Egypt. J. Aquat. Biol. & Fish., Vol.7, No.4 : 21 - 44 (2003) *ISSN 1110 – 6131*

NEUROSECRETORY CELLS IN SOME PRINCIPAL GANGLIA OF THE SEA-HARE, *APLYSIA OCULIFERA*

Setaita H. Sleem
Department of Zoology, Faculty of Science, Ain Shams University.

Key words: *Aplysia*, Opithobranchia, anatomy, histology, ultrastructure, neurosecretion, nervous system.

ABSTRACT

The principal ganglia of the central nervous system (CNS), of the sea hare, *Aplysia oculifera*, are concentrated around the oesophagus, caudally from the buccal mass. The usual complement of such ganglia comprises paired cerebral, buccal, pleural and pedal ganglia. The neurosecretory cells (NSCs) were variably observed in all the above mentioned ganglia. They were differentiated according to size and shape into three cell types namely: large (type A), medium (type B) and small (type C) cells. The A cell type is less common and has a large size with an oval or flattened shape. It measures from 116.6 x 69.9 μ to 34.8 x 19.5 μ, with a centrally located irregularly shaped or polymorphic nucleus, with many small intensely colored nucleoli. The B cell type is more numerous and has an oval or rounded shape, with a rounded and centrally located nucleus, which has many small intensely colored nucleoli. It measures from 59.5 x 40.8 μ to 22.2 x 18.1 μ. Meanwhile, C cell type constitutes the majority of the NSCs. It is small rounded or ovoid cell, measuring from 35.6 x 31.5 μ to 5.6 x 5.0 μ, with a large rounded nucleus, having a sharply defined eccentrically placed nucleolus.

The ultrastructure of NSCs of these ganglia was also investigated. Generally, it showed active Golgi body, rough endoplasmic reticulum and a great number of free ribosomes. The electron dense neurosecretory granules were observed. These were attributed to the activity of both Golgi apparatus and rER that have the main role in their formation.

INTRODUCTION

The definition of neurosecretion rests in part on the existence of signs of secretion in cells that are otherwise definable as neurons. Regardless of the nature of the stain employed, revelation of granules or globules, which in epithelial cells would be referred to as "secretion" marks a nerve cell as possibly neurosecretory. Gabe (1966) and Simpson *et al.* (1966) have published reviews of the early literature on neurosecretory phenomena in many species of gastropods.

Many investigators recorded the presence of neurosecretory cells in the principal ganglia of some stylommatophore gastropods (Krause, 1960; Pelluet & Lane, 1961; Lane, 1964; Smith, 1966 & 1967; Boer *et al.*, 1977; Wijdenese *et al.*, 1980; El-Saadany, 1995; Sleem & Aly, 1998 and Heiba & Mahran, 1999) and many prosobranch species (Nagabhushanam & Muley, 1974; Joosse, 1975; Narian & Singh, 1982; Francesco, 1989; Ibrahim *et al.*, 1993; Sleem & Aly, 1997 and Aly, 2001). In contrast, neurosecretion in the opisthobranchs has received a relatively little attention (Soinila & Mpitsos, 1991; Moroz & Gillette, 1996; Moroz *et al.*, 1996; Diaz-Rios *et al.*, 1999, Lechner *et al.*, 2000 and Li *et al.*, 2001).

The ultrastructural studies of the neurosecretory ganglia of gastropods were mainly restricted to certain pulmonate species (Simpson *et al.*, 1963; Boer *et al.*, 1977; Wendelaar-Bonga, 1970 & 1971; El-Saadany, 1995 and Aly & Sleem, 2000). However, few ultrastructural studies were conducted on neurosecretory cells of the opisthobranch species, that were mostly restricted to the abdominal ganglion (Rosenbluth, 1963 a&b; Simpson *et al.*, 1963; Coggeshall, 1967; Frazier *et al.*, 1967 and Musio & Bedini, 1990).

Thus, the present work aimed to investigate the histology and ultrastructure of the neurosecretory cells in some principal ganglia of the sea hare, *Aplysia oculifera,* collected from the Red Sea, Egypt.

MATERIALS AND METHODS

Some adult specimens of the sea hare, *Aplysia oculifera* were collected from the coast of the northern part of the Red Sea at Hurghada, Egypt. After dissection of such specimens, the nervous system was drawn under a binocular dissecting microscope, using a camera Lucida. For histological examination by light microscope, the

principal ganglia of the nervous system (cerebral, pleural, pedal and buccal) were excised and fixed immediately in aqueous Bouin's fluid. The fixed tissues were dehydrated, cleared, embedded and sectioned at 5μ thickness. These sections were stained with hematoxylin-eosin, the routine neurosecretory stain, or Mallory's triple stain (Mallory, 1944). Sections were examined and photographed using 35mm camera, mounted on Olympus microscope. The neurosecretory cells were identified and their different types were measured under the light microscope using an ocular micrometer, and transformed to microns.

For the ultrastructural examination, the principle ganglia were fixed immediately in 4% phosphate buffered glutraldhyde solution for 2 hours, washed in phosphate buffer for 30 minutes "2 changes". Then post fixed in 2% osmic acid for 1-2 hours at 4C and washed in phosphate buffer. The tissues were dehydrated and embedded in pure resin and kept in an oven for 48 h. at 60 C. Semi-thin sections of these tissues were cut at 1μ, and stained with toludine blue for the light microscope investigation. The specimens were trimmed and ultra-thin sections were cut and mounted on copper grids, then stained with a drop of saturated solution of uranyl acetate mixed with ethyl alcohol (1:1) for 5 min., transferred to distilled water for 5 min. "many changes" and left to get dried. Finally, the grids were examined and photographed under Joel transmission electron microscope.

RESULTS AND DISCUSSION

Nervous system anatomy:

The present study revealed that the nervous system of the sea hare, *Aplysia oculifera,* consists of the principal ganglia, which form the central nervous system and the abdominal ganglion, which are composed of the fused parieto-visceral ganglia. The principal ganglia of the central nervous system (CNS) are concentrated around the esophagus, caudally from the buccal mass. The usual complement of such ganglia comprises a pair of cerebral ganglia, a pair of buccal ganglia, a pair of pleural ganglia and a pair of pedal ganglia (Fig. 1).

Cerebral ganglia are located supra-esophageal and they are ovoid in shape and connected with each other by a broad short commissure. Each cerebral ganglion is also connected with the buccal, pleural and pedal ganglia by separate connectives, namely cerebrobuccal, cerebropleural and cerebropedal connectives,

respectively. These cerebrals innervate eyes, statocytes, head tentacles, skin, head muscles and penis (Fig. 1).

The buccal ganglia are the most anteriorly placed ones, ventrally to the gut. They have a thin short commissure, and innervate the pharynx, salivary glands, esophagus and stomach.

The pedal ganglia are node-like masses. They are connected with each other by two commissures, pedal and parapedal, and innervate the foot muscles and skin. Pleural ganglia have no commissure, but they receive three connectives from cerebral and pedal ganglia and from the visceral loop (Fig. 1).

The present findings are in accordance with those recorded on the anatomy of the nervous system of various other species of *Aplysia* by Bullock & Horridge (1965) and Soliman (2001).

