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The gas transfer system in alvinellids (Annelida Polychaeta, Terebellida). Anatomy and ultrastructure of the anterior circulatory system and characterization of a coelomic, intracellular, haemoglobin.

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Abstract : In *Alvinella pompejana*, *A. caudata* and *P. grasslei*, the vascular blood which contains an extracellular haemoglobin is propelled through the anterior gills by a branchial heart, in which a rod-like heart-body increases the pumping efficiency of the heart. Behind the heart, the dorsal vessel runs back to the major part of the body and contains an intravasal haematopoietic heart-body. Coelomic erythrocytes, not previously known in alvinellids, contain an intracellular haemoglobin. These erythrocytes are clustered together with granulocytes in a perioesophageal pouch which encloses also a well developed plexus of thin blood capillaries. In the pouch, the diffusion distance between the extracellular and the intracellular haemoglobins is short (0.5 µm) and the association of blood capillaries and erythrocytes represents in alvinellids a complex respiratory gas transfer system previously unknown in polychaetes. Histological observations of dark granules in the blood vessels' wall also suggest that, in *P. grasslei,* sulfide enters the body by diffusion across the branchial surface area, is transported by the blood and immobilized mainly in the coelomic epithelium lining the blood vessels. It is suggested that the respiratory gas transfer system of the perioesophageal pouch should also participate in sulfide detoxification and that it could have been selected in these species in relation with the varying physico-chemical conditions of the deep hydrothermal environment.

Résumé : Chez *Alvinella pompejana*, *A. caudata* et *P. grasslei*, le sang est propulsé dans les branchies par un cœur branchial, dans lequel un corps cardiaque en forme de baguette accroît la force de propulsion du cœur. En arrière du cœur, le vaisseau dorsal présent tout le long du corps contient un corps cardiaque hématopoiétique. Des érythrocytes cœlomiques, jusqu'ici non décrits chez les alvinellidés, renferment une hémoglobine intracellulaire. Ces érythrocytes sont concentrés, mélés à des granulocytes, dans une poche péri-œsophagienne qui recouvre aussi un plexus capillaire sanguin. Dans la poche, la distance de diffusion entre les hémoglobines extracellulaire et intracellulaire est courte (0,5 µm) et l'association capillaires sanguins-érythrocytes représente chez les Alvinellidés un système de transfert respiratoire complexe, nouveau chez les Polychètes. L'observation sur coupes histologiques de grains noirs dans la paroi des vaisseaux sanguins suggère que, chez *P. grasslei*, le sulfure d'hydrogène du milieu extérieur pénètre dans le corps par diffusion à travers la surface branchiale. Transporté par le sang, il serait immobilisé principalement dans l'épithélium cœlomique formant la paroi des vaisseaux sanguins. Le système de transfert respiratoire de la poche péri-œsophagienne pourrait participer à la détoxification du sulfure d'hydrogène et représenterait un dispositif avantageux chez ces animaux soumis aux conditions physico-chimiques variables du milieu hydrothermal profond.

Keywords : Circulatory system, erythrocytes, haemoglobin, Alvinellidae, Polychaeta, hydrothermalism.

Introduction

Most species of Polychaeta from the order Terebellida are characterized by a limited number of well developed and

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highly vascularized anterior gills. In such species of terebellids, ampharetids and trichobranchids for example, the blood is pushed into the vascular system of the gills by a contractile dorsal vessel running on the oesophagus, the so-called branchial heart (Picton, 1899), which contains an intravasal tissue, the heart-body (Fauvel, 1897; Picton, 1899; Kennedy & Dales, 1958). Heart-bodies are present, with a variable extension, in the anterior dorsal vessel of Terebellida as well as in other polychaetes including cirratulids and flabelligerids (Meyer, 1887; Picton, 1899; Spies, 1973). The haematopoietic function of this intravasal tissue, *i.e.* the synthesis of the extracellular haemoglobin dissolved in the plasma, has been suggested (Meyer, 1887) and later demonstrated (Kennedy & Dales, 1958; Dales, 1965; Mangum & Dales, 1965; Dales & Pell, 1970; Spies, 1973; Friedman & Weiss, 1980; Braunbeck & Dales, 1985). In addition, in some species, a mechanical function of the heart-body has also been observed (Fauvel, 1897; Picton, 1899).

Alvinellids are recently discovered polychaete annelids of the order Terebellida that are endemic to the deep sea hydrothermal vents in the Pacific Ocean (Desbruyères & Laubier, 1986). They possess four pairs of well developed, anterior gills, whose organization and fine structure have been described in *Alvinella pompejana* Desbruyères & Laubier, 1980, and *Paralvinella grasslei* Desbruyères & Laubier, 1982 (Jouin & Gaill, 1990). The blood irrigating these gills contains an extracellular haemoglobin whose properties have already been studied (Terwilliger & Terwilliger, 1984; Toulmond *et al.*, 1990). The aim of the present study was to investigate the pattern of the anterior circulatory system of these alvinellids and to compare our data with those obtained in other families of the order Terebellida. In addition, the ultrastructure of coelomic erythrocytes, and the features of the intracellular haemoglobin they contain, were examined.

Material and methods

Specimens of *Alvinella pompejana*, *A. caudata* Desbruyères & Laubier, 1986 and *Paralvinella grasslei* were collected in October 1991 and March 1992 during the French-American "HERO" scientific cruises on the "13°N" hydrothermal vent site (East Pacific Rise, 12°48'N, 103°56'W). Large pieces of black and white smokers were plucked off, using the manipulators of the submersibles *Nautile* or *Alvin*, and maintained at collection temperature (2.4°C) in an insulating plastic box during the trip to the surface (2-3 h). On board the surface ship, selected worms were relaxed in a mixture of sea water and 7% magnesium chloride solution, dissected for anatomical study and fixed with 10% sea water-buffered neutral formalin. Other relaxed specimens were directly fixed with an intracoelomic injection of 10% sea water-buffered neutral formalin. For histological study, the specimens were post-fixed with Bouin and 6 µm thick paraffin sections were stained with the periodic acid-Schiff (PAS) method and Groat haematoxylin.

For scanning electron microscopy (SEM), formalin fixed animals were dissected, pinned on a small piece of thin sheet of cork, then dehydrated in ethanol and critical point dried with carbon dioxide. The specimens were mounted, gold coated, and examined with a Jeol 840A scanning electron microscope. For transmission electron microscopy (TEM), the specimens were post-fixed with 1% osmium tetroxide in 0.2 M sodium cacodylate buffer, pH 7.4, for 1 h, dehydrated and embedded in Epon. The specimens were sectioned with a Reichert Ultracut microtome, and the grids covered with pioloform were examined with a Philips 201 electron microscope.

In a few live, selected specimens of each species, the coelomic fluid was carefully sampled through the body wall with a syringe and hypodermic needle and centrifuged at 12000 rpm and 4°C for 10 min. The colorless supernatant and the whitish upper part of the pellet, essentially composed of white coelomocytes and gametes, were discarded. The reddish, lower part of the pellet was resuspended in sea water at 4°C and centrifuged in the same conditions as above. This operation was repeated twice and the final red pellet, mainly composed of red coelomocytes, was resuspended in 1 ml distilled water at 4°C, centrifuged at 12000 rpm and 4°C for 20 min, and the red supernatant immediately frozen in liquid nitrogen. U.V./vis. absorption spectra of these samples, prepared as described in Van Assendelft (1970), were obtained using a Bausch and Lomb Spectronic 2000 spectrophotometer.

Results

General Anatomy of the circulatory system

In alvinellids the circulatory system follows the basic normal pattern of annelids and comprises two main vessels extending the length of the body, *i.e.* a well developed dorsal vessel, closely applied to the gut, and a ventral vessel, well separated from the gut, a vessel applied to the nerve cord (*Alvinella*), a gut sinus, and segmental vessels. This pattern is modified in the anterior region of alvinellids by the presence of four pairs of gills located on the first four body segments.

Approximately at the same level in the two genera (at a short distance from the oesophagus - stomach junction) the dorsal vessel divides in two parts: a contractile branchial heart, well separated from the oesophagus and running anteriorly to the gills, and a thinner supra-oesophageal vessel connected anteriorly to the head region (Figs. 1-3).

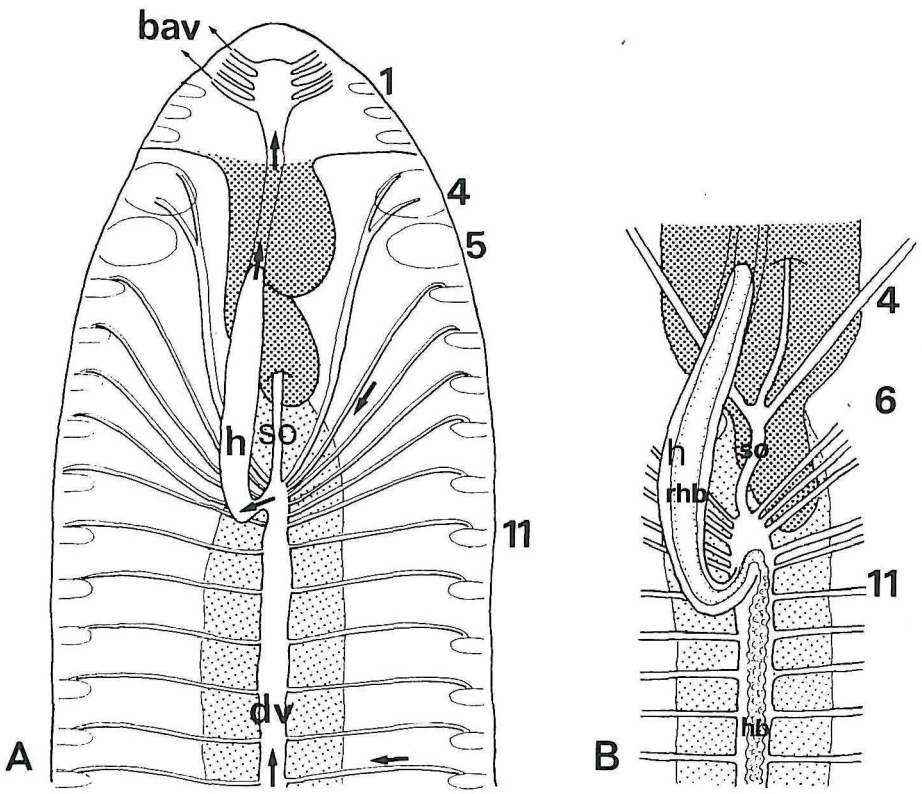


Figure 1, 2 and 3: Semi-diagrammatic drawings of the anterior circulatory system of alvinellids, dorsal view. The dorsal vessel (dv) divides in two parts: a contractile branchial heart (h) ending up anteriorly in four pairs of branchial afferent vessels (bav) and a supra-oesophageal vessel (so). Both penetrate into a perioesophageal pouch (darker dotted area). Clear dotted area, stomach. Arrows: direction of blood circulation.

