The Ultrastructure of Neurons and Neuroglia in the Cerebral and Pleuro-Pedal Ganglia of *Haliotis asinina* Linnaeus

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ABSTRACT The ultrastructure of neurons and neuroglia in the cerebral and pleuro-pedal ganglia of *Haliotis asinina* are described. There are four types of neurons (NR_{1-4}) and three types of neuroglia. (NG_{1-3}). The NR_1, which is the largest nerve cell, has a round nucleus with a thin rim of heterochromatin attached to the nuclear envelope. The cytoplasm contains numerous rough endoplasmic reticulum, mitochondria, but only a few small elementary granules. The nuclei of NR_2 , NR_3 and NR_4 have increasingly condensed heterochromatin. The cytoplasm of these cells contain relatively few organelles such as rough endoplasmic reticulum, mitochondria and elementary granules. The NG_1 is spindle-shaped with little perinuclear cytoplasm. The NG_2 and NG_3 are highly ellipsoid in shape. The cytoplasm of NG_1 and NG_3 is very thin and contains ribosomes, a few rough endoplasmic reticulum and mitochondria. The cytoplasm of NG_2 is extremely thin and contains only ribosomes. There are no elementary granules in the neuroglia.

KEYWORDS: *Haliotis asinina*, neuron, neuroglia, ultrastructure.

INTRODUCTION

Bullock and Horridge[1] classified the neurons in the ganglia of gastropods on the basis of morphology (size and nuclear - cytoplasmic ratio of the cell body and perikaryon). In the pulmonate snails such as *Achatina fulica* Bowdich, *Helix pomatia* Linnaeus, *Arion ater* Linnaeus and *Limax maximus* Linnaeus, the classification of neurons is based on cell size.[2-4] They are giant neurons, ordinary neurons and globuli cells. The giant neurons are characterized by their large size and irregularly-shaped nucleus.[4] The nuclei are often flattened or lobated.[5] The nucleoli are usually large and annular.[6] The surface of the giant neuron is often deeply indented by processes of glial cells.[7-8] The ordinary neurons are divided into large, medium and small cells.[1-4] They are similar to the giant neurons in having large clear nuclei with one or several nucleoli, abundant cytoplasm and thick processes.[1] The ultrastructure of ordinary neurons has been described in many gastropod species such as *Aplysia californica* Cooper,[7] *Lymnaea stagnalis* Lamarck[8]. *Archachatina marginata* Lamarck,[9] *Helix aspersa* Müller,[10] *A. fulica,*[11] *Bithynia tentaculata* (Linnaeus)[12] and *Haliotis rufescens* Swainson.[13] The cytoplasm contains a large number of small mitochondria, innumerable cisternae of rough endoplasmic reticulum, free ribosomes, polyribosomes, lysosomes, numerous Golgi complexes and elementary granules. The free ribosomes and cisternae of the rough endoplasmic reticulum are especially concentrated near the nucleus and in the region of the axon hillock.[1] The globuli cells contain chromatin-rich nuclei. The cytoplasm is scant and contains organelles similar to those found in the ordinary neurons.[11]

Glial cells are distributed between the neurons and their sheath cells and along the outer surfaces of blood vessels.[7,14,15] The nuclei are round or oval. There are two types of glial cells in *L. stagnalis*.[8,14] The first type of neuroglia indents into the perikaryon and into the axon of the large neurons. Cell organelles are scarce in the cytoplasm of those glial cells except for mitochondria. Usually some glycogen is present. A second type of glial cells (filamentous glial cells) is characterized by a large number of thin filaments (5 nm) which are comparable to tonofilaments. Moreover, these cells contain abundant mitochondria, a rather extensive rough endoplasmic reticulum, numerous Golgi complexes, and lysosome-like structures.[8] The ultrastructure of glial cells of *L. stagnalis* is similar to those of *A. marginata*[9] and *A. californica.*[7]

From the previous reports on the ultrastructure of neurons and neuroglia in the ganglia of various species of gastropods, it is apparent that the knowledge of ultrastructure of neurons and neuroglia is still lacking in *Haliotis asinina* Linnaeus.

Therefore, the present study reports on the fine structure of neurons and neuroglia in the cerebral and pleuro-pedal ganglia of *H. asinina*.

