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Lymphoid Organ and Its Developmental Property of Larval Prawn *Penaeus japonicus*

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Abstract

The larval prawn *Penaeus japonicus* has been reported to experience mass mortality during its limited period of development, especially at such stages as early postlarvae.

In this experiment, the lymphoid organ was detected histologically in th larvae and its differentiation process was observed from the basal area of the 2nd antenna. Its shape was a tubular body possessing a connective-tissue sheath and a central lumen, differing from the adult organ in its structure and distribution. A variation was recognized in its size during developmental stages of the larvae. By transcription of the histological sections of the organ and weighing those of copied figures, it was revealed that the size value was high at the mysis 3 and postlarva 1-3. Reversely, lower values were obtained at the stages of the postlarva 4 and 10. In relation to these changes in the size of the organ at each stage, its involvement in the resistance to diseases was suggested.

Prawn culture in Japan has increased its productivity under stable management and effective improvements of the diet. However, it was considered recently to initiate a prevention against the large numbers of larval deaths due to bacterial or virus diseases of the midgut gland. The symptoms of these diseases were reported to occur during successive stages of larval growth[1]. This has been observed primarily in the early stages up to the postlarva 10.

Larval tolerance for the diseases during the development has not been well investigated. Also the larval morphology of internal organs related to a defence mechanism has been lacking in scientific research. For the adult prawn *Penaeus japonicus*, only the lymphoid organ has been histologically determined as one of the sites involved[2].

In this report, an organ similar to that of the adult is presented for the first time in the stages of mysis and postlarvae along with its developmental change in size during the larval period.

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Material and Methods

Larvae of the prawn *Penaeus japonicus* were sampled on each day after hatching from eggs in 500 *l* tank. Normal development of larvae was checked by observation of their external characters at each stage under a dissecting microscope. For feeding, *Chaetoceros*, *Brachionus* and yeast were used according to the routine of method. During the rearing, water temperature was 22.5-27.0℃ (Apr. 10-May 10).

After fixation with BOUIN solution for about 1 h, 5 individuals of each stage from mysis 3 to postlarva 30 were dehydrated through ethanol series and embedded in paraffin. Cross sections of 5 μm in thickness were stained by PAS-hematoxylin. Then, the preparations were subjected to the histological observation.

All of the contour lines of the lymphoid organ recognized in the preparation were transcribed individually on the copy paper by a camera lucida. After cutting out each drawing along the lines, the pieces of one organ were weighed in the lump and the value was used as an index of the size of the individual organ in each larva. This index was expressed as an exchanged value of the weight of the lymphoid organ.

Results and Discussion

From the external observation, larvae of all stages treated in this experiment were determined to be normal. Contrary to the adult prawn, there was no organ in the larvae which showing similarities of shape and position to the lymphoid organ described at the anterior region just adjacent to the midgut gland[2]. The organ of the adult has been reported as a pair of flattened globes which consist of many tubular units. Each of these components possesses a lumen at its center. In the intertubular tissue, the loose connective-tissue is spread. Instead of such a mass structure, the lymphoid organ of the larvae is a long tubule with a thin connective-tissue around the external surface and a central lumen (Figs. 1 and 2). As for the central lumen, it becomes more distinct at the late postlarvae than early stages.

The lymphoid organ of the larvae is stained weakly with PAS, and its margins facing the central lumen and external sheath shows a somewhat strong reaction. It contains numerous cells with very little cytoplasm (Fig. 2). The scattered nuclei are less than 3 μm in diameter, and not all of them shows hematoxylin-positive. Some observed nuclei are grayish in color. These aspects of the tissue, especially of the cells and/or nuclei, seem to correspond to those of one tubular tissue in the adult organ.

The lymphoid organ of the larvae is attached to the antennal sac named so by the author during this study. The sac is situated at the basal area of the 2nd antenna, possessing simple layered nuclei and a ciliary border toward its lumen. It is thought to bear some function analogous to the antennal gland of the adult. Such a configuration is recognized in the mysis 3 as shown in Figs. 3.1 and 3.2. Trunks of both sides of the lymphoid organ run dorso-posteriorly along each side of the lateral wall of the stomach, decreasing their

