Bull. Fac. Fish. Hokkaido Univ.
23 (3), 135-143. 1972.

Demonstration of Possible Neurosecretory Cells in the Nervous System of Neptune Whelk, *Neptunea arthritica*

Takehiro YAHATA* and Hiroya TAKAHASHI*

Abstract

The central nervous system of neptune whelk, *Neptunea arthritica*, was studied histologically. The nervous system composed of paired cerebral, pleural and pedal ganglia together with a single supra- and sub-oesophageal ganglion forms a compact circumoesophageal ring which is characterized by being deformed to lie on its left side with slight distortion. In the ganglia, four types of cells, termed as types A, B (subtypes B-I and B-II), C and D cells, are distinguished histologically. Among those, the types B-I, C and D cells show a wide distribution over all the ganglia observed, whereas the type B-II cells are not found in the pedal ganglia and the type A cells are noticed rather restrictedly in the cerebral and pedal ganglia.

With the exception of the type B-II cells which are evidently acidophilic in stainability, the cytoplasmic granular materials of the ganglion cells are stained positively with AF. The perineurium and the medulla of the ganglia, in which the axons and their endings of ganglion cells are distributed, also accumulate the stainable materials. The AF-positive cells excluding some of the type D cells show a seasonal change in their stainability with a maximum intensity attained by February, which is accompanied with similar changes in amount of the AF-positive materials present in the perineurium and the ganglional medulla. These phenomena are considered as suggesting a possible occurrence of neurosecretion in the central ganglia of the neptune whelk.

Since B. Scharrer[1] reported in 1935 the occurrence of neurosecretory cells in the opisthobranchs *Aplysia limacina* and *Pleurobranchaea meckeli*, a morphological demonstration of possible neurosecretory phenomena has been done in a variety of gastropod molluscs. Much interest of these work has so far been centered in the neurosecretion found in pulmonates (refer to Bullock[2]) and opisthobranchs[3][4]. Comparatively little is known about the neurosecretion in prosobranchs in which *Bithynia tentaculata*[5][6] and *Nordotis discus*[7] have recently proved to display possible neurosecretory activities in their central ganglia.

In the present paper, the writers report the results of histological observations on the central nervous system of neptune whelk, *Neptunea arthritica*, with a note on seasonal changes in the histological characteristics of possible neurosecretory cells found in the central ganglia.

Before going further, the writers wish to express their sincere thanks to

* *Laboratory of Fresh-Water Fish-Culture, Faculty of Fisheries, Hokkaido University*
(北海道大学水産学部淡水増殖学講座)

Professor Kiichiro Yamamoto, Hokkaido University, for his invaluable criticism and encouragement throughout this study. Thanks are also due to Dr. Ryogo Yuki, Hokkaido Marine Cultivation Center, for his helpful suggestion and to the Kamiiso Fisheries Cooperation for friendly facilities in collecting the material used in the present study.

Material and methods

Neptune whelk, *Neptunea arthritica*, employed in the present study were collected in the coast of Kamiiso in the suburbs of Hakodate. Monthly samplings of the whelk of 7–12 cm in shell length, more than 8 each month, were performed during the period from May 1971 to April 1972.

They were carried to the laboratory and sacrificed as soon as possible. Their central ganglia were dissected out and mainly fixed with Zenker-formol solution or Bouin's fluid. Serial paraffin sections were cut at 10 μ in thickness and were alternately distributed into three sets of histological slides. One of these sets was stained with Gabe's aldehyde-fuchsin (AF) with counterstains, and the other two were treated each with any one of the other staining procedures such as Gomori's chrome hematoxylin (CH)-phloxine, Nissl staining with thionine and PAS reaction.

Results

The central nervous system of *N. arthritica* forms a compact circumoesophageal ring which is somewhat distorted and is leaning toward the left side of the body (Fig. 1). The ring is composed of paired cerebral, pleural and pedal ganglia which adjoin very closely to one another. Two other single ganglia, which are presumed to be a supraoesophageal ganglion and a suboesophageal one on referring to the descriptions made by Bullock[2] and Andrews[5], are also present nearby the ring, connecting with the pleural ganglia. The central ganglion system is seen to lie on its left side, that is, the right cerebral and pleural ganglia with the supraoesophageal ganglion are dorsal to the oesophagus and aorta, the left cerebral and pleural ganglia are situated ventrally, and the two pedal and the suboesophageal ganglion are on the right side of the body.