Figure 1 *Alvinella pompejana.* **A:** The efferent vessels from the parapodia and body wall of segments 4 to 10 flow into a small confluence area of the dorsal vessel (dv) just at the outset of the heart (h). From segment 11 backwards, each efferent vessel flows into the dorsal vessel (dv). **B:** in some specimens of *A. pompejana* the efferent vessels of segment 4 merge with the supra-oesophageal vessel (so). hb: heart-body in the dorsal vessel; rhb: rod-like heart-body in the heart.

Figure 1, 2 and 3 : Dessins semi-schématiques du système circulatoire antérieur des alvinellidés en vue dorsale. Le vaisseau dorsal bifurque en un cœur branchial (h), se terminant à l'avant par quatre paires de vaisseaux afférents branchiaux (bav), et un vaisseau sus-œsophagien (so). Le cœur et le vaisseau sus-œsophagien pénètrent dans une poche périœsophagienne (pointillé sombre). Pointillé clair : estomac. Les flèches indiquent le sens de la circulation.

Figure 1 *Alvinella pompejana.* **A :** Les vaisseaux efférents des parapodes et de la paroi du corps des segments 4 à 10 se jettent dans une courte région du vaisseau dorsal (dv) située au niveau du départ du cœur (h). A partir du segment 11, vers l'arrière, chaque vaisseau efférent se jette dans le vaisseau dorsal. **B :** chez certains specimens de *A. pompejana* les vaisseaux efférents du segment 4 se jettent dans le vaisseau sus-œsophagien (so). hb : corps cardiaque dans le vaisseau dorsal ; rhb : corps cardiaque du cœur branchial.

In all three species, the heart ends anteriorly with four pairs of branchial afferent vessels, each penetrating a gill. Symmetrically, eight efferent vessels leave the gills and enter into the ventral vessel which extends backwards and gives rise in each segment to a pair of afferent vessels running mainly to parapodia and body wall.

It must be noted that the body cavity in alvinellids is not divided by septa and that there is only one anterior septum forming a perioesophageal pouch (see below). Nevertheless, all along the body, septal rudiments are present ventrally along the segmental afferent vessels, and a small discontinuous dorsal mesentery attaches the dorsal vessel to the body wall.

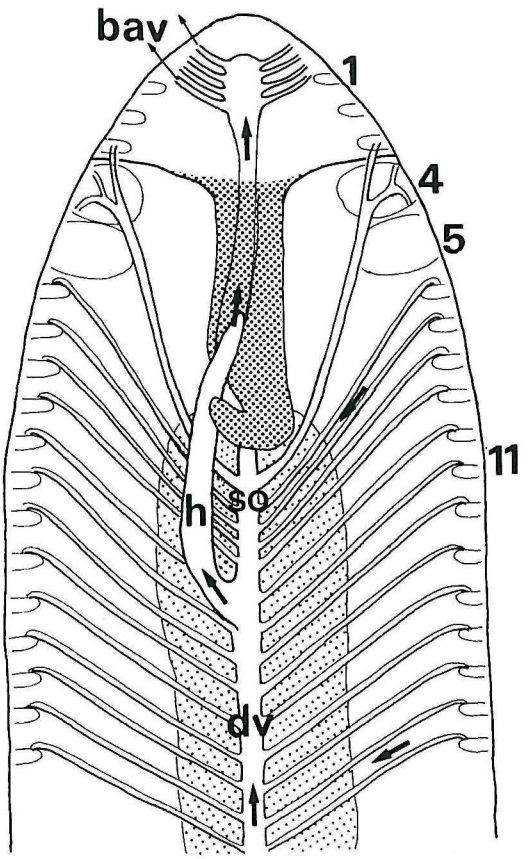


Figure 2. *A. caudata.* All the efferent vessels from segments 4 to 10 are well separated from each other and flow into the supra oesophageal vessel. Behind the ouset of the heart, from segment 11 backwards, each efferent vessel flows into the dorsal vessel (dv).

Figure 2. *A. caudata.* Tous les vaisseaux efférents des segments 4 à 10 sont séparés les uns des autres et se jettent en avant du cœur dans le vaisseau sus-œsophagien. A partir du segment 11 vers l'arrière, chaque vaisseau efférent se jette dans le vaisseau dorsal (dv).

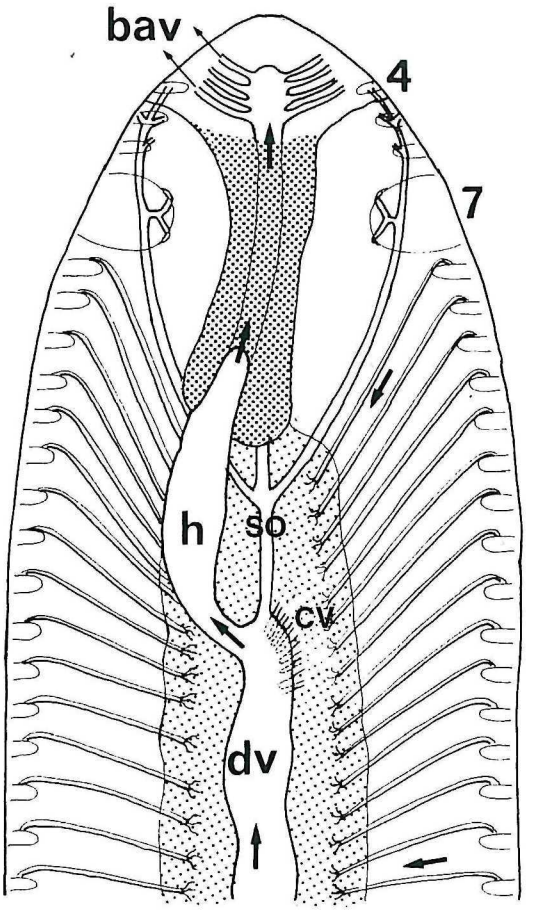


Figure 3 *Paralvinella grasslei.* The metameric efferent vessels are connected to a perienteric blood sinus, except for the common vessels of setigers 4 to 7 which merge with the supra-oesophageal vessel (so). The perienteric blood sinus is connected to the dorsal vessel (dv), by 6-7 unpaired vessels (cv) on the dorsal part of the stomach, at the level of the heart outset.

Figure 3 *Paralvinella grasslei.* Les vaisseaux efférents métamériques se jettent dans un sinus péri-entérique, sauf les vaisseaux efférents communs aux sétigères 4 à 7 qui se jettent dans le vaisseau sus-œsophagien (so). Le sinus péri-entérique est relié au vaisseau dorsal (dv) par 6-7 vaisseaux impairs (cv) sur la partie dorsale de l'estomac au niveau du départ du cœur.

The arrangement of the segmental efferent vessels

It was not possible to observe the circulation of the blood in the vessels, except in the heart, but owing to the presence of the anterior cardiac pump, all the segmental vessels connected to the dorsal vessel or to the supra-oesophageal vessel most probably drive the blood toward the heart. The distribution of these vessels, coming mainly from parapodia and body wall (efferent vessels), is different according to species.

In *Alvinella pompejana* (Fig. 1A-B) the efferent vessels from segments 6 to 10 are separated from each other and enter into a small confluence area of the dorsal vessel (approximately at level of segment 10) where the heart begins. The efferent vessels of segment 4 (and probably segment 5, but these vessels were not visible) have a common branch, wider than the other vessels, and according to specimens they merge either with the confluence area (Fig. 1A) or with the supra-oesophageal vessel anterior to the confluence area (Fig. 1B). The vessels of the first segments could not be studied. Behind segment 10, each segmental efferent vessel connect with the dorsal vessel (Fig. 1A-B).

In *A. caudata* (Fig. 2) the segmental efferent vessels have a more regular arrangement. They connect with the supra-oesophageal vessel: those of segment 4 (and probably 5) have a common branch, with a wide diameter and those of segments 6 to 10, are well separated from each other. The efferent vessels of segments 10 and 11 join with the dorsal vessel just at the outset of the heart. From segment 12 to segment 57 all the efferent vessels connect with the dorsal vessel. From segment 57 to segment 96 they enter into the circum-enteric sinus, laterally to the dorsal vessel. They have not been studied in the most posterior part of the body (from segment 97 backwards). It must be noted that, as in *A. pompejana,* there is a variable arrangement of the efferent vessels according to specimens and that some specimens of *A. caudata* exhibit an arrangement of the efferent vessels from segments 4 to 10 similar to that of some specimens of *A. pompejana* (Figure 1B).

