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Comparative Anatomy on the Cardiac Organs of Crustacea, *Penaeus japonicus* BATE (Decapod) and *Ligia exotica* ROUX (Isopod)

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Abstract

Comparative morphological investigations of the hearts of the prawn and the sea louse with the recognitions of its: location, cell number, and innervation; together with the observations of inner structures such as: ostia, arterial valves, and other appendages were carried-out. Hearts were extirpated from intact or formalin-fixed specimens, then prepared for binocular and histological examinations.

In the prawn, a paired aorta diverges forward from the anterior of the heart. Pericardium occurs on the base of this aorta, and envelops it completely. Posteriorly, an aorta runs backward, leaving the heart, and branches off another aorta which descends near its proximal area. Ventrally, the heart provides a paired arteria hepatica. In such aortae and arteriae there exist valvular systems at their proximal lumens. As for ostia, four on the ventral surface and six on the dorsal surface of the heart exist. The cardiac ganglion is situated within a nerve trunk that lies on the midline of inner surface of the dorsal wall of the heart. It contains nine cells, irregularly arranged. A cardiac nerve connects with the former after penetrating into dorsal wall at the middle point of the heart. In the sea louse, the posterior end of a tubular heart is blinded, and its anterior lengthens to become an anterior aorta. Near the proximal end of this aorta, a paired arteria lateralis anterior derives from the cardiac wall. In addition, three pairs of arteriae are provided along each side of the heart ventro-laterally. Each of their proximal lumens has a thin concave valve. Two paired ostia are distributed possessing distinct thick borders in the latter half of the heart along each of its lateral surfaces. The nerve trunk, containing the cardiac ganglion, runs along the midline of the inner surface of the dorsal wall of the heart. Cells of the ganglion are six in number, arranging regularly along the length of the trunk.

Crustacea possess an open vascular system for its hemolymph circulation, and cardiac organs which function as important pumping centers. These are classified generally into two types based on morphology alone. One is a compact or globular shape observed commonly in the Decapoda. The other is a slender or tubular one observed in many orders, such as: the Stomatopoda, Amphipoda, Isopoda, Mysidacea, and Nebaliacea in Malacostraca, except the Decapoda¹⁾. Anatomical investigations of structures of cardiac organs have been undertaken on the Decapoda by BAUMANN²⁾, GADZIKIEWICZ³⁾, LEMOINE⁴⁾, MILLER⁵⁾, and others, concerning myocardium, ostia and other appendages. As for a mechanism of a nervous control in the decapod heart,

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investigations have been progressively undertaken since the discovery of cardiac ganglion cells by BERGER at the end of nineteenth century⁶). Further innervations have been reported by ALEXANDROWICZ⁷⁾⁸⁾⁹, DOGIEL¹⁰, MAYNARD¹¹⁾¹²), NUSBAUM¹³), SUZUKI¹⁴), and others, concerning: a local system, regulatory fibers, and nerves to cardiac and arterial valves. On the other hand, in the Isopoda anatomical investigations of structures and innervations concerning the organs have been dealt with by ALEXANDROWICZ¹⁵) and SUZUKI¹⁶). In this experiment, the cardiac organs of the prawn, *P. japonicus*, has been investigated morphologically, comparing it with that of the sea louse, *L. exotica*, an example of the tubular type.

Materials and Methods

Prawns, 10–20 cm in body length were used in this study. Specimens were brought from culturists at Nobeoka, in Miyazaki Prefecture, and at Yakushima, in Kagoshima Prefecture. As for the sea louses, they were collected at the sea shore near the laboratory. Their body length was about 4 cm. In both cases the cardiac organs were extirpated from intact or formalin-fixed specimens and, 1) the tissues were dissected under a dissecting microscope, stained sometimes with methylene blue for a gross anatomy concerning the arrangements of aortae, arteries, ostia and trunks or the cardiac ganglions, or 2) were re-fixed with BOUIN's solution and, following dehydration through an ethanol series, were embedded in paraffin and sectioned for the recognition of detailed structures. The sections were 4–8 μ m in thickness and stained with hematoxylin-eosine or periodic acid – SCHIFF properly.

Results and Discussion

The prawn and the sea louse have similar open vascular systems; however, in external appearance of the heart, the structures of the myocardium, arteries, ostia, valves and other appendages are largely different between these two species.

Penaeus japonicus I.1) External appearance

The heart situated adjoining the inner surface of the midline of the carapace posteriorly is holded on each side by a paired gonad, and contacts the dorsal face of the mid-gut gland. A paired aorta (a. anterior) diverge forward at the anterior of the heart, sending out two branches at its ventro-interior, just apart from its proximal. The aortae run forward along the front of the mid-gut gland, passing by the pylorus on either side, to the cephalic region. Pericardium occurs at the proximal end of the aortae, enveloping the heart completely. An aorta (a. posterior) runs backward along the dorsal surface of the intestine, leaving the heart and branching off another aorta (a. descends) near its proximal area. The latter descends to a ventral artery, penetrating the middle of a ventral nerve cord. Ventrally, the heart is provided with a paired artery (a. hepatica) running into the dorsal surface of the mid-gut gland.