Materials and Methods

The adult abalone *H. asinina*, with a shell length of 4-5 cm, were obtained from the Coastal Aquaculture Development Center, Klong Wan, Prachuap Khiri Khan Province, Thailand. Abalone were anesthesized with 5% MgCl, after which their shells were removed. The cerebral and pleuro-pedal ganglia were dissected out and fixed in a mixture of 4% glutaraldehyde and 2% paraformaldehyde in 0.1M Millonig buffer (pH 7.8) at 4°C for 24 hours. Specimens were washed six times with 0.1 M Millonig buffer. They were postifixed in 1% OsO_4 in 0.1 M Millonig buffer for 2 hours, then dehydrated through a graded series of ethanol. They were embedded in Spurr's resin. The sections were stained with uranyl acetate in 70% ethanol and lead citrate and examined with a Hitachi H-300 transmission electron microscope operating at 75 KV.

Results

From the histological observation, , there are four types of neurons (NR_{1-4}) and three types of neuroglia (NG_{1-3}) in the cerebral and pleuro-pedal ganglia[16,17].

Type 1 neuron (NR_1). The cell body is round or oval with very large size (14x28 µm). The nucelus is round (9 µm in diameter) with a thin rim of heterochromatin attached to the nuclear envelope. Most of the remaining chromatin is finely dispersed euchromatin that is scattered uniformly throughout the nucleus (Fig 1A). The cytoplasm contains numerous mitochondria and stacks of rough endoplasmic reticulum (Fig 1A). Only few small elementary granules are present.

Type 2 neuron (NR_2). The cell body is oval and about 4-6 µm in diameter. The nucleus also has an oval shape (4-6 µm in diameter), with thick patches of heterochromatin along the periphery and in the central area (Fig 1B). The cytoplasm is very thin and contains a few rough endoplasmic reticulum, mitochondria, polyribosomes and some elementary granules (Fig 1B).

Type 3 neuron (NR_3). The cell body and nuclear size of these cells are similar to those of NR_2. However, in the nucleus, the heterochromatin is increased greatly in comparison to NR_2 (Fig.1C). The cytoplasm is relatively thin comparing to the size of the nucleus, and contains sparing amount of rough endoplasmic reticulum and mitochondria, a few polyribosomes and small elementary granules.

Type 4 neuron (NR_4). These cells are round in shape and about 4-6 µm in diameter. The nucleus contains thin patches of heterochromatin along the nuclear embrane, while most of the nucleoplasm in the central area is clear (Fig 1D). The cytoplasm is thin and contains the usual organelles such as, rough endoplasmic reticulum, mitochondria, ribosomes, along with some elementary granules (Fig 1D). These cells are rarely observed in the cerebral ganglia, while they are more numerous in the pleuro-pedal ganglia.

Type 1 neuroglia (NG_1). The cell body is spindle-shaped with little perinuclear cytoplasm. The nucleus is also spindle-shaped with patches of heterochromatin attached to the periphery of the nuclear membrane, and few large blocks of heterochromatin occur in the central area (Fig 2A). The cytoplasm contains a few rough endoplasmic reticulum, mitochondria and ribosomes (Fig 2A). No elementary granules were observed. These neuroglia are intermingled with neurons in all layers of the cortex.

Type 2 neuroglia (NG_2). The cell body (3x8 µm in diameter) and nucleus (3x5 µm in diameter) are highly ellipsoid in shape. The nucleus contains a dense heterochromatin strip along the nuclear envelope, continuing into large blocks in the central region (Fig 2B). The cytoplasm is extremely thin and contains only ribosomes (Fig 2B). These neuroglia form a single sheet of continuous cell layer adjacent to the basement membrane, which is surrounded in turn by ganglionic connective tissue capsule.

Type 3 neuroglia (NG_3). The cell body and nuclear characteristic of these neuroglia are similar to those of NG_2, but they are smaller in size and the nuclear membrane is more indented (Fig 2C). The nucleus contains dense heterochromatin (Fig 2C). The cytoplasm is very thin and contains ribosomes, a few rough endoplasmic reticulum and mitochondria (Fig. 2C). These neuroglia are interspersed amongst the nerve tracts in the neuropil.