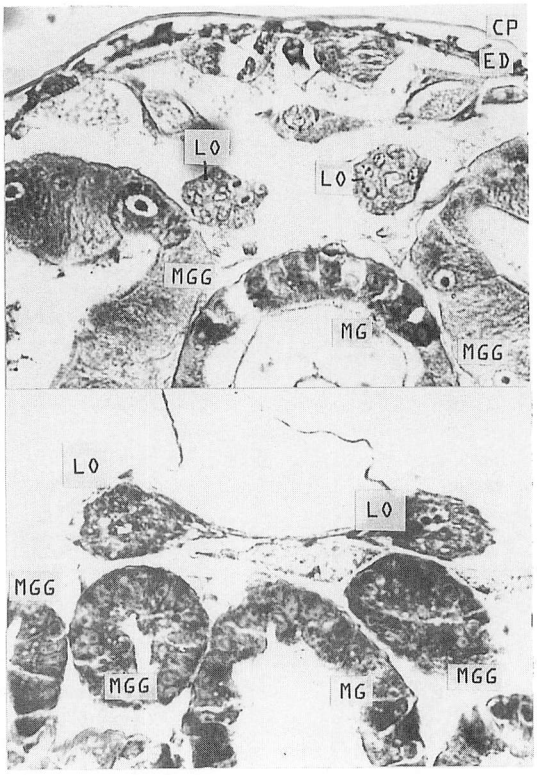

Fig. 1. Comparison of the lymphoid organ between the stages of mysis 3 and postlarva 30. Nuclei of the organ's cells at the mysis show larger than those of the postlarva. The central lumen has developed at the postlarva. CP, carapace; ED, epidermis; LO, lymphoid organ; MG, midgut; MGG, midgut gland.

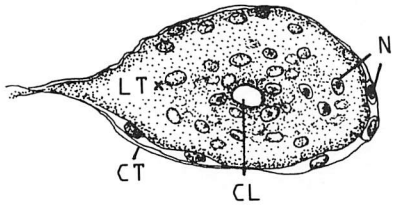

Fig. 2. Cross section of the posterior lymphoid organ, drawn from the postlarva 30. CL, central lumen; CT, connective-tissue sheath; LT, lymphoid tissue; N, nucleus.

diameter, and reach the dorsal surface of the midgut (Figs. 3.11 and 3.12). Just before the heart, they come gradually in contact with each other at the midline of the dorsal midgut. Before the dorsal surface of the midgut, they pass under the midgut cecum[3] which is a specific projection of the antero-dorsal midgut in the larvae of the prawn (Figs. 3.4-3.7), and traverse the anterior portion of the midgut gland (Figs. 3.8-3.10). Stereographical distribution of them is represented diagrammatically in Fig. 4.