In *Paralvinella grasslei* (Fig. 3) the vessels' arrangement is quite different: the efferent blood of segments 4 to 7 is collected by two large vessels, connected with the supra-oesophageal vessel. From segment 8 backwards the efferent vessels, well separated from each other, enter into the circum-enteric blood sinus, except in the ten last segments (S52-S62) where they connect with the dorsal vessel. Anteriorly, just at the outset of the heart, six or seven short median vessels connect the peri-stomachal sinus with the dorsal vessel. Such connecting vessels are absent in *Alvinella* since the efferent vessels communicate directly with the dorsal vessel.

The dorsal vessel and haematopoietic heart-body

Based on its morphology, the tissue in the lumen of the dorsal vessel correspond to an intravasal haemoglobin secreting tissue. Present along the major part of the dorsal vessel, it is attached along its medio-ventral line and it occupies a large part of the vessel lumen in the middle body region (Fig. 1B, 4 A-D). In a specimen of *A. pompejana* with about 100 setigers, the major part of the dorsal vessel contained such a brown intravasal tissue which was lacking only in the last 20 segments of the body. In a specimen of *A. caudata* with 200 setigers, the posterior flattened part of the body began at segment 50 and the dorsal vessel contained an intravasal tissue from segment 10 to segment 80 where the dorsal vessel became thinner.

The folded intravasal cords comprise a pseudostratified epithelium, surrounded by an extracellular matrix or *vascular lamina* (0.5 µm in thickness), without a *lamina densa* (Fig. 4 A, B). There is probably only one cell type, with a round nucleus, a well developed rough endoplasmic reticulum and a Golgi apparatus poorly developed on the specimens examined (Fig. 4 C, 9 A). Dark granules, similar to haematin granules are present (Fig. 9 A) together with conspicuous large compound granules (Fig. 9 B) sometimes surrounded by concentric membranes (Fig. 9 A). The mitochondria are mainly located at the intravasal cord periphery along the luminal sides (Fig. 4 B). Moderately dense paracristalline arrangements, free in the cytoplasm, are similar to ferritin molecules. Haemoglobin molecules are present in the intercellular spaces, before they reach the vessel lumen and the blood (Fig. 4 C, D).

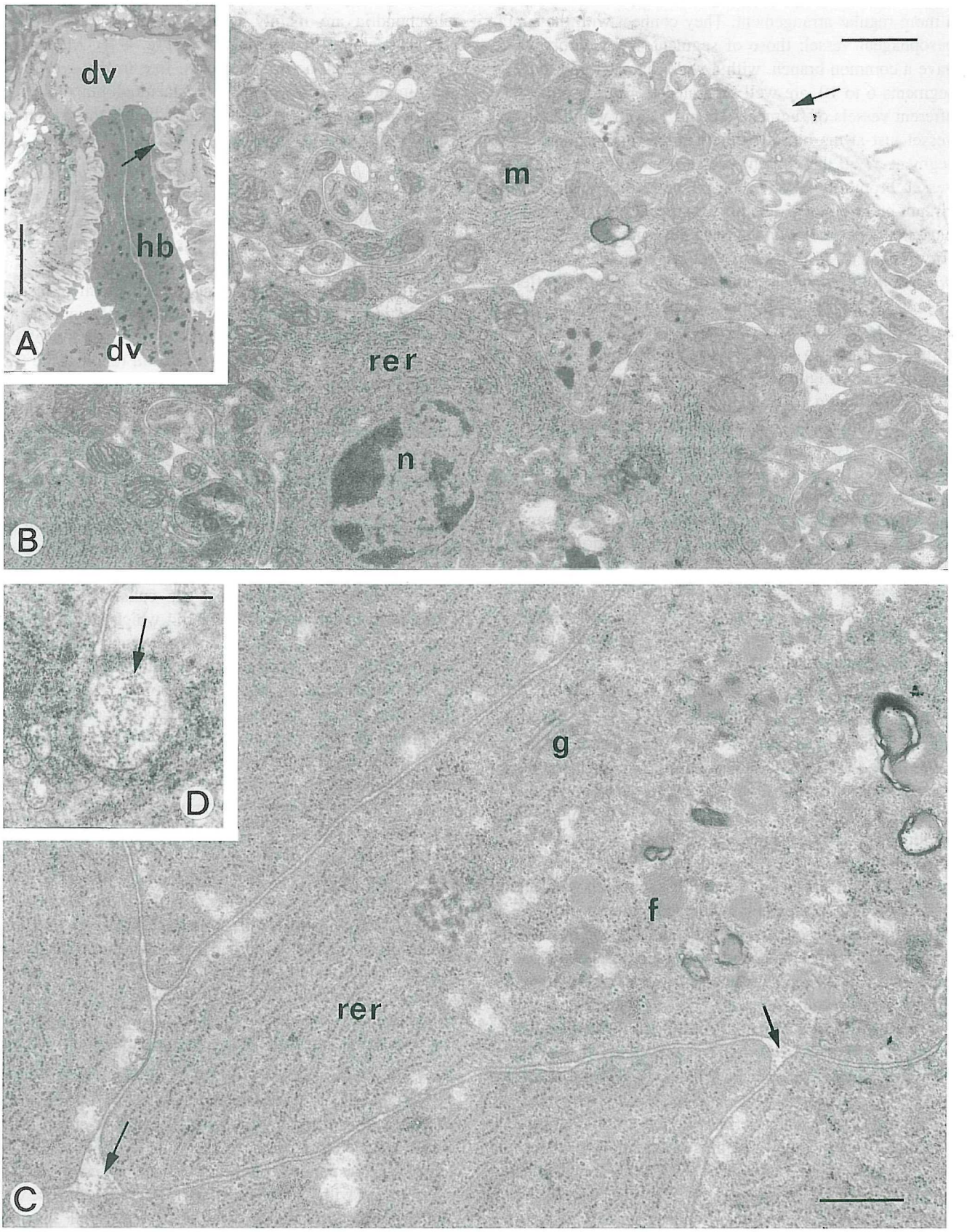
The tubular branchial heart and its rod-like heart-body

The muscular wall of the heart is well supplied, on its coelomic surface, with capillaries originating from the dorsal vessel. In the three species the heart has a thick muscular wall, arranged in two layers: - on the coelomic side, the cell bodies of the muscle cells with numerous mitochondria, - on the luminal side, the myofilaments laying on a basal lamina (*lamina densa*) and a thick extracellular matrix, both forming the *vascular lamina* (about 1.5 µm in thickness) which borders the heart lumen (Fig. 5 B).

The branchial heart contains in the three species a rather translucent rod-like heart-body, all along its length (Figs. 5 A, 6 D). At its posterior end this heart-body is curved and continuous backwards with the brown and folded intravasal tissue of the dorsal vessel (Fig. 1B). The rod-like heart-body, formed by a compact tissue, is solid; it is free in the blood space and comprises only one cell type forming a pseudostratified epithelium. Elongate and radially arranged at the periphery of the organ, the cells form a cortex different from the middle of the rod which has no central lumen and where the cells have no particular arrangement. In the middle of the cortical zone, the thin cell processes are united to each other by small desmosomes (Fig. 5 C). The cells have around the median nucleus a well developed rough endoplasmic reticulum and few Golgi elements (Fig. 5 C), while adjacent parts of the cytoplasm, devoid of such organelles are electron lucent and contain abundant granules which are probably glycogen granules since the rod-like organ shows APS positive grains on histological sections. Haemoglobin molecules were rarely observed in the intercellular spaces. At the rod periphery, the cells have numerous thin processes laying on a thick *vascular lamina* (with no *lamina densa*), 1-2 µm in thickness (Fig. 5 B). The radial appearance of the organ is also due to collagenous strands, continuous with the surrounding matrix, which separate adjacent cells from place to place. This extracellular matrix is much more abundant in *A. pompejana* (Fig. 5 D) and *A. caudata* than in *P. grasslei.*

The perioesophageal erythrocyte-containing pouch

The anterior part of the heart disappears into a pouch which encloses also the oesophagus, the supra-oesophageal vessel and a "tissue", colored red on living animals, which actually corresponds to a huge amount of coelomocytes intermingled with a blood capillary plexus (Fig. 6). The wall of the pouch corresponds to an anterior septum, attached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laterally, in *Paralvinella grasslei* between setiger 4 and 5, and in *A. pompejana* and *A. caudata*, in front of the setiger 4. This extended septum is attached posteriorly on the gut, at the oesophagus-stomach junction (Fig. 1-3). In both species of *Alvinella* the perioesophageal pouch has posteriorly a small lobe, which is lacking in *Paralvinella*. The pouch is larger in *A. pompejana*. The wall of the pouch is a septum, made of two coelomic epitheliums placed side by side. It separates a small anterior coelomic compartment and the large, extended, posterior one. The wall is riddled in *P. grasslei* with small holes, allowing the coelomocytes to migrate from the pouch into the posterior, perivisceral, cavity and probably *vice versa* (Figs. 7, 8 A).

The anterior cœlom probably communicates with the coelomic cavities of the peribuccal tentacles in which clusters of coelomocytes are regularly present. It must be noted by the way, that the tentacles in alvinellids are able to retract entirely inside the oesophagus, whereas it is generally admitted that alvinellid tentacles could only retract into the buccal cavity; a similar ability of tentacles retraction into the oesophagus was mentionned by Fauvel (1897) in ampharetids.

