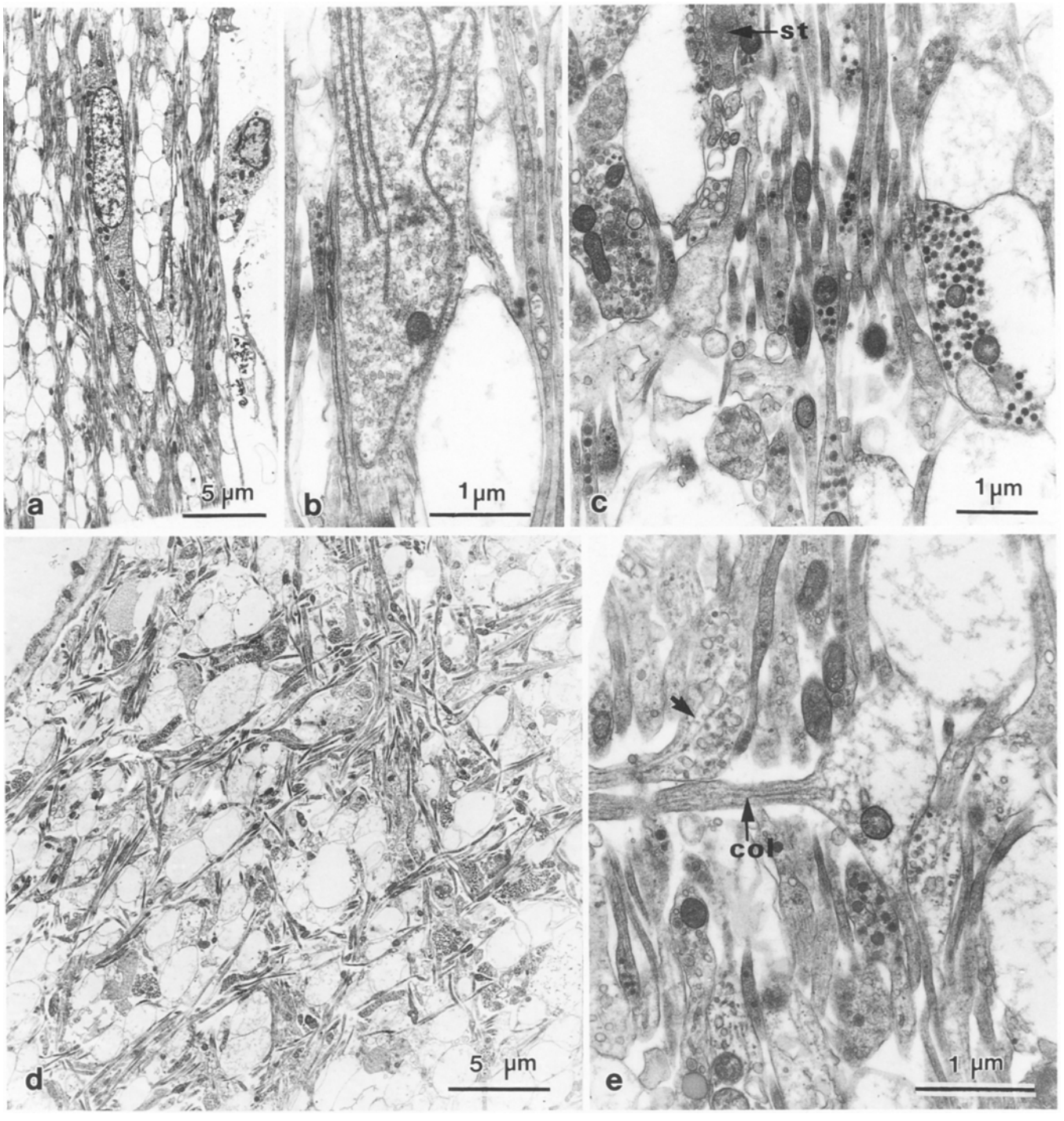

Fig. 6a–e. Horizontal sections of the radial nerve cord: **a** Nerve cell body positioned within the trunk of nerve fibres which is densely filled with vesicles. **b** Partial view of **a**. **c** Varicosities of nerve fibres filled with vesicles. **d** Low-magnification figure of part of the neuropile. **e** Collaterale (*col*) and nerve fibre (*arrow*) deflecting towards the lateral nerve cord

closed. A distinct area of neuropile is not present in the off-branching region of the lower half. The lowermost part of the side strip (Figs. 1e, 8a, *ss*) is obviously not involved in the formation of the lateral nerve cord.

