Ultrastructure of the spines in the copulatory organ of some Monocelididae (Turbellaria, Proseriata).

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SUMMARY.

A comparative study of the ultrastructure of the copulatory organ has been done in four genera of the Monocelididae. In all of these genera the male copulatory organ belongs to the conjuncta-duplex type provided with a cirrus armed with spines.The fine structural analysis of the cirrus spines revealed that these structures are specializations within the basement lamina of the cirrus.In this part of the male canal the basement lamina has a trilamellar structure.The spines are formed by a local thickening of the middle electron dense layer and show a structural similarity in all the Monocelididae investigated here.
The systematic value of this character within the family of the Monocelididae is discussed.

INTRODUCTION

In a previous paper (MARTENS and SCHOCKAERT, 1981) a detailed description was given of the copulatory organ of Archilopsis unipunctata, a representative of the Monocelididae (Proseriata). The copulatory organ in this species is of the so-called conjuncta-duplex type: a seminal vesicle, a prostate vesicle and a cirrus armed with spines. The cirrus is enclosed (as is also the seminal vesicle) in a cirrus bulb lined with a fibrous septum and surrounded with muscles (see KARLING, 1956 ; MARTENS and SCHOCKAERT, 1981 for electron microscopy). Data were presented which supported the view that these spines are differentiations of the basement lamina of the epithelium lining the cirrus lumen and that this epithelium disappears in the adult stage.
We have now studied the ultrastructure of the copulatory organ of six other species of Monocelididae with a spiny cirrus. A description of the ultrastructure of the spines is given and compared with those in Archilopsis unipunctata. The species involved are: Promonotus schultzei AX,1943; P. marci AX,1954; Archiloa westbladi AX, 1954; A. petiti AX, 1956; Monocelopsis otoplanoides AX, 1951 and "Archilopsis" n.spec. (Description will be given later on. This species is similar to Archilopsis unipunctata but with a stylet in the cirrus.)

MATERIAL AND METHODS

Specimens of Promonotus schultzei were collected in a shallow brackish waterpond, called Dievegat, in the North of Belgium; Archiloa petiti and "Archilopsis" spec. in the Zwin-estuary also in northern Belgium; Archiloa westbladi in the Slack-estuary in Ambleteuse in the North of France; Promonotus marci and Monocelopsis otoplanoides from the belgian sandy beach in De Panne.
The animals were extracted by decantation with a $MgCl_2$-solution isotonic to seawater. Fixation occured in

2% glutaraldehyde in 0,1M phosphate buffer with 10% sucrose (pH 7,3) at 4°C for 2-3 h. After rinsing in the same buffer they were postfixed in 1% phosphate buffered osmium-tetroxide at 4°C for 2 h. Dehydration occured in graded aceton series and flat embedding in Araldite. Ultrathin sections were cut with a Reichert OMU 3 ultramicrotome and mounted on pioloform coated cupper grids. The sections were stained with 2% aqueous uranyl acetate and 1,2% aq. lead citrate, and examined in a Philips EM 400.

RESULTS.

In none of the species studied a structurised epithelium was found lining the lumen of the cirrus. The cytoplasmic elements occuring in the lumen differ from the zone of the cirrus (proximal versus distal part) and from individual to individual (related to their stage of maturity ?). The material in the lumen shows the aspect of a highly degenerating and desintegrating tissue (Fig. 1 and 5), or consists of a very vesiculated cytoplasm (Fig. 11) with some recognisable remnants as mitochondria, or appear to be filled with a fine granular material (Fig. 3). In the individual of Archiloa petiti of which the electron micrograph in Figure 3 was taken , cytoplasmic remnants can be seen between granules from the prostate glands also occuring in the lumen. In Monocelopsis otoplanoides shown in Figure 11 there are some degenerating cells still adhering to the basement lamina between clusters of spermatozoa. Often bacteria can be seen (Fig. 5 and Fig. 7). In some specimens no epithelial elements at all were found in some zones of the cirrus. Those different aspects of the cytoplasmic elements in the cirrus lumen thus correspond with those described earlier in A. unipunctata (MARTENS and SCHOCKAERT, 1981). These observations seem to confirm the idea that the epithelium lining the cirrus lumen in the juvenile (subadult) individual disappears in the adult and that the lining of this lumen now consists of the

