

The Neurosecretory system of the Pholad, *Diplothyra smithii* (Mollusca : Lamellibranchiata)

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(With 1 Text-figure)

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I—INTRODUCTION

While a great deal of information was gathered on the cytology, distribution and the role of the neurosecretory cells in the neuroendocrine regulation in both invertebrates and vertebrates, similar studies on the bivalve molluscs were started only in 1955. Gabe (1955) was the first investigator to report the occurrence of secretory neurons in 20 species of lamellibranchs. Lubet (1955, 1956) using the mussel, *Mytilus edulis* and the clam, *Chlamys varia*, showed a definite correlation between neurosecretion and sexual cycle. Fahrman (1961) reported two types of neurosecretory granules in the freshwater mussel, *Unio tumidus*, and Nagabhushanam (1962 a, b, c, 1964 a) observed two neurosecretory cell types in the oyster, *Crassostrea virginica*, the surf clam, *Spisula solidissima*, and the shipworm, *Bankia gouldi*, and one cell type in *Modiolus demissus*.

The present paper deals with the distribution and structure of the neurosecretory cells in the central nervous system of the pholad, *Diplothyra smithii*, with a view to extend our knowledge on the phenomenon of neurosecretion in the lamellibranchs.

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II—MATERIAL AND METHODS

Freshly collected animals were used. They were frequently collected by breaking open the valves of the oysters obtained from Gulf Coast near New Orleans, U.S.A. The shell valves were carefully removed and the whole body was transferred to one of the following fixatives: formalin, Bouin's or Helly's fluid. The formalin-fixed material was post-chromated with 2-5 per cent Potassium dichromate to improve fixation. The material was then dehydrated in alcohol, cleared in xylol and embedded in Tissuemat. Serial sections were cut at 6-8 μ . The following staining techniques were employed :

1. Mallory's triple stain (MS),
2. Gomori's chromalum-hematoxylin-phloxin (CHP),
3. Periodic acid-Schiff (PAS) reaction for polysaccharides after diastase digestion (Pearse, 1960),
4. Sudan Black B, mounted in an aqueous medium, and
5. Einarson's gallocyanin-chromalum method for nucleic acids (Pearse, 1960).

III—OBSERVATIONS

General Description of the Nervous System.—The central nervous system of *Diplothyra smithii*, which conforms to that of *Martesia striata* (Nagabhushanam, 1962d), consists of three pairs of ganglia: cerebral, visceral and pedal. The cerebral ganglia are paired, situated one on each side near the epidermis, above the base of the labial palps and in front of the anterior end of the inner demibranch. The cerebral ganglia are smaller than the visceral ganglia. From each cerebral ganglion arises, besides the cerebral commissure, the anterior pallial nerve, anterior adductor nerve and a small labial nerve. Besides these 3 nerves, each cerebral ganglion gives off a cerebro-pedal connective and a cerebro-visceral connective.

The two pedal ganglia form a small cylindrical mass lying close to the mouth, a little below the antero-dorsal surface of the visceral mass. From the ventral surface of the pedal ganglia small nerves are given off into the foot.

The two visceral ganglia are enclosed in a fibrous capsule. Immediately in front of the visceral ganglia, the two cerebro-visceral connectives are joined by a transverse connective and this is slightly swollen centrally to form an accessory visceral ganglion. From each visceral ganglion arises the ctenidial nerve, renal nerve, posterior adductor nerve, posterior retractor nerve and a large nerve to the siphons.

Types of Neurosecretory Cells.—The distribution of the neurosecretory cells in the cerebral and visceral ganglia is shown in Text fig. 1a and b. Two types of neurosecretory cells, differing in size and staining ability with chromalum-hematoxylin-phloxin and Mallory's triple stains, were seen in the various ganglia (Table 1). These two types of neurons are designated as Cell Type I and Cell Type II.