Histology of the ganglia:

Histologically, the principal ganglia of the central nervous system of *Aplysia oculifera* are surrounded by the connective tissue, the perineurium. The distribution of the neurosecretory cells within the studied ganglia is generally outlined in Figure (2). The neurosecretory cells (NSCs) were observed in all examined principal ganglia. These observations are in agreement with the results obtained by Sleem & Aly (1997) in prosobranch snail, *Lanistes carinatus* and Sleem & Aly (1998) in the land snail, *Eobania vermiculata*. However, in *Lymnaea stagnalis*, no neurosecretory cells have been observed in the buccal and pedal ganglia (Wendelaar Bonga, 1970). On the other hand, Diaz-Rios *et al.* (1999) found that Gamma-aminobutyric acid immunoreactive (GABAi) neurons were located in all ganglia of the central nervous system of *Aplysia californica*.

The neurosecretory cells of the present opisthobranch, *A. oculifera* are arranged in radiating groups. They are mainly localized at the peripheral regions of the ganglia. They are unipolar and sending their processes to the central zone occupied by the neuropile. The same results were also recorded in other gastropods such as *Arion hortensis*, *Deroceras reticulatum* and *Helix aspersa* (Wijdenes *et al.*, 1980), *Lymnaea caillaudi* (Sleem, 1993), *Biomphalaria alexandrina* (Aly, 1995), *Lanistes carinatus* (Sleem & Aly, 1997) and *Eobania vermiculata* (Sleem & Aly, 1998).

Generally, observations of the stained sections of different principal ganglia revealed that these neurosecretory cells differ from each other in size and general shape. According to these differences,

they are classified into three types of cells, namely large (type A), medium (type B) and small (type C) cells.

Similarly, three types of neurosecretory cells were reported by Ibrahim *et al.* (1993) in the freshwater prosobranch, *Bellamya unicolor*, Sleem & Aly (1998) in the land snail, *E. vermiculata* and Aly (2001) in freshwater prosobranch, *Cleopatra bulimoides*. On the other hand, variable numbers of NSCs were described in other gastropods by many authors. Wendelaar Bonga (1970) distinguished seven types of these cells in *Lymnaea stagnalis*, while, Shylaja & Alexander (1977) demonstrated only one cell type in *Pila virens* and Sleem & Aly (1997) recorded 2 cell types in *L. carinatus*.

The A cell type is characterized by its large size with oval or flattened shape (Figures 3, 4, 7, 8, 11 and 13). It is fewer in number, and measures from 116.6 x 69.9 μ to 34.8 x 19.5 μ, with an average of 63.14 x 45.32 μ (Table 1). It has a centrally located irregular-shaped or polymorphic nucleus, which has many small intensely colored nucleoli. The medium or B cell type is characterized by its oval or rounded shape, with a rounded and centrally located nucleus, which has many small intensely colored nucleoli. This cell type being more numerous in number and measures from 59.5 x 40.8 μ to 22.2 x 18.1 μ, with an average of 34.58 x 24.4 μ (Table 1 and Figures 5, 7, 9, 12 and 14). On the other hand, the small or C cell type constitutes the majority of the NSCs. It is small rounded or ovoid in shape, measuring from 35.6 x 31.5 μ to 5.6 x 5.0 μ in size, with an average of 16.32 x 13.67 μ. Each has a relatively large and rounded nucleus that occupies most of the cell body (Table 1 and Figs. 4, 6, 7, 10 and 11-14), and having a sharply defined eccentrically placed nucleolus.

The present study revealed that the sea hare, *Aplysia oculifera* showed so far the largest neurosecretory cells, that reached to 116.6 x 69.9 μ, while, the greatest diameter of such cells reached to 20 μ in *L. carinatus* (Sleem & Aly, 1997), and to 61 x 39 μ in *E. vermiculata* (Sleem & Aly, 1998).

There is a great similarity in the affinities of the above mentioned cell types to hematoxylin-eosin stain and the routine neurosecretory stain, Mallory's triple stain (orange color). Similar results were reported by Nagabhushanam & Muley (1974); Shylaja & Alexander (1977); Rajalakshmi Bhanu *et al.* (1983); Ibrahim *et al.* (1993) and Sleem & Aly (1997).

A. Cerebral ganglia:

The neurosecretory cells in these ganglia are localized in three groups (Figs. 2 & 3). The first group is mainly composed of the A & B cell types and extends from the latero-dorsal area of each cerebral ganglion to the mid-dorsal of the cerebral commissure. The second group is composed mostly of the C cell type and is localized at the latero-ventral sides of the ganglion, around the bases of the cerebropleural and cerebropedal connectives, and extends towards the neuropile of the ganglion. Meanwhile, the third group is located at the mid-ventral of the cerebral commissure and composed of a fewer number of A cell type.

In cerebral ganglia, the cells of type A measure from 83.4 x 67.1 μ to 34.8 x 19.5 μ, with an average of 61.15 x 43.05 μ. Their nuclei are flattened and measure from 48.9 x 22.2 μ to 19.5 x 11.7 μ, with an average of 36.78 x 15.89 μ. The cells of type B measure 23.63 x 16.82 μ in average size, and having an oval nucleus with an average size of 15.15 x 8.89 μ, while, the small cells of type C are rounded in shape and measure 16.62 x 13.42 μ, and having a rounded nucleus with average size of 10.28 x 7.15 μ (Table 1 and Figs. 5, 6&15).

Dorsal bodies:

There is a pair of rounded dorsal bodies, located at the latero-dorsal sides of the cerebral ganglia. Each body contains a single cell of type A and many of small ovoid cells of type C, with rounded or ovoid nuclei. These dorsal bodies connect to the cerebral ganglia through the long cell processes (Figs. 3&4). This result disagrees with that recorded by Wijdenes *et al.* (1980) in *Helix aspersa*; Ezzughayyar & Wattez (1989) in *Arion rufus* and Sleem & Aly (1997) in *E. vermiculata.*

B. Buccal ganglia:

The neurosecretory cells are distributed in a circular manner, having a few number of the large cells (type A) and many of type B at the peripheral layer beneath the perineurium, which covers the ganglia. The small cells (type C) constitute the majority of NSCs in the buccal ganglia and located under the peripheral layer, as nearly as the cerebro-buccal connectives (Fig. 7). The presence of NSCs was previously reported in other gastropods such as *Arion ater* (Smith, 1967); *Arion hortensis*, *Deroceras reticulatum* and *Helix aspersa* (Wijdenes *et al.*, 1980); *Lanistes carinatus* (Sleem & Aly, 1997) and *Eobania vermiculata* (Sleem & Aly, 1998). On the other hand, these NSCs were totally absent from the buccal ganglia of *Lymnaea*

stagnalis (Hekstra & Lever, 1960) and *Bulinus trancatus* (Banna, 1985).

In the buccal ganglia, cells of type A measure 69.06 x 51.67 μ in average size. Their nuclei are oval in shape and measure 43.86 x 39.53 μ. The cells of type B measure 32.58 x 21.66 μ in average size, and having an oval nucleus with an average size of 21.16 x 13.89 μ, while, the small cells of type C are rounded in shape and measure 8.27 x 6.6 μ, and having a rounded nucleus with average size of 6.05 x 4.66 μ (Table 1 and Figs. 8-10).