In the upper as well as in the lower half the cell bodies of the support cells touch the lumen of the central canal and cloth its lumen with adluminal sheets (Fig. 8c, *as*). Occasionally, a cell body reaches from the lumen to the basal lamina, but most of the support cells are provided with a short and delicate stem that may be branched. A cross-striation of the few intracellular fibrils is not detectable. The stems terminate in the proximal cytoplasmic sheets (Fig. 9b, *ps*) that cover the basal lamina. Cell bodies of nerve cells are relatively rare and do not form a closed layer. They are also provided with a cilium (Fig. 8b, *ci*).

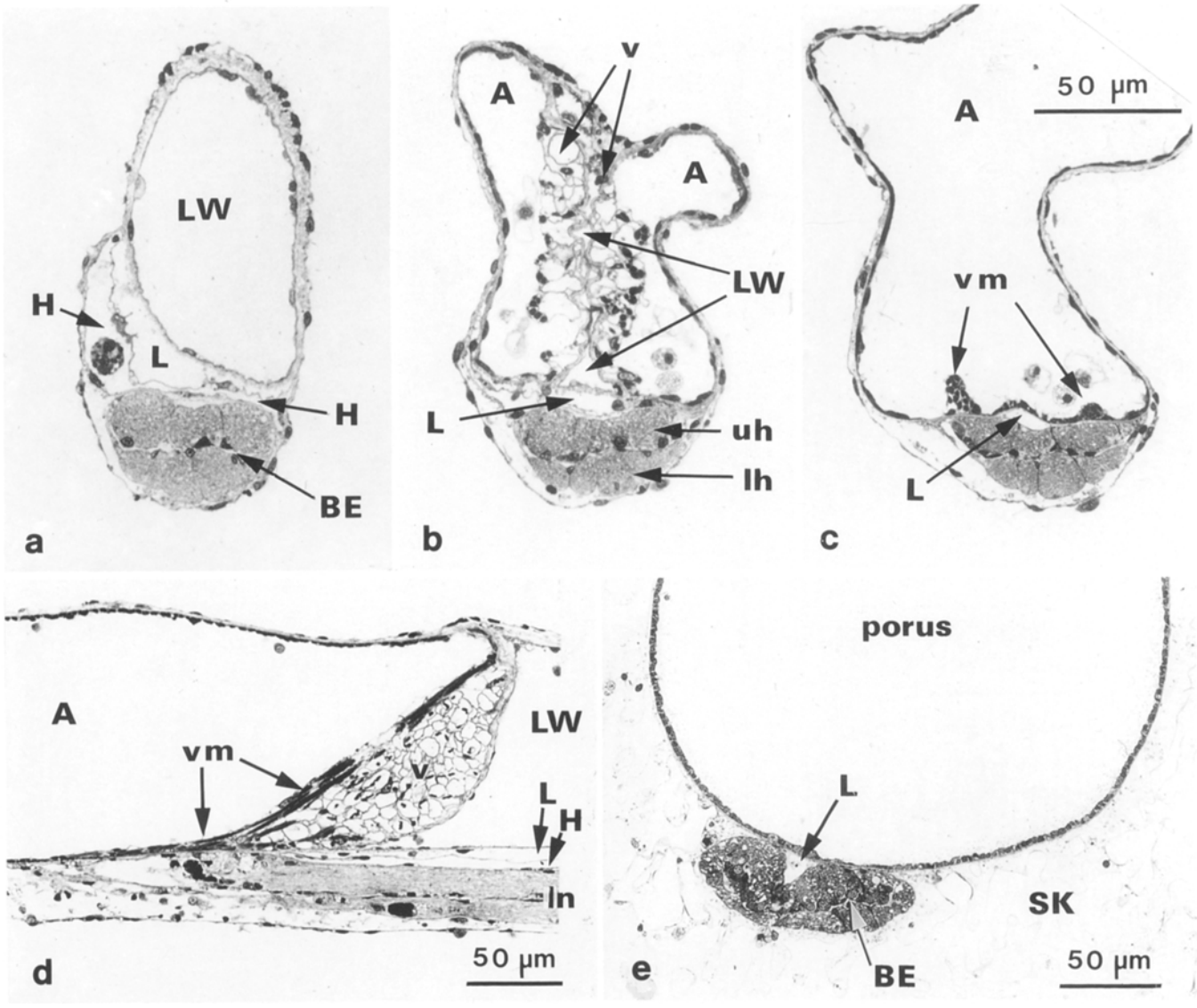

Fig. 7a–e. Semithin sections of the lateral branches of the radial organ complex: **a–c** A series of cross-cut sections from the lateral water vessel (*LW*), the valve (*v*), up to the ampulla (*A*). **d** Lengthwise cut lateral nerve cord (*ln*) that meets the valve in the attachment area of the valve muscles (*vm*). **e** Cross-cut pore of the ambulacral plate accompanied by the lateral nerve cord. *BE* branch of the epineural canal; *uh*, *lh* upper and lower half of the lateral nerve cord. Further abbreviations see Fig. 1

