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SCANNING ELECTRON MICROSCOPY OF THE DORSAL VESSEL OF *PANSTRONGYLUS MEGISTUS* (BURMEISTER, 1835) (HEMIPTERA: REDUVIIDAE)

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In this study we analyzed the microanatomy of the dorsal vessel of the triatomine Panstrongylus megistus. *The organ is a tubule anatomically divided into an anterior aorta and a posterior heart, connected to the body wall through 8 pairs of alary muscles. The heart is divided in 3 chambers by means of 2 pairs of cardiac valves. A pair of ostia can be observed in the lateral wall of each chamber. A bundle of nerve fibers was found outside the organ, running dorsally along its major axis. A group of longitudinal muscular fibers was found in the ventral portion of the vessel. The vessel was found to be lined both internally and externally by pericardial cells covered by a thin laminar membrane. Inside the vessel the pericardial cells were disposed in layers and on the outside they formed clusters or rows.*

Key words: dorsal vessel – scanning electron microscopy – *Panstrongylus megistus* – Hemiptera – Reduviidae

Panstrongylus megistus is a haematophagous bug, which may transmit to man the protozoan *Trypanosoma cruzi*, the causative agent of the Chagas' disease in South and Central America (Chagas, 1909). The insect is the most important invertebrate host of *T. cruzi* in Brazil, due to its large geographic distribution, being the main vector of the disease in the major endemic foci in the states of Minas Gerais and Bahia (Brener & Andrade, 1979).

Considering the participation of the triatomines in the dissemination of the Chagas' disease, studies about the physiology of these insects are of great importance. A research line has been developed in our laboratory to study the dorsal vessel of *P. megistus*. This organ is the only partially closed portion of the circulatory system through which the haemolymph flows in the haemocel, since in the insects no capilaries or veins are found. The dorsal vessel is a hollow and contractile tubule, closed in the caudal extremity and opened in the cephalic end, which runs along all the dorsal portion of the body. The terminal region of the organ is wider and is connected to the body wall through the alary muscles. It presents lateral openings (ostia) and is called heart. The portion of the vessel which extends from the heart to the head is denominated aorta (Barth, 1972). The dorsal vessel functions not only as a pump to the haemolymph. It was recently shown that its integrity is fundamental in the eggs production by the female adults of *Rhodnius prolixus*, a closely related insect (Chiang et al., 1990).

The anatomy of the dorsal vessel shows great diversity among the different orders of the Insecta class (Locy, 1884; Pantel, 1913; McIndoo, 1939; Nutting, 1951; Jones, 1954; Hinks, 1966). Lacombe & Santos (1986) in an anatomical and hystological study on the dorsal vessel of triatomines, including *P. megistus*, described this organ as being formed by an anterior aorta, and a heart which occupies the 6th, 7th and 8th abdominal segments. In the latter they found 8 pairs of alary muscles. These authors observed that this part of the organ is divided in 3 chambers, delimited by 3 pairs of ostiolar valves. They also described the presence of many pericardial cells inside and outside all the vessel (heart and aorta). Chiang et al. (1990) described in *R. prolixus* 7 pairs of alary muscles and 2 groups of longitudinal muscular fibers running along the ventro-lateral region of the heart. In the abdominal portion of the aorta the longitudinal fibers

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formed 3 distinct bundles. The cells found inside the vessel were denominated by these authors as endocardial cells.

In this paper we analyze the microanatomy of the dorsal vessel of *P. megistus* by scanning electron microscopy.

MATERIALS AND METHODS

Insects – Adults of *P. megistus* (10 days after moult) of both sexes were used. The insects were kept at 28 °C and 70% RH at the Department of Entomology of the Instituto Oswaldo Cruz, Rio de Janeiro-RJ, Brazil. Three days after a blood meal on swiss albino mice the bugs were decapited and sticked with entomological pins to Petri dishes containing solidified paraffin. The wings, lateral conexives, legs and tergits were removed. The dissection was carried out under an estereoscopic microscope.