C. Pedal ganglia:

Histologically, each pedal ganglion is composed of 3-4 small rounded masses. Inside each mass, the neurosecretory cells are distributed in a circular manner, having mostly a large cell (type A) and many of type B cells (Fig. 11). Meanwhile, numerous cells of the type C are aggregated at the base of cerebropedal and pleuropedal connectives (Fig. 12). These NSCs were also recorded in other gastropods by many investigators (Simpson *et al.*, 1966; Smith, 1967; Boer *et al.*, 1977; Wijdenes *et al.*, 1980; Sleem, 1993; Aly, 1995; Sleem & Aly, 1997 and Sleem & Aly, 1998). However, Lever *et al.* (1961) and Wendelaar Bonga (1970) reported the total absence of these NSCs from the pedal ganglia in *Lymnaea stagnalis*.

The three types of neurosecretory cells in the pedal ganglia are considered the largest cells (Table 1). The cells of type A measure from 116.6 x 69.9 μ to 41.9 x 30.3 μ, with an average of 71.33 x 52.21 μ. Their nuclei are flattened and measure from 93.2 x 34.9 μ to 23.3 x 18.7 μ, with an average of 53.61 x 29.84 μ. The cells of type B measure 39.18 x 28.03 μ in average size, and having an oval nucleus with an average size of 29.31 x 18.17 μ. The small cells of type C are rounded in shape and measure 20.93 x 17.61 μ, and having a rounded nucleus with average size of 13.66 x 10.88 μ (Table 1 and Figs. 11, 12&16).

D. Pleural ganglia:

Histologically, pleural ganglion is composed of 2-3 small masses. Neurosecretory cells are distributed at the rim of each mass, having a large cell of type A and many of type B cells. But, numerous cells of the type C are concentrated at the base of connectives in the ganglion center (Fig. 13). In the pleural ganglia, cells of type A measure 48.96 x 32.64 μ in average size. Their nuclei are flattened and measure 33.81 x 18.36 μ in average size. The cells of type B

measure 38.41 x 28.28 μ in average size, and having an oval nucleus with an average size of 22.86 x 15.83 μ. Meanwhile, the small cells of type C are rounded in shape and measure 15.69 x 14.37 μ, and having a rounded nucleus with an average size of 9.86 x 8.78 μ (Table 1).

Electron microscopy:

Generally, the neurosecretory cells showed active Golgi body, rough endoplasmic reticulum and a great number of free ribosomes. The electron dense neurosecretory granules were observed throughout the cytoplasm.

The neurosecretory cell of A type possesses a large elongated and irregularly outlined nucleus with heterochromatin aggregations and many small nucleoli (Figs. 17&18). Its cytoplasm is packed with scattered strands of rough endoplasmic reticulum and Golgi bodies, which appeared giving off electron dense secretory granules (Figs. 19&20). These secretory granules aggregate forming large globules between the lamellae of rough endoplasmic reticulum, which is distributed evenly in the cytoplasm. The free ribosomes and polysomes are most conspicuous of the cell components in the cytoplasm of this cell type. The multivesicular bodies, lysosomes and oil droplets are also observed in this neurosecretory cell body (Figs. 19&20).

The neurosecretory cell of B type possesses a rounded or oval nucleus with heterochromatin aggregations (Fig. 21). Its cytoplasm is packed with secretory granules, which aggregate forming large accumulations between the lamellae of the rough endoplasmic reticulum. These accumulations of secretory granules are distributed throughout the cytoplasm of the cell. The multivesicular bodies and lysosome-like bodies are found in these cells (Fig. 22).

The neurosecretory cell of C type is characterized by possessing a relatively large, oval or rounded nucleus with heterochromatin aggregations and having an eccentric and high electron dense nucleolus (Figs. 23&24). This cell contains Golgi bodies, long and short strands of rough endoplasmic reticulum, free ribosomes and mitochondria. It is characterized by the presence of secretory droplets, which are probably of lipid nature (Figs. 24-26).

Several studies have been carried out on the neurosecretory cells of the gastropod nervous ganglia at the ultrastructural level to investigate the products and the cell organelles (Wendelaar Bonga, 1971; Van Minnen & Reichelt, 1980; Roubos *et al.*, 1981; Al Yousuf, 1988; Aly, 1995; El-Saadany, 1995; Sleem & Aly, 1997; Aly &

Sleem, 2000; and Thongkukiatku, 2000). They found that the rough endoplasmic reticulum and Golgi apparatus have the main role in their neurosecretion.

Moreover, Wendelaar Bonga (1970 & 1971), Sleem & Aly (1998) and Thongkukiatku (2000) described also the increasing size of secretory granules by their accumulation to form globules. The presence of oil droplets in neurosecretory cells was also reported in *Lymnaea stagnalis* (Wendelaar Bonga, 1970); *Lanistes carinatus* (Sleem & Aly, 1997) and *Eobania vermiculata* (Sleem & Aly, 1998).

REFERENCES

Aly, R. H. (1995). Biological, histological and histochemical studies of the effect of a molluscicide and a fertilizer on the nervous system and gonads of *Biomphalaria alexandrina* and its host parasitic relationship. Ph D. Thesis, Zool. Dept., Fac. Sci., Ain Shams University.

Aly, R. H. (2001). Morphological studies on the freshwater snail *Cleopatra bulimoides* (Olivier) (Prosobranchia-Thiaridae) 2- The nervous system. Egypt. J. Aquat. Biol. & Fish., *5*(3): 213-225.

Aly, R. H. and Sleem, S. H. (2000). Ultrastructure studies on the nervous system of the land snail *Eobania vermiculata* (Gastropoda: Stylommatophora). J. Egypt. Soc. Parasitol., *30*(1): 197-209.

Al Yousuf, S. (1988). Origin of neurosecretory granules in the cerebral ganglion of the earthworm *Aporrectoda caliginosa*. Egypt. J. Histol., *11*(2): 287-294.

Banna, B. M. (1985). Observations on neurosecretion in *Bulinus truncates* (Audouin) with special reference to the effect of a molluscicide. J B S R, *16*(1): 57-71.

Boer, H. H. ; Roubos, E. W. ; Van Dalen, H. and Groesbeek, J. R. F. Th. (1977). Neurosecretion in the basommatophoran snail *Bulinus trancatus* (Gastropoda: Pulmonata). Cell Tiss. Res., *176*: 57-68.

Bullock, Th. H. and Horridge, G. A. (1965). Structure and Function in the Nervous Systems of Invertebrates. Vol. II, Chapter 23 (Mollusca: Gastropoda). W. H. Freeman and Company, San Francisco and London.

Coggeshall, R. E. (1967). A light and electron microscope study of the abdominal ganglion of *Aplysia californica*. J. Neurophysiol., *30*: 1263-1287.

Diaz-Rios, M. ; Suess, E. and Miller, M. W. (1999). Localization of GABA-like immunoreactivity in the central nervous system of *Aplysia californica*. J. Comp. Neurol., *413*(2): 255-270.

El-Saadany, M. M. (1995). Studies on the neurosecretory cells of the freshwater snail, *Biomphalaria alexandrina*. J. Egypt. Soc. Zool., *16*(C): 71-84.

