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Histological and ultrastructural characterization of the intravasal body in Vestimentifera (Siboglinidae, Polychaeta, Annelida)

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Introduction

An intravasal or heart-body is a strand of tissue, generally located inside the dorsal contractile blood vessel and attached to its ventral luminal surface. It is separated from the blood by a basal lamina. Among the polychaetes, an intravasal body is present in several groups among the Terebellida (Dales & Pell, 1970), such as the Flabelligeridae (Spies, 1973) and the Alvinellidae (Jouin-Toulmond et al., 1996) as well as in frenulate and vestimentiferan Siboglinidae (Ivanov, 1963; Southward, 1993; Gardiner & Jones, 1993). Kennedy & Dales (1968) showed that the heart-body in *Neoamphitrite figulus* is involved in haemoglobin synthesis.

In Vestimentifera, the intravasal body starts behind the heart, formed by the dorsal vessel in the anterior vestimental region (Gardiner & Jones, 1993). The present study provides information on the histology and ultrastructure of the vestimentiferan intravasal body. To date, the origin of haemoglobin in the vestimentiferan circulatory system and coelom is unknown. The demonstration that the vestimentiferan intravasal body is ultrastructurally similar to the heart-body of other polychaetes suggests that it is also involved in the vascular haemoglobin metabolism.

Material and methods

The dorsal blood vessel of the following species was examined with light microscopy: *Arcovestia ivanovi* Southward & Galkin, 1997, *Escarpia laminata* Jones, 1985, *Lamellibrachia* cf *luymesi* Van der Land & Nørrevang, 1975, *Oasisia alvinae* Jones, 1985, *Paraescarpia echinospica* Southward et al., 2002, *Ridgeia piscesae* Jones, 1985, *Riftia pachyptila* Jones, 1981, *Tevnia jerichonana* Jones, 1985, *Seepiophila jonesi*, Gardiner et al., 2001. In addition, *Ridgeia piscesae* was examined with transmission electron microscopy.

For light microscopy (LM), specimens previously fixed in 7% buffered formalin were cut into pieces of up to 1 cm length. The pieces were rinsed with running tap water for 4-5 h and then left in tap water overnight. They were dehydrated in an ethanol series up to 100%. JB-4 plastic resin was used for embedding, using the instructions of the manufacturer. Serial cross sections of 3 µm were made with glass knifes on a Sorvall Porter-Blum JB-4 microtome. The sections were stained with toluidine blue, Wright's stain and eosine/haematoxylin and mounted using Permount, DPX Mountant or Entellan. Photographs were taken with a Zeiss microscope equipped with a 35 mm camera.

For Transmission Electron Micrsocopy (TEM) examination, specimens were fixed in 2.5% glutaraldehyde in 0.2 M Millonig's phosphate buffer with 0.14 M NaCl (pH 7.4), rinsed with a 1:1 mixture of Millonig's phosphate buffer and 0.6 M NaCl, postfixed in 1% OsO_4 and dehydrated in an ethanol series up to 100%. Propylene oxide was used as a transitional agent. Specimens were infiltrated in a 1:1 mixture of Epon resin and propylene oxide for approximately 8 h at room temperature in a rotary mixer, followed by a 3:1 mixture of resin and propylene oxide for the same amount of time. They were placed in embedding molds containing pure resin and infiltrated for another 8-10 h. Polymerization was accomplished at 60°C for 24 hrs. Semithin sections (1 µm) were cut with glass knives on a Reichert OM U2 microtome and stained with Richardson's stain. Thin sections were cut with glass knives or a diamond knife and mounted on 200 mesh grids. Staining was performed with uranyl acetate (2%, pH 4.5,

1.5 h) and lead citrate (0.1%, 15 min). The sections were viewed with a Hitachi 7000 transmission electron microscope at 75 kV.