In *P. grasslei*, the perioesophageal pouch surrounds a network of thin and abundant blood capillaries, forming a plexus, located all around the oesophagus, mainly laterally (Fig. 6 A, D). The capillaries, which contain the extracellular haemoglobin, are interconnected with each other (Fig. 6 B, C) and connected to the supra-oesophageal vessel by four small vessels. In both species of *Alvinella* the network of capillaries is present mainly in contact with the coelomic wall and appears less developed than in *Paralvinella*. The wall of the capillaries comprises a thin coelomic epithelium, about 0.5 µm in thickness (Fig. 8 D) and its basal lamina.

Two types of coelomocytes, granulocytes and erythrocytes, are to be found in the pouch (Fig 8). The granulocytes have various shapes, and obviously a phagocytic activity; they have a size of about 10 µm and a nucleus of about 3 µm in diameter. Their cytoplasm contains numerous electron-dense granules (diameter 0.3 µm), endocytotic vacuoles (Fig. 8 B, C), microtubules, bundles of microfilaments and few RER cisterns. The erythrocytes with a diameter of about 9 µm have an electron-dense cytoplasm with few organelles. Their round central nucleus, about 3 µm in diameter, is surrounded by a few mitochondria and, on sections, an electron-dense body is generally present near the mitochondria (Fig. 8 C). The Golgi apparatus is reduced and some peripheral endocytotic vacuoles are often present (Fig.. 8 C).

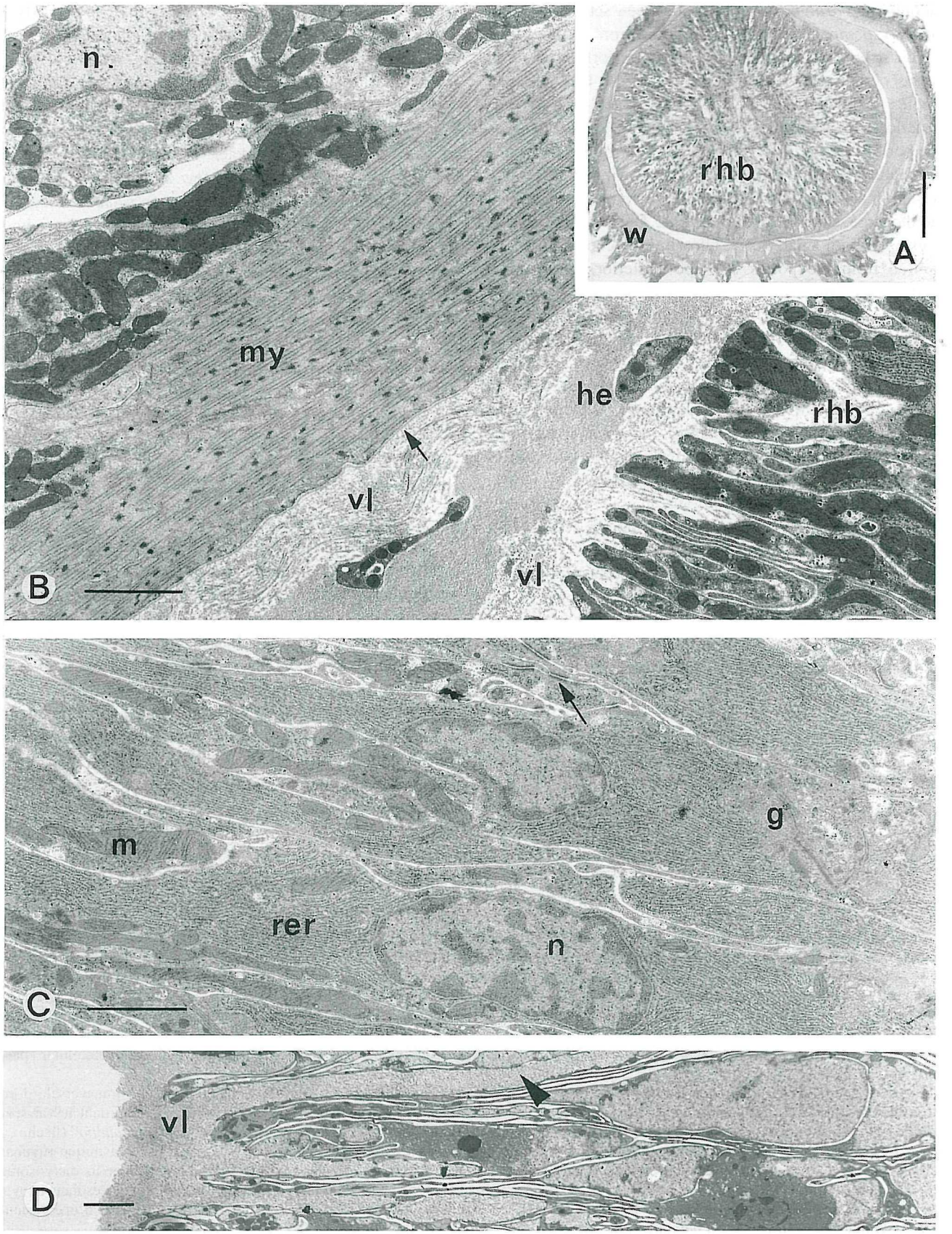
The existence of an intracellular haemoglobin has been demonstrated in these cells (see below); in the perioesophageal pouch the diffusion distance between this haemoglobin and the extracellular one in the capillaries is very short, about 0.5 µm (Fig. 8 D). Gas transfer probably occur between the vascular blood in the capillary plexus, which represents a very large surface area, and the surrounding abundant erythrocytes.

Sulfide contamination

On histological sections of *Paralvinella grasslei,* numerous dark granules are present in the coelomic epithelium lining different vessels and compartments in the anterior body part (Fig. 9 C). The most conspicuous concentration of dark granules occurred in the coelomic walls of the branchial efferent vessels and the ventral vessel, but in some specimens, branchial afferent vessels are also marked. The coelomic epithelium lining different organs (perioesophageal pouch, blood vessel capillaries inside the pouch, wall of the heart), different blood vessels, for example those running along the gonoducts, the cells of rod-like and haematopoietic heart bodies, and the coelomocytes, also contained numerous intracellular dark granules (Fig. 9C).

Figures 4 A-D. Haematopoietic heart-body of *Alvinella pompejana* ; **B**, **C**, **D** TEM. **A:** semi-thin cross section of a part of the dorsal vessel (dv) showing the intravasal haematopoietic heart-body (hb). Arrow points to the vessel's *vascular lamina*. Scale bar: 50 µm. **B:** cross section through the peripheral part of the heart-body, bordered by a the thin *vascular lamina* (arrow). Near the luminal side are convoluted cell processes with numerous mitochondria (m). n: nucleus, rer: rough endoplasmic reticulum. Scale bar: 1 µm. **C:** the heart-body cells contain a well developed rough endoplasmic reticulum (rer) small dictyosomes (g), moderately dense granules and paracristal-line arrangements of probable ferritin molecules (f), free in the cytoplasm. Haemoglobin molecules are present in the intercellular spaces (arrows). Scale bar: 0.5 µm. **D:** detail of a vesicle (arrow) containing accumulated haemoglobin molecules near an intercellular space. Scale bar: 0.5 µm.

Figures 4 A-D. Corps cardiaque hématopoiétique d'*Alvinella pompejana* ; **B**, **C**, **D** MET. **A :** coupe semi-fine transversale d'une partie du vaisseau dorsal (dv) montrant le corps cardiaque intravasal (hb). La flèche indique la "*vascular lamina*" bordant le vaisseau. Echelle : 50 µm. **B :** coupe transversale au niveau de la périphérie du corps cardiaque bordé par une mince "*vascular lamina*" (flèche). A ce niveau les prolongements cellulaires sinueux sont riches en mitochondries (m). n : noyau, rer : réticulum endoplasmique rugueux. Echelle : 1 µm. **C :** les cellules du corps cardiaque ont un réticulum endoplasmique rugueux (rer) très developpé, de petits dictyosomes (g) des granules moyennement denses aux electrons et des arrangements paracristallins, probablement de ferritine (f), libres dans le cyto-plasme. Des molécules d'hémoglobine sont visibles dans les espaces intercellulaires (flèches). Echelle : 0,5 µm. **D :** détail d'une vésicule (flèche) contenant des molécules d'hémoglobine, près d'un espace intercellulaire. Echelle : 0,5 µm.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By contrast, the connective tissue surrounding the nerve cord and the basal lamina of the oesophagus had only a small number of dark granules. The walls of nephridia, gonoducts and oesophagus had generally few granules, even when the oesophagus lumen contained large dark ingested deposits. In the coelom, the large oocytes had no dark granules, while, surprisingly, the young oocytes had generally conspicuous dark granules. The epidermal cells appeared devoid of large dark granules.

The coelomic intracellular haemoglobin

In all three species, the oxy-U.V./vis. absorption spectra of the red supernatant obtained by haemolysis of the erythrocytes were similar. In *A. pompejana*, both the oxy-, deoxy- and carboxy-absorption spectra were typical of an intracellular haemoglobin. Specifically, the ratio of absorbance of the α to the β peaks in the oxyhaemoglobin spectra was higher than one, clearly different from the α/β ratio of the corresponding extracellular haemoglobin dissolved in the blood, which is significantly lower than one (Table 1). From these observations, it is clear that alvinellids possess a coelomic, intracellular haemoglobin in addition to a vascular, extracellular haemoglobin.

Table 1. Spectral position in nm of intra- and extracellular HbO_2 α and β peaks, and ratio of absorbance of the α to the β peaks in *Alvinella pompejana*.

Tableau 1. Valeurs spectrales en nanomètres (nm) des pics α et β des hémoglobines intra- et extracellulaires et rapports d'absorbance des pics α etβ chez *Alvinella pompejana*.