Ezzughayyar, A. and Wattez, C. (1989). Relationship between dorsal bodies activity and the female reproductive activity in the slug *Arion rufus* (Mollusca, Gastropoda, Pulmonata). C. R. de l' Acad. Sci., *309*(3): 505-511

Francesco, M. (1989). A comparative study of the neurosecretory system in two gastropod Mollusca, *Amyclina cornicuium* Olivi and *Cyclope neritea* L. (Nassariidae, Prosobranchia: Stenoglossa). Zool. Anz., *223*(5-6): 341-350.

Frazier, W. T. ; Kandel, E. R. ; Kpfermann, I. Waziri, R. and Coggeshall, R. E. (1967). Morphological and function properties of identified neurons in the abdominal ganglion of *Aplysia californica*. J. Neurophysiol., *30*: 1288-1351.

Gabe, M. (1966). Neurosecretion. Intern. Ser. Monogr. Biol. 28, Oxford-London- New York: Pergamon Press.

Heiba, F. N. and Mahran, H. A. (1999). Demonstration of the neurosecretory cells in the cerebral ganglia of the land snails, *Eobania vermiculata* and *Monacha contiana*. J. Egypt. Soc. Zool., *28*(C): 175-184.

Hekstra, G. P. and Lever, J. (1960). Some effects of ganglion extirpation in *Lymnaea stagnalis*. Proc. Kon. Ned. Akad. Wet., *63*: 271-282.

Ibrahim, A. M. ; Saad, A. A. and Sleem, S. H. (1993). The neurosecretory system in the freshwater prosobranch, *Bellamya unicolor*. J. Faculty of Education, *18*: 315-337.

Joosse, J. (1975). Endocrinology of mollusks. Coll. Intern. C.N.R.S. No. 251, Actualites sur les hormones d'invertebres.p. 107-123.

Krause, E. (1960). Untersuchungen uber die neurosekretion in Schlundring von *Helix pomatia* L. Z. Zellforsch, *51*: 748-776.

Lechner, H. A. ; Baxter, D. A. and Byrne, J. H. (2000). Classical conditioning of feeding in *Aplysia*: II. Neurophysiological correlates. J. Neurosci., *20*(9): 3377-3386.

Lane, N. J. (1964). Elementary neurosecretory granules in neurons of the snail, *Helix aspersa* Quart. J. Microscope. Sci., *105*: 31-34.

Lever, J. ; Kok, M. ; Meuleman, E. A. and Joosse, J. (1961). On the location of Gomori-positive neurosecretory cells in the central ganglia of *Lymnaea stagnalis*. Proc. Kon. Ned. Akad. Wet., *64*(5): 640-647.

Li, L. ; Floyd, P. D. ; Rubakhin, S. S. ; Romanova, E. V. ; Jing, J. ; Alexeeva, V. Y. ; Dembrow, N. C. ; Weiss, K. R. ; Vilim, F. S. and Sweedler, J. V. (2001). Cerebrin prohormone processing, distribution and action in *Aplysia californica*. J. Neurochem., *77*(6): 1569-1580.

Mallory, F. (1944). Physiological Techniques. W.B. Saunders Co. Philadelphia.

Moroz, L. L. ; Chen-Dong; Gillette, M. U. and Gillette, R. (1996). Nitric oxide synthase activity in the molluscan CNS. J. Neurochem., *66*(2): 873-876.

Moroz, L. L. and Gillette, R. (1996). NADPH-diaphorase localization in the CNS and peripheral tissues of the predatory sea-slug, *Pleurobranchaea californica*. J. Comp. Neurol., *367*(4): 607-622.

Musio, C. and Bedini, C. (1990). Fine structure and axonal organization in the buccal ganglia nerves of *Aplysia* (Mollusca: Gastropoda). Zoomorphology, *110*(1): 17-26.

Nagabhushanam, R. and Muely, E. V. (1974). Studies on the neurosecretory system of a prosobranch, *Melania scabra*. Marathwada Univ. J. Sci., *13*(6): 147-152.

Narian, A. S. and Singh, B. B. (1982). Neurosecretory cells in some principal ganglia of the common Indian apple-snail, *Pila globosa*. Arch. Biol. (Bruxelles) *93*: 353-361.

Pelluet, D. and Lane, N. J. (1961). The relation between neurosecretion and cell differentiation in the ovotestis of slugs (Gastropoda: Pulmonata). Can. J. Zool., *39*: 789-805.

Rajalakshmi Bhanu, R. C. ; Shyamasundari, K. and Hanumantha Rao, K. (1983). The structure and cytochemistry of the neurosecretory cells in the Intertidal gastropod *Thais bufo* (Lamarch). Z. Mikrosk. Anat. Forsh. Leipzig., *973*(S): 535-546.

Rosenbluth, J. (1963a). The visceral ganglion of *Aplysia californica*. Z. Zellforsch., *60*: 213-236.

Rosenbluth, J. (1963b). Fine structure of epineural muscle cells in *Aplysia californica*. J. Cell. Biol., *17*: 455-460.

Roubos, E. W. ; Schmidt, E. D. and Moorer-Van Delft, C. M. (1981). Ultrastructural dynamics of exocytosis in the ovulation neurohormone producing caudo-dorsal cells of the freshwater snail *Lymnaea stagnalis* (L.). Cell Tissue Res., *215*: 63-73.

Shylaja, R. and Alexander, K. M. (1977). Neurosecretion in the freshwater prosobranch, *Pila virens*. I. Neurosecretion in the normal and aestivating snails. Proc. Indian Acad. Sci., *86B*(5): 323-327.

Simpson, L. ; Bern, H. A. and Nishioka, R. S. (1963). Inclusions in the neurons of *Aplysia californica* (Cooper, 1863) (Gastropoda: Opithobranchiata). J. Comp. Neurol., *121*: 237-258.

Simpson, L. ; Bern, H. A. and Nishioka, R. S. (1966). Examination of evidence for nerosecretion in the nervous system of *Helisoma tenue* (Gastropoda: Pulmonata). Gen. Comp. Endocr., *7*: 525-548.

Sleem, S. H. (1993). Studies on the reproductive biology and some factors affecting reproduction of certain freshwater snails. Ph. D. Thesis. Zool. Dept., Fac. Sci., Ain Shams University.

Sleem, S. H. and Aly, R. H. (1997). Neurosecretory cells in some principal ganglia of the prosobranch snail, *Lanistes carinatus* Olivier. Egypt. J. Histol., *20*(1): 47-56.

Sleem, S. H. and Aly, R. H. (1998). Histological studies of the nervous system of the land snail, *Eobania vermiculata* (Gastropoda: Pulmonata). Egypt. J. Zool., *31*: 95-105.

Smith, B. (1966). The structure of the central nervous system of slug, *Arion ater* (L.), with notes on the cytoplasmic inclusions of the neurons. J. Comp. Neurol., *126*(3): 437-452.

Smith, B. (1967). Correlation between neurosecretory changes and maturation of the reproductive tract of *Arion ater* (Stylommatophora: Arionidae). Malacologia, *5*(2): 285-298.

Soinila, S. and Mpitsos, G. J. (1991). Immunohistochemistry of diverging and converging neurotransmitter systems in mollusks. Biol. Bull. Mar. Biol. Lab. Woods-Hole, *181*(3): 484-499.