Cell Type I.—The cells are somewhat pyriform in shape ; the cell bodies range from 18 to 22 μ in length and 12 to 15 μ in width. The nucleus is round or oval measuring 8 to 10 μ wide ; it may be either central or eccentric in position (Text-fig. 1c and d). The nucleus generally contains one large nucleolus but in certain instances two or three nucleolus-like bodies occur inside the nucleus. The secretory material stains red with Mallory's and blue-black with CHP. The granules always appear as very fine particles. Different Type I cells in the same animal are not always filled to the same extent with neurosecretory granules. For example, in several cerebral or visceral ganglia of some pholads, Type I cells which were packed with neurosecretory granules were found adjacent to cells which contained only a small number of granules. It is not improbable that this appearance is correlated with different phases of secretory activity. Vacuoles are generally absent. In certain cells the secretory material could be seen in the axons. This cell type is represented in all the ganglia.

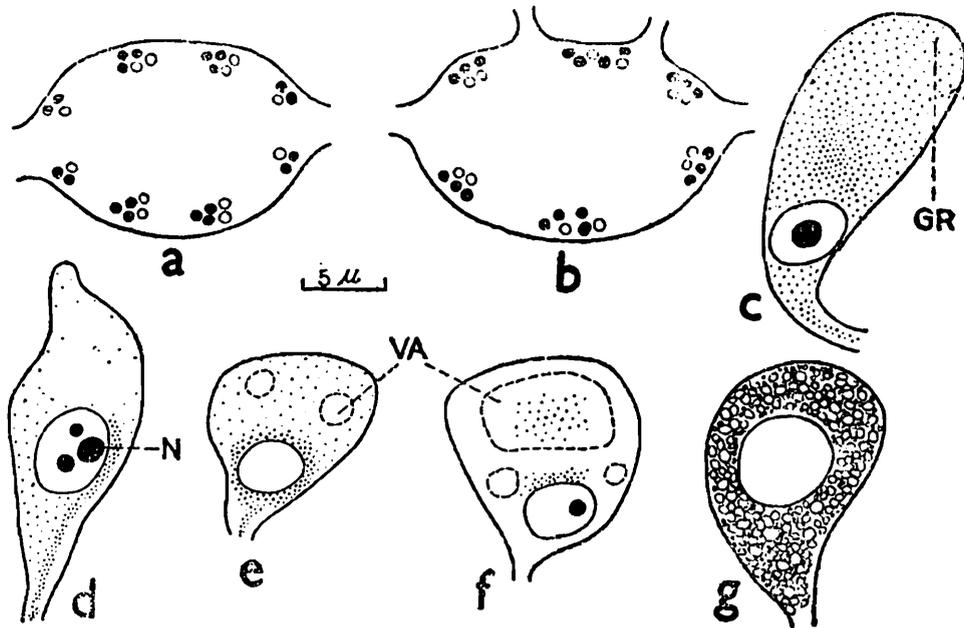
TABLE I.—*Characteristics of cells present in the central nervous system of Diplothyra smithii*

Description	Type I	Type II
Shape of the cell body	Pyriform	Oval
Size of the cell body	18—12 μ	12—18 μ
<i>Staining reaction :</i>		
Mallory's stain	Red	Pinkish
Gomori's technique	Blue-Black	Gray
Vacuoles	Absent	Abundant

Cell Type II.—These cells are smaller than the Type I Cells and restricted to the cerebral and visceral ganglia. The cell body is somewhat oval in shape, measuring 12-18 μ in diameter. Their nuclei are similar to those of Type I Cells and the fine granules in the cytoplasm stain pinkish with Mallory's and gray with CHP. In certain cells the neurosecretory granules are particularly concentrated around the nucleus. The vacuolisation of these cells is very striking ; the vacuoles do not possess a characteristic shape (Text-fig. 1e and f). Occasionally very fine particles are observed in the vacuoles. Just as in Type I Cells the secretory material leaves the perikaryon by way of the axons.

Observations on the living Type I Neurosecretory Cells.—Under the phase contrast microscope, the granules appeared as dark masses filling the cytoplasm. The nucleus is transparent with low refractive index. The nuclear membrane is distinctly visible. Towards the periphery

of the cell, the granules showed Brownian movements. Inside the cytoplasm small spheroids of different sizes are visible besides the granules (Text-fig. 1g).



TEXT-FIG. 1.—Cerebral and visceral ganglion of *Diplothyra smithii* Tryon, showing the distribution and location of neurosecretory cells.