The ganglia are covered by a perineurium which is loaded with AF- or phloxine-positive materials. Moreover, they are enveloped with networks of a connective tissue containing gelatinous substance which is strongly PAS positive (Figs. 2 and 3). The nerve cell bodies are mostly located in the cortical portion of the ganglia, and almost all of them seem to be of an unipolar type, sending their axons inwards. In the medullary portion of ganglia where a neuropile structure is composed, a various amount of stainable material is detectable enclosing irregular

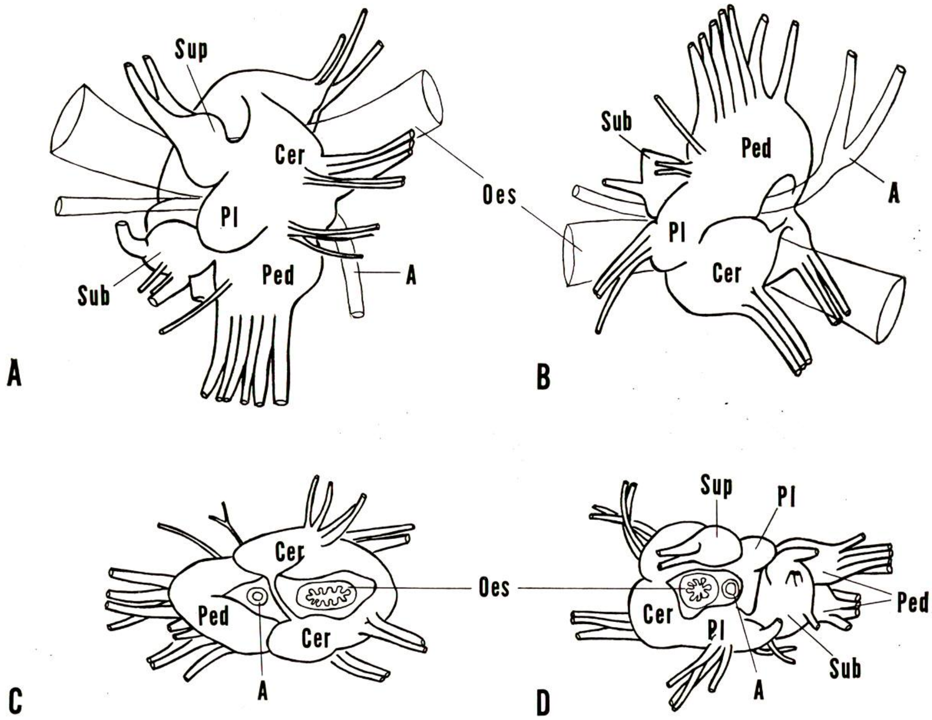
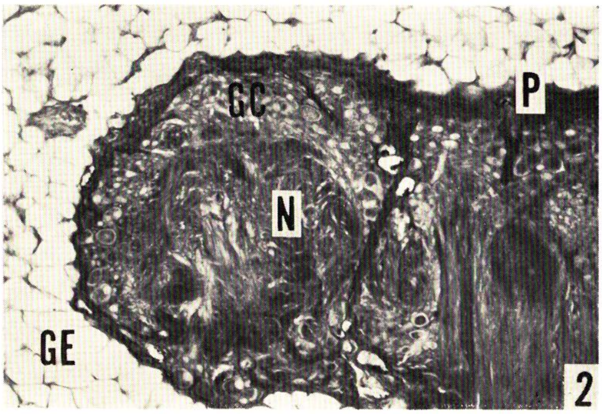


Fig. 1. Diagrammatic drawings of the central nervous ring of neptune whelk in dorsal (*A*), ventral (*B*), anterior (*C*) and posterior (*D*) views; the anterior side of the nervous ring to the right side in *A* and *B*, the dorsal side of the nervous ring to the upside in *C* and *D*. *A*, aorta; *Cer*, cerebral ganglion; *Oes*, oesophagus; *Ped*, pedal ganglion; *Pl*, pleural ganglion; *Sub*, suboesophageal ganglion; *Sup*, supraoesophageal ganglion.



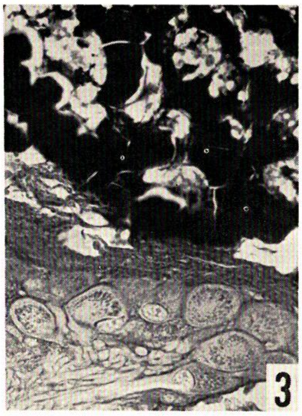


Fig. 2. Frontal section through the ganglia surrounded by the perineurium (*P*) and connective tissue with gelatinous substance (*GE*), showing the cortical arrangement of ganglion cells (*GC*) and the medullary neuropilar zone (*N*). Zenker-formol, AF. × 120.

