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FINE STRUCTURE OF VAS DEFERENS WALL IN THE MITE,
PERGAMASUS VIATOR HALAS. (MESOSTIGMATA, PARASITIDAE)

BY

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ABSTRACT

The vas deferens wall of Pergamasus viator Halas is built by epithelial cells, striated muscle cells and basement membrane. The epithelial cells, 35 to 40 µm in diameter, contain in their cytoplasm the endoplasmic reticulum surrounding the nucleus in a characteristic arrangement, numerous Golgi complexes, mitochondria, microtubules, and two types of vacuoles. The luminal part of plasmalemma forms scarce microvilli. The mononuclear muscle cells with the maximal thickness of 5 µm, as measured at the level of nucleus, possess numerous processes and in their cytoplasm there are mitochondria, microtubules, cisternae of sarcoplasmic reticulum, lysosome-like vacuoles, and myofibrils composed of thick and thin myofilaments. Each of thick filaments (200 Å) is surrounded by 10 to 12 thin filaments (50 Å). The Z lines show irregular contours. The basement membrane enclosing from the outside the whole vas deferens, consists of several layers, each 200 Å thick.

The secretory role of epithelial cells in the vas deferens is discussed.

RÉSUMÉ

La paroi du vas deferens de Pergamasus viator Halas est composée de cellules épithéliales, de muscles striés et d'une membrane basale.

Les cellules épithéliales, d'une diamètre de 35 à 40 mm contiennent un reticulum endoplasmique disposé d'une manière caractéristique autour du noyau. Dans ces cellules il y a aussi de nombreux complexes de Golgi, des mitochondries, des microtubules et deux sortes de vacuoles.

La partie du plasmalemme qui est dirigé vers la lumière du vas forme de nombreux microvilli. Les cellules musculaires d'une épaisseur maximale de 5 µ (mesurée au niveau du noyau) forment de nombreuses ramifications et dans leur cytoplasme on voit des mitochondries, des microtubules, des cisternes de reticulum sarcoplasmique, des vacuoles ressemblant à des lysosomes ainsi que des myofibrilles, composées de myofilaments épais et minces.

Chaque filament épais (200 Å) est entouré par 10 à 12 filaments minces (50 Å). La ligne Z a un contour irrégulier. La surface extérieure du vas est entourée d'une membrane basale dans laquelle on voit de nombreuses couches, chacune d'entre elles ayant 200 Å d'épaisseur.

L'auteur discute enfin du rôle secréteur des cellules épithéliales du vas deferens.

INTRODUCTION.

The ultrastructure of vasa deferentia in mites has not been studied hitherto, as opposed to male genital ducts in insects (Bairati, 1968; Riemann, 1973), crustaceans (Radu and Criciun, 1973; Reger and Fain-Maurel, 1973), and myriapods (Breucker, 1970), on which
a good deal of information is available. The male genital system in *P. viator* (Fig. 1), similarly to other representatives of parasitidae (BelozeroV, 1957) is composed of unpaired testis localized in the posterior part of idiosoma and two vasa deferentia which meet at the level of IV coxae to form a singular ductus ejaculatorius ended with the genital orifice frontally to the anterior border of sternum. An unpaired accessory gland is connected with the initial part of ductus ejaculatorius. Vasa deferentia are filled with spermatozoa embedded in an electron-dense material (Witalinski, 1975).

**Material and methods.**

Males of *P. viator* were collected from the forest bedding during winter and spring. The dissected vasa deferentia were in part studied under the phase-contrast microscope, part of material fixed in 4 % formaldehyde was submitted subsequently to one of following light microscopic procedures: staining with Mayer's haematoxylin and eosin, Sudan B staining for lipid content, and reaction for acid phosphatase activity after Gomori (Pearse, 1968).

![Diagram of the genital system of *P. viator*](image)

**Fig. 1:** A schematic drawing presenting the genital system of male *P. viator*: 1) genital orifice; 2) sternum; 3) ductus ejaculatorius; 4) vas deferens; 5) testis; 6) accessory gland.