The nerve fibres run strictly lengthwise in the direction of the lateral nerve cord (Fig. 9a, b). They are separated from the basal lamina by the proximal cytoplasmic sheets of the support cells. The sole exceptions are found in the part of the lateral nerve cord that runs along the bottom side of the ampulla. On both sides of the heamal lacuna (*L*) nerve processes (*nt*) occur that push the proximal sheets aside (Fig. 9b, c, *arrows*) and immediately invest the basal lamina. They exhibit the features of nerve terminals described by Florey and Cahill (1977) from the podial nerve plexus. The nerve terminals are filled with clear vesicles (diameter 30–70 nm). Structures of this sort were also observed by Cobb (1970). Nerve cell bodies which obviously produce clear vesicles are seen in the lateral part (Fig. 9b, *x*). In one of the nerve terminals (Fig. 9b, *inset*) the base of a cilium is present. It is obviously the axon hillock region which is cut rather than an axon.

The branch of the epineural canal opens to the exterior at the base of the podium. The orifice is bordered by epidermal cells with well-developed microvilli in place of the smooth adluminal sheets of support cells. The microvilli form a sieve that keeps the entry clear of foreign matter which might pass into the epineural canal.

D. Discussion

I. Gross survey

The nerve cord of the eleutherozoan echinoderms is an epidermal neuroepithelium. In the naked nerve cords of

