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Studies on the neurosecretion of thoracic ganglion in relation to reproduction of female *Macrobrachium lanchesteri* (de Man)

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Abstract. The changes in the histology of thoracic ganglion in females of *Macrobrachium lanchesteri*, prior to spawning and post parturial moult, have been described. Based on the differences in size, cell inclusions and differential staining with CHP, four distinct types of neurosecretory cells have been identified in the ganglion. The activity of these cells has been correlated with the ovarian condition of the prawn and evidence for the elaboration of a gonad-stimulating hormone (GSH), by the ganglion has been derived. The probable regulation of ovarian maturation during a normal ovarian cycle and that during the ovigerous condition of *M. lanchesteri* have been illustrated.

Keywords. *Macrobrachium*; thoracic ganglion; NSC; GSH; ovarian regulation.

1. Introduction

The neurosecretory cells (NSC) of the thoracic ganglion have been described to a considerable extent in reptantians (Enami 1951 ; Matsumoto 1954, 1958 ; Parameswaran 1956 ; Miyawaki 1960 ; Nagabhushanam and Ranga Rao 1966 ; Gorgees and Rashan 1977). However, similar studies on the natantian decapods are meagre (Nagabhushanam and Vasantha 1972). Only recently, the types of NSC have been described in the thoracic ganglion of two species of penaeids (Ramadan and Matta 1976).

In brachyuran crustaceans, morphological (Matsumoto 1958, 1962) and experimental (Otsu 1963 ; Gomez 1965; Hinsch and Bennet 1979) evidences have indicated that the thoracic ganglion is the major site of neuroendocrine factors with gonad accelerating effects and this principle is generally termed the gonad-stimulating hormone (GSH). In their review, Adiyodi and Adiyodi (1970) have pointed out that further critical studies are needed to confirm the suggestions of Otsu (1963) that in adult female crustaceans, GSH is produced even during the sexually quiescent periods. While the exact nature and activity of the NSC during gonad maturation of natantians is not known, some evidence for GSH activity has been indicated in *Caridina rajadhari* (Shirwadkar 1977).

In the ovigerous state, considerable amount of ovarian growth has been reported in the freshwater natantian *Macrobrachium lanchesteri* even during the lull reproductive season (Rao *et al* 1981). Presently, an attempt has been made to identify and characterize the NSC of thoracic ganglion of *M. lanchesteri* and correlate their activity with the ovarian maturation.

2. Material and methods

Adult females of *M. lanchesteri* (de Man) were collected from the Agaram tank (near Bangalore) during April, May 1979. The prawns were stocked in the laboratory, in glass aquaria containing aerated freshwater, at a temperature of 26·6 ± 1·06° C. From this stock, active females (total length more than 35 mm) were grouped into the following two stages :

Stage I. Premoult individuals prior to spawning ; ovary occupying $\frac{3}{4}$ of the carapace area, with a number of maturing oocytes, measuring 711·63 μ in length (L) and 435·74 μ in width (W) and

Stage II. Premoult individuals prior to the post parturial moult, after release of larvae ; ovary indicating a number of growing oocytes, measuring 357·83 μ in L and 260·23 μ in W.

The ovarian histology in the above stages has been described by Rao *et al* 1981.

The thoracic ganglion of the above females were dissected out and fixed in Bouin's fluid for purposes of histology. Paraffin sections cut at 6 μ were stained with Bargman's chrome-haematoxylin (CHP) (Pearse 1977).

3. Results and discussion

A knowledge of the precise location of NSC in the thoracic ganglion of natantians is a prerequisite for studying their nature and secretory activity. Figure 1 indicates the distribution of NSC in the thoracic ganglion of *M. lanchesteri*. The NSC

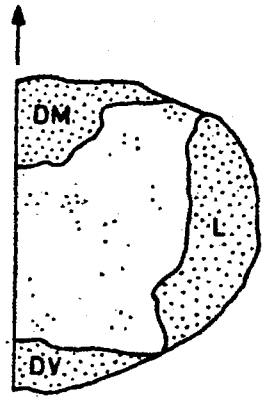

Figure 1. *Macrobrachium lanchesteri* : Diagrammatic representation indicating the distribution of neurosecretory cell aggregations in the thoracic ganglion. DM-Dorsc-median; DV-Ventro-median; L-Lateral.

are aggregated into bilaterally arranged discrete groups along the mid-dorsal, mid-ventral and lateral regions. Very few cells are distributed in between these cell groups and in the mid axis of the ganglion. This arrangement is in conformity with that described for another natantian *Caridina weberi* (Nagabhushanam and Vasantha 1972).

It is most important to know the cytological details of each type of NSC, before attempting to study its physiological role. NSC can be easily distinguished from ordinary neurons, by the larger size, large nuclei and elaboration and discharge of neurosecretory substance (NSS). Depending on the size, the NSC in the thoracic ganglion of *M. lanchesteri* are classified into four types, viz, *A*, *B*, *C* and *D* cells.

A cells are largest in size, measuring 61·6 μ (SE = 5·23 ; $n = 4$) in diameter (figure 2a). These cells are comparable to the giant cell (Miyawaki 1960) and *A*/*A'* cells (Matsumoto 1958 ; Nagabhushanam and Ranga Rao 1966) described for brachyuran crabs and *A* cells of the penaeid prawns (Ramadan and Matta 1976). These cells are generally round with ovoid nuclei (25·6 μ in diameter; SE = 1·13; $n = 4$). The cells are CHP positive, the NSS staining dark purple. A few reddish spherules are also found in the periphery of the cells.

The second type-*B* cells are medium sized (measuring 30·1 μ in diameter ; SE = 1·52; $n = 10$) ; oval to pear-shaped with round or oval nuclei (16·6 μ in diameter ; SE = 0·79 ; $n = 10$) having deeply stained chromatin bodies (figure 2b). The NSS is CHP positive. Some cells display intense staining with CHP, the entire cytoplasm taking up dark purple colour. These cell types are comparable to the median cells of brachyuran crabs (Miyawaki 1960) and type *B* cells of *Scylla serrata* (Nagabhushanam and Ranga Rao 1966).

C cells are comparatively smaller in size, measuring 16·6 μ (SE = 1·31 ; $n = 12$) in diameter (figure 2c). They are round or oval in shape. The cell boundary of some is not so distinct as was described in penaeid prawns (Ramadan and Matta 1976). In the periphery of the nucleus (12·5 μ in diameter ; SE = 0·62 ; $n = 12$), darkly stained, pinkish-red agglutinated chromatin is seen. These cells correspond to the type-VI cells described in the eyestalk of another freshwater prawn *Palaemon paucidens* (Hisano 1974).

The fourth type-D cells are the smallest, measuring 6·4 μ in diameter (figure 2d). They are either oblong or irregular in shape, without a distinct nucleus. The cells stain red with CHP and sometimes small pinkish-red granules are observed inside the cell. The cells are distributed throughout the thoracic ganglion of *M. lanchesteri*. Such cells have also been described in brachyurans, where no secretory function has been attributed (Miyawaki 1960).

Changes in the morphological and cytological characteristics of NSC in the thoracic ganglion of the two stages of *M. lanchesteri* in relation to ovarian maturation are represented in table 1. It is evident that while the *A* cells are totally absent in the thoracic ganglion of I stage females, their presence is conspicuous in the II stage. *B*, *C* and *D* types of cells are present in both the stages. Though the *B* and *C* cells and their nuclei increase in size and number there is no marked difference in either the cell number/size and/or cell inclusions in the *D* type of cells.

The *B* and *C* cells of I stage females contained moderate CHP positive material, grey in colour and their nuclei included some light-pink chromatin granules. In this stage, the cells were distributed along the periphery of the thoracic ganglion, while only few cells were distributed as aggregations in the dorso-lateral regions

(figure 3). The cellular activity in this stage may represent a stage subsequent to the active state of the thoracic ganglion where the NSS (may be GSH ?) might have been used up by the maturing oocytes of the ovary.