Discussion

The neurons in the cerebral and pleuro-pedal ganglia of *H. asinina* can be divided into 4 types (NR_{1-4}) based on the nuclear characteristic. NR_1 is the largest neuron and exhibit the ultrastructural features similar to typical motor neurons of vertebrates such as ventral horn motor cells of the spinal cord and

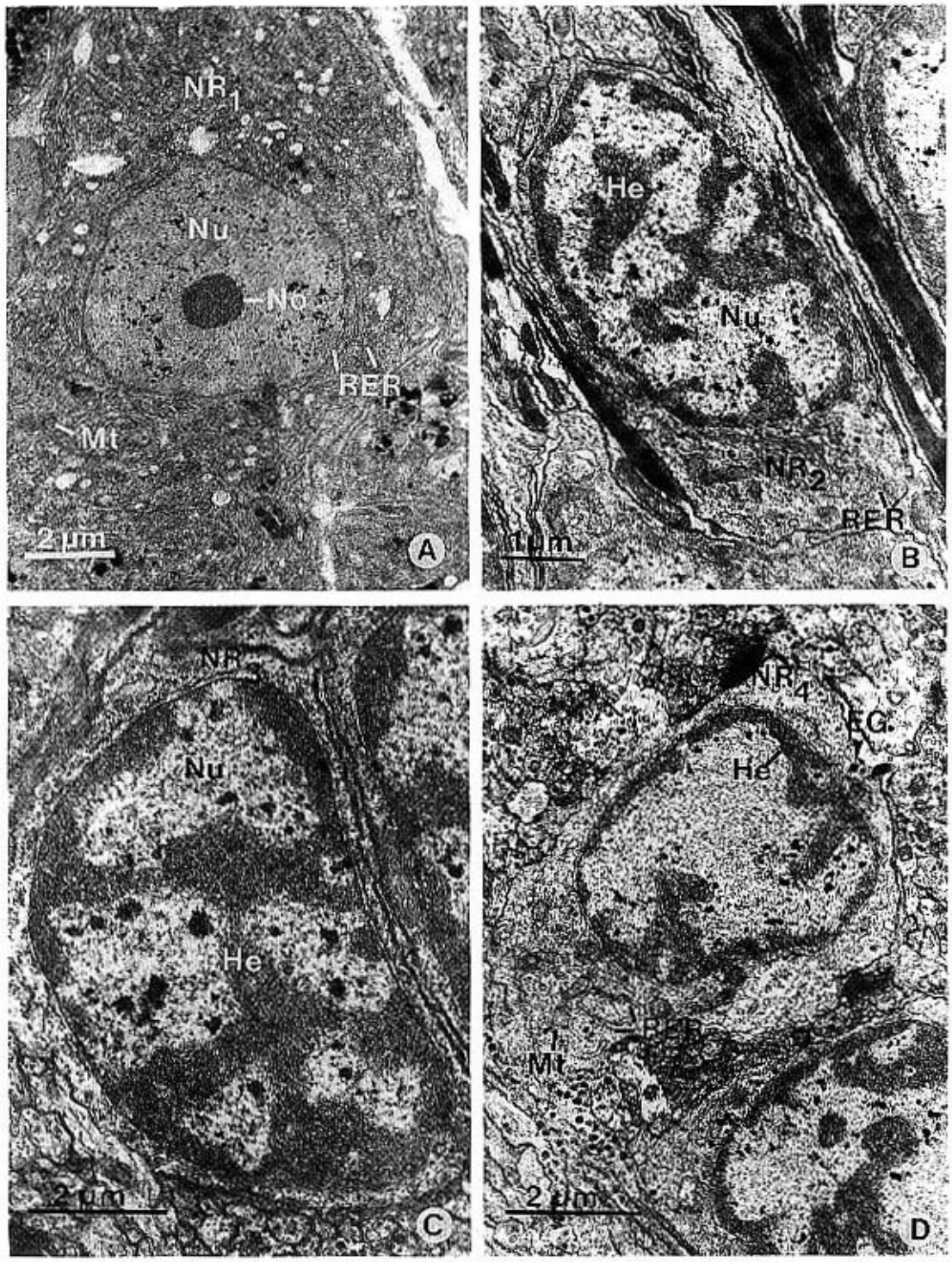

Fig 1. A. A low power micrograph of type 1 neuron (NR_1) in the cerebral ganglia showing a round nucleus (Nu) with a prominent nucleolus (No). The cytoplasm contains numerous mitochondria (Mt) and stacks of rough endoplasmic reticulum (RER).
B. A medium power micrograph of type 2 neuron (NR_2) in the cerebral ganglia showing an oval nucleus (Nu) with patches of heterochromatin (He) along the nuclear envelope and in the central area. C. A medium power micrograph of type 3 neuron (NR_3) in the cerebral ganglia showing an oval nucleus (Nu) with completely dense heterochromatin (He) and a thin rim of cytoplasm. D. A medium power micrograph of type 4 neuron (NR_4) in the pleuro-pedal ganglia showing a round nucleus (Nu) with thin patches of heterochromatin (He) along the nuclear membrane. The cytoplasm contains a few rough endoplasmic reticulum (RER), mitochondria (Mt) and elementary granules (EG).