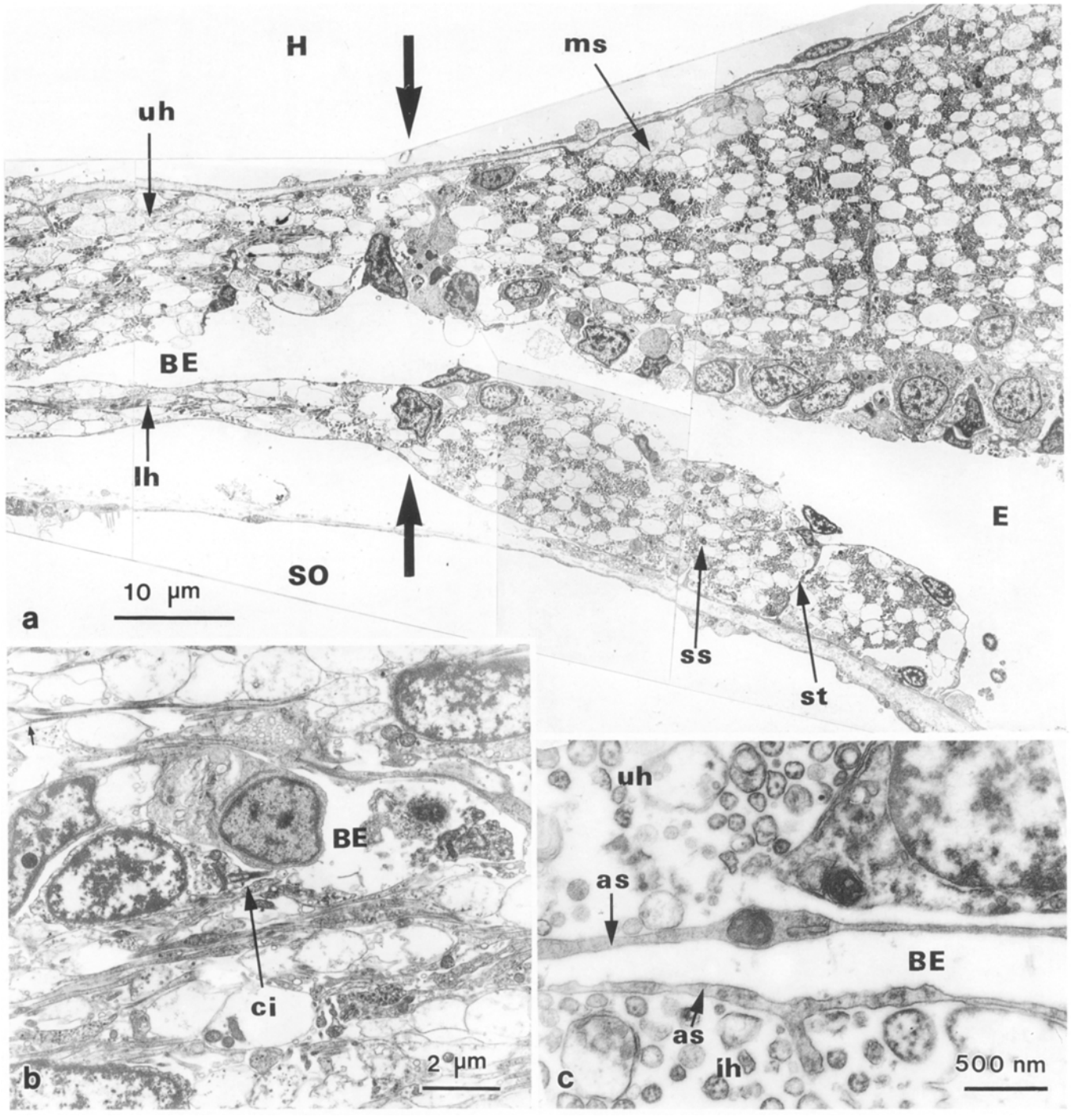

Fig. 8. **a** The origin of a lateral nerve cord (cf. Fig. 1a), *arrows* indicate the border between the radial and the lateral nerve cord. **b** Central part of a lengthwise cut lateral nerve cord. A nerve cell with a cilium (*ci*). The nerve fibres show varicose swellings (*small arrows*). **c** Partial view of a cross-cut lateral cord; the branch of the epineural canal (*BE*) is clothed with adluminal sheets (*as*)

asteroids, the ectoneural cord is provided with a cuticle (Bargmann et al. 1962; Cobb 1970) which is a general feature of the echinoderm epidermis (Holland and Nealson 1978). In ophiuroids, holothuroids and echinoids the nerve cord is shifted into the interior and accompanied by the epineural canal. A "subneural cuticle" was observed by Wilkie (1978) in ophiuroids, but in echinoids we did not discern it.

Asteroids, ophiuroids and holothuroids are provided with a well-developed hyponeural motor system positioned in the haematocoel (Smith 1965). Cobb (1985, 1990) presumes that the hyponeural system is of mesodermal nature. In echinoids the "hyponeural tissue is found in ten ganglia off the circumoral ring" (Cobb and Lavarack 1966, Cobb 1987). It innervates the strong muscles of the lantern. But in the radii the hyponeural