The dorsal vessel was withdrawn and the heart separated from the aorta. All dissection work was done with the animals immersed in phosphate buffered saline (0.15N NaCl, 0.01M phosphate buffer, pH 7.2). The fragments were thereafter transfered to the fixative.

Scanning electron microscopy – Fixation of the fragments was carried out for 2 h at room temperature in 2.5% glutaraldehyde diluted in either 0.1 M phosphate or cacodylate buffer, pH 7.2. The material was then washed, post-fixed in 1% OsO_4, dehydrated in ethanol and immediately critical-point dried with CO_2. The fragments were adhered to adequate specimen supports prior to gold sputtering in a Balzers apparatus, where a coating about 20 nm thick was deposited. All micrographs were taken with a Jeol 25-S-II scanning electron microscope.

RESULTS

The caudal portion of the dorsal vessel, corresponding to the heart, had a length of about 3-4 mm. By scanning electron microscopy we observed that this part of the organ was internally divided in 3 chambers, each one with approximately 1.2 mm in length. These chambers were delimited by 2 pairs of 32 μm thick structures located on the lateral walls of the organ. These structures correspond to the cardiac valves (Fig. 1).

In the centre of each chamber we observed a pair of lateral ostia, which allow the flow of haemolymph from the haemocel into the heart. The edge of these apertures was projected to the interior of the vessel forming the ostiolar valves, about 6.4 μm thick. The distance between a cardiac and an ostiolar valve was approximately 0.6 mm (Fig. 1).

The heart presented large amounts of rounded pericardial cells, arranged either in two or more layers at the inner surface of the vessel, or in clusters at the external surface of the organ. Due to the presence of the pericardial cells, the lumen of the heart had approximately 20 μm in diameter. Connecting the heart to the body wall 8 pairs of alary muscles were found. The external surface of the heart was hardly visualized, due to the insertion of the alary muscles. In the sites of insertion of the alary muscles the thickness of the wall of the organ was increased from 14 μm to 21 μm (Fig. 2). The alary muscle bundles were covered by large clusters of pericardial cells, wrapped up by a thin laminar structure which delaminates at the points of contact with the muscle, forming several projections involving these muscular bundles (Fig. 3).

The external surface of the aorta had a wrinkled appearance (Figs 4, 5). On this part of the vessel, disposed parallely to the major axis of the organ, a long nervous bundle (in the dorsal portion) and groups of longitudinal muscular fibers (in the ventral portion) were found (Fig. 6). The nervous bundle presented branching fibers which inervated the vessel wall (Fig. 4). When the nerve was removed it could be seen that these fibers formed motor plates on the vessel wall (Fig. 5). The longitudinal muscle fibers appeared not to fuse with the aorta wall and did not display adhered pericardial cells (Fig. 6). They were not observed in the heart.

In the aorta, pericardial cells were also found inside and outside the vessel. In this region they were more oval-shaped than in the heart and were also found in smaller amount. In the lumen of the aorta they were usually arranged in one or two layers. As a consequence the lumen was wider, attaining 50 μm in the vessel thoracic portion. In the external face, the pericardial cells were arranged in rows always longitudinaly disposed in relation to the major axis of the vessel. These external rows