Soliman, G. N. (2001). Invertebrate Zoology. The Mideastern Invertebrate Fauna. II. The Coelomates. The Palm Press, Cairo, Egypt.

Thongkukiatku, A. ; Kruatrachue, M. ; Upatham, E. S. ; Sobhon, P. ; Wanichanon, C. Chitramvong, Y. and Pumthong, T. (2000): Ultrastructure of neurosecretory cells in the cerebral and pleuropedal ganglia of *Haliotis asinine* Linnaeus. J. Shellfish-Res., *19*(1): 539.

Van Minnen, J. and Reichelt, D. (1980). Photoperiod dependent neural control of the activity of the neurosecretory cells in the lateral lobes of the cerebral ganglia of the freshwater pulmonate snail *Lymnaea stagnalis* (L.). Cell Tissue Res., *208*: 457-465.

Wendelaar-Bonga, S. E. (1970). Ultrastructure and histochemistry of neurosecretory cells and neurohaemal areas in the pond snail *Lymnaea stagnalis* (L.). Z. Zellforsch., *108*: 190-224.

Wendelaar-Bonga, S. E. (1971). Formation, storage and release of neurosecretory materials studied by quantitative microscopy in the freshwater snail *Lymnaea stagnalis* (L.). Z. Zellforsch., *113*: 490-517.

Wijdenes, J. ; Van Minnen, N. and Boer, H.H. (1980). Study on the neurosecretion demonstrated by the alcian blue-alcian yellow technique in three terrestrial pulmonates (Stylommatophora). Cell. Tiss. Res., *210*: 47-56.

LEGENDS OF FIGURES

Figure (1): A schematic drawing of the principal ganglia in the central nervous system of sea hare, *Aplysia oculifera.*

Figure (2): A drawing of the principal nervous ganglia of sea hare, *Aplysia oculifera*, showing the distribution of different types of neurosecretory cells in these ganglia.

⬭ :Type A cell. ◯ : Type B cell. ● : Type C cell

Figure (3): A photomicrograph of T. S. of cerebral ganglion showing locations of neurosecretory cells and dorsal body. (Mallory's triple, X 100).

Figure (4): A photomicrograph of dorsal body enlargement showing NSCs and cell processes. (Mallory's triple, X 400)

Figure (5): Cell of B type in cerebral ganglion. (Mallory's triple, X 1000)

Figure (6): Cells of C type in cerebral ganglion. (Mallory's triple, X1000)

Figure (7): T. S. of buccal ganglia showing the distribution of different types of NSCs. (H&E, X 100)

Figure (8): Cell of A type showing irregular shaped nucleus. (Mallory's triple, X400)

Figure (9): A photomicrograph showing cell of B type in buccal ganglion. (Mallory's triple, X 1000)

Figure (10): Cells of C type in buccal ganglion. (Mallory's triple, X 1000)

Figure (11): A photomicrograph of T S of pedal ganglion masses showing the distribution of different types of NSCs. (Mallory's triple, X100)

Figure (12): A photomicrograph of T S of pedal ganglion masses near the connectives showing distribution of NSCs. (Mallory's triple, X100)

Figure (13): A photomicrograph of T S of pleural ganglion showing the distribution of different types of NSCs. (Mallory's triple, X100)

Figure (14): Enlargement of figure (13), showing B and C types of NSCs. (Mallory's triple stain, X400)

Figure (15): A photomicrograph of semi-thin section of A type cell in cerebral ganglion. (X 400)

Figure (16): A photomicrograph of semi-thin section of B and C cell types in pedal ganglion. (X1000)

Figure (17): Electron micrograph of A cell in cerebral ganglion. (X1500)

Figure (18): Electron micrograph of A cell in pedal ganglion. (X1500)

Figure (19 & 20): Enlarged parts of Figure (18), showing different organelles of the cell and secretory granules. (X 20000)

Figure (21): Electron micrograph of B cell in pedal ganglion. (X 2500)

Figure (22): Enlarged pert of the above micrograph, showing different organelles of the cell. (X 10000)

Figure (23): Electron micrograph of C cell in pedal ganglion. (X2500)

Figure (24): Electron micrograph of C cell in cerebral ganglion. (X5000)

Figure (25 & 26): Enlarged parts of Figure (24), showing different organelles of the cell, secretory granules and oil droplets. (X 30000 and 20000 respectively)

LIST OF ABBREVIATIONS

A: Type A cell
B: Type B cell
B cm: *Buccal commissure*
Bg: Buccal ganglion
C: Type C cell
CB cn: Cerebrobuccal connective
C cm: Cerebral commissure
Cg: Cerebral ganglion
CPd cn: Cerebropedal connective
CP: cell processes
CPl cn: *Cerebropleural connective*
DB: dorsal body
GB: Golgi body
M: mitochondrion
mvb: multivesicular body
N: nucleus
nsm: neurosecretory material
nu: neucleolus
od: *oil droplet*
Pd cm: Pedal commissure
Pd g: Pedal ganglion
Pl g: Pleural ganglion
Pn: perineurium
PPd cm: Parapedal commissure
rer: rough endoplasmic reticulum
sg: secretory granules