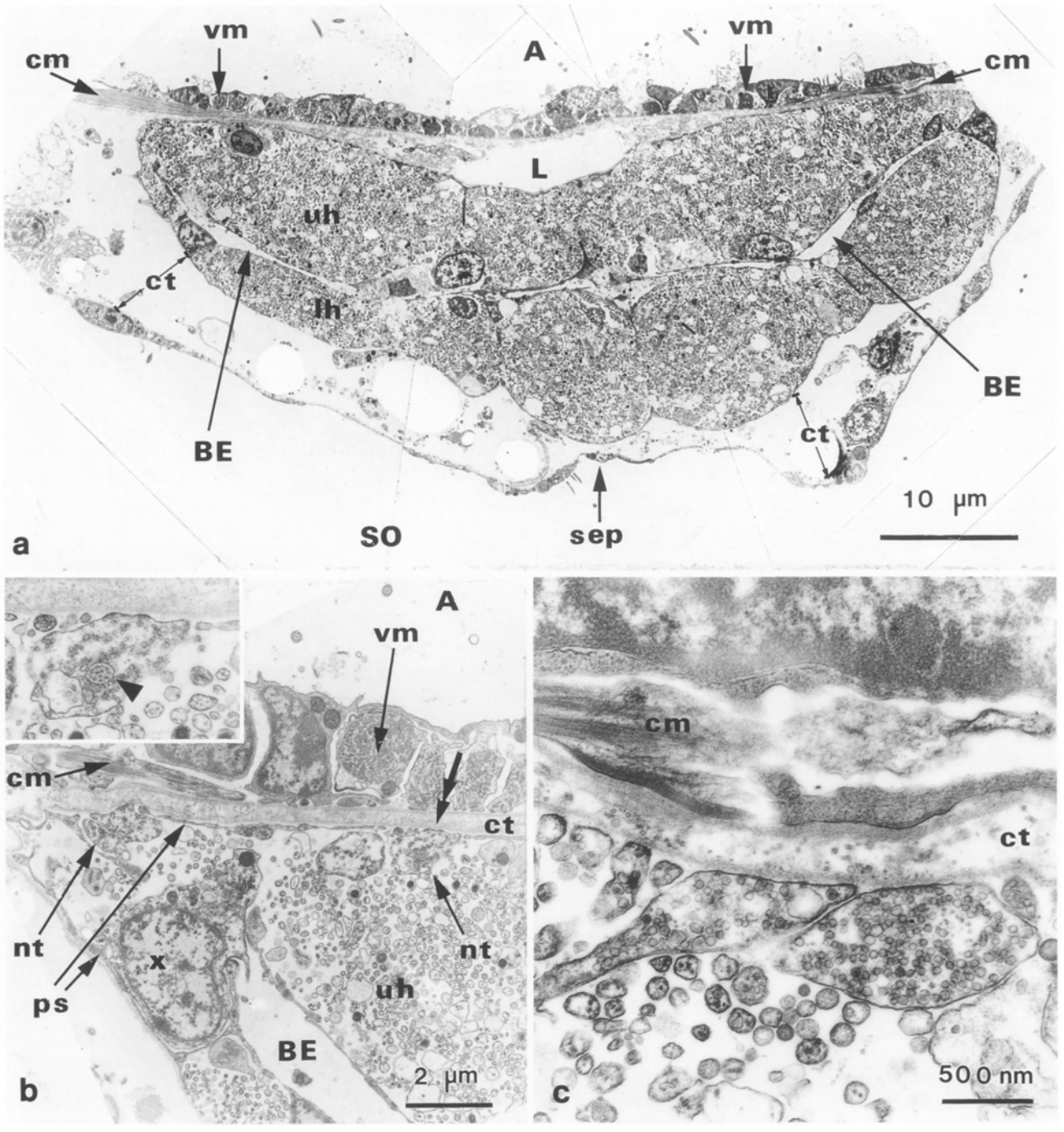

Fig. 9. **a** The cross-cut lateral nerve cord and its contact with the ampulla (*A*) in the attachment region of the valve muscles (*vm*). **b** Nerve cell body (*X*) and the nerve terminals (*nt*) in the upper left edge of the lateral nerve cord. *Double arrow* indicates the position of the inset which shows a cilium (*arrow head*). **c** Nerve terminals from the upper right edge of the section shown in **b**. *cm* circular muscle of the ampulla