Results

An intravasal body was observed in the dorsal vessel of all the species examined, except in *Tevnia jerichonana*. Only the anterior vestimentum of this species was studied, and it is possible that there is an intravasal body elsewhere in the dorsal vessel. In all other species, the intravasal body is a strand of tissue in the dorsal vessel that starts behind the heart in the anterior vestimentum. As observed in *Ridgeia piscesae* and *Riftia pachyptila*, it continues through the trunk and into the opisthosome. In the opisthosome, it could not be observed with LM, but with TEM, a small strand of intravasal tissue was detected. Whereas the intravasal body in the vestimentum is small and relatively constant in diameter in *Escarpia laminata, Seepiophila jonesi* and *Arcovestia ivanovi* (Fig. 1A), in *Riftia pachyptila* (Fig. 1B), *Ridgeia piscesae* and *Oasisia alvinae* it can almost obstruct the lumen of the dorsal vessel in the vestimentum and trunk. The intravasal body is located ventrally inside the dorsal vessel, overlying the line where the mesentery splits to form the walls of the blood vessel (Fig. 1C). In *Riftia pachyptila, Ridgeia piscesae* and *Oasisia alvinae* it is not uniform in diameter but forms a series of swellings throughout the length of the animal. In a *Riftia pachyptila* with a vestimentum diameter of 4.1 mm, the diameter of the intravasal body in cross section alternates between 375 µm and approximately 10 µm (Fig. 1 B, C). Along the 4 mm length of one vestimentum, six swellings were present. Distances between the swellings are variable and are larger in the trunk than in the vestimentum.

Histologically, the intravasal body of *Riftia pachyptila* has a structure slightly different from that of the other vestimentiferan species. Its wall is a squamous epithelium that is thickened at its dorsal side (Fig. 1B). In this area, it is pseudostratified and up to 100 µm thick. The basal lamina in this region is approximately the same thickness as the vascular lamina of the dorsal vessel and it penetrates deeply between the epithelial cells (Fig. 1B). The basal lamina is thinner in the thinner epithelial regions. The apical surface of the intravasal body epithelium in the thickened region shows globular bodies. With every stain applied, the lumen of the intravasal body always has a different colour than the blood or coelomic fluid, indicating different fluid compositions in these compartments.

In *Ridgeia piscesae, Oasisia alvinae* and *Paraescarpia echinospica*, the basal lamina of the intravasal body is thinner but it invaginates also between the epithelial cells. The intravasal body epithelium lining the lumen is spongy in appearance with an irregular apical surface. In all the species examined, the large epithelial cells are histologically similar. They have a clear cytoplasm and a large nucleus (diameter up to 5 µm) with a distinct nucleolus.

Ultrastructurally, the basal lamina that lines the intravasal body of *Ridgeia piscesae* (Fig. 2A) differs from the vascular lamina of the dorsal vessel. In areas where it is not invaginated, it is less than 1 µm thick, while the vascular lamina is 4.5 µm thick. Collagen fibres are less abundant in this basal lamina than in the vascular lamina. However, the invaginated areas of the basal lamina sometimes contain striated collagen fibres and crystalline structures (Fig. 2B). The crystals show a pattern of electron-dense and electron-lucent stripes, repeating approximately every 70 nm. The