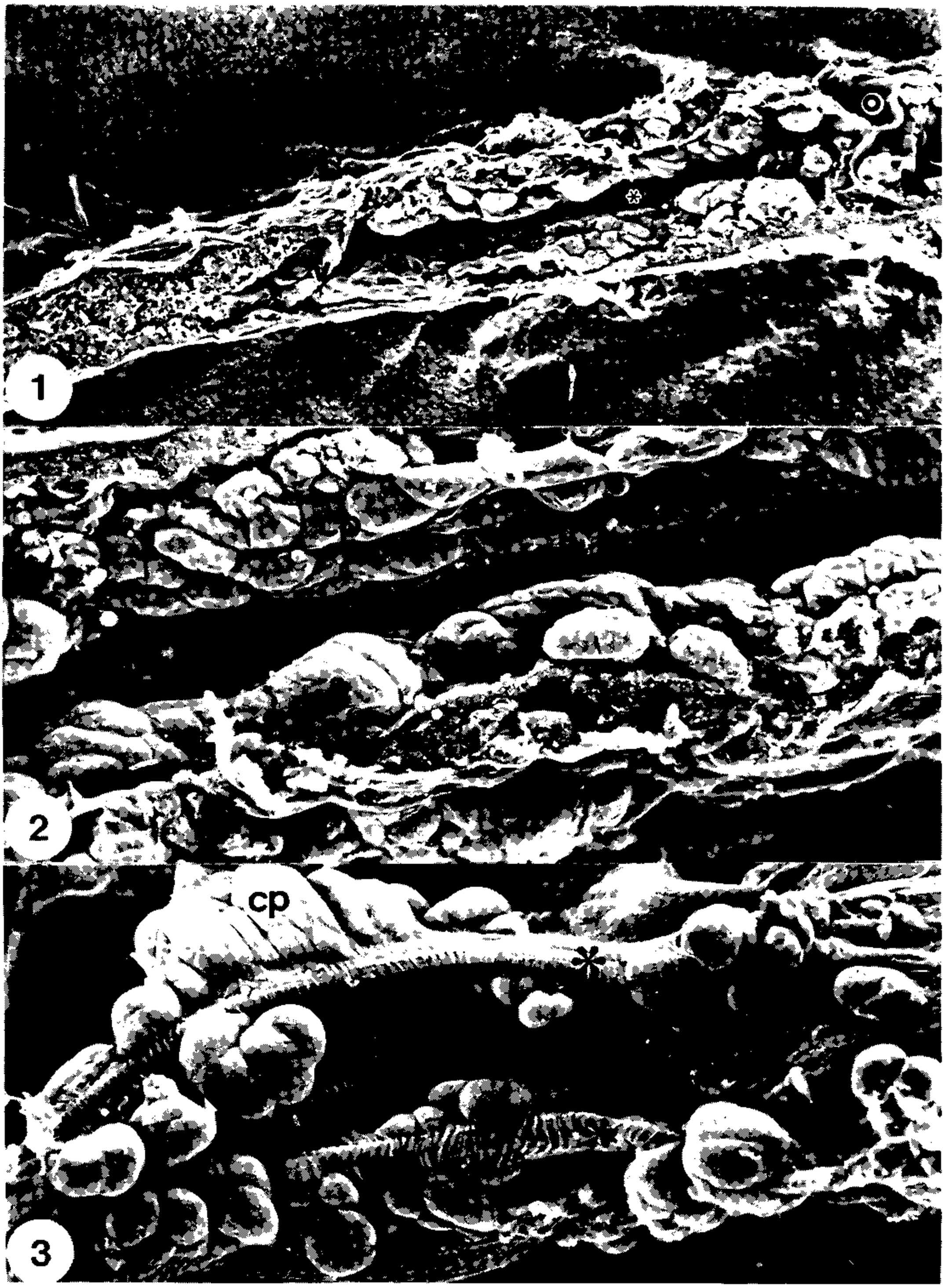

Fig. 1: longitudinal section of the heart showing part of a chamber, limited by a cardiac valve (V). An ostium (O) with its ostiolar valve (arrow) can be seen to the right. The asterisk marks the narrow lumen of the chamber. 280 X. Fig. 2: detail of the heart. Groups of pericardial cells (CP) can be observed inside and outside the vessel. The asterisk marks the lumen of the heart. 840 X. Fig. 3: bundles of alary muscles (asterisk) enveloped by clusters of pericardial cells (CP). A thin pelicle covers all structures. On the muscle cells the pelicle forms paralel arrays (arrowheads). 1,000 X.