	α peak	β peak	A_α/A_β
Intracellular HbO_2	576.3	542.0	1.035
*Extracellular HbO_2	574.5	539.8	0.920

*Values from Toulmond *et al.*, 1990

Discussion

General pattern of the circulatory system

As in terebellids, ampharetids and trichobranchids a branchial heart, that is dorsal to the oesophagus and sends the blood to the gills, is present in alvinellids. The well developed dorsal vessel all along the body suggests a more primitive pattern in alvinellids than in the three other families, where a dorsal vessel is present only as a supra-oesophageal vessel, the branchial heart (Picton, 1899). In these polychaetes the heart-body is located only in the branchial heart which is, for example in *Amphitrite ornata*, only about 10% of body length (Friedmann & Weiss, 1980). By contrast, in alvinellids two functionally different heart-bodies are present: a haematopoietic heart-body in the long dorsal vessel, and a rod-like heart-body, with a mechanical function, in the anterior branchial heart.

The arrangement of the anterior blood vascular system, although variable in a small extent according to specimens, is similar in *Alvinella pompejana* and *A. caudata*. That of *Paralvinella grasslei* is different since the efferent vessels indirectly join the dorsal vessel, with the correlative development of connecting vessels between the perienteric blood sinus and the dorsal vessel, at the outset of the heart.

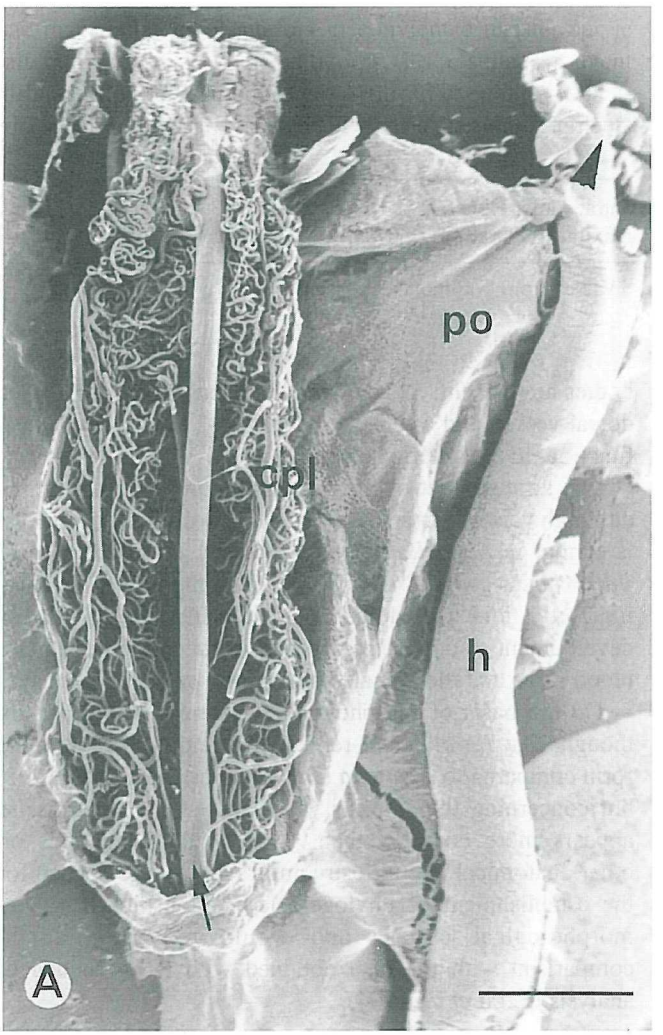
On the basis of morphological features, it is generally thought that *Paralvinella* represents a more plesiomorphic form compared to *Alvinella* (Desbruyères & Laubier, 1991) but concerning the anterior vascular system, *Paralvinella* appears more complex than *Alvinella*. Investigations on other anatomical and ultrastructural features are needed for the establishment of phylogenetic relationships based on morphological criteria and would be useful for a comparison with the data obtained with RNA sequences analysis (Feral *et al.*,1994).

The heart-bodies

We examined by TEM only a small number of specimens, and the haematopoietic heart-bodies observed

Figure 5 A-D. Cross sections of the rod-like heart-body. **B**, **C**, **D**, TEM. **A:** *Alvinella pompejana*, semi-thin cross section of the branchial heart, containing the rod-like heart-body (rhb); w: muscular wall of the heart. Scale bar: 100 µm. **B-C** *Paralvinella grasslei.* **B:** cross section through the muscular heart and the periphery of the rod-like heart-body (rhb) both of them lined, on the luminal side, by a *vascular lamina* (vl). Arrow points to the *lamina densa*. he: haemocyte, my: myofilaments, n: nucleus of a cell body in the muscular wall of the heart. Scale bar: 2 µm. **C:** the rod-like heart-body cells have a well developed rough endoplasmic reticulum (rer), Golgi elements (g) and mitochondria (m). Adjacent cells are united in some places by desmosomes (arrow); n nucleus. Scale bar: 2 µm. **D:** *Alvinella pompejana*, note the thick collagenous extracellular matrix (vl) surrounding the organ and forming collagenous strands inside the heart-body (arrow head). Scale bar: 3 µm.

Figure 5 A-D. Corps cardiaque du cœur branchial, coupes transversales. **B**, **C**, **D**, MET. **A :** *Alvinella pompejana*, coupe semi-fine montrant le corps cardiaque en baguette (rhb) ; w: paroi musculaire du cœur. Echelle : 100 µm. **B-C** *Paralvinella grasslei.* **B :** paroi musculaire du cœur et périphérie du corps cardiaque (rhb) bordées chacune par une *vascular lamina* (vl). La flèche indique la *lamina densa*. he : hémocytes ; my : myofilaments, n : noyau d'une cellule musculaire de la paroi du cœur. Echelle : 2 µm. **C :** les cellules du corps cardiaque ont un réticulum endoplasmique rugueux (rer) très développé, des dictyosomes (g) et des mitochondries (m). Les cellules adjacentes sont unies par endroits par des desmosomes (arrow) ; n noyau. Echelle : 2 µm. **D :** *Alvinella pompejana*, remarquer la matrice extracellulaire périphérique épaisse (vl) formant des cloisons au sein de l'organe (tête de flèche). Echelle : 3 µm.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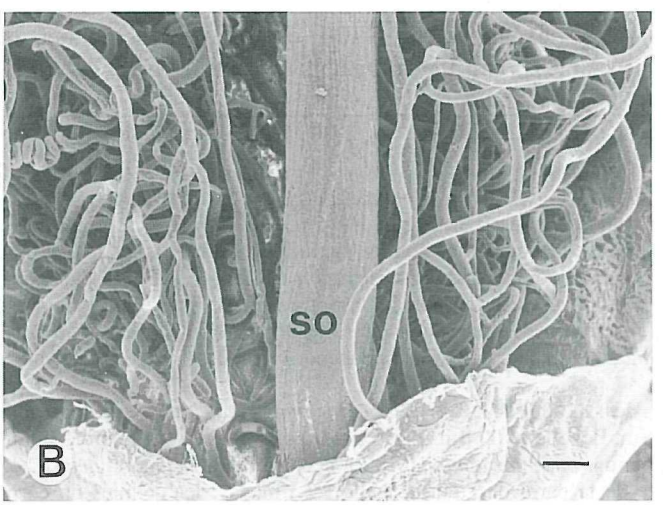
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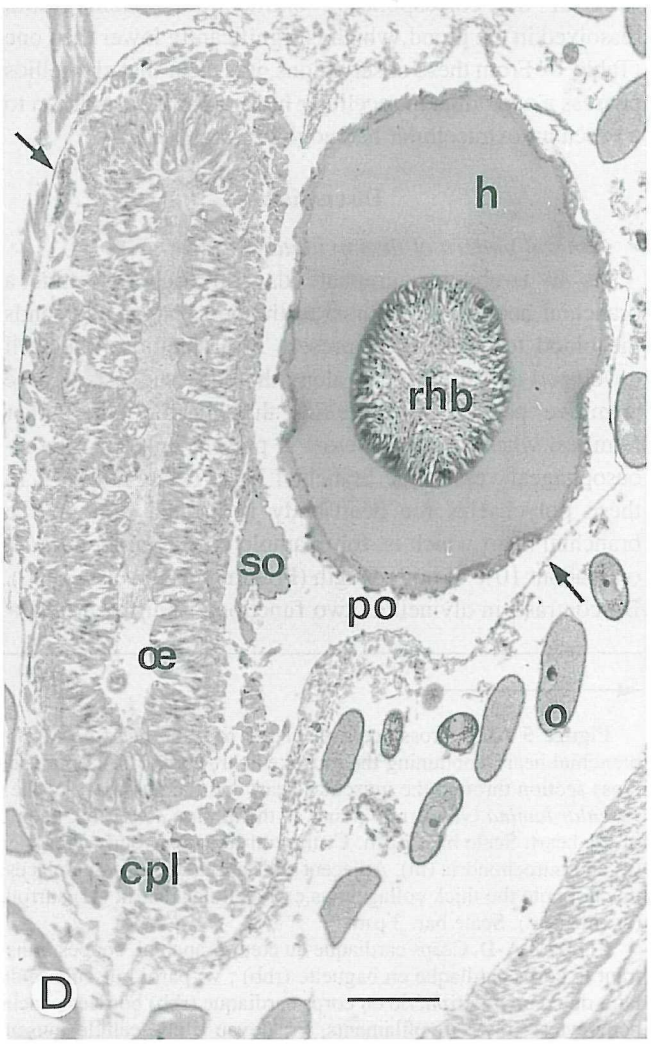
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did not exhibit an intense activity of haemoglobin synthesis; it is very likely that there is in alvinellids, as in other polychaetes such as *Neoamphitrite figulus* (Braunbeck & Dales, 1985), a cycle of production of extracellular haemoglobin. The whole process of haemoglobin secretion could not be observed here, the Golgi apparatus of the heart-body cells being weakly active. Nevertheless, the extensive RER, the presence of small molecules, arranged into paracrystals in the cytoplasm and similar to ferritin molecules (Ouaghi & Grasset, 1991), and of haemoglobin molecules free in the extracellular spaces, are in agreement with a haematopoietic function of this intravasal tissue. As in other polychaetes (review in Fransen, 1988), vacuoles containing haemoglobin molecules migrate to the cell periphery and discharge their contents in the extracellular spaces, then the haemoglobin molecules may easily cross the extracellular matrix which surrounds the heart-body and reach the vessel lumen. The presence of dense granules and of large heterogenous dense bodies, similar to the compound haematin granules described in *Neoamphitrite* (Braunbeck & Dales, 1985), are also typical features of haematopoietic tissues, and correspond to by-products of haemoglobin synthesis combined with iron (Kennedy & Dales, 1958; Mangum & Dales, 1965; Dales & Pell, 1970).