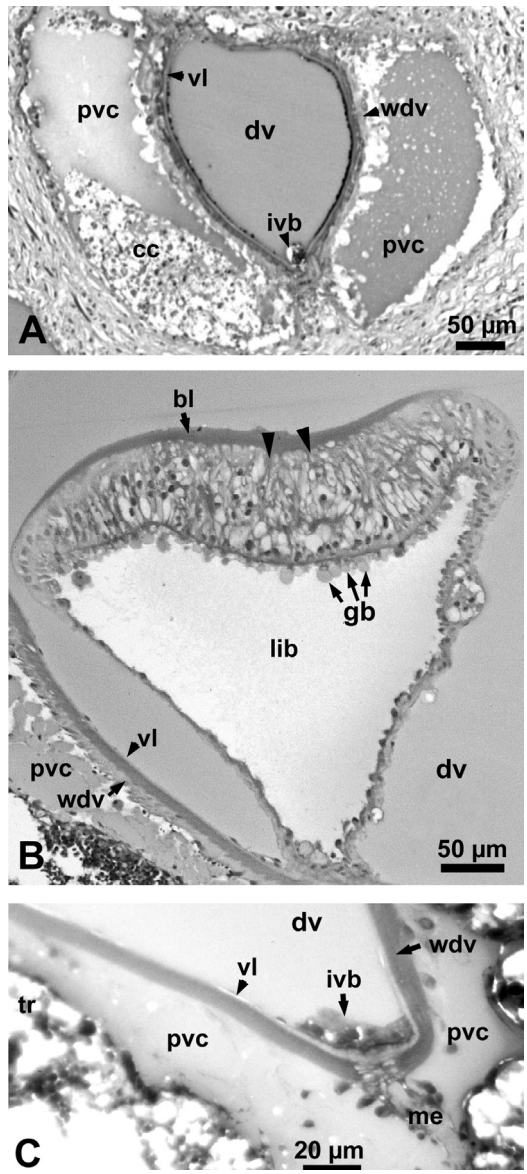

Figure 1. Cross sections (LM) of dorsal blood vessel, intravasal body, perivascular coelom. **A.** *Arcovestia ivanovi*, with a small intravasal body (approximately 10 µm in diameter). **B.** *Riftia pachyptila*, with an enlarged intravasal body in the trunk region (arrowheads: basal lamina invaginating between epithelial cells). **C.** *Riftia pachyptila*, intravasal body as a small strand of cells in the trunk region. (*bl*) basal lamina; (*cc*) coelomocytes; (*dv*) dorsal vessel; (*gb*) globular bodies in the lumen of intravasal body; (*ivb*) intravasal body; (*lib*) lumen of intravasal body; (*me*) mesentery; (*pvc*) perivascular coelom; (*tr*) trophosome; (*vl*) vascular lamina; (*wdv*) wall of dorsal vessel.