For electron microscopic purposes vasa deferentia were fixed in 5 % glutaraldehyde and 0,7 % osmium tetroxide at 15°C, dehydrated in ethanol and propylene oxide, and embedded in Epon 812. Semithin sections prepared for light microscopy were stained with Richardson's dichrome (Richardson et al., 1960), or with PAS procedure (Nevalainen et al., 1972). Ultra-thin sections were cut on LKB 4800 microtome fitted with glass knife, and after staining with uranyl acetate and lead citrate (Venable and Coggshall, 1965), or with chromic acid and phosphotungstic acid (CA-PTA) (Rambourg, 1967) were examined under Tesla BS 500 electron microscope.

**Results.**

The vasa deferentia of *P. viator* have 1,5 mm in length and 30 to 70 µm in diameter — which is dependent on the amount of spermatozoa present in the duct. Vasa deferentia which contain
small number of spermatozoa show distinct, regularly distributed strangulations. The thickness of the wall ranges usually from 9 to 15 µm, but in vasa deferentia closely packed with spermatozoa the distended wall can be much thinner. The electron microscopic observations revealed three components, which build the wall of vas deferens: epithelial cells, muscle cells, and basement membrane. The epithelial cells form a single layer, with the scarcely distributed muscle cells adjoicing to it from the haemocoel side and with the basement membrane enclosing both cellular elements and separating them from the haemocoel (Fig. 2).

**Epithelial cells.**

The lumen of vas deferens is outlined by a single layer of flattened cells, 35-40 µm in diameter, with a centrally localized discoidal nuclei (10 µm in diameter) in which single prominent nucleoli can be observed. The cytoplasm of those cells contains scarce, big PAS-positive granules, similarly reacting to PAS-positive substance filling the lumen of vas deferens, in which spermatozoa are embedded. The reaction for acid phosphatase activity reveals in the cytoplasm of epithelial cells numerous positively reacting vacuoles of various size. No evidence was found for the presence of Sudan B — positive material within the cells.

Under the electron microscope the membranes of neighbouring cells appear to form numerous interdigitations, with septate desmosomes present on the area between the luminal surface and 1/3 to 1/2 of the wall thickness. The luminal plasmalemma develops scarce microvilli which adhere to the cell surface. Fuzzy coated vesicles or other endocytosis-related structures were not observed. The nuclear envelope shows the presence of numerous pores, and the perinuclear space is often continuous with the cisternae of rough endoplasmic reticulum, which demarcates a characteristic ring of ground cytoplasm surrounding the nucleus (Fig. 3). In the remaining areas of cytoplasm the endoplasmic reticulum forms big, dilated cisternae, between which there are numerous Golgi complexes, with the diktyosomes 0.6 µm in diameter, composed of 4 to 7 lamellae. Numerous vacuoles of various size can be found in the cytoplasm. The first type, with lysosomal appearance, 40 to 1,500 nm in diameter, is filled with an electron-lucent,
FIGS. 3-5: 3) Fragment of epithelial cell. Note also two layers of basement membrane. H) haemocoel; N) nucleus; NB) nerve bundle; S) fragment of spermatozoon. × 15 800; 4) Fragment of epithelial cell with the Golgi complexes (GA) and the lysosome-like vacuoles (LV). × 22 000; 5) Fragment of epithelial cell: LVD) lumen of vas deferens; MC) process of muscle cell; MV) vacuole containing myelin-like structures. × 21 500.
fine granular material and internally to the enclosing membrane fragments of another membrane are often visible (Fig. 4). Some of those granules contain typical myelin-like structures and electron-dense bodies (Fig. 5). Near the lumen of vas deferens another type of smaller vacuoles with a diameter of up to 1,000 nm, and different appearance can be observed. They are filled with an electron-dense material and often contain centrally localized less electron-dense core formed of slightly granular substance, in this respect very similar to the material observed between the spermatozoa within the lumen of vas deferens (Fig. 6). These two materials also strongly stain in CA-PTA method (Fig. 7). Such dense vacuoles are particularly numerous in the epithelial cells of vasa efferentia filled with big amount of spermatozoa. Apart from the described

Figs. 6-7: 6) Fragment of epithelial cell with two types of vacuoles: the lysosome-like vacuoles (LV) and dense vacuoles (DV). The luminal surface of the cell shows scarce microvilli (arrow). LVD) lumen of vas deferens. × 27,500;

7) Similar fragment as on figure 6 — stained with CA-PTA, material in the lumen of vas deferens and dense vacuoles are darkly stained. EC) epithelial cell; H) haemocoel; S) spermatozoon. × 18,000.
above structures, the cytoplasm of epithelial cells contains numerous free ribosomes, a few elongated mitochondria and microtubules, about 220 Å in diameter, running in various directions. In some cases, in areas where degenerating spermatozoa adhere to the wall of vas deferens, specific epithelial cells with anastomosing microvilli and bigger than usual number of lysosome-like vacuoles (200 to 500 nm in diameter) can be observed (Fig. 8).