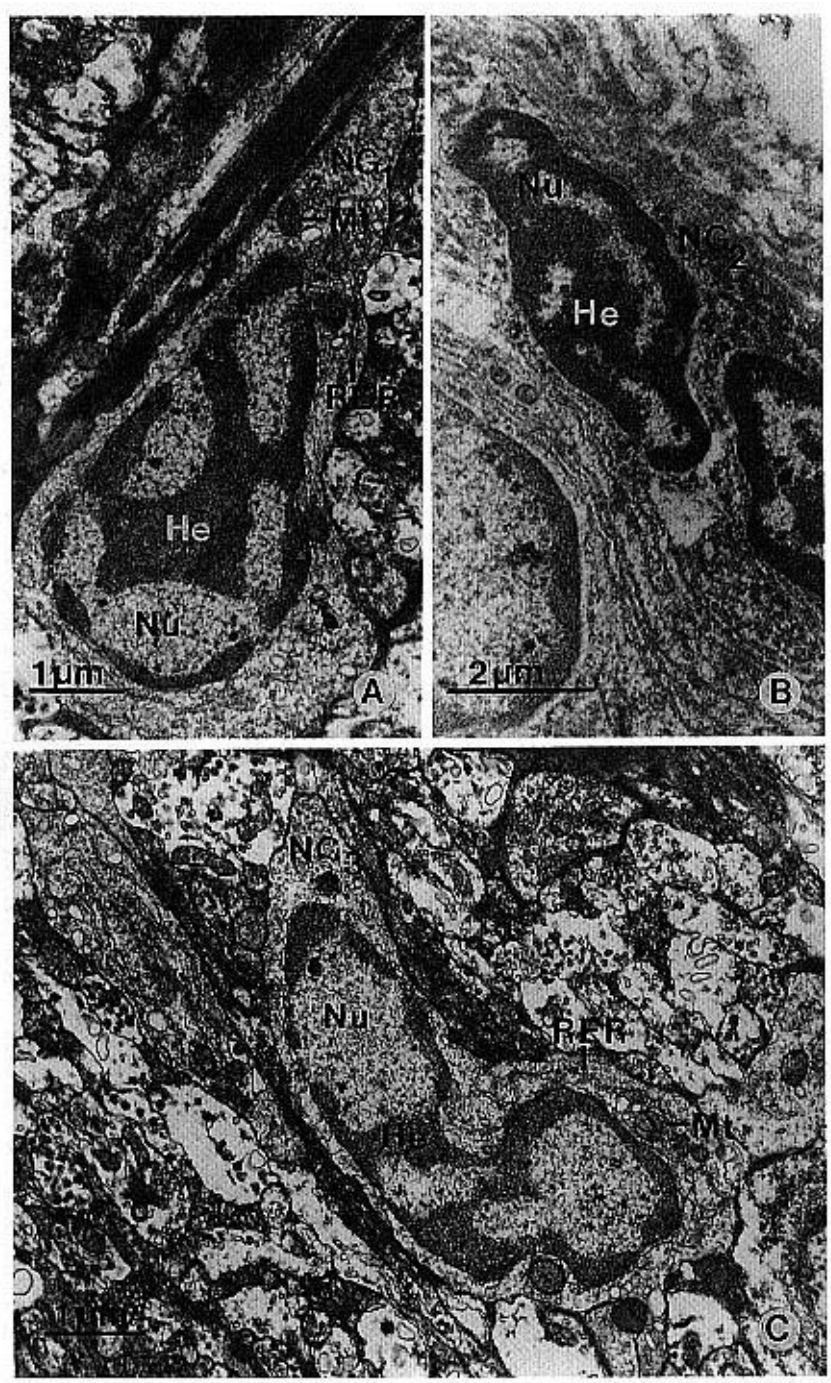

Fig 2. A. A medium power micrograph of type 1 neuroglia (NG_1) in the pleuro-pedal ganglia showing a spindle-shaped nucleus (Nu) with patches of heterochromatin (He) in the peripheral and central regions. The cytoplasm contains a few rough endoplasmic reticulum (RER) and mitochondria (Mt). B. A medium power micrograph of type 2 neuroglia (NG_2) in the pleuro-pedal ganglia showing a nucleus (Nu) with very dense heterochromatin (He). The cytoplasm is extremely thin and contains only ribosomes. C. A medium power micrograph of type 3 neuroglia (NG_3) in the pleuro-pedal ganglia showing a nucleus (Nu) with dense heterochromatin (He). The cytoplasm contains a few rough endoplasmic reticulum (RER) and mitochondria (Mt).

Purkinje cells in the cerebellum. Their chromatin is completely euchromatic with a prominent nucleolus, while the cytoplasm contains abundant rough endoplasmic reticulum and mitochondria, but only a few typical elementary or neurotransmitter granules.

Other types of neurons (NR_2, NR_3, NR_4) may belong to the same group. They are characterized by increasing condensation of heterochromatin. The cytoplasm is small but contains sizable numbers of organelles particularly polyribosomes and mitochondria. There are small elementary granules present in the cytoplasm of these neurons. These features are similar to association neurons in the vertebrate nervous system, such as small neurons in the molecular layer of cerebellum of mammals.

The ultrastructure of ordinary neurons had been studied extensively in the ganglia of several pulmonates such as *L. stagnalis*,[18] *Helisoma tenue* (Phillippi),[19] *A. californica*,[7] *H. pomatia*,[3] *H. aspersa*,[10] *A. marginata*[9] and *A. fulica*.[11] The studies on the neurons of prosobranchs were reported in *H. rufescens*[13] and *B. tentaculata*.