nerve is absent in echinoids, although the radial haematocoel is well developed. This probably results from the reduction of the radial musculature which is due to the development of the firm test. In all sea urchins examined [*Eucidaris tribuloides* (Lamarck, 1816), *Diadema setosum* (Leske, 1778), *Arbacia lixula* (Linné, 1758) and *Sphaerechinus granularis* (Lamarck, 1816)] the radial nerve cord is subdivided into the main and two side strips. The evolutionary trend possibly goes towards a closed neural tube which at present only exists in the lateral nerve cords. Of course, the tubular structure of the echinoid lateral nerve cord evolved independently from the chordate nerve cord which it resembles. But there is possibly a common trend in the Deuterostomia to form hollow nerve cords. It is noteworthy that Cuénot (1891) already figured a double chain of nuclei in the lengthwise cut

lateral nerve cords of echinoids. Figures and descriptions given by later authors (e.g. Smith 1965) are less accurate.

The presence of the side strips in the radial nerve cord and the connections between the epineural canal and the sea water were not observed by previous authors, and they are not known from non-echinoid echinoderms. Reinvestigations of the nerve cords and epineural canals of non-echinoids seem desirable. This especially applies to the holothuroids. Several authors (Fell 1965; Smith 1988) speculate that echinoids and holothuroids are more closely related to each other than to the other echinoderm groups.

At least in echinoids the epineural canal is not closed off from the environment. Whether there is a considerable exchange of sea water via the lateral branches of the epineural canal or a water current within the epineural canal is an important but unsolved question. It is not to be excluded that it plays a role in the respiratory exchange of the nerve cord.

II. The nerve cord is a neuroepithelium

The nerve cord shows some analogies to the myoepithelia which are well knwon from echinoderms (Rieger and Lombardi 1987). The interpyramidal muscle of the sea urchin lantern is an extremely developed example (Märkel et al. 1990). The support cells may be compared with the adluminal cells (peritoneocytes) of the myoepithelia. In both instances the cell bodies lie near the surface, the cilium is encircled by the horseshoe-shaped nucleus and cytoplasmic processes reach to the basal lamina. The nerve and the muscle tissues respectively lie within the interstices.

1. Support cells. The nerve cord is a derivative of the epidermis and its support cells are surely homologous with the support cells of the epidermis, although they have lost the cuticle. The echinoderm epidermis is built up of ciliated as well as non-ciliated support cells according to Holland (1984). The ciliated cells are said to contain only a few kinds of fibres. The non-ciliated cells contain intracellular filaments running from the apex to the base of the cell. The filaments are smooth (Florey and Cahill 1977). Harris and Shaw (1984) stated that the bundle of filaments is associated with microtubules. Because of the cross-reaction with a monoclonal antibody against desmin, the filaments were thought to be related to vertebrate intermediate filaments.

The cursory examination of the radial nerve cords of other echinoid species showed that the fibrils within the support cells are probably smooth. According to Bargmann et al. (1962) this also applies to the majority of the support cells in the naked nerve cord of *Asterias rubens* (Linné, 1758), but several cells contain cross-striated fibrils of different stages of complexity. The striation is dissimilar to that found in *M. globulus*. Our own perfunctory examination of the nerve cord of the ophiuroid *Ophiothrix fragilis* (Abildgaard, 1789) showed that cross-striated fibrils occur in the support cells.

The ultrastructural details and the functional reasons that cause the conspicious striation of the filament bundles within the ciliated support cells of the nerve cord of *M. globulus* are still enigmatic; especially since the support cells of the lateral nerve cord contain the usual bundles of non-striated fibrils. The stems of the support cells attach in the centre of a depression that comprises the proximal sheet and the basal lamina (Fig. 2a). This phenomenon may result from a certain contraction of the cross-striated fibrils. Cross-striated fibrils are often thought to be contractile (Bouland et al. 1982). But the phenomenon can likewise result from a swelling of the axon layer during the process of fixation. The spacious vacuoles which are present in the perinuclear area of the support cells indicate that these cells are not restricted to the support function but also play a role in phagocytosis.