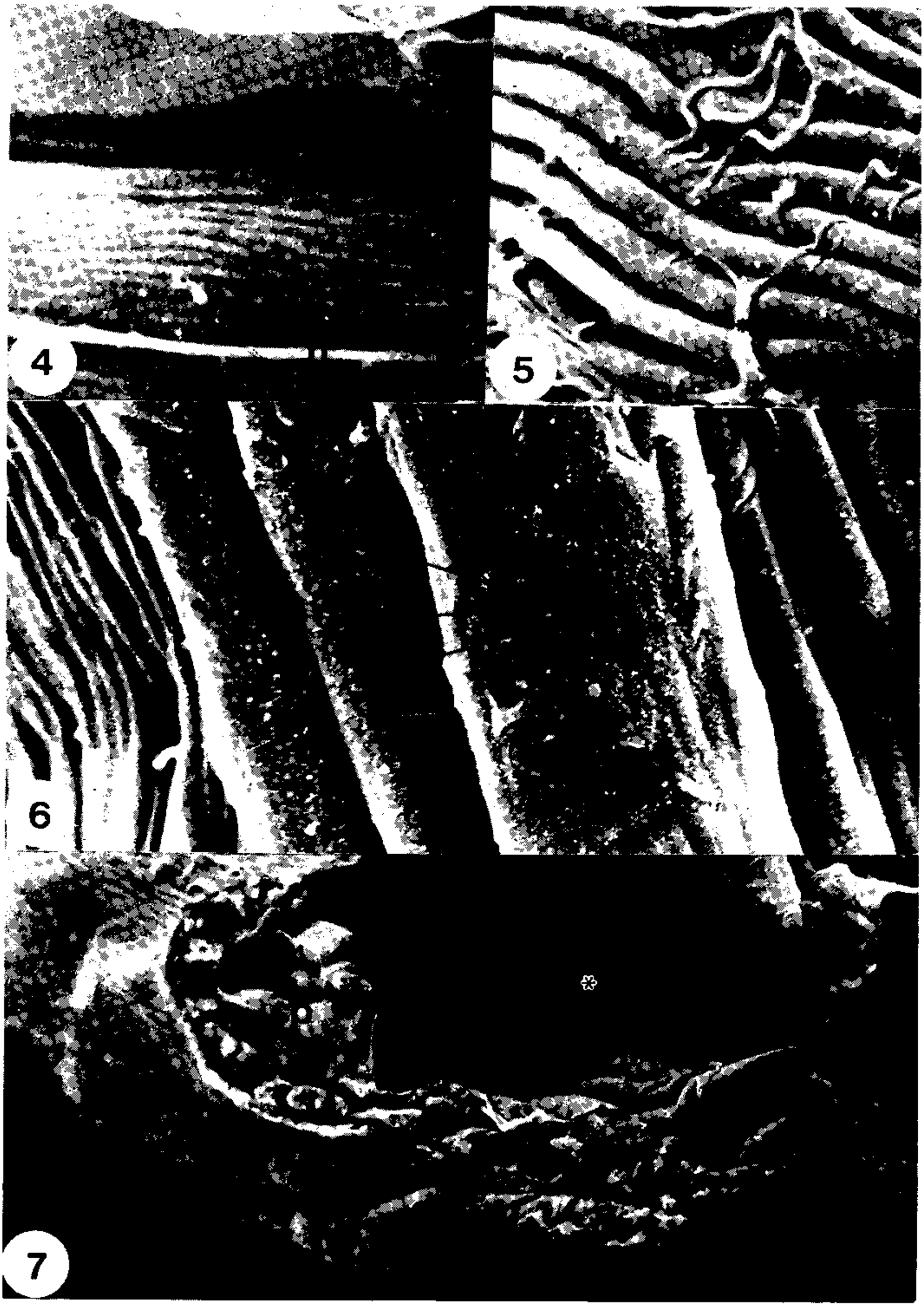

Fig. 4: the recurrent nerve (n) can be seen on the wrinckled surface of the aorta (a), running along its major axis. Nerve fibers (arrowheads) branch from the recurrent nerve. 3,600 X. Fig. 5: removal of the recurrent nerve allows a better visualization of the ramifications of the small fibers (asterisks) which inervate the dorsal vessel. 7,700 X. Fig. 6: longitudinal muscle fibers (star) on the ventral surface of the aorta. 3,600 X. Fig. 7: transversal section of the aorta. The pericardial cells (CP) form layers inside and paralel rows outside the vessel. A thin laminar membrane (arrows) covers the pericardial cell layers inside the vessel. The asterisk marks the lumen of the aorta. 1,600 X.