basal lamina of this epithelium.
This basal lamina has a trilamellar structure: a thin (3-7 nm) outer very electron dense layer (on which adhere remnants of the basal plasmalemma of the degenerating or desintegrated epithelium), an inner fibrous to fine granular layer and a more homogeneous layer in between of moderate to high electrondensity (see electron micrographs of higher magnification).
The spines themselves are in fact protuberances of the underlaying tissue covered by a thickened basement lamina. In all the species the middle homogeneous electron dense layer becomes much thicker on the spines than between them. This thickening arrises abruptly at the base of the spines in both Promonotus species (Figs.2 and 4) and in Archiloa westbladi (Fig. 8), but it grows gradually thicker in A. petiti (Fig. 10), Archilopsis n.sp.(Fig. 5) and in Monocelopsis otoplanoides (Fig. 11).
The thickness of the three layers between and on the spines is given in Table I.

TABLE I.

Species	outer layer (in nm)	middle layer between spines (in nm)	middle layer in spines (in µm)	inner layer (in nm)
Promonotus schultzei	3	15	0,50-0,65	60-500
P. marci	4	16	0,15-0,25	25-300
Archiloa westbladi	5	16	0,75- 1	30-250
A. petiti	3	17	0,45-0,80	20-160
Monocelopsis otoplanoides	4	14	0,20-0,30	70
"Archilopsis" n.sp.	7	18	0,40-0,80	100-300

The core of the spines is formed by the sarcoplasm of the underlaying muscle cells in M. otoplanoides (Fig. 11), P. schultzei (Fig. 1), both Archiloa (Figs. 7 and 9) and both Archilopsis species (Fig. 5). Myofibrils of the circular muscles are found in the base of the spines. This results in a regular arrangement of the spines in circular rows, very appearant in P. schultzei. The tip of the spine core in the Archiloa species, M. otoplanoides and especially in P. marci (Fig. 3) is filled with very loose and vesicular cytoplasmic material. Moreover, in the latter

species muscles surrounding the cirrus are very poorly developed: only some longitudinal fibrils are found underneath the base of the spines. The main component of the tissue between the basement lamina of the cirrus and the septum of the cirrus bulb consists of what we consider as parenchymatous elements which most probably also penetrate the spines (Fig. 3). The muscle layer around the septum on the other hand is extremely thick compared to that in the other species. In P. marci this parenchymatous core may fill almost completely the inside of the spine or may be very narrow. In the latter case the spine is filled with the loosely packed fibrous material of the inner layer of the basement lamina. From the micrographs in Figure 1 and 3 it can be seen that the cirrus spines in both Promonotus species are arranged in alternating longitudinal rows. In P. schultzei it can be observed that another distinct zone is formed in the upper layer of the spines by a further condensation of the electron dense material (Fig. 2).

DISCUSSION.

The analysis of the ultrastructure of the spines in the cirrus of the six species presented here is consistent with the description given for Archilopsis unipunctata by MARTENS and SCHOCKAERT (1981).
The variations in the spine structure of the different species, i.e. variations in the thickness and in the electrondensity of the different layers in the basement lamina, are minor quantitative differences to be considered as species characters. The spine structure alone does not give any clues to clarify phylogenetic relationships within the family (see however below). On the other hand, this study confirms that in all the Monocelididae studied so far the spines are derivatives of the basement lamina of the epithelium that lined the cirrus in the subadult and that this epithelium disappears at adulthood. This basement lamina shows a characteristic trilamellar structure and is thickened on the spine by the thickening of the middle homogeneous electron dense layer