2. Nerve cells. The nerve cells of the nerve cord obviously correspond to the nerve cells of the epidermis. Weber and Grosmann (1977) differentiate between ciliated primary sense cells and unciliated multipolar ganglion cells which belong to the basal nerve plexus. The present study indicates that cilia are a primary constituent of all kinds of nerve cells. Hitherto, cilia were rarely described in nerve cells other than sensory cells. Cobb and Stubbs (1981) found a cilium in the axon hillock region of a nerve cell in the ectoneural nerve cord of an ophiuroid. Cobb (1970) figured a cilium in the hyponeural nerve of an asteroid. Peters (1985) observed a basal body in the axon hillock region of a neurone in the basal nerve ring of a sea urchin spine.

Depending upon the polarity of the nerve cell bodies three types of nerve cells are discerned: (1) Nerve cells whose cilium protrudes into the epineural canal whereas the axon hillock region is at the opposite pole. In their structure these cells resemble the primary sensory cells of the epidermis described by Weber and Grosmann (1977), Burke (1980) and Peters and Campbell (1987). They are surely true sensory cells. They probably control the chemistry or the water current of the sea water within the epineural canal. (2) Subluminal nerve cells whose cilium originates in the axon hillock region. They are probably homologous to the ganglion cells of the nerve plexus and resemble the nerve cells described by Cobb and Stubbs (1981) from the ophiuroid nerve cord. There are possibly several axons that arise from the axon hillock region, but they cannot be discerned with certainty. (3) Neurones that are embedded in the trunk of nerve fibres. They are likewise provided with a cilium. The axons probably arise in bundles from the narrow sides of the stretched cell bodies. The three types of nerve cells surely fulfil different functions, but speculations concerning this seem premature.

III. The supposed function of the nerve terminals

According to Cobb (1970, 1987, 1990) the ectoneural nervous system does not penetrate the basal layer and also does not directly innervate non-ectodermal tissue. This hypothesis obviously does not apply to the peripherical part of the ectoneural system. Peters (1985) and Stauber (1990) did convincingly show the direct ectoneural innervation of the muscles of spines or pedicellaria. But it obviously applies to the radial and lateral nerve

cords. In echinoids Cobb found that the muscles of the ampulla "end against the basement membrane but in position opposite ectoneural nerve endings on the other side". In his opinion the muscles are indirectly innervated by ectoneural nerve endings across the basal laminae of the two epithelia and the connective tissue in between. Florey and Cahill (1977) likewise assume the diffusion of transmitter substances across the connective tissue in the podia. This hypothesis is strengthened by the present investigation.

The ampulla and the tube foot are clothed with a simple myoepithelium. In the walls of the ampulla the contractile processes run in a rectangular direction to the narrow base of the ampulla (Fig. 9, *cm*). At the base itself the contractile processes run in lengthwise direction, i.e. parallel to the lateral nerve cord (own observation). Near the pore the contractile processes are small (Fig. 9, *vm*); towards the valve they become more and more enlarged, and in the end, they form the musculature of the valve (Fig. 7). The very first contact between the lateral nerve cord and the ampulla is in the attachment area of the valve musculature (Fig. 7d), and just in this area the first nerve terminals occur. The ampulla and the tube foot are components of a semi-autonomous hydraulic system which can be closed off from the radial water vessel by the valve. Nerve terminals are present in the lateral nerve cord as well as in the podial nerve. They are absent in the part of the nerve cord that passes the ambulacral pore. The hydrocoel epithelium that clothes the pore is a simple, non-muscular epithelium (apart from a minute bundle of "muscle tails" which obviously come from the valve muscles and will be described elsewhere). The occurrence of nerve terminals in the lateral nerve cord on the one hand and the podial nerve on the other leads to the assumption that they are part of the system which controls the co-operation between the ampulla and the tube foot.