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Fig. 1. Diagrams of the external appearances of the heart and its appends of the prawn, *Penaeus japonicus*. A paired aorta diverges at the front of the heart, and also ventrally a paired arteria runs to the mid-gut gland leaving behind the latter. Posterior of the heart possesses an aorta that runs backward, branching another aorta at the proximal. The latter descends and reaches to a ventral artery at the distal. The heart provides itself with ostia, which are 4 and 6 in number at the ventral and the dorsal, respectively. Abbrev., ao.ant.: aorta anterior, ao.des.: aorta descends, ao.post.: aorta posterior, ar.m.gl.: artery of the mid-gut gland or arteria hepatica, os.: ostium.

It seems that a cord of slender tissue, coming from anterior region to the midline of the ventral base of the paired aorta, may be a nervous bundle which runs backward along the midline of a pericardial membrane, ventrally. Such a cord is recognized also on the ventral surface of the aorta posterior.

I.2) Inner structure

The heart is basically a single-chambered sac of striated muscle. The cardiac walls consist of an inner muscularis and outer layers of connective tissue. The former muscle bands form an irregular three-dimensional network. Some valvular systems are recognized in the lumens at the origins of the aortae and arteriae. Anterior valves (v. anterior) are situated at the divergent points of each aorta anterior. Each consists of a paired thick flap, forming a membranous pocket which has free edges projecting horizontally into the lumen in the direction of hemolymph flow. Each of flaps possesses a girder protruded from the vessel wall along its midline; the latter connecting proximally with the wall of the heart. The previous cord passes under this valve, and becomes suddenly indistinguishable after that. An other valve (a.



Fig. 2. Diagrams of expected structures of the valvular system at the proximal of the aorta posterior in *Penaeus japonicus*. Upper: dorsal view of the inferior half of the valve, Lower: lateral view of the sagittal cut-end. The valve of aorta posterior is a projection of the dorsal wall of the vessel into its proximal lumen. Its basal part fuses dorsally with posterior extension of the cardiac wall and ventrally its convex surface is in contact with inner wall of the vessel. Further, a nerve trunk, coming along the inner surface of the dorsal wall of the heart, is innervated on the frontal base of the valve. The nerve trunk contains 3 cells of the cardiac ganglion at its ending bulge. Abbrev., ao.des.: aorta descends, ao.post.: aorta posterior, car.cav.: cardiac cavity, car.w.: cardiac wall, gan.c.: ganglion cell, ner.tr.: nerve trunk, val.post.: valva posterior. posterior) is situated at the origin of the aorta posterior, just at the proximal lumen to the branching of the aorta descends. It seems that it separates itself to an upper larger piece and a lower one. The former shows a concave shape distally, and connects with vessel walls at the upper and both of the lateral sides. Preparing narrow openings at both lower sides, the bottom of the upper piece has contact with the lower piece and the vessel wall. It seems, therefore, that after passing through each lateral side of the valve, hemolymph joins again under the upper piece, then flows into the proximal lumen of the aorta posterior. In addition, each arteria hepatica provides itself with a valve of thin conical shape (v. arteria hepatica). As for ostia, they number four on the ventral and six on the dorsal surface of the heart, regularly and symmetrically arranged. They are lens-shaped openings into the heart from a pericardial cavity. The latter is surrounded by a pericardial membrane which possesses, at least on its dorsal surface, narrow openings corresponding to ostia. For the prawn, the ostia are thought to be only slits provided in the myocardium because of lack of muscular structures on their perimeters. It seems that their functions are probably subordinate to myocardium, against BAUMANN's report on the crayfish²⁾.

I.3) Innervation and the ganglion cells

It has been recognized that nervous elements directly supplying the cardiovascular system comprise four distinct components: 1) the local system, whose neurones and fibers are limited to the myocardium; 2) the regulator fibers running from the ventral cord to the neurones of the local system and possibly also to the myocardium; 3) the nerves supplying the arterial values; and 4) the nerves supplying the alary muscles¹⁾. This experiment treats only the local system as follows. Dorsal view of the innervation shows generally like a tree, branching symmetrically to its main trunk which is a median nervous bundle containing many neurones and fibers in it. Anteriorly, the trunk connects with the cardiac nerve at the inner dorsal surface of the myocardium. That is, the latter has its connection with the former after penetrating into the cardiac wall at the middle. The trunk runs backward descending along the midline of the inner surface of the wall and arrives at the basal of the valva posterior. On the other hand, the nerve derived from the front of the trunk becomes to be indistinguishable before reaching to the valva anterior. Nerves branching laterally from the trunk descend distally along the inner surface of the cardiac wall. Further, a nervous relation with the ostia is not observed distinctly in their neighbourhood. Total cell number of the cardiac ganglion is recognized to be nine, in the ten specimens observed. These cells are elliptical and $15-30 \,\mu m$ in size. All of them are situated within the sheath of the trunk and show irregular arrangements. That is, the larger three crowded at the receiving area of the cardiac nerve, three separating each other along the length, and three crowded at the posterior end of the trunk. This result agrees with a general pattern of decapodean ganglion reported previously⁶). The latter three cells seem to engage in controlling the function of the valva posterior.



