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STUDIES ON THE SHRIMP *CARIDINA LAEVIS* (HELLER)

IV. The Neurosecretory System

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INTRODUCTION

APART from attributing a function of transmission of nerve impulses, that the nerve cells produce a secretion was demonstrated by Scharrer (1928) and other scientists thenceforward. Recently the neurons showing glandular properties have come into prominence and these have been described as neurosecretory cells by Scharrer and Scharrer (1954). Neurosecretory cells are distributed throughout the central nervous system of Crustacea. Localised clusters are arranged in the brain and the eyestalks (Bliss, Welsh and Durand 1954, Enami 1951, Durand 1956). The precise localisation of the source of the hormones and their influence on particular physiological processes have not been investigated fully. Passano (1951) has shown a hormone that is capable of inhibiting moult, synthesised inside the X-organ and Durand (1956) has shown that the 'type 2' neurosecretory cells are the source of the moult-inhibiting hormone. The neurosecretory cells have been mapped in a few Crustacea (Enami 1951 a, Bliss and Welsh 1952, Bliss, Durand and Welsh 1954, Knowles 1953, Durand 1956, Parameswaran 1956). These based on their size, shape and staining reactions have also been classified into separate Cell Types (Enami 1951 b, Matsumoto 1954, Parameswaran 1956, Durand 1956).

METHODS

The study of the neurosecretory system was carried out mainly by the help of cut material. The entire head was fixed in Bouin's fluid, Heidenhain's Susa and Zenker-formol. The brain and thoracic ganglia with the eyestalks were dissected out in fixing fluids and kept in the same for the required time. Out of these, it was found that Heidenhain's Susa was best suited as it gave clear pictures without shrinkage, while the brain fixed in Bouin's fluid was liable to shrinkage which obscured the details of the cell types. Sections cut at 5 to 10 μ were stained in Heidenhain's Azan, Mallory's triple stain, Heidenhain's iron haematoxylin and Gomori's chrome-alum-haematoxylin-phloxine (Gomori 1941 adapted by Bargmann 1949). The latter though extensively used in the study of vertebrate neurosecretory cells, did not give as clear pictures as Heidenhain's Azan. Surgical removal of the sinus gland as was carried out by Kleinholz (1947) in the cray fish is not feasible due to the minute size of the animal.

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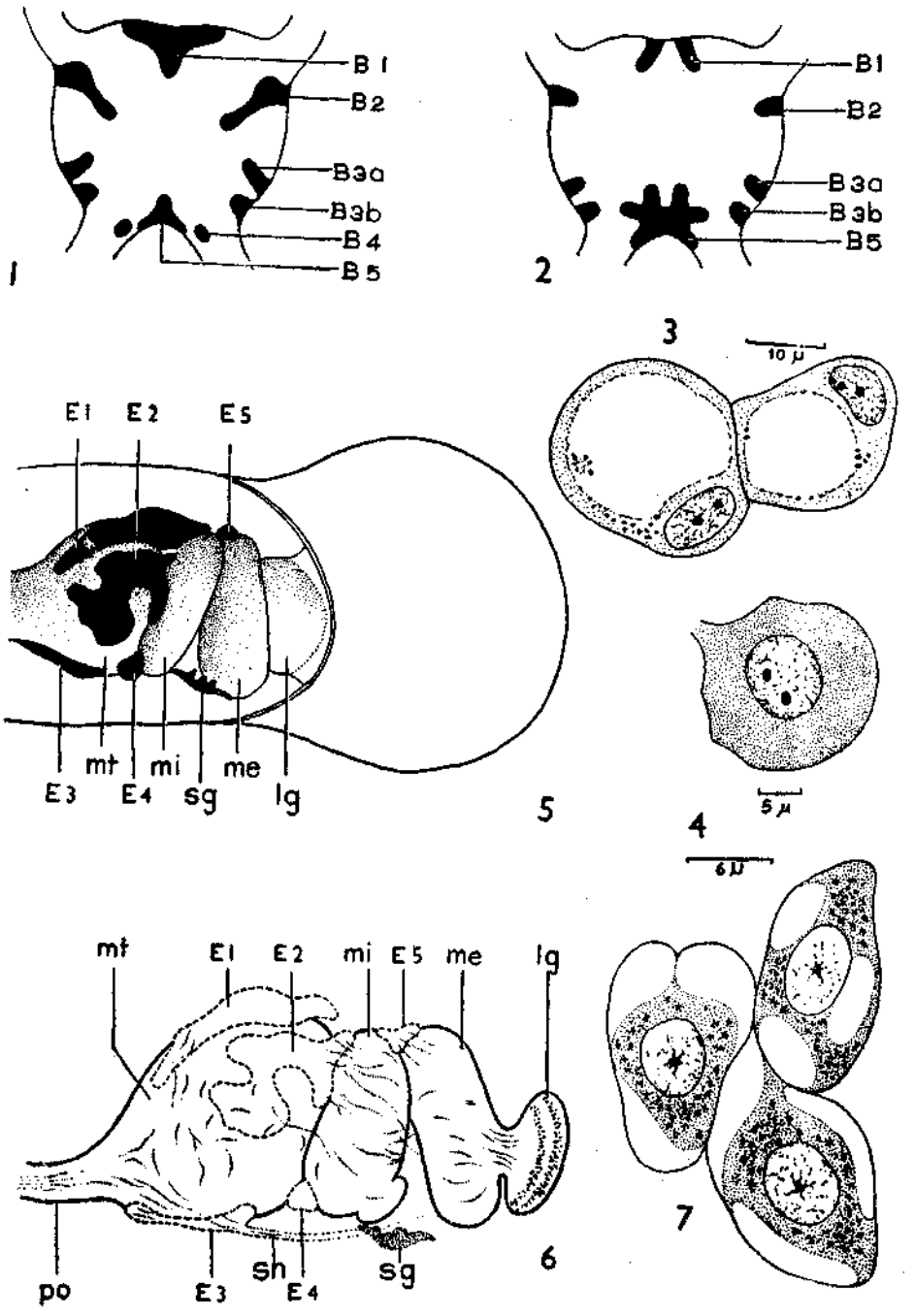