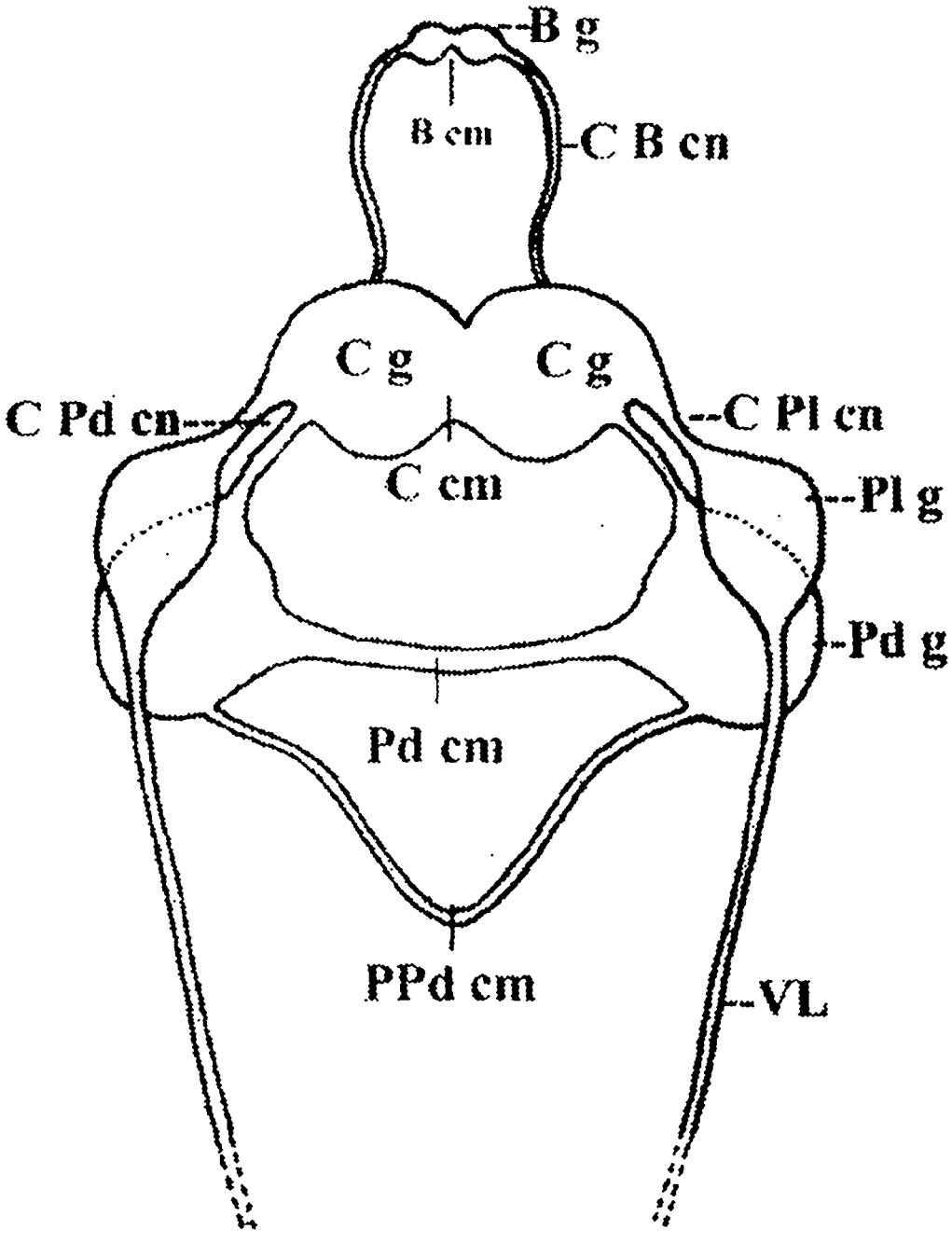


Figure (1)

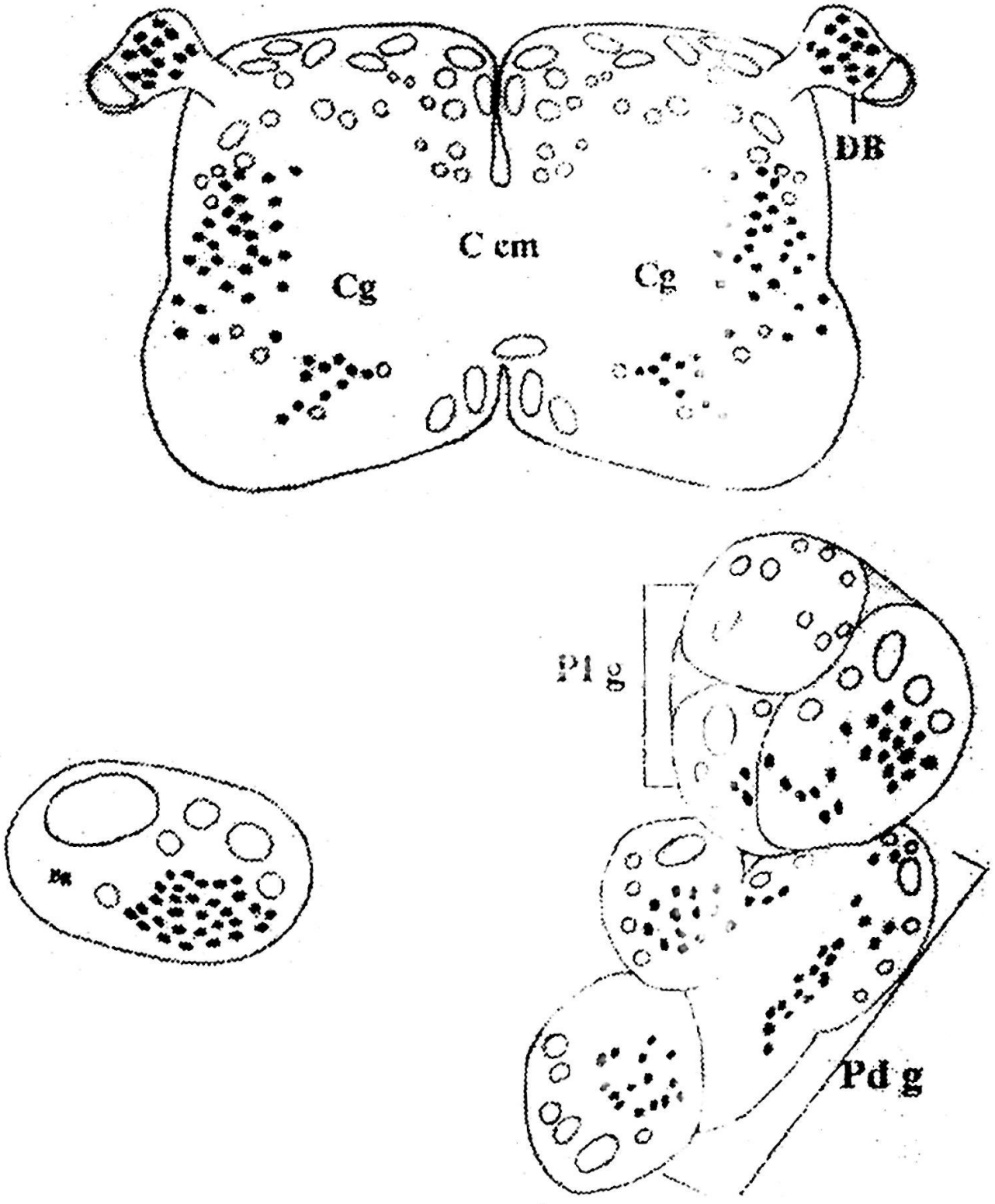
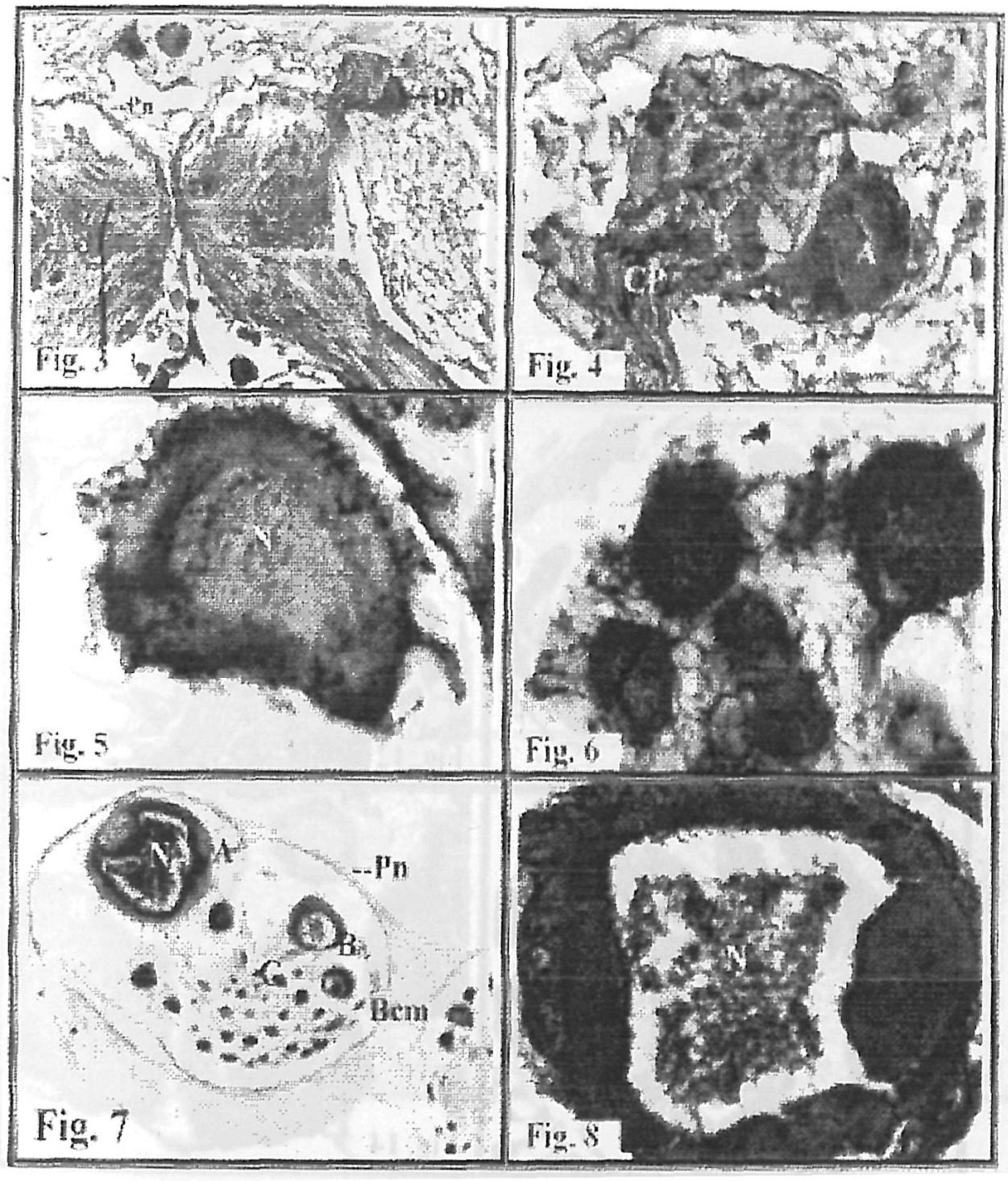


