NEUROSECRETORY CONTROL OF RED CHROMATOPHORES IN THE SHRIMP, *ALPHEUS MALABARICUS*

NEUROSECRETORY cells were described in Crustacea, for the first time, by Enami (1951) who distinguished three types of cells in the brain of *Sesarma*. Observations of Passano (1951), Bliss and Welsh (1952), Passano (1953) and Carlisle and Passano (1953) contributed to the establishment of the neurosecretory X-organ-sinus gland system in Crustacea. Among the Brachyura, the works of Enami (1951), Bliss and Welsh (1952), Matsumoto (1954, 1956, 1958), Potter (1954), Parameswaran (1956), Miyawaki (1956, 1960) and Maynard (1961) are the most imporant, who distinguished more than one kind of neurosecretory cells in various parts of the central nervous system.

In Natantia very few studies were made on the neurosecretory cells. The presence of secretory cells in the eyestalk had been reported in *Crangon, Lysmata, Leander* and *Pandalus* (Gabe, 1966). Of these perhaps only in *Pandalus borealis* an attempt was made to classify the neurosecretory cells in the eyestalk.

The present investigation was aimed to study (1) the neurosecretory system of *Alpheus malabaricus* and (2) to describe the effects of eyestalk removal upon the remaining neurosecretory system in *Alpheus*. Observations were also made on changes that occurred in the titer of the red pigment concentrating hormone in the brain of eyestalkless shrimps.

MATERIAL AND METHODS

Alpheus malabaricus, used in the present study, were collected from the Vellar estuary at Porto Novo. Adult specimens were fixed *in toto* in Bouin's fluid as soon as they were brought to the laboratory. The eyestalks were separated from the body and processed separately. The supraoesophageal ganglia, thoracic ganglion and abdominal ganglia were dissected out carefully from intact animals and kept in separate dishes. After dehydration in alcohol and clearing in xylol, the material was embedded in paraffin and serial sections were cut at 8-10 μ in thickness and stained with Gomori's chrom-haematoxylin-phloxin (CHP) or Mallory's triple stain. Transverse and sagittal sections were made to reconstruct the distribution of the neurosecretory cells.

To study the effect of eyestalk removal upon the remaining neurosecretory system, the brains and optic stubs of animals whose eyestalks were removed 2 weeks earlier were fixed in Bouin's fluid, sectioned and stained with Mallory's triple stain to observe the distribution of neurosecretory cells. Extracts of brains of eyestalk-less animals were made according to the method of Sandeen (1950).

Results and Discussion

Histology of the neurosecretory system

The neurosecretory cells are noticed in groups in the eyestalk, supraoesphageal ganglia, tritocerebral commissural ganglia, thoracic ganglion and abdominal ganglia.

The eyestalk is a compact structure. The lamina ganglionaris is cup-shaped and enclosed the medulla interna and medulla externa ventrally and laterally. In life the sinus gland is clearly seen through the dorsal cuticle of the eyestalk as a bluish-white cup-shaped body surrounding the blood sinus. Fig. 1 shows a reconstruction of eyestalk ganglia and related structures. The X-organ constitutes a few cluster of cells lying on the ventral aspect of the eyestalk between the medulla terminalis and extending upto the optic peduncle. The cells of the X-organ are larger than the ordinary nerve cells. The neurosecretory cell groups are also seen in medulla externa and medulla interna (Fig. 1).

The distribution of neurosecretory cell groups in the supraoesophageal ganglia was shown in Fig. 2. The darkened areas are those where neurosecretory cell bodies are found in stained sections. Five major groups of neurosecretory cells were found in supraoesophageal ganglia, namely (1) an unpaired group between the optic nerve peduncles, (2) a paired group lateral to the left and right olfactory lobes, (3) a pair mesial to the left and right olfactory lobes, (4) a small pair lateral to the base of each circumoesophageal commissure and (5) an unpaired group between the routes of the two circumoesophageal commissures.