The hard parts in the copulatory organ of all other turbellarian taxa studied so far, have been identified as intracellular differentiations: MAINITZ (1977) for Acoela, DOE (1982) for Macrostomida, EHLERS and EHLERS (1980) for Coelogynoporidae (Carenscoilia) and Otoplanidae, LANFRANCHI (1978) for Otoplanidae. In the genus Cirrifera, a coelogynoporid with a cirrus, and in Archimonocelis, a monocelid with a stylet and needles, the cirrus spines and the stylet and needles are also intracellular structures (unpublished data).
The Monocelididae (exclusive Archimonocelis) are thus so far the only taxon of the Proseriata (and of the Turbellaria) known with "basement membrane-spines" in the cirrus. This character may be considered as an argument (an apomorphy) for the monophyletic origin of the family. In that context the position of the genus Archimonocelis within the Monocelididae will be revised: Considering the intracellular localisation of the copulatory hard structures the genus Archimonocelis must be removed from the family as apparently more related to the Coelogynoporidae and the Otoplanidae than to the Monocelididae. Observations on representatives of the subfamily Minoninae done so far reveal that also in these Monocelididae the cirrus spines are derivatives of the basement lamina.
We do however expect that other turbellarian taxa may be shown to have basement membrane derivatives in the copulatory organ. In the Kalyptorhynchia it has been shown that the spines on the proboscis of Carcharodorhynchus and Cheliplana (Schizorhynchia) are derivatives of the basement lamina, while the proboscis hooks in Gnathorhynchus (Eukalyptorhynchia) are intracellular specializations (DOE, 1976 ; RIEGER and DOE, 1975).

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ABBREVIATIONS.

b - bacteria
bl - basement lamina
cr - cell remnants
g - granules of prostate glands
hd - hemidesmosomes
L - lumen of cirrus
m - muscles of cirrus
mi - mitochondria
p - parenchymal elements
S - spine
sp - septum
st - stylet
sz - spermatozoa

PLATE I

Figs. 1-2: Promonotus schultzei.

Fig.1: Electron micrograph of a sagittal section through the cirrus showing that the spines are arranged in alternating rows. Note the stratification in the basement lamina and the cell remnants in the cirrus lumen. Muscle cells filling the core of the spines are attached to the inner fibrous layer by hemidesmosomes.(scale: 0,5µm)

Fig.2: Higher magnification of the onset of a spine seen in 1. Note the trilamellar structure of the basement lamina (each layer indicated by an arrow) and the abrupt thickening of the middle layer. In the spine layer itself another electron dense zone can be seen.(scale: 0,2µm)

Figs. 3-4: Promonotus marci.

Fig.3: Electron micrograph of a sagittal section through the cirrus showing cross sectioned spines (on the left), longitudinally sectioned spines (in the middle) and also sections through the base of some spines which is due to their arrangement in alternating rows. The core is filled with loosely packed fibrous material of the inner layer and/or parenchymatous elements.(scale: 0,5 µm)

Fig.4: Electron micrograph of the onset of a spine showing the three layers in the basement lamina (arrows). The middle dense layer shows an adrupt thickening at the base. Note the cytoplasmic remnants in the lumen adhering to the basement lamina.(scale: 0,2 µm)

Figs 5-6: "Archilopsis" sp.

Fig.5: Electron micrograph of a sagittal section through the cirrus showing two spines and a part of the stylet. The core of the spines is filled with muscle cells. The material in the stylet wall below the basement lamina as seen here consists of stacks of the sarcoplasmic parts of muscle cells which are situated at the base of the stylet. Note the presence of bacteria in the cirrus lumen. (scale: 0,5 µm)

Fig.6:Higher magnification of the three layers in the basement lamina as seen between the spines (small arrows, in upper part of micrograph) and in the spine (large arrows). (scale: 0,2 µm)