[12] The ultrastructure of the ordinary neurons in *H. asinina* is rather simple compared with those in the pulmonates. They contain the usual cytoplasmic organelles such as rough endoplasmic reticulum, mitochondria, ribosomes, polyribosomes, similar to the neurons of pulmonates but in smaller numbers. Their Golgi bodies and lysosomes are not observed as frequent as those of the pulmonate neurons. In addition, the ordinary neurons of pulmonates usually contain a large number of elementary granules. There are only a few elementary granules in *H. asinina* neurons. These granules are not stained by chrome-hematoxylin and paraldehyde-fuchsin. It is possible that the small vesicles of the neurons might incorporate neurotransmitters, which are non-peptides or glycoprotein.[18] Similar granules, thought to contain neurotransmitters have been described in the neurons of *L. stagnalis*[18] and *A. marginata*.[9]

In addition to the elementary granules, special types of inclusions and secretory granules have been reported in the neurons of *H. rufescens* and *B. tentaculata*.[12,13] In *H. rufescens*, the cerebral neurons contain large membrane-bound inclusions showing various degrees of organization such as packed membranes or filaments, clumps of strongly osmiophilic material, and homogeneous pale material. Frequently, a single inclusion shows all three features.[13] It is possible that these inclusions are secretory in function.[13] No such inclusions were observed in *H. asinina* neurons. Andrews[12] reported the presence of lipofuscin spherules containing carotenoid pigments in *B. tentaculata* neurons. We did not observe these spherules in the neurons of *H. asinina*.

The neuroglia of the cerebral and pleuro-pedal ganglia of *H. asinina* contain spindle-shaped nuclei and little perinuclear cytoplasm. The NG_1 contains a few rough endoplasmic reticulum, mitochondria and ribosomes, while the NG_2 and NG_3 contain only ribosomes. These neuroglia of *H. asinina* are different from those described in *B. tentaculata*,[12] *L. stagnalis*[14] and *A. marginata*[9] which have several mitochondria, rough endoplasmic reticulum, Golgi complexes and lysosome-like structures. Furthermore, they are interspersed amongst the neurons, but do not indent into the cytoplasm of the neurons like those described for the neuroglia of pulmonates.[1]