FIGS. 1 and 2. Maps showing distribution of neurosecretory cells in the brain ; dorsal and ventral views respectively. *B1* to *B5* represent areas of neurosecretory distribution.

FIGS. 3 and 4. Neurosecretory cell of Type I from X-organ and brain respectively.

FIG. 5. Map showing the distribution of neurosecretory cell groups in the left eye. Dorsal aspect (Reconstruction). *E1* to *E5*. Neurosecretory cell groups. *lg*. Lamina ganglionaris. *me*. Medulla externa. *mi*. Medulla interna. *mt*. Medulla terminalis. *sg*. Sinus gland.

FIG. 6. Optic centres, with secretion laden axonic fibres super-imposed therein showing their course. *po*. Peduncle lobus opticus. *sn*. Sinus gland nerve. (Other lettering as in fig. 5.).

FIG. 7. Neurosecretory cells of Type III form brain.

OBSERVATIONS

The maps showing the distribution of the neurosecretory cells for the brain and optic ganglia in *Caridina laevis* are represented in figures 1 and 2. In addition to these, neurosecretory cell groups have been observed in the stomatogastric ganglion

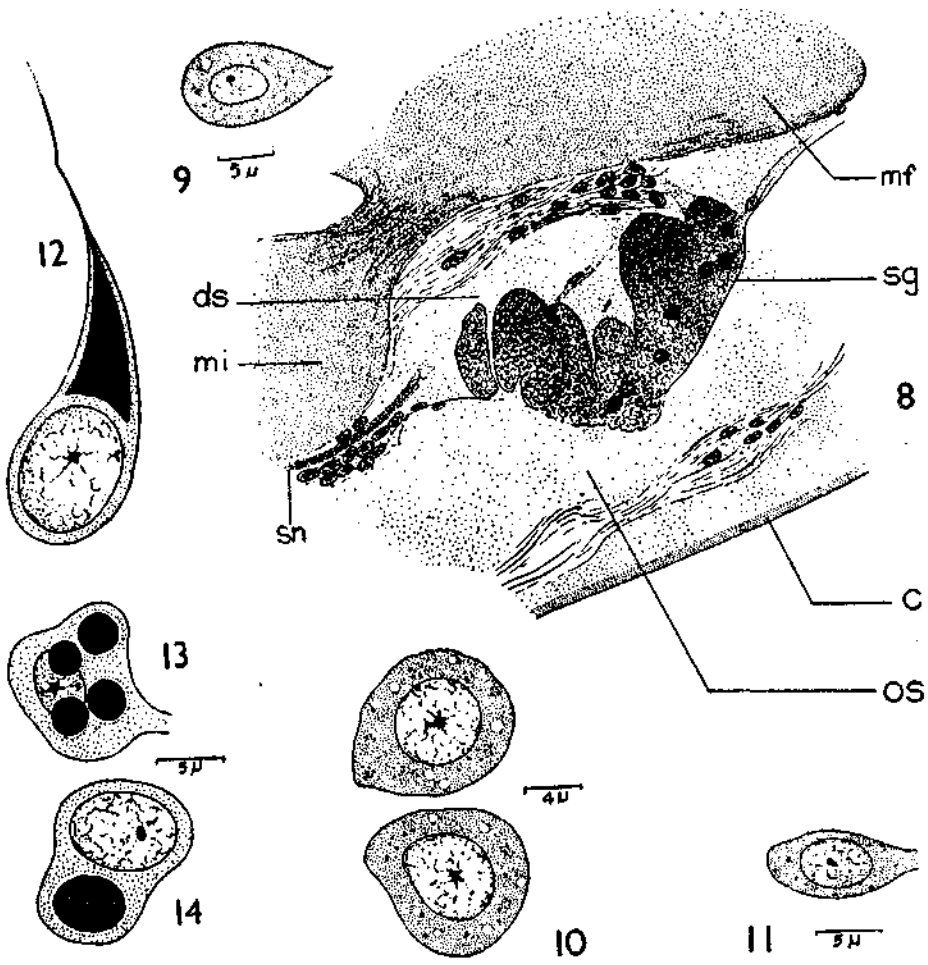

FIG. 8. Longitudinal section through sinus gland. *c*. Cuticular wall of the eyestalk. *ds* Dorso-lateral sinus. *os*. Outer optic sinus. Other letterings as in fig. 5.
FIG. 9. Neurosecretory cell Type III from brain.
FIG. 10. Neurosecretory cell from stomatogastric ganglion.
FIG. 11. Neurosecretory cell Type IV from eyestalk.
FIGS. 12, 13 and 14. Neurosecretory cells of Type II from X-organ showing secretory droplets in the cytoplasm.

and the ventral nerve cord. The stomatogastric ganglion which projects into the carotid dilatation and bathed by blood is enveloped by a cluster of neurosecretory cells. The tritocerebral connective ganglion demonstrated a few neurosecretory

cells on its median aspect. The 17 pairs of ganglia of the ventral nerve cord are also surrounded by neurosecretory cells.

The Sinus Gland (Figs. 5, 8)

The sinus gland is situated on the dorso-distal part of the eyestalk between the medulla externa and medulla interna which position has been noticed to shift usually towards the proximal part. It is formed as a differentiation of the epineurium and encloses the dorso-lateral blood sinus which communicates with the outer optic sinus. The sinus gland is 95 μ long and is 15 μ thick. Where the secretory product is sparse, fine striations of an eosinophil nature are noticeable. In others the syncytial wall of the sinus gland is laden with secretory droplets stainable red, blue or lilac in Heidenhain's Azan. The nuclei are placed more towards the outer side and are small and quite similar to the nuclei of other nerve cells. It is innervated from the medulla terminalis through the axons of which the secretory products from many neurosecretory cells could be traced as ultimately reaching the sinus gland which is looked upon as a storage depot.

X-organ

Hanstrom (1931) first detected the X-organ in Crustacea and has put forward the hypothesis that it represents the transformed sensory cells of a rudimentary eye papilla, which has taken on a secretory function (1939). Though strictly derived from sensory cells and not from true ganglionic cells, they are considered as neurosecretory cells.

The X-organ (figure 5 E1) constitutes a few clusters of cells lying on the ventral aspect of the eyestalk between the medulla terminalis and the medulla interna and extending as far as the peduncle lobus opticus. The X-cells are larger than the ordinary nerve cells and are arranged around the nerve starting from the proximal end of the medulla terminalis and innervating the rudimentary sensory papilla. Some of the cells constituting the X-organ are incretory in that they have been found to develop secretory granules stainable red in Azan and Mallory's, and blue in Gomori's chrome-alum-haematoxylin-phloxine. Groups of larger spherical structures 2 to 3 μ across have been noticed inside many of the cells of the X-organ. Hanstrom (1939) has interpreted these as irregularly shaped spherical concretions of secretion.

Neurosecretory cell types

Durand (1956) has described 4 cell types in *Orconectes virilis*. The cell types found in *Caridina* more or less conform to these.

Cell type I (Figs. 3, 4)

These are very few and found in the distal portion of the X-organ and in the anterior cell group of the brain. Those in the brain measure about 23 μ across, with nuclei half their size. The cytoplasm is granular. This type of cells in the X-organ is seen to develop large vacuoles containing secretory granules and resemble the ' a ' type described by Enami (1951 b).