In polychaetes, the rod-like and the haematopoietic heart-bodies are made of cells with a common origin, i.e. the infolding of an extravasal coelomic epithelium (Picton, 1898; Kennedy and Dales, 1958). In alvinellids these cells, radially arranged in the rod-like organ, store glycogen granules and secrete a framework of extracellular collagenous matrix, but haemoglobin molecules were rarely observed and it seems likely that these cells have lost their haematopoietic function. A similar cylindrical heart-body, with radially arranged cells, is present in some ampharetids such as *Melinna* (Meyer, 1887), *Ampharete grubei* (Fauvel, 1897) and in the small ampharetid from hydrothermal vent sites, *Amphisamytha galapagensis* (C.J.-T. unpublished data). In the trichobranchid *Terebellides stroemi* such a rod-like organ, according to Steen (in Picton, 1899), has a valvular function preventing a back flow of blood when the gills contract. The mechanical function of such a cylindrical heart-body has also been observed on living *Ampharete* by Fauvel (1897) and on terebellids by Picton (1899). We agree with Kennedy & Dales (1958) and Spies (1973) who stressed that, at systole, the lumen of the contractile heart must be greatly reduced, the wall of the heart coming into contact with the rod-like heart-body; this consequently increases the pressure which is then strong enough to drive the blood into the branchial afferent vessels.

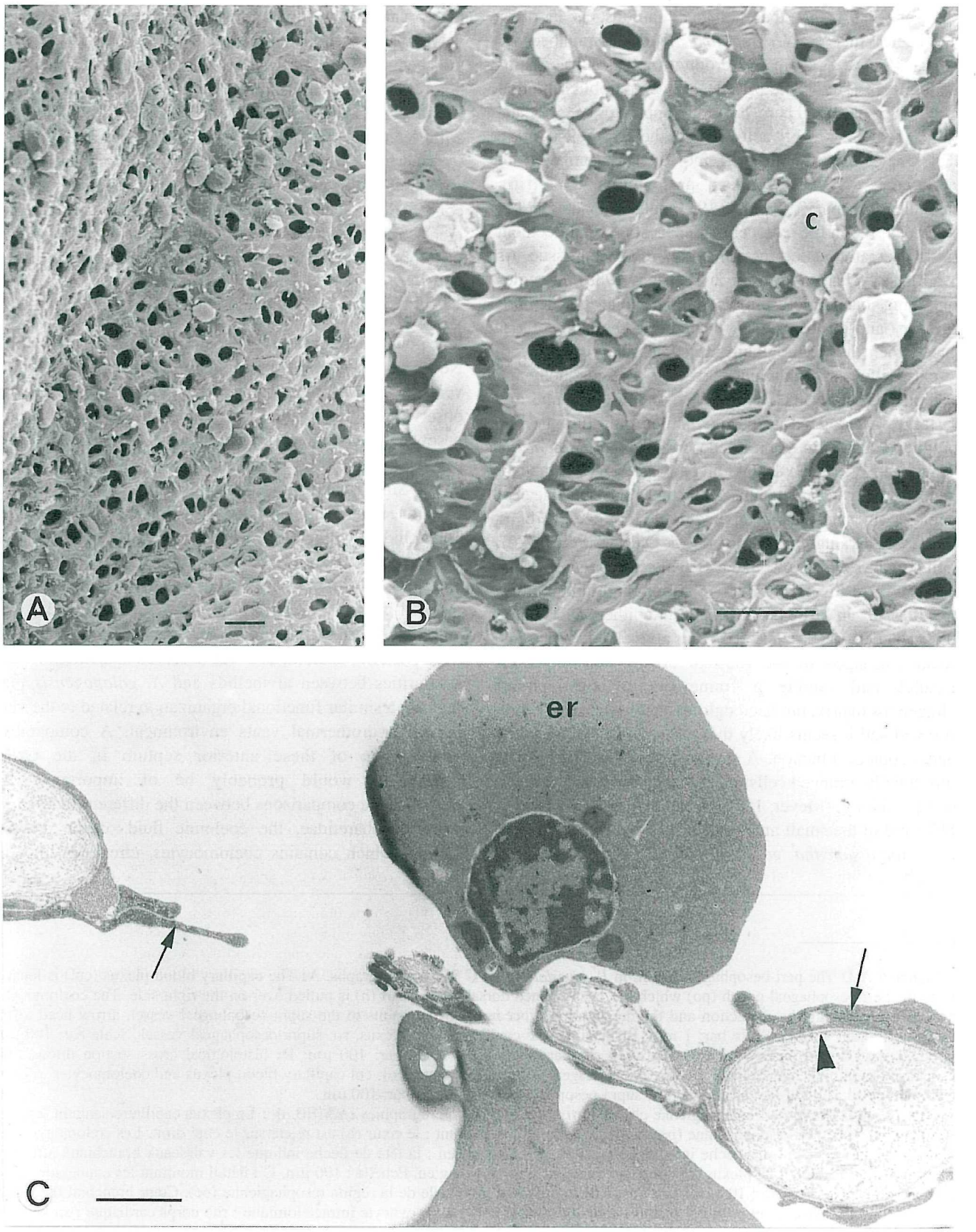
The erythrocyte containing pouch

With its capillary plexus and content of coelomocytes, this pouch appeared as a peculiar feature of alvinellids. Nevertheless, in the small ampharetid from hydrothermal vent sites *Amphisamytha galapagensis* Zottoli 1983, we observed a similar pouch surrounding oesophagus, supra-oesophageal vessel and branchial heart, and filled also with blood capillaries and coelomic cells (C. J.-T. unpublished data). Similar and probably homologous formations are mentioned in Ampharetidae by Fauvel (1897) and in Terebellidae by Picton (1899): a fenestrated diaphragm is present in ampharetids, attached laterally in front of the anterior nephridia, extending around the oesophagus and inserted at the posterior limit of the oesophagus. The anterior coelom communicates through small holes with the large posterior coelom, as it was observed in alvinellids. The similarities between alvinellids and *A. galapagensis* may indicate a similar functional organization related to the very special hydrothermal vents environment. A comparative examination of these anterior septum in the Order Terebellida would probably be of importance for phylogenetic comparisons between the different families.

In Ampharetidae, the coelomic fluid of the anterior cœlom, which contains coelomocytes, circulates into the

Figure 6 A-D The peri-oesophageal pouch in *P. grasslei;* **A**, **B**, **C** SEM micrographs. **A:** The capillary blood plexus (cpl) is located inside the peri-oesophageal pouch (po) which has been opened dorsally; the heart (h) is pulled over on the right side. The coelomocytes have been lost during the dissection and the dehydration procedure. Arrow points to the supra-oesophageal vessel, arrow head to the branchial afferent vessels. Scale bar: 1 mm. **B:** detail of the capillary blood plexus. so: supra-oesophageal vessel. Scale bar: 100 µm. **C:** detail showing the interconnections between capillaries (arrows). Scale bar: 100 µm. **D:** histological cross section through the oesophagus (oe), branchial heart (h) and peri-oesophageal pouch (arrows and po); cpl capillary blood plexus and coelomocytes, o intra-coelomic oocyte; rhb rod-like heart-body; so supra-oesophageal vessel. Scale bar: 100 µm.

Figure 6 A-D Poche péri-œsophagienne chez *P. grasslei;* **A**, **B**, **C** micrographies en MEB. **A :** Le plexus capillaire sanguin (cpl) est situé dans la poche péri-œsophagienne (po) qui a été ouverte dorsalement ; le cœur (h) est rejeté sur le côté droit. Les cœlomocytes ont disparu lors du traitement. La flèche indique le vaisseau sus-œsophagien ; la tête de flèche indique les vaisseaux branchiaux afférents. Echelle : 1 mm. **B :** détail du plexus capillaire. so : vaisseau sus-œsophagien. Echelle : 100 µm. **C :** détail montrant les connexions entre capillaires (flèches). Echelle : 100 µm. **D :** Coupe histologique transversale de la région œsophagienne (oe). Cœur branchial (h), poche péri-œsophagienne (flèches et po) ; cpl plexus capillaire et cœlomocytes ; o ovocyte intracœlomique ; rhb corps cardiaque ; so vaisseau sus-œsophagien. Echelle : 100 µm.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coelomic cavities of the tentacles (Fauvel 1897). Since we regularly found clusters of coelomocytes in the feeding tentacles of alvinellids, the cœlom of the pouch likely communicates with the coelomic cavities of these tentacles which are, as in other Terebellida, always devoid of any blood vessel. Like the gills, these tentacles spread out into the surrounding medium, so gas exchanges might occur, not only at the gill level between the blood and the external milieu, but also through the tentacle wall, between the coelomic erythrocytes and the external milieu. A similar respiratory function for feeding tentacles via erythrocytes has been demonstrated in *Enoplobranchus sanguineus*, a Terebellidae devoid of a vascular system (Mangum *et al.*, 1975).