(a) Cerebral ganglion, showing the distribution of the neurosecretory cells. (b) Visceral ganglion, showing the location of the neurosecretory cells. (c) and (d) Type I neurosecretory cells in the cerebral ganglion. (e) and (f) Type II neurosecretory cells in the visceral ganglion. (g) Living Type I neurosecretory cells showing the spheroids.

NOTE.—Dots represent Type I cells

Circles represent Type II cells

GR granules ; N., Nucleolus ; VA., Vacuole.

Cytochemistry of Type I Neurosecretory cells.—Type I neurosecretory material was poorly preserved by alcoholic fixatives. This loss of granules was appreciably prevented by post-fixing in 10 per cent formalin after initial fixation in 80 per cent ethanol prior to paraffin embedding. This loss of secretory material appears to be due to the solvent action of alcohol. Careful examination of Helly's fixed adjacent paraffin sections of the ganglia, one series stained with Mallory's and the other with Sudan Black B, revealed that the Type I neurosecretory material is strongly sudanophilic. The secretory material gave positive test with PAS after digestion with diastase. In sections fixed in Bouin's fluid and stained with gallocyanine-chromalum the cytoplasm of the neurosecretory cells showed blue colouration indicating a concentration of RNA.

IV—DISCUSSION

Among the molluscs several observations have been made concerning the neurosecretory cells of gastropods, but rather few about the central nervous system of other groups. In gastropods, the presence of neuro-

secretory cells in different ganglia was reported by Gabe (1953a, b). Lever (1957) distinguished 5 cell types in the pulmonate, *Ferrissia* sp. while Krause (1960) observed only 2 types of cells in *Helix*. Among the lamellibranchs, Gabe (1955) observed only one cell type in the cerebral and visceral ganglia of 20 species. Lubet (1955) also described one cell type in the cerebral and visceral ganglia of *Mytilus* and *Chlamys*. Both these investigators failed to notice any secretory neurons in the pedal ganglia. Later, Fahrmann (1961) in *Unio* and Nagabhushanam (1962a, b, c) in *Crassostrea*, *Spisula* and *Bankia* reported two types of neurosecretory cells present in all the ganglia.

In the present investigation, two neurosecretory cell types were found in the central nervous system of *Diplothyra smithii* and they were designated as Type I and Type II Cells. The most exact resemblance is between the cells which are here described as Type I and those designated as grana II by Fahrmann (1961) in *Unio*, the pyriform-shaped cells of *Teredo* (Gabe and Rancurel, 1958) and to the Type I Cells in *Crassostrea* and *Bankia* (Nagabhushanam, 1962a, c). Grana I described by Fahrmann (1961) in *Unio* and Cell Type II of *Crassostrea*, *Spisula* and *Bankia* (Nagabhushanam, 1962a, b, c) agree very closely with Type II Cells of *Diplothyra*.

Concerning the distribution of the neurosecretory cells, Type I cells are found in all the ganglia while Cell Type II is observed only in the cerebral and visceral ganglia. Similar observations were made by Nagabhushanam (1962a, b, c) in *Crassostrea*, *Spisula* and *Bankia*. From a study of the histological sections, various authors (Scharrer, 1965, Lever, 1957; Nagabhushanam, 1962a, b, c) concluded that the neurosecretory material is transported along the axons. The observations in *Diplothyra* support this view, the secretory material being traced along the axons.

Cytochemical observations on the Type I Cells reveal that the cytoplasm of the neurosecretory material contains a high concentration of RNA and the positive tests for PAS and lipid seem to be due to the presence of glycolipid. This is in agreement with the observations of Nagabhushanam (1962a) in *Crassostrea*. Fahrmann (1961) also obtained a strong positive test for lipids in the neurosecretory material of *Unio*.

V—SUMMARY

1. A detailed morphological description of the neurosecretory cells in the pholad, *Diplothyra smithii*, is given. The general anatomy of its central nervous system conforms to that of *Martesia striata*.

2. On the basis of size and staining properties, two types of neurosecretory cells are distinguished which are characterized as follows: *Type I*: pyriform-shaped cells, measuring 18 to 22 μ in length and 12 to 15 μ in width; the secretory material stains red with Mallory's stain and blue-black with Gomori's technique. *Type II*: oval cell, measuring 12 to 18 μ in diameter and the granules stain pinkish with Mallory's and gray with Gomori's stain; vacuoles are abundant.