Figure (2)


--Pn
--DR
Fig. 3
A
Cp
Fig. 4
N
Fig. 5
Fig. 6
N
A
--Pn
Bm
C
Bcm
Fig. 7
N
Fig. 8

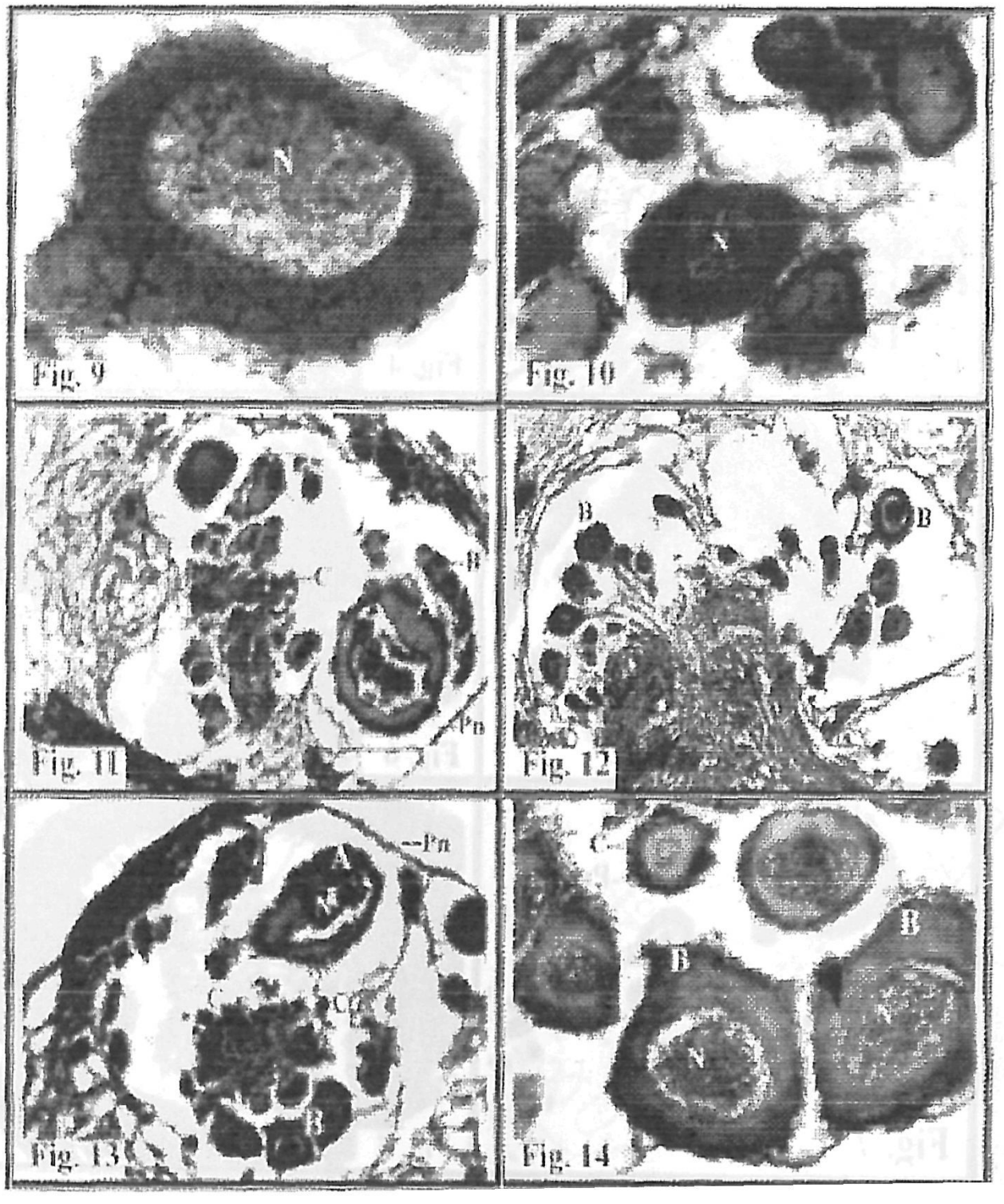


Fig. 9

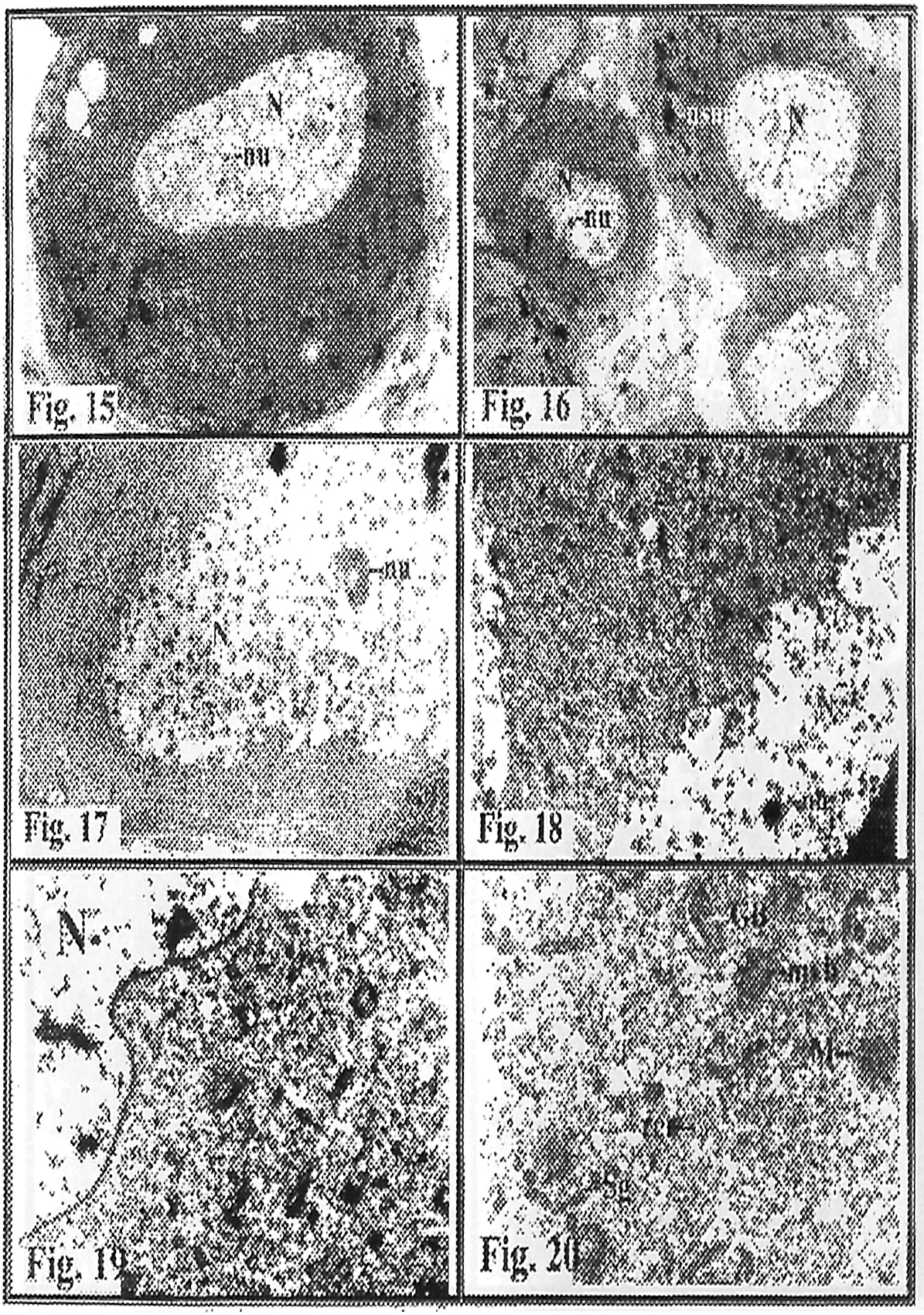
Fig. 10