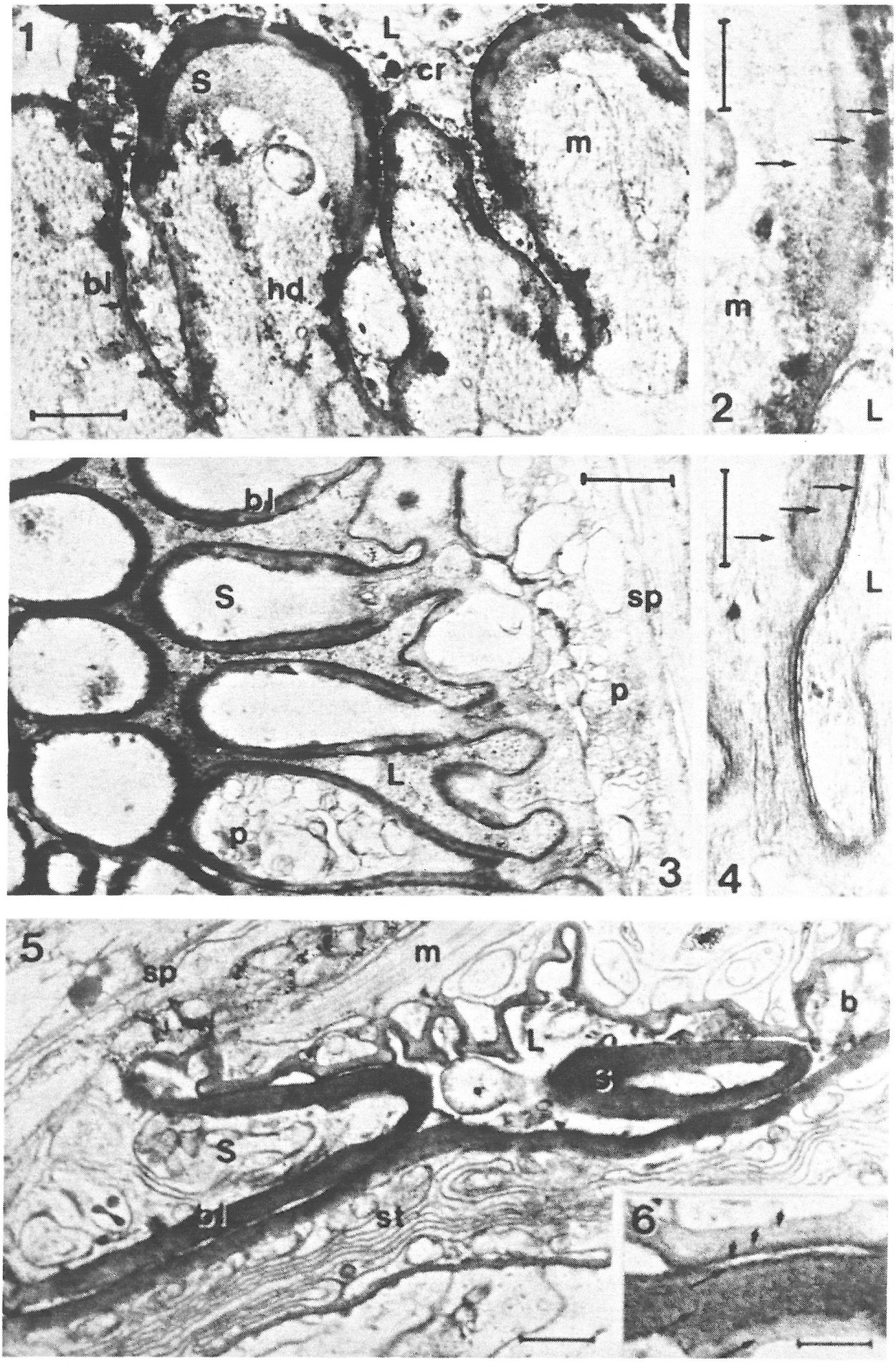
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PLATE II
Figs. 7-8: Archiloa westbladi.

Fig. 7: Electron micrograph of a sagittal section through a partially everted cirrus showing a spine between epithelium cells lining the atrial lumen. The spine core is filled with parenchymatous elements and muscle cells. Note the bacteria in the lumen around the spine.(scale: 0,5 µm)

Fig. 8: Electron micrograph of the onset of a spine. Note the trilamellar structure in the basement lamina and the abrupt thickening of the middle layer forming the spine(scale:0,2µm)

Figs. 9-10: Archiloa petiti.