3. The secretory material in both the cell types is transported through the axons.

4. The cytoplasm of the Type I neurosecretory cells contains a high concentration of RNA. A positive test for PAS and lipid seems to indicate that the secretory material probably contains glycolipid.

VI—REFERENCES

- FÄHRMANN, W. 1961. Licht- und electronenmikroskopische untersuchungen des nervensystems von *Unio tumidus* (Philipsson) unter besonderer berücksichtigung der neurosekretion.—*Z. Zellforsch.*, Berlin, **54**, pp. 689-716.
- GABE M. 1953a. Particularites morphologiques des cellules neurosécrétrices chez quelques Prosobranches monotocardes.—*C. R. Acad. Sci.*, Paris, **236**, pp. 323-325.
- GABE, M. 1953b. Particularités histologiques des cellules neurosécrétrices chez quelques Gasteropodes opisthobranches.—*Ibid.*, **236**, pp. 2166-2168.
- GABE, M. 1955. Particularites histologiques des cellules neurosécrétrices chez quelques Lamellibranches.—*Ibid.*, **240**, pp. 1810-1812.
- GABE, M. & RANCUREL, P. 1958, Caracteres histologiques des cellules neurosécrétrices chez quelques *Teredo*.—*Bull. Inst. franc, Afr. noire*, Paris., **20**, pp. 73-79.
- GOMORI, G. 1941. Ovservations with differential strains on human islets of Langerhans.—*Amer. J. Path.*, Boston, **17**, pp. 395-406.
- KRAUSE, E. 1960. Untersuchungen über die neurosekretion im schlundring von *Helix pomatia* L.—*Z. Zellforsch.*, Berlin, **51**, pp. 748-776.
- LEVER, J. 1957. Some remarks on neurosecretory phenomena in *Ferrissia* sp. (Gastropoda, Pulmonata).—*Proc. Kon. Ned. Ak. Wtensch. Ser. C*, **60**, pp. 510-522.
- LUBET, P. 1955. Cycle neurosécrétoire de *Chlamys varia* et *Mytilus edulis* (Mollusques, lamelibranches).—*C.R. Acad. Sci.*, Paris, **241**, pp. 119-121.
- LUBET, P. 1956. Effects de l'ablation des centres nerveux sur l'émission des gametes chez *Mytilus edulis* Let *Chlamys varia* L.—*Ann. Sci. nat. (Zool.)*, Paris, (II) **18**, pp. 175-183.
- NAGABHUSHANAM, R. 1962a. Neurosecretion in the oyster, *Crassostrea virginica*.—*Amer. Zool.*, **2** (3), p. 433.
- NAGABHUSHANAM, R. 1962b. The histology of the neurosecretory system of the clam, *Spisula solidissima*.—*Ibid.*, **2** (4), p. 543.
- NAGABHUSHANAM, R. 1962c. On the neurosecretory system in the wood boring mollusc, *Bankia (Bankiella) gouldi*.—*Proc. 2nd All India Cong. Zool. (Varanasi)*, **17** (Abst).
- NAGABHUSHANAM, R. 1962d. The anatomy and histology of the wood boring mollusc, *Martesia striata*.—*Ibid.*, **18-19** (Abst).

- NAGABHUSHANAM, R. 1964. Note on the neurosecretory cells in the nervous system of the mussel, *Modiolus demissus*.—*Curr. Sci.*, Bangalore, **33** (1), pp. 20-21.
- NAGABHUSHANAM, R. 1965. Neurosecretory cell types in the central nervous system of the lamellibranch mollusc, *Rangia cuneata*.—*Sci. & Cult.*, Calcutta, **31** (3), pp. 156-157.
- PEARSE, A. G. E. 1960. Histochemistry, theoretical and applied.—London (J.A. Churchill Ltd.).
- SCHARRER, B. 1965. Über das Hanstromsche Organ X bei Opisthobranchiern.—*Pubbl. Staz. Zool. Napoli, Napoli*, **15**, pp. 132-142.