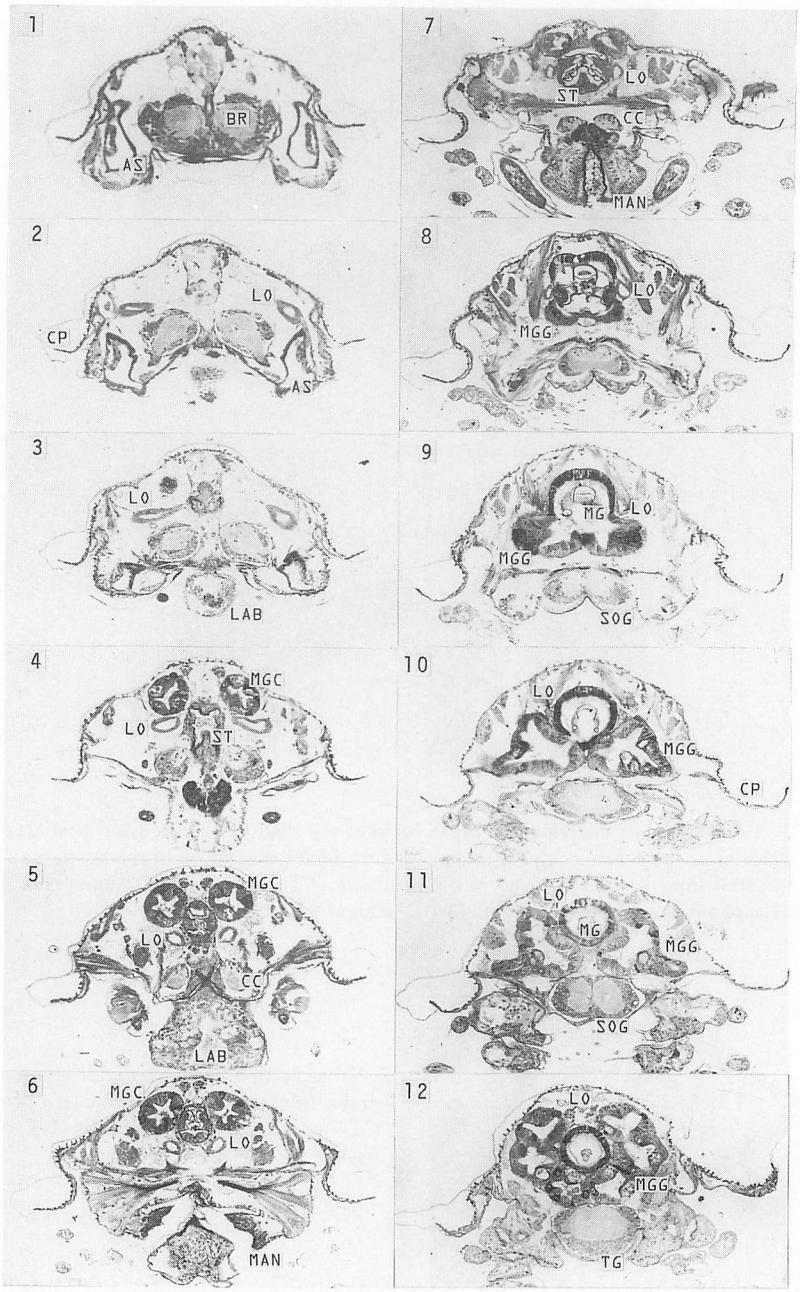

Fig. 3. Histological section of the thoracic area of the larva at the stage of mysis 3, representing the deriving site and distribution of the lymphoid organ. Sections are arranged from anterior to posterior. AS, antennal sac; BR, brain; CC, circumoesophageal connectives; CP, carapace; LAB, labrum; LO, lymphoid organ; MAN, mandible; MG, midgut; MGC, midgut cecum; MGG, midgut gland; SOG, suboesophageal ganglion; ST, stomach; TG, thoracic ganglion.

The volume of the lymphoid organ of the larvae showed a drastic variation according to the developmental stages. At the stages of mysis 3, postlarva 1, 2 and 3, its size was comparatively larger than that of successive stages until the postlarva 20. After postlarva 20, it greatly increased in volume. In Fig. 5, such a transition of index is demonstrated. For the stages past postlarva 12, the index was only calculated for the postlarva stages of 15, 20, 25 and 30, respectively. It would be recognized that remarkably low values occur in the postlarva 4 and 10. These stages would lose the power of resistance to diseases, if indeed this organ functioned as a member of the lymphatic system during the larval period. This proposition could explain the corresponding mass mortality of larvae at these stages.

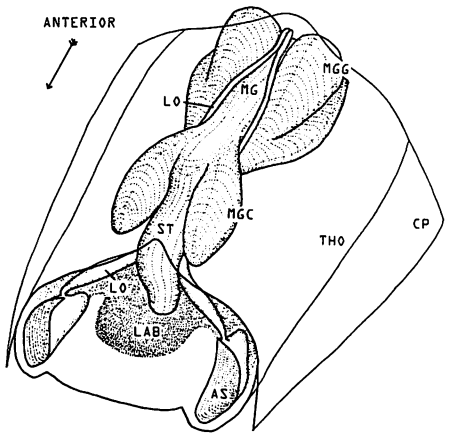

Fig. 4. Three dimensional diagram of the lymphoid organ of the mysis larva, drawn from Fig. 3. AS, antennal sac; CP, carapace; LAB, labrum; LO, lymphoid organ; MG, midgut; MGC, midgut cecum; MGG, midgut gland; ST, stomach; THO, thoracic area.

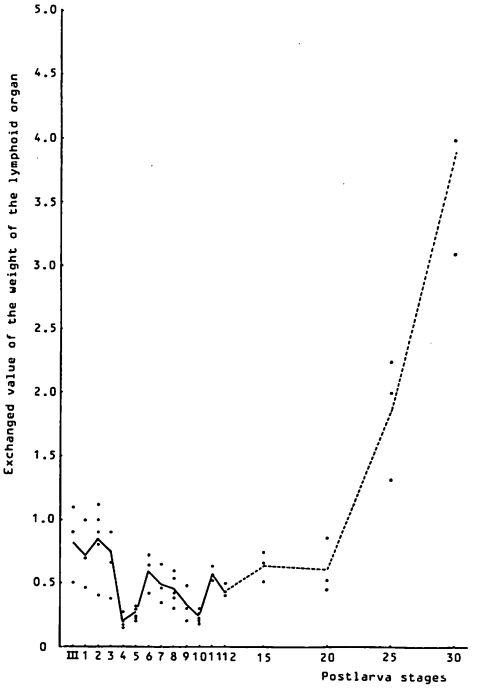

Fig. 5. Developmental change of the size value of the lymphoid organ during larval stages. Abscissa, larval stages; Ⅲ=mysis 3 and 1-30=stage No. of postlarvae. Ordinate, index of the relative size of the lymphoid organ. The index calculated for each prawn is represented as a dot at its corresponding stage. The dotted line in the figure indicates the relationship of averages at the intermittent stages.

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