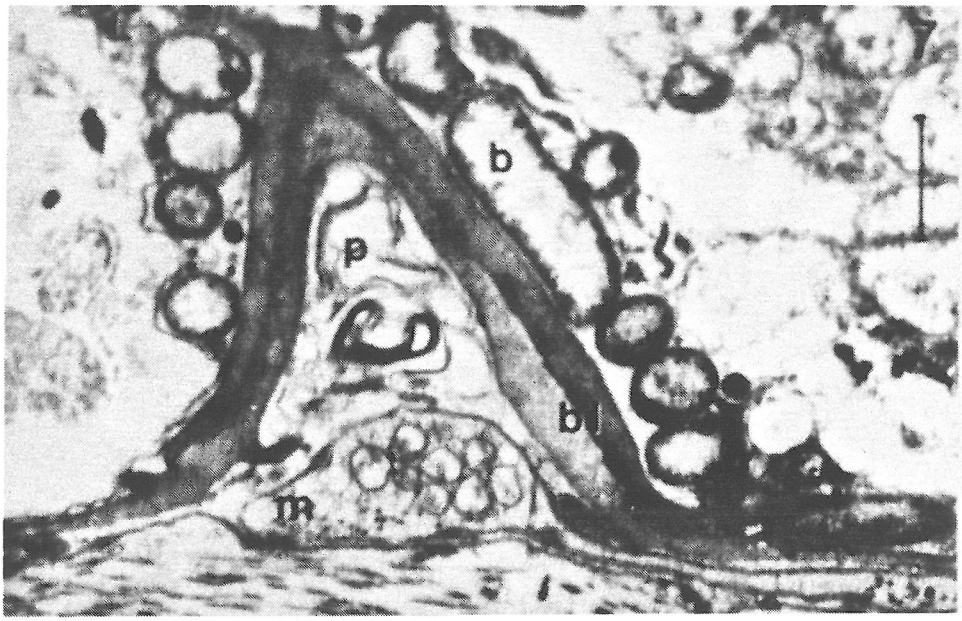
Fig. 9: Electron micrograph of a sagittal section through the cirrus showing two spines protruding in the lumen. The cores are partially filled with muscle cells. Note the mitochondria of degenerating epithelium between granules of the prostate glands in the lumen.(scale: 0,5 µm)

Fig. 10: Electron micrograph of a spine showing the trilamellar structure of the basement lamina(arrows) and the gradually thickened middle layer.(scale: 0,2 µm)

Figs. 11-12: Monocelopsis otoplanoides

Fig 11: Electron micrograph of a cross section through the cirrus. In the cirrus lumen degenerating epithelium cells, still adhering to the basement lamina, can be seen between clusters of spermatozoa.(scale: 0,5 µm)