In *M. lanchesteri,* at the end of the incubation period following spawning, the egg hatches out into a larva and subsequently, after releasing all the larvae, the mother undergoes a post parturial moult. During the ovigerous state, the immatureova gradually increase in their size due to fat deposition and by the end of incubation period, before the post parturial moult, the ovaries in the stage II females contain growing oocytes (Rao *et al* 1981). The NSC of the thoracic ganglion in this stage exhibited a high secretory activity, the secretions staining dark purple with CHP. Apart from this, the *A* cells showed few phloxinophil spherules in the periphery. The nuclei of *A*, *B* and *C* cell types were observed to possess rich chromatin. In the II stage, the *A* cells were localized in the ventro-lateral region, *B* cells dorso-medially and ventro-medially. Two aggregations of *C* cells were distributed in the lateral region of the thoracic ganglion (figure 3). The presence or absence of *A* cells, the increase in the cell number, size, nuclear diameter, staining intensity of *B* and *C* cells, in the II stage as compared to that in I stage suggest the probable production of increased NSS. A secretory activity and relative abundance of secretory substances in the gaint-*A* cells of the thoracic ganglion have also been reported in the ovigerous *Paratelphusa hydrodromous* (Parameswaran 1956).

The NSC activity observed in the thoracic ganglion of the above stages of females, reflect the nature of their elaboration and perhaps the production of GSH. From the above data, it is evident that there is continued production of GSH by the NSC of thoracic ganglion even in the ovigerous state of *M. lanchesteri,* accounting for the marked growth of oocytes in the stage II ovaries. This GSH might be acting (1) directly on the ovary in bringing about ovarian growth and/or (2) on the maintenance of secondary sexual characters, viz, plumose ovigerous setae and brood pouch. The present observations in *M. lanchesteri* provide evidence for the suggestions of Adiyodi and Adiyodi (1970) that GSH promotes oocyte growth.

The extended ovigerous state of *M. lanchesteri* represents the long intermoult period when the *Y*-organ is suppressed in its activity (Rao *et al* 1981). This further testifies the hypothesis of Adiyodi and Adiyodi (1970) that GSH might also be responsible for the suppression of the *Y*-organ. In the II stage females of *M. lanchesteri,* the GSH elaborated in the thoracic ganglion might be acting on the secondary sexual characters mentioned above, probably through a sex hormone produced in the ovary. In this connection, it is interesting to note that an ovarian hormone (OH) produced in the amphipod, *Orchestia gammarella,* is known to control similar secondary sexual characters (Charniaux-Cotton 1952, 1957). Presuming that a similar OH is elaborated by the ovary of *M. lanchesteri,* it is now possible to suggest that the GSH mediated ovarian growth/suppression of *Y*-organ activity, may be due to the cues provided by the sensory elements in the pleopods (figure 4 ; see also Passano 1960). It is interesting to note that GSH is produced in the thoracic ganglion of *M. lanchesteri* in the lull reproductive season, and this offers the first histological proof to the statement of Otsu (1963).

In the absence of cues from the sensory elements of the secondary sexual characters, in the I stage females of *M. lanchesteri,* the extensive maturation of ovary can be presumed to be due to the elaborate production of GSH (from thoracic ganglion