The presence of erythrocytes was not previously known in alvinellids. Different types of erythrocytes may occur in polychaetes, and those of alvinellids, although they are of a smaller size, are morphologically similar to those of Glyceridae (Seamond & Schumacher, 1972; Sean & Boilly, 1980). They appear to have no nutritive reserves contrary to that of the terebellid *Amphitrite johnstoni* (Dales, 1964) and to be more specialized and probably more highly evolved than those of Terebellidae (Terwilliger *et al.*, 1985). They are found everywhere in the cœlom, especially in contact with developing gametes which are thus provided with oxygen. The granulocytes are widespread in polychaetes and several studies have demonstrated that these coelomocytes are involved in the phagocytosis of small particles or larger foreign material, and in the antibacterial defence (see review in Dhainaut & Porchet-Henneré, 1988).

The perioesophageal blood plexus of alvinellids does not seem similar to the blood plexus of the anterior blood system in ophelids, the main role of which is to enhance the pressure of the prostomial hydrostatic skeleton and then assist locomotion through the sand (Harris, 1994). It does not seem similar either to the blood plexus of *Sabella* (Koechlin, 1966), which represents an ultrafiltration site, since we did not observe podocytes along the wall of the peri-oesophageal capillaries. The association of blood capillaries and erythrocytes represents in alvinellids a complex respiratory gas transfer system unique in polychaetes. The functional properties of the intracellular haemoglobin and the possible gas transfer between extracellular and intracellular haemoglobins remain to be investigated.

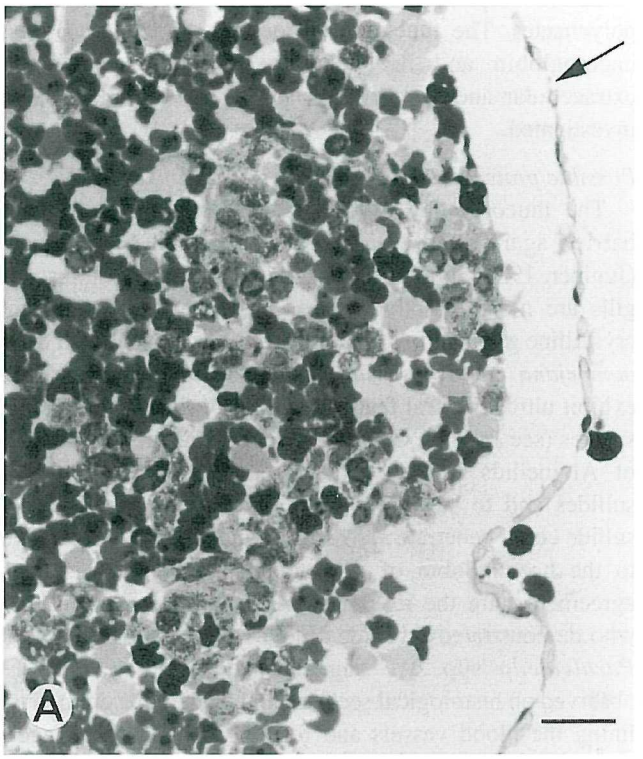
Possible protection from sulfide and detoxification

The mucous tube of *P. grasslei* represents a passive barrier against sulfide diffusion across the body wall (Juniper, 1988; Juniper & Martineu, 1995). By contrast the gills are more directly exposed to the external medium: crystalline granules are abundant in the gill epithelium of *A. pompejana* and *P. grasslei* and mitochondria in the gills exhibit ultrastructural features similar to those of thiobiotic species (see Jouin & Gaill, 1990). The branchial epithelium of Alvinellids is then probably able to partly detoxify sulfides and to store less toxic compounds. Nevertheless sulfide could penetrate across the branchial surface and bind to the haemoglobin of the branchial vessels. This is in agreement with the results of Martineu & Juniper (1995) who demonstrated a sulfide binding ability of the blood of *Paralvinella* spp. We suggest that the dark granules observed on histological sections in the coelomic epithelium lining the blood vessels and forming the perioesophageal pouch, are related to sulfide detoxification, an hypothesis also in agreement with the demonstration by Juniper & Martineu (1995) of a sulfide oxidation by tissue homogenates in *Paralvinella sulfincola* and *P. palmiformis*. The nature and chemical composition of the dark granules of the coelomic epithelium are still unknown. It is possible that this epithelium, which has the same origin as the intravasal haematopoietic heart body, accumulates granules of haematin which may be formed in the blood and, as in other invertebrates, catalyze sulfide oxidation (Patel & Spencer, 1963, Powell & Arp, 1989). Metals can also penetrate through the gill epithelium and the dark granules concentrated in the coelomic epithelium could also represent a metal detoxification process.

In the coelomocytes (erythrocytes) of *Urechis*, an echiuran, haematin forms brown granules which catalyzed

Figure 7 A-B: *P. grasslei,* SEM micrograph of the wall of the coelomic pouch (internal surface), riddled with holes and with some remaining adherent coelomocytes (c). Scale bars: 10 µm. **C:** *Alvinella pompejana*, cross section through the wall of the coelomic pouch. The two layers of flat coelomic epithelial cells (arrows), separated by a collagenous matrix, are discontinuous allowing coelomocytes, here an erythrocyte (er), to cross. Arrow head points to the *lamina densa*. TEM, Scale bar: 1 µm.

Figure 7. A-B : *P. grasslei,* micrographies en MEB de la paroi de la poche péri-œsophagienne (face interne) criblée de trous, avec quelques cœlomocytes (c). Echelles : 10 µm. **C :** *Alvinella pompejana*, coupe transversale de la paroi de la poche. Les deux épithéliums cœlomiques (flèches) séparés par une matrice extracellulaire forment une paroi discontinue qui permet le passage des cœlomocytes, ici un érythrocyte (er). La tête de flèche indique la *lamina densa*. MET, Echelle : 1 µm.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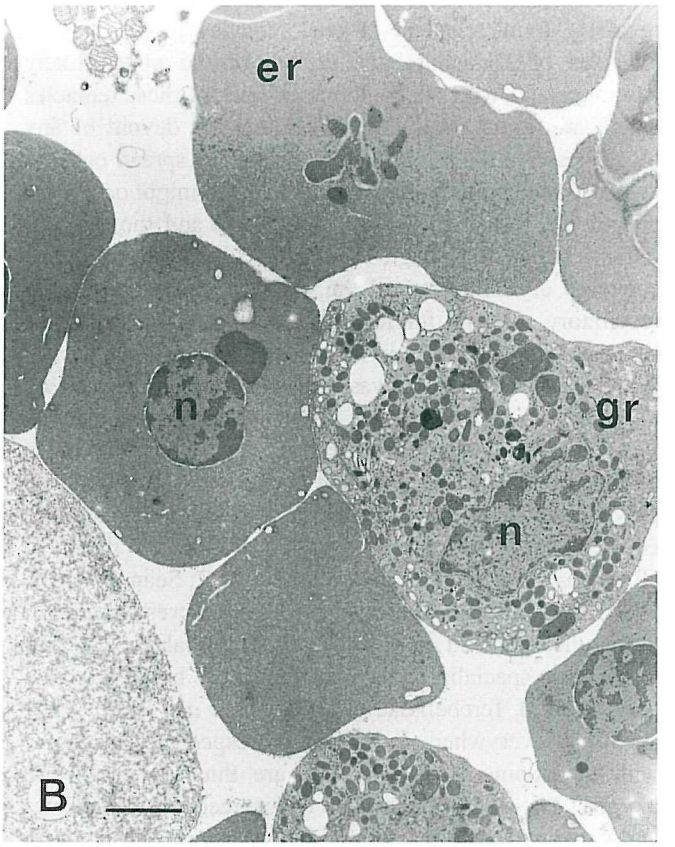
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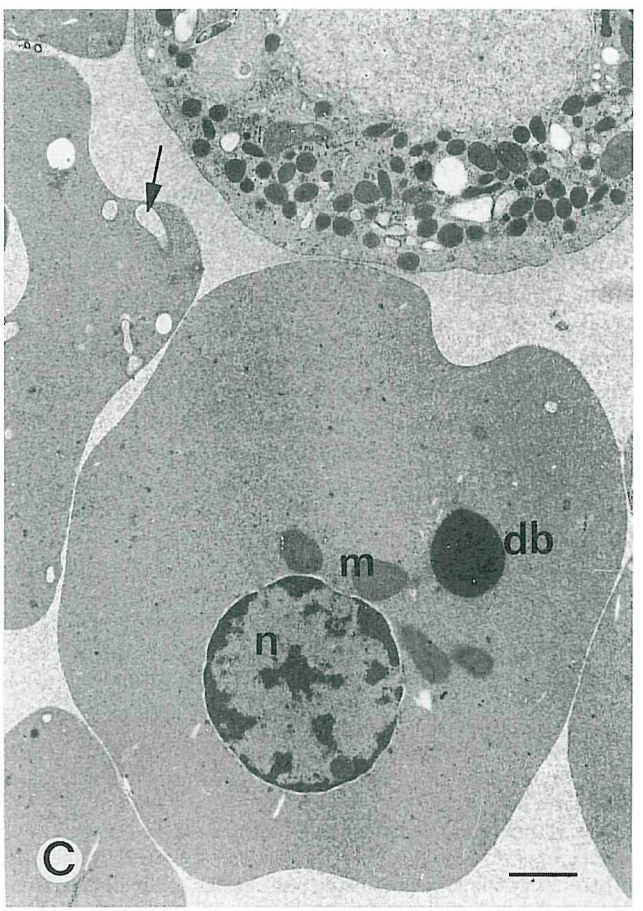
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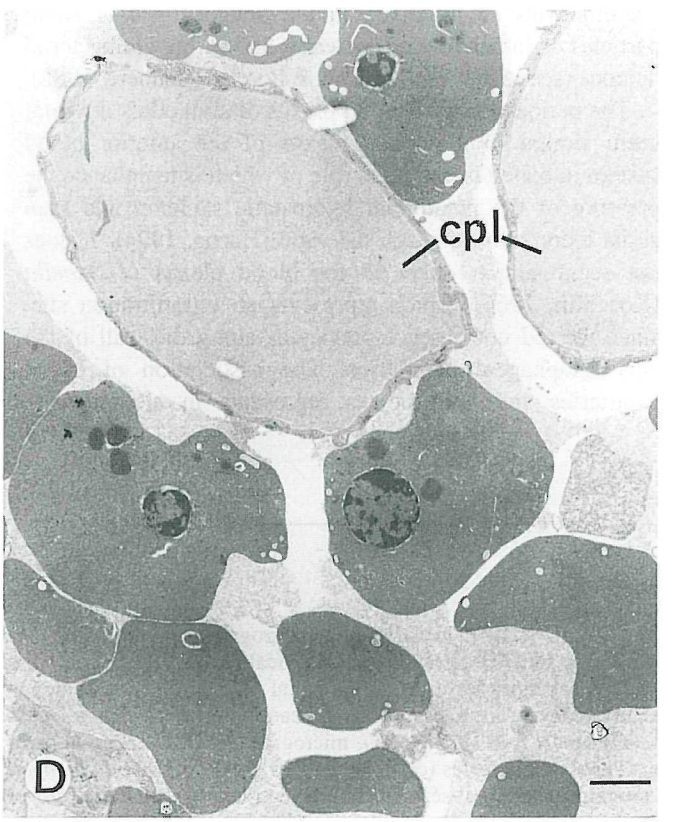
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Figure 8. A-D. *Alvinella pompejana*, coelomocytes and capillary blood plexus inside the perioesophageal pouch. **A:** semi-thin cross section; note the blood capillaries (clear areas) intermingled with coelomocytes (darker) and the discontinuous wall of the pouch (arrow). Scale bar: 20 µm. **B:** granulocyte (gr) and erythrocyte (er) the last characterized by its electron dense, haemoglobin containing cytoplasm. TEM, Scale bar: 2 µm. **C:** erythrocytes often contain peripheral endocytotic vesicles (arrow) and dense bodies (db) near the perinuclear mitochondria (m). TEM, Scale bar: 1 µm. **D:** erythrocytes surround blood capillaries (cpl) inside the pouch. TEM, Scale bar: 2 µm.