Fig. 11

Fig. 12

Fig. 13

Fig. 14


N
Fig. 15
N
Fig. 16
N
Fig. 17
N
Fig. 18
N
Fig. 19
Fig. 20

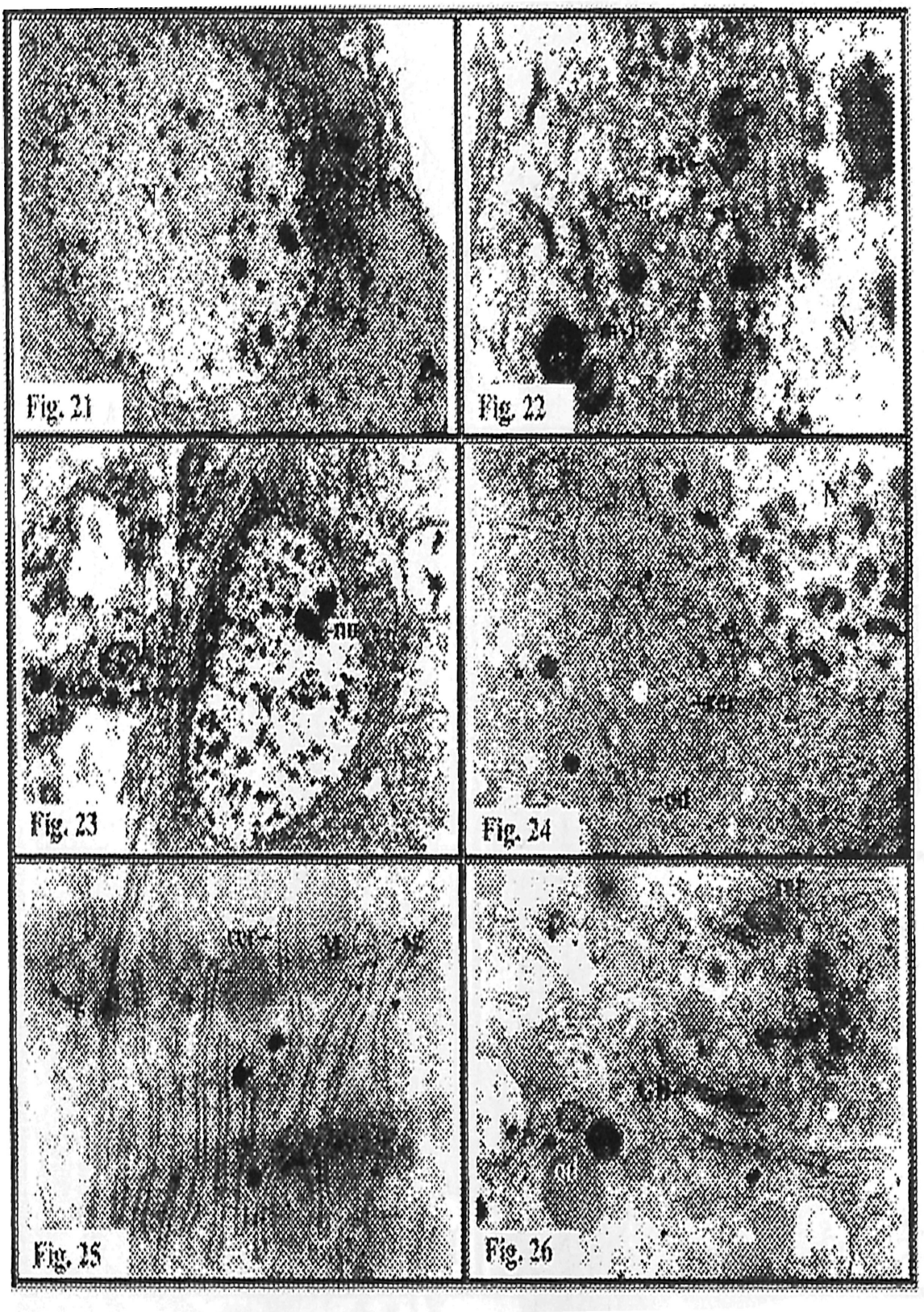


Fig. 21

Fig. 22

Fig. 23

Fig. 24

Fig. 25

Fig. 26