In the thoracic ganglion the secretory cells appear as discrete groups along the mid-dorsal, mid-ventral and dorsolateral regions. In the abdominal ganglia the cells are concentrated in the posterio ventral parts of the ganglia.

The neurosecretory cells measure about 20 to 30 μ in length and 10-15 μ in diameter. The cell body is pear-shaped and possesses much cytoplasm and contain a small nucleus (Fig. 3). The secretory material appears as minute granules or droplets that stain red with Mallory's or blue-black with Gomori's stain. In some sections the secretory material was seen at the point where the axon leaves the cell body and along the axon for some distance.

The morphology of the neurosecretory system of *Alpheus* is broadly similar to that of *Palaemon serratus* (Carlisle and Knowles, 1959). There appears to be one cell type in the eyestalk and in other central nervous organs. These cells bear close resemblance to the cell type II described by Durand (1956) in *Orconectes*. The distribution of neurosecretory cells in the eyestalk and brain more or less confirm the observations made by Pillai (1961) in *Caridina laevis*.

Effect of eyestalk ablation upon neurosecretion

The brains were dissected from the shrimps whose eyestalks had been removed 14 days previously and also from intact animals. Sections of brains of intact and 14-

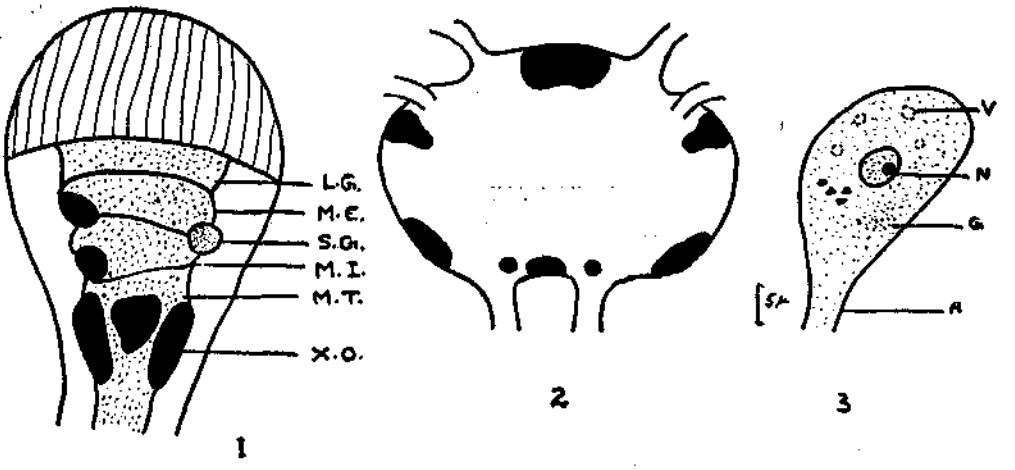

FIG. 1. L. S. eyestalk of *Alpheus* (diagrammatic). Regions of neurosecretory cells are shown in solid black. L.G., lamina ganglionaris; S.G., sinus gland; M.E., medulla externa; M.I., medulla interna; M.T., medulla terminalis; X.O., X-organ.

FIG. 2. Diagrammatic representation of the supraoesophageal ganglion showing the neurosecretory cell groups (darkened areas).

FIG. 3. Neurosecretory cell. A., axon; N., nucleus; V., Vacuole; G., granules.