Figure 8. A-D. *Alvinella pompejana*, cœlomocytes et plexus capillaire sanguin dans la poche péri-œsophagienne. **A :** coupe semi-fine ; noter les capillaires sanguins (sections claires) entremêlés avec les cœlomocytes (cellules sombres) et la paroi discontinue de la poche (flèche). Echelle : 20 µm. **B :** un granulocyte (gr) et des érythrocytes (er) ces derniers caractérisés par leur cytoplasme dense, contenant de l'hémoglobine. MET, Echelle : 2 µm. **C :** les érythrocytes présentent souvent des vacuoles d'endocytose périphériques (flèche) et des corps opaques aux électrons (db) près des mitochondries (m) voisines du noyau. MET, Echelle : 1 µm. D: les érythrocytes entourent les capillaires sanguins (cpl) dans la poche péri-œsophagienne. MET, Echelle : 2 µm.

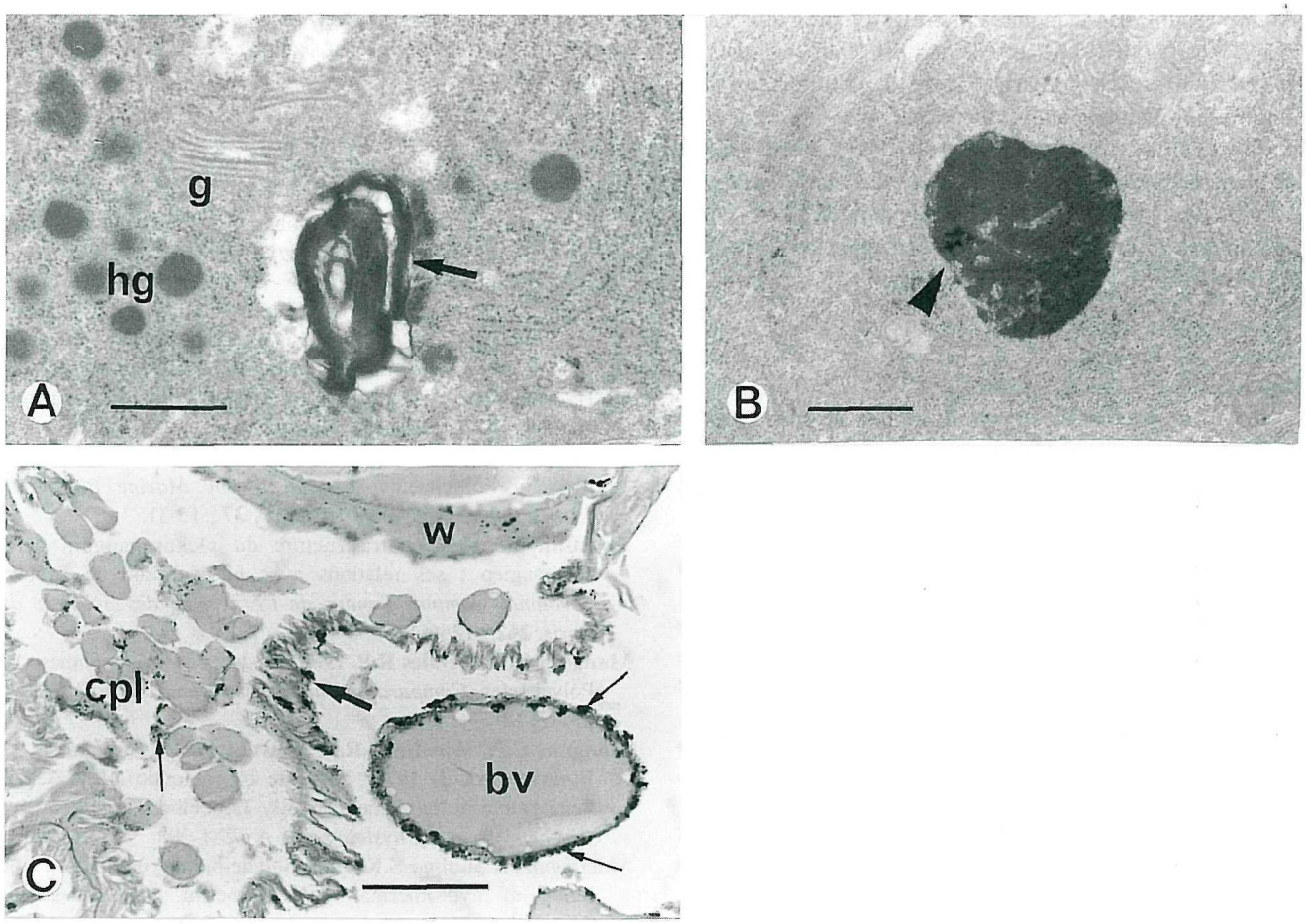

Figure 9. A-B *Alvinella pompejana*: TEM section of the haematopoietic heart-body showing dictyosomes (g), haematin granules (hg) compound granule (arrow head) sometimes surrounded by concentric membranes (arrow). Scale bars A 0.5 µm, B 1 µm. **C**: *Paralvinella grasslei,* histological cross section at the level of setiger 9, showing accumulated dark granules in the coelomic epithelium of the perioesophageal pouch (arrow) and of the vessels' walls (small arrows): capillary blood plexus (cpl) and efferent vessel of setigers 4-7 (bv). w: wall of the heart. Scale bar: 50 µm.

Figure 9. A-B. *Alvinella pompejana* : Coupes (MET) du corps cardiaque hématopoiétique montrant les dictyosomes (g), les granules d'hématine (hg), les granules composés (tête de flèche) parfois entourés de membranes concentriques (flèche). Echelles A 0.5 µm, B 1 µm. **C** : *Paralvinella grasslei,* coupe histologique transversale au niveau du segment sétigère 9 montrant l'accumulation de grains noirs dans l'épithélium cœlomique de la poche péri-œsophagienne (flèche) et de la paroi des vaisseaux sanguins (petites flèches): capillaires péri-œsophagien (cpl) et vaisseau efférent des sétigères 4-7 (bv). Echelle : 50 µm.

oxidation of hydrogen sulfide (Powell & Arp, 1989). It is possible that the electron dense granules observed in the alvinellids erythrocytes contained also haematin; in this case the oxidation of hydrogen sulfide could occur not only in the vascular system but everywhere in the coelom, since erythrocytes are free coelomic cells which spread through the body cavity. We suggest that the complex respiratory gas transfer system of the perioesophageal pouch may also be involved in sulfide detoxification and that it could have been selected in alvinellids in relation with the varying physico-chemical conditions of the deep hydrothermal environment.

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