Muscle cells.

The light microscope permits to distinguish only the nuclei of muscle cells, which with their oval shape differ from the nuclei of epithelial cells. The electron microscopic observations reveal that muscle cells are lying separately on the epithelial layer at the haemocoel side, and possess numerous processes with a diameter ranging from 1 to 5 µm, dipped in the recesses of epithelial cells. Such recesses are visible as strangulations of vas deferens in the light microscope. The
Figs. 10-12: 10) Transversal section through the process of muscle cell lying in the recess of epithelial cell (EC). BM) basement membrane; H) haemocoel; LVD) lumen of vas deferens. × 39 700; 11) Transversal section through the process of muscle cell. Note the arrangement of thick and thin myofilaments and the presence of microtubules (Mt). × 54 000; 12) Longitudinal section through the process of muscle cell: EC) epithelial cell; Sp) sarcoplasm; Z lines are indicated by arrows. × 9 800.
central part of muscle cell (about 5 µm in diameter) contains the nucleus with a small amount of heterochromatin. In the perinuclear cytoplasm are found the vacuoles (about 500 nm in diameter) embracing the membranes arranged concentrically (as in lysosome-like vacuoles of epithelial cells) or parallelly (Fig. 9). Scarce mitochondria with not numerous cristae observed in muscle cells differ from the mitochondria from epithelial cells in respect of having a lesser electron density. The contractile apparatus is composed of myofibrils localised mainly in the areas of cytoplasm at the side of the lumen of vas deferens (Fig. 10). Each process of muscle cell contains a single myofibril, and the myofibrils from different processes are seen near the nucleus running not parallelly. The myofibrils consist of thick myofilaments (200 Å) surrounded by orbitals of 10 to 12 thin myofilaments (50 Å) (Fig. 11). The longitudinal sections reveal quite irregularly contoured Z lines (Fig. 12), separated from each other by the distance of about 10 µm. The length of thick myofilaments is about 8 µm. In the direct neighbourhood of myofilaments microtubules with a diameter of 220 Å are observed. The sarcoplasmic reticulum appears in form of a few big, smooth-surfaced cisternae with an electron-lucent content.

Basement membrane.

The wall of vas deferens is separated from the haemocoel by the basement membrane composed of several layers, each about 200 Å thick, built of electron-dense, amorphous material and rather loosely connected with the cells. Between the layers of basement membrane are occasionally found the unmyelinated nerve fibres, innervating the vas deferens (Fig. 3).

DISCUSSION.

The wall of vas deferens in *P. viator* is composed, similarly to the vas deferens wall described in ticks (BALASHOV, 1964), of two cell types: epithelial cells and muscle cells.