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References

Bargmann W, von Harnack M, Jacob K (1962) Über den Feinbau des Nervensystems des Seesternes (*Asterias rubens* L.). Z Zellforsch 56:573–594

Bouland C, Massin C, Jangoux M (1982) The fine structure of the buccal tentacles of *Holothuria forskali*. Zoomorphology 101:133–149

Burke RD (1980) Podial sensory receptors and the induction of metamorphosis in echinoids. J Exp Mar Biol Ecol 47:223–234

Cobb JLS (1970) The significance of the radial nerve cords in asteroids and echinoids. Z Zellforsch 108:457–474

Cobb JLS (1985) The neurobiology of the ectoneural/hyponeural synyptic connection in an echinoderm. Biol Bull 168:432–446

Cobb JLS (1987) Neurobiology of the Echinodermata. In: Ali MA (ed) Nervous systems in invertebrates. Plenum Press, New York, pp 438–525

Cobb JLS (1990) The significance of a non-centralized nervous system to general studies on echinoderm biology. In: De Ridder C, Dubois P, Lahaye CM, Jangoux M (eds) Echinoderm Research. Balkema, Rotterdam, pp 3–7

Cobb JLS, Lavarack MS (1966) The lantern of *Echinus esculentus* (L.). II. Fine structure of hyponeural tissue and its connexions. Proc R Soc B 164:641–650

Cobb JLS, Stubbs T (1981) The giant neuron system in ophiuroids. I. The general morphology of the radial nerve cords and circumoral ring. Cell Tissue Res 219:197–207

Cuénot L (1891) Etudes morphologiques sur les échinodermes. Arch Biol 11:303–680

Fell HB (1965) The early evolution in the Echinozoa. Breviora 219:1–19

Florey E, Cahill MA (1977) Ultrastructure of the sea urchin tube feet. Evidence for connective tissue involvement in motor control. Cell Tissue Res 177:195–214

Hamann O (1887) Beiträge zur Histologie der Echinodermen. Heft 3; Anatomie und Histologie der Echiniden und Spatangiden. Fischer, Jena

Harris P, Shaw G (1984) Intermediate filaments, microtubules and microfilaments in epidermis of sea urchin tube foot. Cell Tissue Res 236:27–33

Holland ND (1984) Echinodermata: Epidermal cells. In: Bereiter-Hahn J, Matoltsy AG, Richards KS (eds) Biology of the integument, vol 1. Invertebrates. Springer, Berlin Heidelberg New York Tokyo, pp 756–774

Holland ND, Nealson KH (1978) The fine structure of the echinoderm cuticle and the subcuticular bacteria of echinoderms. Acta Zool (Stockholm) 59:169–185

Hyman LH (1955) The invertebrates. IV. Echinodermata. McGraw-Hill, New York

Kawaguti S (1965) Electron microscopy on the ampulla of the echinoid. Biol J Okayama Univ 11:75–86

Märkel K, Röser U (1983) The spine tissues in the echinoid *Eucidaris tribuloides*. Zoomorphology 103:25–41

Märkel K, Röser U, Stauber M (1990) The interpyramidal muscle of Aristotle's lantern: its myoepithelial structure and its growth. Zoomorphology 109:251–262

Peters BH (1985) The innervation of the spines in the sea urchin *Echinus esculentus* L. Cell Tissue Res 239:219–228

Peters BH, Campbell AC (1987) Morphology of the nervous and muscular systems in the heads of pedicellariae from the sea urchin *Echinus esculentus* L. J Morphol 193:35–51

Rieger RM, Lombardi J (1987) Ultrastructure of coelomic lining in echinoderm podia: significance for concepts in the evolution of muscle and peritoneal cells. Zoomorphology 107:191–208

Smith AB (1988) Echinoid Palaeobiology. Allen and Unwin, London

Smith JE (1965) Echinodermata. In: Bullock TH, Horridge GA (eds) Structure and function of invertebrate nervous systems, vol II. Freeman, San Francisco London, pp 1519–1558

Stauber M (1990) Untersuchungen zur Histologie und Ultrastruktur der Echinodermen-Muskeln. Dr-Thesis, Bochum, 113 p

Weber W, Grosmann M (1977) Ultrastructure of the basiepithelial nerve plexus of the sea urchin *Centrostephanus longispinus*. Cell Tissue Res 175:551–562

Wilkie IC (1978) Functional morphology of the autotomy plane of the brittle star *Ophiocomina nigra* (Abildgaard). Zoomorphology 91:289–305

Note added in proof. The "podial pit" [Millot and Coleman (1969) The podial pit – a new structure in the chinoid *Diadema antillarum* Philippi. Z Zellforsch 95:187–197] is the obliquely cut and misinterpreted opening of the epineural canal (own reinvestigation).