Fig. 3. Densely stained, PAS positive gelatinous substance in the connective tissue abutting on the perineurium. Zenker-formol, PAS. × 200.

branches of the connective tissue with blood lacunae invaded from the perineurium (Fig. 2).

The nerve cells of the ganglia may be classified into four types on the basis of the differences in their size and cytological characteristics. In the following description, they will be termed each as types A, B, C and D.

Type A cells

The ganglion cells of this type are situated in the postero-lateral region of the cerebral ganglia and in the inner lateral region of the pedal ganglia. A few of them are encountered also in the suboesophageal ganglion. The cell body is roundish pyriform in shape, measuring more than 35 μ in width, and is provided with a clear nucleus near the axon hillock. The nucleus is spherical to oval in shape, with a diameter approximately 20 μ in length, and contains one or a few distinct nucleoli. In the cytoplasm, a large amount of AF-positive granules are accumulated in the perinuclear zone (Fig. 4). On some occasions, the cells display a volute aspect in the cytoplasm especially when they have scanty stainable granules (Fig. 5).

Type B cells

The nerve cells of this category have round or pear-shaped contour of 17–28 μ in width. The nucleus with a prominent nucleolus is round or oval, about 15 μ in size, and lies in the eccentric region of the cytoplasm as in the type A cells. The type B cells can be divided into subtypes B-I and B-II according to their affinity to the histological dyes employed. The B-I cells are charged in their cytoplasm with materials which are stainable with AF (Fig. 6, B-I). They reveal a preponderant distribution in all the ganglia exmained in this study. On the other hand, the B-II cells are conspicuous for their acidophilic products packing the cytoplasm (Fig. 6, B-II). They occur mainly in the pleural ganglia and, though fewer in number, in the cerebral, supra- and sub-oesophageal ones.

Type C cells

The cells of this type predominate in the posterior region of the cerebral ganglion, although they are distributed rather widely over all the central ganglia. The cells are of round or oval shape, 13–17 μ in size, and are provided with larger nuclei in proportion to cellular size. Moreover, prominent nucleoli are hardly observable in these cells. Granular materials occurring in the cytoplasm of these cells are definitely positive to AF (Fig. 7).

Type D cells

The cells belonging to this category are slender in contour, being about 17 μ in length and 8 μ in width. They are present in all the central ganglia observed.