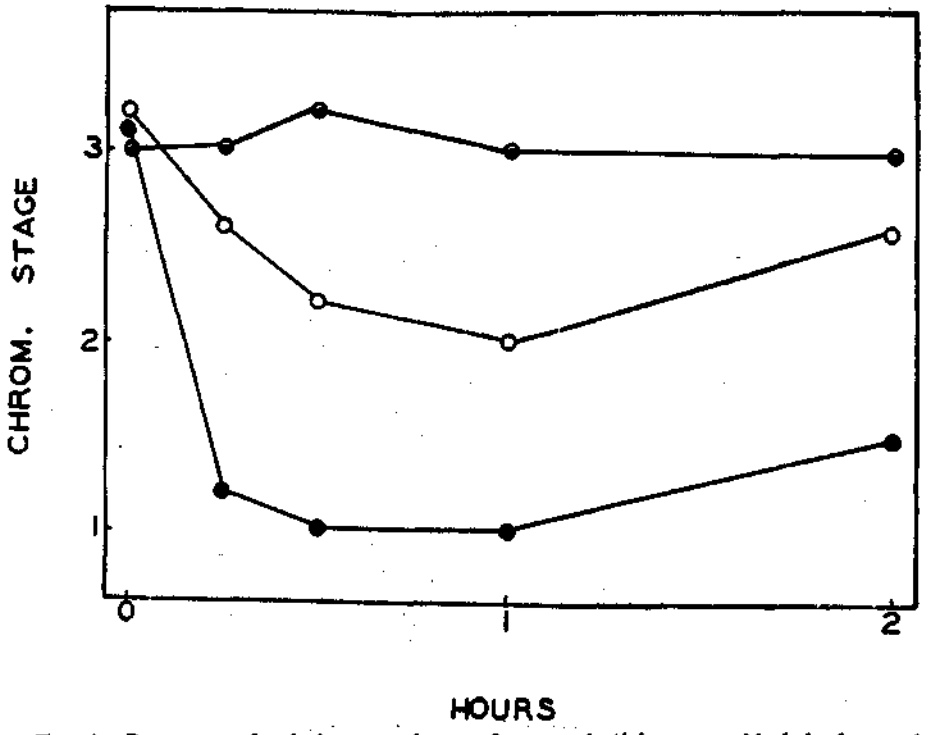

FIG. 4. Responses of red chromatophores of one-eyed *Alpheus* on a black background to extracts of brain of 14-day eyestalkless (dots), and intact individuals (circles). Control animals received sea water injections (circles half-filled).

day eyestalkless animals and the optic stubs of these eyestalkless shrimps were stained with Mallory's stain, for comparison with each other. The regions of neurosecretory cells in the brain of eyestalkless and intact animals contained dark red droplets. However, the concentration of droplets was much greater in the brain of eyestalkless shrimps than in those of intact forms. Droplets have also accumulated at the swollen ends of the optic nerves whereas optic nerves of intact shrimps had no droplets.

In an effort to identify at least a part of the accumulated material, brains of intact animals and of specimens both of whose eyestalks had been removed 14 days previously were assayed for red pigment concentrating hormone. Each extract was injected into ten specimens and the chromatophores were staged according to the system of Hogben and Slome (1931). The experiment was repeated once and the results are shown in Fig. 4. It was observed that the chromatophorotropin was more in the brain of *Alpheus* whose eyestalks had been removed 14 days than in the brain of intact animals. Therefore, apparently at least some of the accumulated droplets contained this red pigment concentrating hormone. Identical results were obtained by Fingerman and Aoto (1960) in the crayfish, *Cambarellus shufeldti*.

Summary

A detailed morphological description of the neurosecretory system in the shrimp, *Alpheus malabaricus*, was given. The supraoesophageal ganglia possess five groups of neurosecretory cells. The optic ganglia, thoracic ganglion and abdominal ganglia also contain neurosecretory cells.

The neurosecretory cells are large (20-30 μ), pear-shaped, possess large cytoplasm and small nucleus. The secretory material stained red with Mallory's and blue-black with Gomori's stain.

Removal of both eyestalks in *Alpheus* resulted in accumulation of neurosecretory material in the brain and in the optic nerve stubs. A portion of the accumulated material in the optic stubs contained red pigment concentrating hormone.

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R. Nagabhushanam

Zoology Department,
Marathawada University,
Aurangabad.

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