Fig. 12: Higher magnification of the basement lamina in a spine showing the three layers (arrows). Note in the lumen the cellular remnants adhering to the basement lamina. (scale: 0,2 µm)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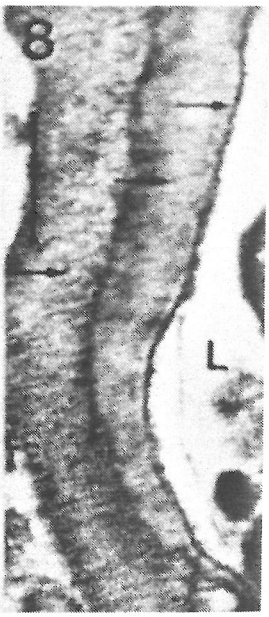
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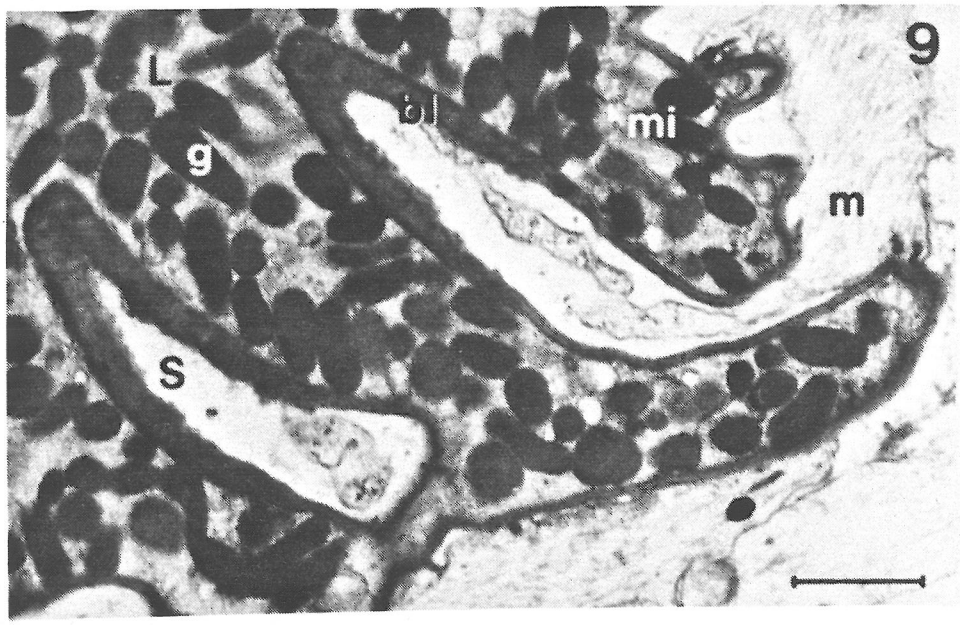
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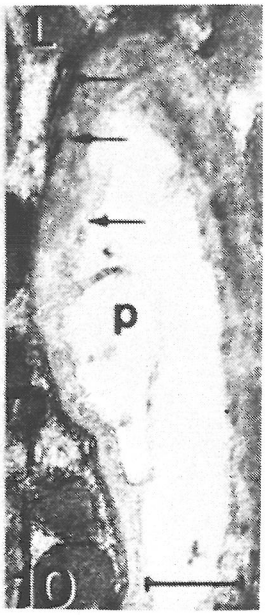
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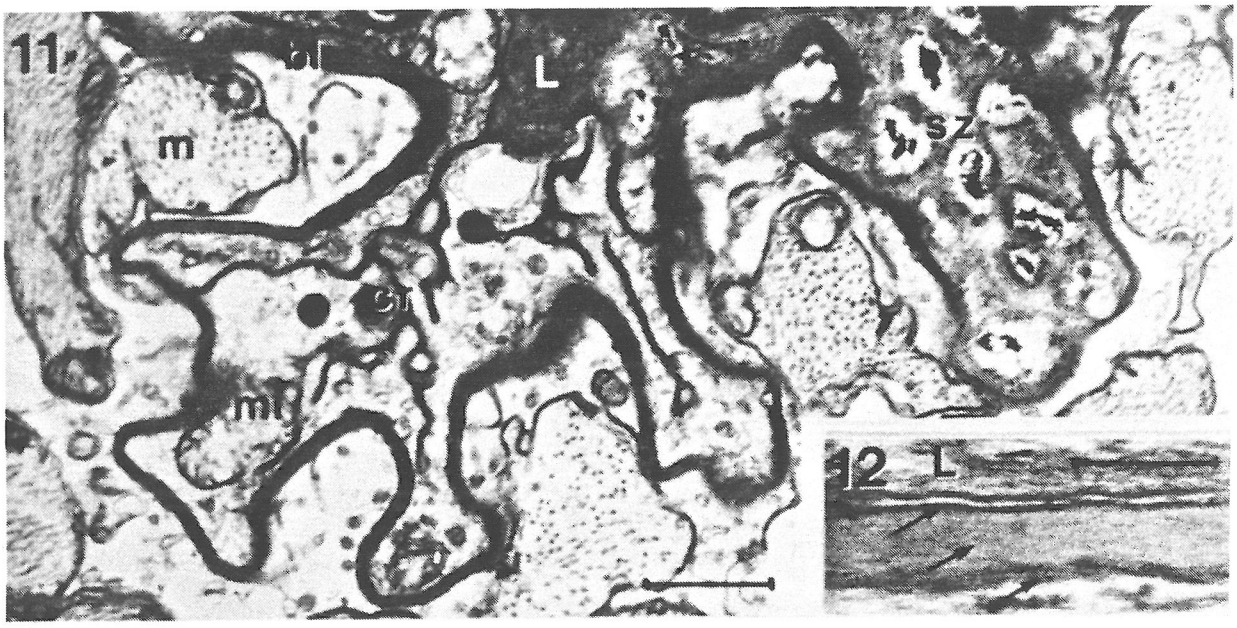
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