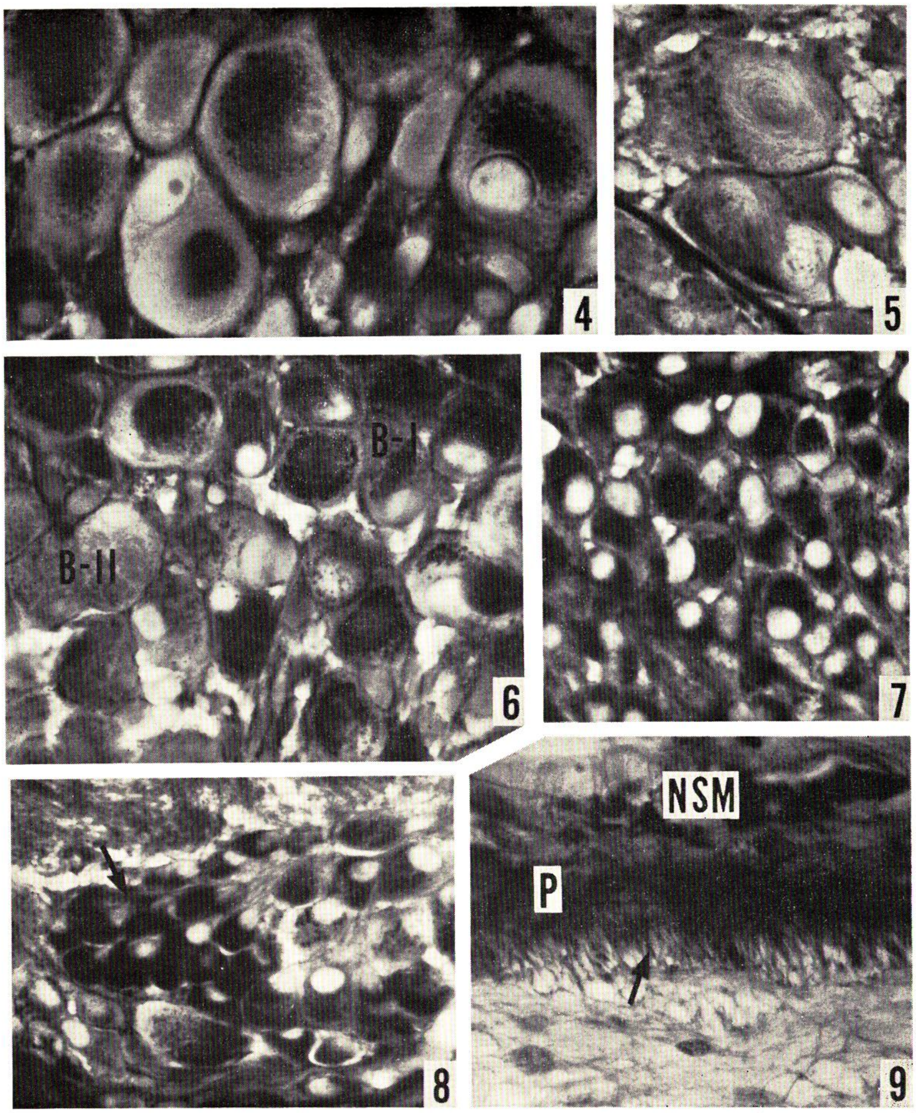


Fig. 4. Type A cells with numerous AF-positive granules in the cytoplasm, in a specimen collected in February. Zenker-formol, AF. × 480.

Fig. 5. Type A cells showing volute structure in a specimen examined in April. AF-positive secretions are hardly present in the cytoplasm. Zenker-formol, AF. × 480.

Fig. 6. AF-positive, type B-I cells (B-I) and acidophilic type B-II cells (B-II) in a specimen collected in February. Zenker-formol, AF. × 480.

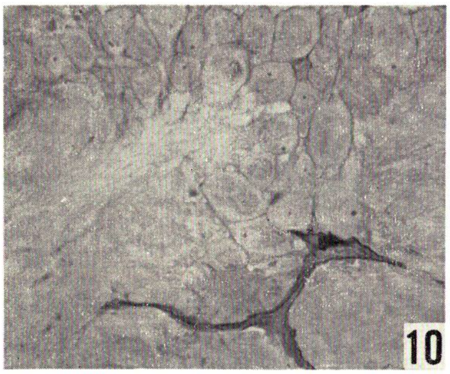
Fig. 7. Type C cells in a specimen examined in February. Zenker-formol, AF. × 480.

Fig. 8. Type D cells (arrow), clustering in the posterior region of cerebral ganglion, in a specimen sampled in February. The cells on the right side of the picture are the type C cells. Zenker-formol, AF. × 480.

Fig. 9. Axon endings (arrow) terminating in the perineurium (*P*) in which neurosecretory materials (*NSM*) are accumulated, in a specimen collected in February. Zenker-formol, AF. × 480.

However, the cells located in groups in the posterior region of the cerebral ganglia are stained intensely with AF (Fig. 8), while the others situated dispersedly just underneath the perineurium of ganglia or in the neighbourhood of the ganglional medulla appear to show more weakened reaction to AF than the former.

The AF-positive cells in the central ganglia, with the exception of the type D cells existing in the posterior region of the cerebral ganglia, exhibit a seasonal periodicity in their staining affinity to AF. The perikarya and axons of these cells


10

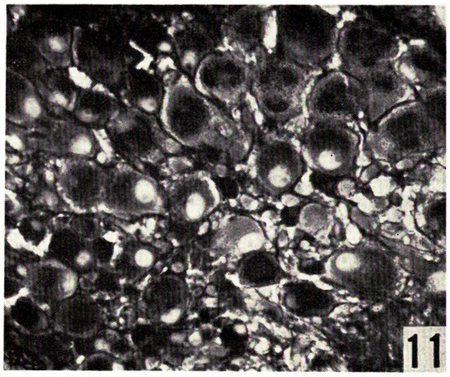

11

Fig. 10. Ganglion cells of the neptune whelk collected in August, revealing weakened affinity of the cells to AF. Zenker-formol, AF. × 200.