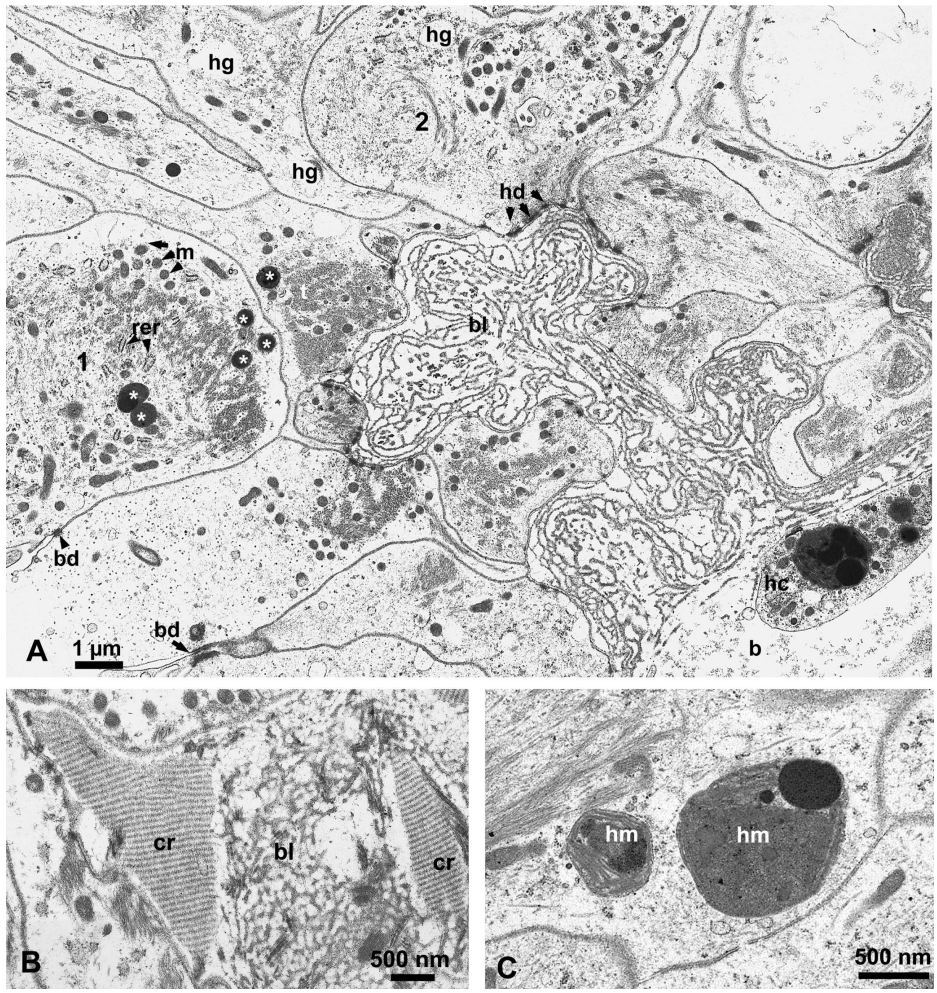

Figure 2. *Ridgeia piscesae*, intravasal body, TEM. **A.** Basal lamina invaginated into the epithelium of intravasal body; cells 1 with several potential haematin granules (white asterisks); cells 2 with presumed haemoglobin vacuoles. **B.** Part of the invaginated basal lamina in intravasal body with striated collagen fibrils and crystals. **C**. Close-up of potential haematin granule. (*b*) blood; (*bd*) belt desmosomes; (*bl*) basal lamina; (*cr*) crystals; (*hc*) haemocyte; (*hd*) hemidesmosomes; (*hg*) electron-lucent vesicles with a potential haemoglobin content; (*hm*) potential haematin granules; (*m*) mitochondria; (*rer*) rough endoplasmic reticulum.

basal parts of the epithelial cells connect to the basal lamina with numerous attachment plaques. From the attachment plaques, a dense network of filaments extends into the basal parts of the cells. The apices of the cells connect via desmosomes. The cytoplasm of the epithelial cells contains numerous mitochondria, rough endoplasmic reticulum, some Golgi vesicles and electron-dense inclusions (Fig. 2A). At high magnification the electron-dense inclusions are of a heterogeneous substructure, partly granular and partly with lamellar structures (Fig. 2C). In addition, small, electron-dense granules of approximately 24 nm diameter are present in the cytoplasm. The cells are often ruptured and open into the lumen of the intravasal body. Whereas some cells contain large numbers of the electron-dense lamellar bodies, others contain a larger amount of electron-lucent vesicles that are apparently derived from the endoplasmic reticulum.

Discussion

As in other polychaetes, the cells in the vestimentiferan intravasal tissue contain electron-dense and electron-lucent vesicles in variable amounts. The electron-dense inclusions in other polychaetes have been identified by chromatographic methods as haematins, mainly Coprohaematin III (Mangum & Dales, 1965; Dales & Pell, 1970). With their partly lamellar and partly granular substructure, the electron-dense vesicles in the vestimentiferan intravasal body resemble the haematin bodies in other polychaetes.

Ultrastructural studies of the extravasal tissue of *Arenicola marina* demonstrated haemoglobin molecules in light grey staining vesicles (Breton-Gorius, 1963, Dales & Pell, 1970). The haemoglobin molecules are approximately 27 nm in diameter, have a molecular weight of 3648 kDa (Zal et al., 1997) and are multimers, arranged as two

hexagonal rings (Terwilliger et al., 1976; Zal et al., 1997). Similar vesicles are also present in the cells of the intravasal body of *Ridgeia piscesae*, but no haemoglobin molecules were detected there. Gardiner & Jones (1993) reported vacuoles with blood-like contents in the intravasal body of the vestimentiferan *Oasisia piscesae* but did not identify haemoglobin. The absence of ultrastructurally recognizable haemoglobin in these vesicles might be an artifact due to a low stability of the molecules. The largest of the vestimentiferan haemoglobin types *(Riftia pachyptila)* has a molecular weight of 3396 kDa and is approximately 27 nm x 18 nm in size (Zal et al., 1996). This is within the size range of the granular bodies found in the cytoplasm of the vestimentiferan intravasal body cells, but these are probably of a different nature because haemoglobin is usually enclosed in vacuoles before it is excreted into blood.