Fig. 3. Innervation of a local system observed in the dorsal wall of the heart in *Penaeus japonicus*. A main nerve trunk is situated at the midline of the inner surface of the dorsal wall, branching 4 paired boughs. The latter diverges into twigs at the periphery. The trunk contains 9 cells of the cardiac ganglion along its length, of which anteriorly and at the posterior ending 3 cells are crowded respectively. The lens-shaped ostia are 6 in number, arranging symmetrically on the dorsal wall. The dotted lines mean parts of muscle bands running in the heart 3-dimensionally, and at the proximal of the aorta posterior they mean a position of the valva posterior. Abbrev., ao.ant.: aorta anterior, ao.des.: aorta descends, ao.post.: aorta posterior, gan.c.: ganglion cell, ner.tr.: nerve trunk, os.: ostia.



Fig. 4. Diagrams of the external appearances of the heart of the sea louse, Ligia exotica. Left: lateral left view, Middle: ventral view, Right: dorsal view. The heart shows a slender or tubular shape, possessing an aorta at its front and 4 paired artery at its ventro-lateral. The cardiac wall shows a spiral pattern of muscle bands. The 2 pairs of ostia are distributed in the latter half of the heart, locating on each lateral side. They are lensshaped and bordered by ostia muscles, their axes showing parallel with the above pattern of the cardiac wall. Abbrev., ao.ant.: aorta anterior, ar.ant.: arteria anterior, ar.lat.: arteria lateralis, os.: ostia.

Ligia exotica II.1) External appearance and inner structure

The heart is a slender or tubular shape occupying a cavity under the tergum, from the 5th to the 13th segment. It possesses symmetrically small paired triangular tissues along its length on each dorso-lateral surface, connecting with each corresponding segment. Such tissues are not muscular but probably adipose. It seems that they work only as suspensory ligaments of the heart, because of lack of a muscular character. As for L. oceanica, similarly shaped tissues have been observed by ALEXANDROWICZ¹⁵) and named as an alary muscle, which locate along each ventrolateral of the heart. Such a muscle is not recognized in this study. Instead of such a muscle the heart touches a spreaded membrane at the ventral surface; on which an adipose tissue is situated segmentally along each side of the heart. This tissue shows remarkable development around the cardiac cavity, indicating some role in metabolism of the heart and the nervous system. The posterior end of the heart is blind, and the anterior lengthens to become an aorta (a. anterior). Near the proximal end of the aorta, a paired artery (a. l) exists that derives from the ventro-lateral of the heart, branching externally into smaller ones, respectively. Posteriorly, three pairs of arteries are prepared along its length on the ventro-lateral surface of the heart. Each of their proximal lumens has a thin conical valve. The aortic and 1st arterial valves especially show complexity, with each lumen extending a little. The cardiac wall shows a single muscle layer and a spiral pattern of muscle bands. This band is continuous, originating at one end and spiraling about the heart, before inserting at the other end. Two pairs of ostia are distributed in the posterior half of the heart, locating on each lateral side. They are lens-shaped and bordered by ostial muscles, which converge at corners of the openings. Their axes show parallel with the above pattern of the myocardium.

II.2) Innervation and the ganglion cells

A main trunk of the nervous system runs along the midline of the inner surface of the dorsal wall of the heart. Peripheral nerve fibers show their distribution all over the myocardium, running sagittally and often possessing mutual connections, transversally. As for ostia, an innervation of the cardiac ganglion is recognized. On the other hand, its innervation is not observed distinctly in the area of each arterial valve. This result is confirmed by previous observations in *L. oceanica*, which treated the control of neurones as derives of the ventral cord, and an inexistence of control of the cardiac ganglion over the valves. Total cell number of the ganglion, is six arranged regularly like a string of beads along the length of the trunk. This result agrees with that of SUZUKI's in the same species¹⁶ and also that of ALEXANDROWICZ's in *L. oceanica*¹⁵. These ganglion cells are spindle-shaped or bipolar and 40–60 μ m in axial size.



Fig. 5. Innervation of the nerve trunk and its peripheral fibers on the inner wall of the heart in *Ligia exotica*. Specimens are spread after cutting along their midlines of the ventral walls. Abbrev., ao.ant.: aorta anterior, ar.lat.: arteria lateralis, ner.tr.: nerve trunk, os.: ostia.



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