Fig. 11. Ganglion cells with prominent secretory materials in the animal sampled in February. Zenker-formol, AF. × 200.

show only a slight or no affinity to AF in August (Fig. 10), whereas they become strongly stained with AF about the beginning of autumn. Henceforth, the intensity of the cells has increased until it attains a maximum by February. At that time all the cells are occupied by a huge amount of AF-positive granules in their cytoplasm (Fig. 11). Thereafter the staining response of these cells weakens in degree, and in April relatively large granules, which are presumed to be lipofuscin in nature, are merely found dispersedly in the cytoplasm. During the successive months, from May to August, the cells appear to retain the condition with scanty AF-stainable materials in the cytoplasm.

As mentioned before, an accumulation of AF-positive materials is noticeable in the perineurium and the medulla of ganglia where the axons and their endings originating from the ganglion cells are terminated (Fig. 9). The materials in these regions also reveal a seasonal change in the degree of staining intensity to AF in close association with the change in the perikarya of ganglion cells. This may imply that the two regions might play the roles of a neurohemal organ in depositing the possible neurosecretory materials and discharging them into the blood lacunae distributed in these regions.

On the other hand, the acidophilic material in the type B-II cells, and that in the perineurium as well, do not display a seasonal periodicity, remaining stained heavily with acidic dyes throughout the seasons. It is remarked, furthermore, that all the above mentioned AF-positive cells exhibit no prominent staining affinity to CH, their cytoplasm being more or less stained grey by the dye.

Discussion

It is generally accepted that, in comparison with the nervous system of higher gastropods, that of prosobranchs is rather simple in anatomical constitution[2]. In terms of structural aspects, however, the central ganglion system in *Neptunea arthritica* assumes much more complexity than that of other prosobranchs such as *Nordotis discus*[7]. The circumoesophageal nerve ring of the neptune whelk is characterized by being deformed to lie on its left side with slight distortion. The features of the nerve ring in the neptune whelk seem to be rather extraordinary, so far as the writers know.

The present study demonstrates that the four types of nerve cells distinguished in the central ganglia of the neptune whelk are provided with AF-positive materials in the cytoplasm, with the exception of the type B-II cells of which the cytoplasmic material is clearly of acidophilic nature. Moreover, it is evidenced that these AF-positive cells show a seasonal change in their staining affinity, though only the type D cells clustering in the posterior region of the cerebral ganglia scarcely display such a prominent change. The AF-positive materials which have been hardly observed in the perikarya and axons of the ganglion cells during spring and summer become remarkably accumulated in late autumn and winter irrespective of sexes, reaching the maximum in amount by February. According to N. Takahashi et al.[8], *N. arthritica*, a gonochoristic species, shows a high gametogenetic activity in winter, executing copulation and spawning by April-June in southern Hokkaido. This may imply that at least some of the AF-positive ganglion cells secrete a certain material or materials which may have a concern with a certain process of reproduction of this species. Similar observations were previously done in *Oncidium verraculatum*[9], *Bithynia tentaculata*[5] and *Nordotis discus*[7], in which remarkable neurosecretory activities were shown by the nerve cells of the cerebral ganglia during the breeding season.

On the other hand, the type D cells existing in the posterior region of the cerebral ganglia preserve remarkable stainability to AF throughout the seasons. As will be reported in the succeeding paper[10], electron microscopy can demonstrate that the cells in question are packed with electron-dense granules of 1100 Å in size which are of Golgi bodies in origin. These granules are quite similar in features to the neurosecretory granules observed so far in various species of molluscs.

Being in sharp contrast to the others, the type B-II cells contain a large amount of acidophilic granular material in the cytoplasm. Axons of the type B-II cells as well as those of the other basophilic cells appear to terminate in the perineurium and ganglional medulla in which the axons may release the neurosecretory materials into blood lacunae. An electron microscopic study on the ganglia of the neptune whelk has revealed that the axon endings with crowded neurosecretory granules are encountered in the connective tissue space of the perineurium[10]. The perineurium serving as a so-called neurohemal organ has been known to exist in the central nervous system of *Lymnaea stagnalis*[11], *Helisoma tenue*[12] and *Nordotis discus*[7]. Furthermore, it has been shown that, in *Aplysia californica*, the perineurium of the parieto-visceral ganglion obviously includes the principle which is capable of inducing egg-laying[13].