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References

1. Bullock TH and Horridge GA (1965) *Structure and Function in the Nervous System of Invertebrate*, Vol 2. W.H. Freeman and Company, San Francisco.
2. Smith BJ (1967) Correlation between neurosecretory changes and maturation of the reproductive tract of *Arion ater* (Stylommatophora : Arionidae). *Malacologia* **5**, 285-98.
3. Pentreath W, Osborne NN and Cottrell GA (1973) Anatomy of giant serotonin-contain neurons in the cerebral ganglia of *Helix pomatia* and *Limax maximus*. *Z Zellforsch Mikrosk Anat* **143**, 1-20.
4. Kruatrachue M, Upatham ES, Seehabutr V, Chavadej J, Sretarugsa P and Sobhon P (1993) General anatomy and histology of the nervous system in *Achatina fulica*. *J Sci Soc Thailand* **19**, 1-24.
5. Dorsett DA (1983) The neuroanatomy of selected gastropod species. In: *The Molluscs* (Edited by Saleuddin ASM and Wilbur KM), pp 101-87. Academic Press, New York.
6. Benjamin PR and Window W (1981) The distribution of three wide acting synaptic input to the identified neurons in the isolated brain of *Lymnaea stagnalis*. *Comp Biochem Physiol* **70A**, 293-307.
7. Coggeshall RE (1967) A light and electron microscope study of the abdominal ganglion of *Aplysia californica*. *J Neurophysiol* **30**, 1263-87.
8. Joosse J (1964) Dorsal bodies and dorsal neurosecretory cells of the cerebral ganglia of *Lymnaea stagnalis*. *Arch Neerl Zool* **15**, 1-103.
9. Amoroso El, Margaret I, Baxter FRS, Chiquoine AD and Nisbet RH (1964) The fine structure of neurons and other elements in the nervous system of the giant African land sail *Archachatina marginata*. *Proc Roy Soc Lond* **160**, 167-80.
10. Fernandez J and Fernandez MS (1972) Nervous system of the snail *Helix aspersa*. 3. Electron microscopic study of neurosecretory nerves and endings in the ganglionic sheath. *Z Zellforsch Mikrosk Anat* 135, 473-482.

11. Kruatrachue M, Sretarugsa P, Seehabutr V, Chavadej J, Upatham ES and Sobhon P. The fine structure of neurons in the nervous system of the giant African snail *Achatina fulica* (Gastropoda : Achatinidae). *J Med Appl Malacol* (in press).
12. Andrews EB (1971) The fine structure of the nervous system of *Bithynia tentaculata* (Prosobranchia) in relation to possible neurosecretory activity. *The Veliger* **14**, 13-23.
13. Miller W, Nishioka RS and Bern HA (1973) The "juxtaganglionic" tissue and the brain of the abalone *Haliotis rufescens* Swainson. *The Veliger* **16**, 125-9.
14. Wendelaar Bonga SE (1970) Ultrastructure and histochemistry of neurosecretory cells and neurohaemal areas in the pond snail *Lymnaea stagnalis*. *Z Zellforsch Mikrosk Anat* **108**, 190-224.
15. Fernandez J (1963) Nervous system of the snail *Helix aspersa*. 1. Structure and function of ganglionic sheath and neuroglia. *J Comp Neurol* **127**, 157-82.
16. Upatham ES, Thongkukiatkul A, Kruatrachue M, Wanichanon C, Chitramvong YP, Sahavacharin S and Sobhon P (1998) Classification of neurosecretory cells, neurons, and neuroglia in the cerebral ganglia of *Haliotis asinina* Linnaeus by light microscopy. *J Shellfish Res* **17**, 737-42.
17. Thongkukiatkul A, Upatham ES, Sobhon P, Kruatrachue M, Wanichanon C, Pumthong T and Nugranad J (1999) Histological studies of the pleuro-pedal ganglion, visceral ganglion and pedal cord ganglia of *Haliotis asinina* Linnaeus. *J Med & Appl Malacol* (in press).
18. Boer HH, Douma E and Koksma JMA (1968) Electron microscope study of neurosecretory cells and neurohemal organs in the pond snail *Lymnaea stagnalis*. *Symp Zool Soc Lond* **22**, 237-56.
19. Simpson L, Bern HA and Nishioka RS (1966) Examination of the evidence for neurosecretion in the nervous system of *Helisoma tenue* (Gastropoda Pulmonata). *Gen Comp Endocrinol* **7**, 525-49.