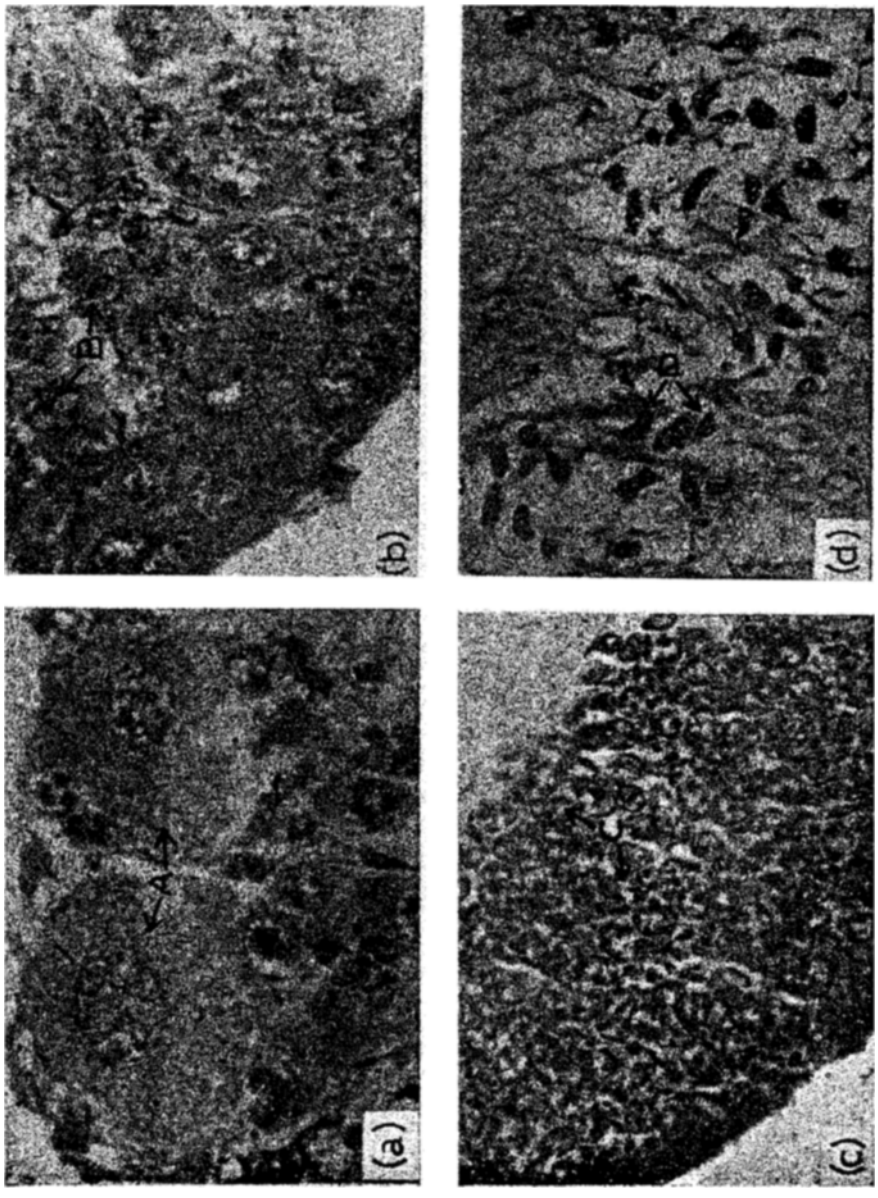

Figure 2. *Macrobrachium lanchesteri*; Neurosecretory cell (NSC) types in the thoracic ganglion (× 650). *A–D* represent the four NSC types described in the text.

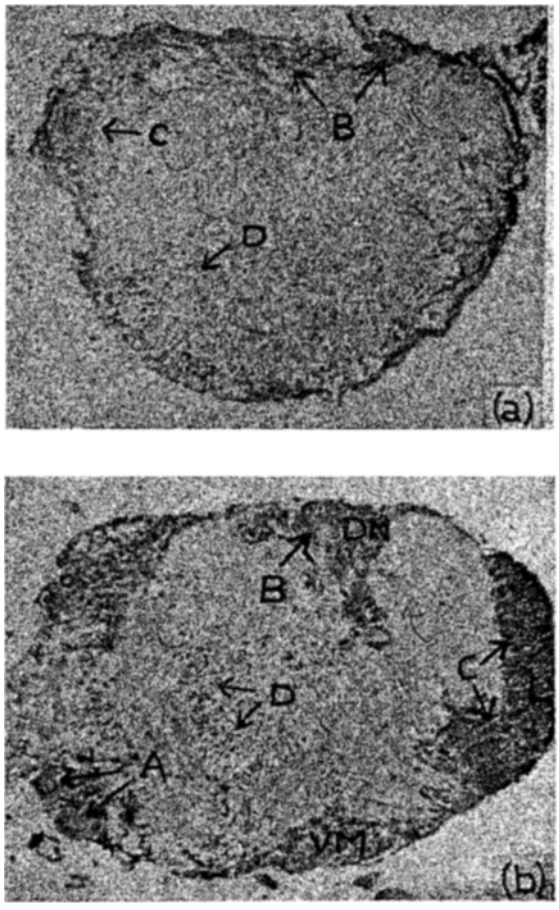

Figure 3. *Macrobrachium lanchesteri* ; Sectional view of the thoracic ganglion of the I and II stage females (× 200) represented as (a) and (b) respectively inside the figures. DM, VM and L—NSC aggregetions as described in figure 1. *A–D*, NSC types as described in figure 2.

Table 1. *Macrobrachium lanchesteri*: Changes in the morphological and cytological characteristics of neurosecretory cells in the thoracic ganglion of the I and II stage females.