The basal lamina of the vestimentiferan intravasal body is similar in structure to the basal lamina of heart-body in other polychaetes. It invaginates between the epithelial cells and is more loosely constructed than the vascular lamina. The basal invagination of the epithelium may serve to increase the surface area for the release of haemoglobin by exocytosis. The loose construction of the basal lamina may facilitate exchange of substances between blood and intravasal tissue. The crystals seen in the basal lamina have a periodicity of 71 nm, which is about the range of the 65-70 nm repeat pattern seen in *Riftia pachyptila* cross-striated interstitial collagen fibrils (Gaill et al.,1991). This observation suggests that the extracellular crystals in the basal lamina are likely collagen.

The ultrastructural similarities of the vestimentiferan intravasal body and the polychaete heart-body, suggest that the two organs perform similar functions. More direct evidence, such as the presence of globin mRNA, haemoglobin complexes or activity of enzymes involved in haeme synthesis are required to confirm that the intravasal body is the site of haemoglobin synthesis in the Vestimentifera.

In addition, Gardiner & Jones (1993) suggest that the vestimentiferan intravasal body serves as a mechanical valve to prevent backflow of blood from the dorsal vessel into the mesenterial vessel. In the vestimentum it might also prevent backflow of blood from the paired, blind-ending, obturacular vessels into the dorsal vessel.

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References

Breton-Gorius J. 1963. Etude au microscope électronique des cellules chloragogènes d'*Arenicola marina* L. Leur rôle dans la synthèse de l'hémoglobine. *Annales des Sciences naturelles,* **5**: 211-271.

Dales R. P. & Pell J. P. 1970. Cytological aspects of haemoglobin and chlorocruorin synthesis in polychaete annelids. *Zeitschrift für Zellforschung,* **109**: 20-32.

Gaill F., Wiedmann H., Mann K., Kühn K., Timpl R. & Engel J. 1991. Molecular characterization of cuticle and interstitial Collagens from worms collected at deep sea hydrothermal vents. *Journal of Molecular Biology* **221**: 209-223.

Gardiner S. L. & Jones M. L. 1993. Vestimentifera. In: *Microscopic Anatomy of the Invertebrates* (F. W. Harrison & Rice M.E. Eds), vol. **12**, pp 371-460. Wiley-Liss: New York.

Ivanov A. V. 1963. *Pogonophora*. Academic Press: London. 479 pp.

Jouin-Toulmond C., Augustin D., Desbruyères D. & Toulmond A. 1996. The gas transfer system in alvinellids (Annelida Polychaeta, Terebellida). Anatomy and ultrastructure of the anterior circulatory system and characterization of a coelomic, intracellular, haemoglobin. *Cahiers de Biologie Marine,* **37**: 135-151.

Kennedy G. Y. & Dales R. P. 1968. Formation of uroporphyrin from porphobilinogen by the heart-body tissue and coelomocytes of the polychaete *Neoamphitrite figulus*. *Journal of the Marine Biological Association of the UK,* **48**: 77-79.

Mangum C. P. & Dales R. P. 1965. Products of haem synthesis in polychaetes. *Comparative Biochemistry and Physiology,* **15**: 237-257.

Southward E. C. 1993. Pogonophora. In: *Microscopic Anatomy of the Invertebrates* (F. W. Harrison & Rice M.E. Eds), vol. **12**, pp. 327-369. Wiley-Liss: New York.

Spies R. B. 1973. The blood system of the flabelligerid polychaete *Flabelliderma commensalis*. *Journal of Morphology,* **139**: 465-490.

Terwilliger R. C., Terwilliger N. B. & Schabtach E. 1976. Comparison of chlorocruorin and annelid hemoglobin quaternary structures. *Comparative Biochemistry and Physiology,* **55A**: 51-55.

Zal F., Lallier F.H., Green B.N., Vinogradov S.N & Toulmond A. 1996. The multi-hemoglobin system of the hydrothermal vent tubeworm *Riftia pachyptila*: II. Complete polypeptide chain composition investigated by maximum entropy analysis of mass spectra. *Journal of Biological Chemistry,* **271**: 8875-8881.

Zal F., Green B. N., Lallier F.H., Vinogradov S.N. & Toulmond, A. 1997. Quarternary structure of the extracellular haemoglobin of the lugworm *Arenicola marina.* A multi-angle-laser-light-scattering and electrospray-ionisation-mass-spectrometry analysis. *European Journal of Biochemistry,* **243**: 85-92.