of cells were also covered by a thin laminar structure. Inside the vessel, this structure appeared as a continuous layer, covering internally all the vessel lumen, separating and preventing a direct contact of the pericardial cells with the lumen of the organ (Fig. 7).

DISCUSSION

The study of the dorsal vessel of *P. megistus* by scanning electron microscopy demonstrated that this organ is a hollow tube possessing the wall thicker in the heart than in the aorta. This happens basicaly due to the insertion in this region of the 8 pairs of alary muscles. This thickening has already been suggested in Reduviidae by Lacombe & Santos (1986). These bundles of muscle cells contract during the diastole of the heart, promoting an even greater distention of the organ wall in this region, facilitating the penetration of the haemolymph in this phase of the cardiac beating. Since no mesenteries are formed during the embryonic development, these muscles and the traqueoles also accounts for maintaining the vessel in position in the haemocel, fastening the vessel to the body wall (Barth, 1972).

In the heart wall, valves were observed dividing it in compartments (chambers) and which are not related to the lateral openings denominated ostia. These valves were previously described by Pantel (1913) and McIndoo (1939). Pantel (1913) called these structures as interventricular valves and the chambers as ventricles. Since the compartments which these valves delimitate function at the same time as atrios (receiving the haemolimph coming from the haemocel) and as ventricles (impelling the haemolimph forward), a fact also observed by McIndoo (1939), we think that the term chambers is more adequate for these compartments than "ventricles". The former denomination was already used in triatomines by Lacombe & Santos (1986). Because the valves which delimit the chambers were localized in the heart we denominated them "cardiac valves". Our data show that the cardiac valves are thicker than the ostiolar valves and that they are not related with the infoldings of the ostia, despite of being also originated in the vessel wall. The cardiac valves are structures which, during the diastole, close and obliterate completely the passage of haemolymph from one chamber to the other.

The pairs of lateral ostia localized in the central portion of each chamber possessed long edges which we denominated ostiolar valves, as previously suggested by other authors (Locy, 1884; Pantel, 1913; Nutting, 1951; Jones, 1954; Hinks, 1966; Lacombe & Santos, 1986). These valves open during the diastole. This opening increases with the contraction of the alary muscles, which expands the heart. The ostiolar valves close during the systole, obstructing the flow of the hemolymph back to the haemocel (Nutting, 1951; Chiang et al., 1990).

The variation in shape of the pericardial cells seems to be related to the quantity of the cells along the vessel. Where they are present in large amounts (heart) they are tightly aposed and round shaped. In the aorta, where they are more sparcely distributed, they are spread. Outside the vessel these cells may be round shaped in compact clusters, or oval shaped in linear arrays. There is a coat covering the pericardial cells, which may function as a selective barrier to the passage of substances (Crossley, 1972).

According to Imms (1970), the nerve which runs along the thoracic and abdominal portions of the vessel corresponds to the recurrent nerve. Contrary to Jones (1954), we observed branched fibers leaving the nervous cord to inervate the aorta muscle. We suggest therefore that the inervation of the thoracic and abdominal aorta is made up of nerve fibers coming from the visceral nervous system, as in other insects (reviewed by Grassé et al., 1976; Barnes, 1984). We could not determinate the whole nerve pathway by scanning electron microscopy.

As suggested by Chiang et al. (1990), probably the ventral longitudinal muscle fibers play an important role in the vessel contraction, thus helping in the propulsion of the haemolymph.

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