The possible functions of epithelial cells include: 1) separation of the vas deferens contents from the haemocoel, 2) production and secretion of intercellular material filling the spaces between the spermatozoa, 3) destruction of some spermatozoa. Numerous Golgi complexes, abundant rough endoplasmic reticulum and the presence of electron-dense vacuoles, probably referable to the PAS-positive vacuoles observed in light microscope — all these features of epithelial cells seem to support their secretory role. Identical reactivity in the PAS and CA-PTA methods, as well as similar ultrastructural appearance of the core of electron-dense vacuoles and of the material surrounding the spermatozoa within the lumen of vas deferens may be regarded as the arguments for their identity, which leads to the conclusion, that in the investigated cells occur the processes of synthesis and secretion of the substance which subsequently fills the vas deferens. The secretory processes have been described in cells of the anterior region of the ejaculatory duct in housefly (RIEMANN, 1973), and in some cells of vasa deferentia in the crustacean, *Porcellio scaber* (NEWSTEAD and DORNFELD, 1965; RADU and CRACIUN, 1973). The second type of vacuoles found in the cytoplasm of epithelial cells in the vas deferens of *P. viator* is more electron-lucent and occasionally contains myelin-like structures. This kind of vacuoles may be most probably referable to lysosomes, which is indirectly confirmed by the presence of numerous acid phosphatase-positive granules in the epithelial cells seen under the light microscope. Myelin-like bodies showing the activity of acid phosphatase have been found i.a. in the taste bud cells of the rabbit (OLIVIERI-SANGIACOMO, 1970) and in mature oocytes of the earthworm, *Eisenia fetida* (LECHENAUT, 1968). Similar structures lacking the acid phosphatase activity have been described in cells of the male accessory genital glands in the colorado-beetle, *Leptinotarsa decemli-
neata (De Loof and Lagasse, 1972) and in amoebocytes of the snail, Limnea stagnalis (Sminia, 1972). The latter data may not necessarily speak against the lysosomal origin of such structures, since the enzymatic activity of lysosomes occasionally may not be detected with histochemical methods. The further fate of the vacuoles observed in *P. viator* is not known, nevertheless, lack of fuzzy coated vesicles or other structures correlated with endocytosis suggests that the vacuoles may be involved in autophagic processes or in the secretion of their content to the lumen of vas deferens. The latter event takes place most probably in the cells to which adhere scarce degenerating spermatozoa, those cells demonstrate the anastomosing microvilli protruding towards the lumen of vas deferens, which may take part in the resorption of products generated during the destruction of spermatozoa. Lack of evidence of active endocytosis indicates, that the resorption in the epithelial cells may be carried out in some other way than the resorption of the material from the destructed extracellular tubules in the vasa deferentia of many crustaceans (Reger and Fain-Maurel, 1973). The connection of epithelial cells by the septate desmosomes mostly localized near the lumen of vas deferens has been also described by Breucker (1970) in the male genital ducts of Chilopoda.

The muscle cells present in the vas deferens wall of *P. viator* are structurally similar to the fibres of visceral muscles described in other arthropoda (Smith et al., 1966; Andersson and Ellis, 1967) from which they differ, however, by the presence of numerous cytoplasmic processes winding round the vas deferens, as well as by the presence of lysosome-like vacuoles in their cytoplasm. The contractile apparatus resembling the one described in the present paper has been observed in the male genital organs of housefly (Riemann, 1974) and Colorado-beetle (De Loof and Lagasse, 1972). The comparison made between the dimensions of thick myofilaments and the relation of the number of thick filaments to the number of thin filaments seems to confirm the hypothesis of Hagopian and Spiro (1968), that those values are correlated. The Z lines show irregular contours, similarly to Z lines described in alary muscles of a cockroach (Adams et al., 1973) or in the giant muscle fibres of barnacle (Hoyle et al., 1973). Near the myofibrils can be found small fragments of sarcoplasmic reticulum known to take part in the accumulation of divalent cations which are involved in the mechanism of contraction in muscle cells (Devine et al., 1973). Considering the low extent of development of sarcoplasmic reticulum, small number of mitochondria with not numerous cristae — the features closely linked with the mode of muscle cell action (Schaefer et al., 1967) — it is tempting to suggest that the muscle cells of *P. viator* vas deferens are only intermittently active, generating the contractions of the duct, responsible for the passage of spermatozoa from vasa deferentia to the ejaculatory duct and subsequently for the refilling of vasa deferentia with a new portion of spermatozoa arriving from the testis.

There are numerous reports concerning the localization of basement membrane between the epithelial and the muscle cells — such situation exist e.g. in male genital ducts of Chilopoda (Breucker, 1970) and Diptera (Riemann, 1973), as well as in alimentary tract of Diptera (Schaefer et al., 1967) and Acarina (Belozerov and Tymopheev, 1973; Bernini, 1973). In some cases, however, the basement membrane does not separate epithelial and muscle cells, but encloses them together. Such arrangement, found in the vas deferens of *P. viator*, and observed also in testis of numerous Echinodermata (Atwood, 1973) suggests particularly close structural and functional relationship between the two types of cells.
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