It seems to be rather common in invertebrate animals that some of the neurosecretions are acidophilic[1][5][6][7][11][14][15][16]. There is a marked similarity in the staining property of intracytoplasmic material between the type B-II cells of *Neptunea* in the present study, the "Gomori-negative", CDC cells of *Lymnaea stagnalis*[11] and the S-1 cells of *Bithynia tentaculata*[6]. According to Andrews[5], a phloxinophilic neurosecretory product may stimulate copulation or oviposition and the AF-positive materials may control gonad maturation, osmoregulation, or other physiological functions. The type B-II cells in the present study differ from the S-1 cells of *Bithynia* in that the former are commonly present in the central ganglia except the pedal ones while the latter locate only in the cerebral ganglion. It has been evidenced, however, that the type B-II cells are provided with fine structural features which are characteristic of neurosecretory cells so far examined[10].

The results of the present observation prove the evidence of the occurrence of more than two different kinds of neurosecretory cells in the central ganglia of the neptune whelk. Further studies including an experimental analysis of the neurosecretory phenomena are to be done to substantiate the physiological significance of neurosecretion in this species.

References

1) Scharrer, B. (1935). Über das Hanströmsche Organ X bei Opisthobranchiern. *Pubbl. Statz. Zool. Napoli* **15**, 132–142.
2) Bullock, T.H. (1965). Mollusca: Gastropoda. p. 1283–1386. In Bullock, T.H. and Horridge, G.A. (eds.), *Structure and function in the nervous systems of invertebrates.* 1719 p. W.H. Freeman and Company, San Francisco & London.
3) Vicente, N. (1969). Corrélations neuroendocrines chez *Aplysia rosea* ayant subi l'ablation de divers ganglions nerveux. *Tethys* **1**, 875–900.
4) Nagabhushanam, R. and Kulkarni, A.B. (1971). Neurosecretion in the slug, *Laevicaulis alte. Proc. Indian Acad. Sci., Sect. B.* **73**, 290–302.
5) Andrews, E. (1968). An anatomical and histological study of the nervous system of

Bithynia tentaculata (Prosobranchia), with special reference to possible neurosecretory activity. *Proc. malac. Soc. Lond.* **38**, 213–232.
6) Andrews, E. (1971). The fine structure of the nervous system of *Bithynia tentaculata* (Prosobranchia) in relation to possible neurosecretory activity. *Veliger* **14**, 13–22.
7) Yahata, T. (1971). Demonstration of neurosecretory cells in the cerebral ganglion of the abalone, *Nordotis discus* Reeve. *Bull. Fac. Fish. Hokkaido Univ.* **22**, 207–214.
8) Takahashi, N., Takano, K. and Murai, S. (1972). Histological studies on the reproductive cycle of the male neptune whelk, *Neptunea arthritica*. *Ibid.* **23**, 65–72. (In Japanese with English abstract).
9) Menon, K.R. (1966). Observations on neurosecretory activity during reproductive period in *Oncidium verraculatum* Cuv. (Mollusca-Gastropoda-Pulmonata). *Gen. Comp. Endocrinol.* **7**, 186–190.
10) Yahata, T. (1972). Fine structure of ganglion cells in the central nervous system of neptune whelk (*Neptunea arthritica*). *Bull. Fac. Fish. Hokkaido Univ.* **23**, 144–149.
11) Boer, H.H., Douma, E. and Koksma, J.M.A. (1968). Electron microscope study of neurosecretory cells and neurohemal organs in the pond snail *Lymnaea stagnalis*. *Symp. zool. Soc. Lond.* **22**, 237–256.
12) Simpson, L., Bern, H.A. and Nishioka, R. (1966). Examination of the evidence for neurosecretion in the nervous system of *Helisoma tenue* (Gastropoda Pulmonata). *Gen. Comp. Endocrinol.* **7**, 525–548.
13) Toevs, L.A. and Brackenbury, R.W. (1969). Bag cell-specific proteins and the humoral control of egg laying in *Aplysia californica*. *Comp. Biochem. Physiol.* **29**, 207–216.
14) Boer, H.H. (1965). A cytological and cytochemical study of neurosecretory cells in Basommatophora, with particular reference to *Lymnaea stagnalis* L. *Arch. Néerl. Zool.* **16**, 313–386.
15) Hagadorn, I.R. (1966). The histochemistry of the neurosecretory system in *Hirudo medicinalis*. *Gen. Comp. Endocrinol.* **6**, 288–294.
16) Bianchi, S. (1969). On the neurosecretory system of *Cerebratulus marginatus* (Heteronemertini). *Ibid.* **12**, 541–548.