Cell Type II (Figs. 12, 13, 14)

These are most numerous in the X-organ. They measure about 9 to 12 μ and contain large secretory droplets about 2-3 μ. The nucleus has a single nucleolus. More or less similar cells have been observed in other clusters in the eyestalk, es-

pecially in the proximal part of the group E2. They differ from others in having a slightly larger nucleus with usually a single large secretory droplet. In some cells the droplets are seen to be transported directly to the medulla terminalis through the axons and finally to the sinus gland.

Cell Type III (Figs. 7, 9, 10)

These cells have a variety of forms probably in connection with their different secretory phases. Some are typically tear drop-shaped ranging in size from 7 to 13 μ and have nuclei half the size with single nucleolus. Around the nucleus are numerous small vacuoles and secretory granules. The stomatogastric ganglion has this kind of cells only. Larger cells measuring about 17 μ and with usually a few large apparently void vacuoles have been observed in the brain. These cells have a very striking resemblance to the 'B' cells described by Matsumoto (1954) and Parameswaran (1956). It is not clear whether they fall into the cell type 3 of Durand (1956).

Cell Type IV (Fig. 11)

These cells are small, oval (6 μ by 10 μ), slightly larger than the ordinary ganglion cells and are grouped together in most of the neurosecretory clusters in the eyestalk (except the X-organ) and the brain. The nucleus almost fills the cytoplasm. Secretory granules are sometimes present in the periphery of the cytoplasm.

The neurosecretory cell map for the eyestalk is given in figures 5 and 6. They have been numbered according to that in *Cambarus* (Bliss, Durand and Welsh 1954 and Durand 1956). The incretory nature of these cells is judged from the demonstrability of stainable secretion in the form of granules and droplets in cytological preparations.

Mode of release of Secretion

The mode of discharge of secretion indicates an axonal transport. The fuchsinophil and eosinophil secretion has been noticed inside the axis cylinder in contradistinction to the light blue-stainable neuropile of the medulla terminalis. All the secretion laden axonic fibres from the X-organ, in a superimposed reconstruction, run more or less parallel towards the origin of the sinus gland where similar accumulation has been noticed. This, on the whole resembles the condition described in various Brachyura by Smith (1948) and Enami (1951). Passano (1952), by phase contrast microscopic studies has traced the spherical system of granules from the X-organ to the sinus gland nerve.

The axonic fibres of the neurosecretory cell groups of the eyestalk, E2 (distal lobe), E5 and possibly E4, enter the neuropiles of the medulla terminalis, medulla interna or the medulla externa independently and are clearly traceable as red tracts in the blue background. The pathways of secretory granules have been observed to cross each other in the region of the thick connective separating the medulla externa and the medulla interna (fig. 6). At the distal extremity of the lamina ganglionaris, a distal and a proximal accumulation of granules have been noticed. It is possible that these represent the terminations of the axonic fibres viz. the 'onion bodies.' From here, the granules are probably liberated into the blood vessels situated below the basal membrane of the compound eyes. A few of these red granules have been observed sticking on to the inner walls of the blood vessels.

Bliss, Durand and Welsh (1954) have studied the axonal transportation of secretion in the neurosecretory cells of *Gecarcinus*. Here all groups of neurosecretory cells including the X-organ have their axons traceable directly to the sinus gland as well defined fibre tracts. The course of the axons directly to the blood sinuses below the basement membrane of the compound eye in the cell groups E2, E5 and possibly E4 in *Caridina* is remarkable. Knowles (1950) has shown that the removal of the sinus gland without blinding leads to an immobility of the distal pigments of the eye in *Leander*. But the reflecting pigment of the eye continues to be affected in response to changes of illumination. This has been interpreted by him (1956) as due to an extra-sinus gland source of the reflecting pigment activator in the eyestalk. From the close association between the blood sinuses surrounding the reflecting pigment and the accumulation and release of secretory products from the apex of the lamina ganglionaris from this extra-sinus gland source, it may be presumed that these have a role in the humoral control of the reflecting pigments of the eye.

Summary

Neurosecretory cell maps for the brain and the optic ganglia of the eyestalks have been constructed and the cell groups numbered. Four types of neurosecretory cells have been distinguished in *Caridina*, comparable to that in *Orconectes*. Axonal release seems to be the mode of transportation of secretory products. Secretion laden axonic fibres from a few cell groups in the eyestalk have been traced directly to the blood sinuses below the basement membrane of the compound eye. It is suggested that these might have a role in the control of the movement of the reflecting pigment in the eye.

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