Cell types		Average cell diameter (μ)	Average nuclear diameter (μ)	Average cell number	Other characteristics
A (>50 μ)	I	..	..	..	..
	II	61·6	25·6	4	Largest, round cells with chromatin rich nuclei. Dark purple CHP positive NSS in the cytoplasm.
B (26–50 μ)	I	28·8	14·9	3	Medium, oval, moderately CHP positive greyish cells. Light pink nuclei with small chromatin granules.
	II	30·6	17·4	23	Medium, oval to pear-shaped cells. Round or oval nuclei with 3–4 deeply stained bodies. Dark purple NSS in the cytoplasm.
C (9–25 μ)	I	16·5	11·7	268	Smaller, round or pear-shaped moderately CHP positive greyish cells with agglutinated light pink chromatin in the nucleus.
	II	16·7	14·4	633	Smaller, round or oval cells with darkly stained pinkish-red agglutinated chromatin in the periphery of the cells.
D (< 8 μ)	I	6·4	..	303	Smallest, oblong to irregular shaped cells without distinct nuclei, red in colour.
	II	6·4	..	285	Same as in stage I.

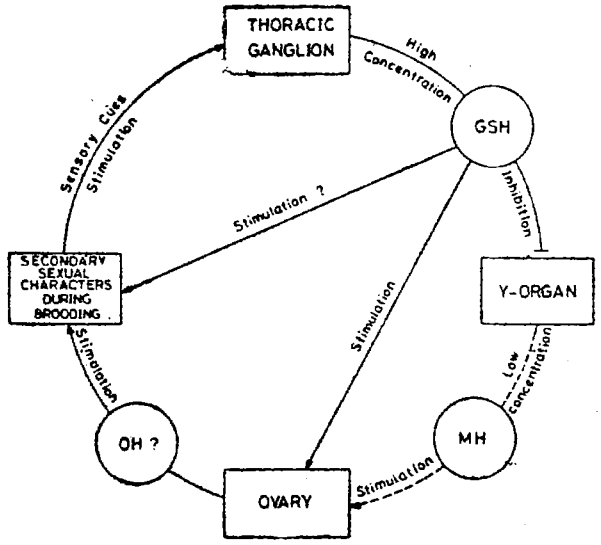

Figure 4. *Macrobrachium lanchesteri*: Diagram illustrating the probable regulation of ovarian maturation during the ovigerous conditions. GSH—Gonad-stimulating hormone; MH—Moulting hormone; OH—Ovarian hormone.

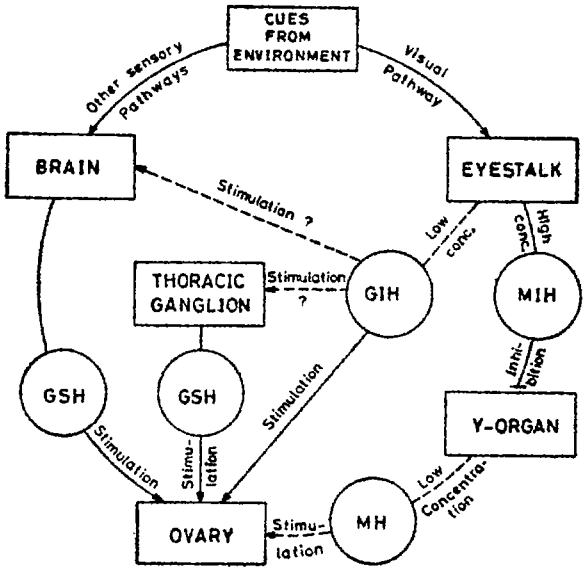

Figure 5. *Macrobrachium lanchesteri* : Diagram illustrating the probable regulation of normal ovarian maturation. GSH—Gonad-stimulating hormone; GIH—Gonad-inhibiting hormone; MH—Moulting hormone; MIH—Moult-inhibiting hormone.

and/or brain ?) and reduction in the gonad-inhibiting hormone (GIH ; from the eyestalk). The triggering factors for the above chain of events are perhaps the environmental cues impinging upon the receptor systems of the prawn (figure 5 ; see also Adiyodi and Adiyodi 1970).

Acknowledgements

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