CHAPTER 3: 
NEUROSECRETION

3.1 INTRODUCTION:

The role of neurosecretion in the reproductive physiology of pulmonate is studied by number of workers. Evidence for the presence of neurosecretory cells in the mollusks was first given by Scharrer (1928) for opisthobranch snails. She observed secretary droplets in the neurosecretory cells of cerebral and visceral ganglia of *Aplysia limacine*. Later on Gabe (1966) went into more details and gave a more precise definition of neurosecretory cell is applied to those structures having a characteristics of neurons and also showing the morphological signs of glandular activity i.e. the intracellular synthesis and discharge of the histological detectable secretory products. The identification of neurosecretory cells, proposed by Bern (1962), is outline to certain principles which may provide criteria for the assessment of neurosecretory status of the animal concerned. He suggested that (I) neurons are possibly neurosecretory on the basis of presence of CHP and PF positive inclusions in their cytoplasm. (ii) they are probably neurosecretory on the basis of histophysiological evidences (iii) on the basis of provision of experimental evidence involving a rigorous demonstration that the elements, concerned are the source of vascular
hormones. Following Bern (1962), Knowles & Bern (1966), have broadened the definition of neurosecretion as “neurons may be considered neurosecretory if they are involved in endocrine regulation either by secretion of vascular hormones or by control of other endocrine organs” to enhance the outskirts of neurosecretion territory. Thus from the physiological viewpoint the neurosecretory cells are considered as connecting link between the nervous system and the endocrine system (Golding 1974).

In Gastropods, distinct neurohaemal organs are not present. Instead of that tracts of neurosecretory axons extend from perikarya situated in various ganglia and from axon terminals either under the perineurium of commissure, connectives and nerves associated with the central nervous system or in the connective tissue surrounding these structures. Release of NSM by exocytosis takes place in these areas (Wendelar Bonga, 1970a).

Simpson, et.al. 1966 and Durchon, 1967, reviewed that in stylommatophoran snails axons of neurosecretory cells situated in the cerebral ganglia, probably from neurohaemal areas associated with the intracerebral commissure, intercommissural nerves and nervi arteriae cerebralis. Groups of neurosecretory cells and their neurohaemal areas have
also been detected in the parietal ganglion of *Helix pomatia* (Sakharov and Salanki, 1971) and in a number of ganglia of the prosobranch *Bithynia tentaculata* (Andrews, 1968). These earlier reports are based on the staining properties of neurons. In most of the above studies the conventional neurosecretory stains such as Gomori’s chromalum-heamatoxylin-phloxin (CHP) and Gomori’s aldehyde fuchsin (AF) have been used for identification of neurosecretory cells. In the earlier studies on the pulmonate gastropods, the neurons have been called neurosecretory mainly on the basis of their stainability with CHP and AF (Krause, 1960 and Kuhlmann, 1963).

Although neurosecretion has been studied histologically in a number of pulmonates detailed maps of the neurosecretory system are available for only two species of basommatophoran Viz. *L.stagnalis* (Wendelaar Bonga, 1970) and *B. truncatus* (Boer et.al., 1971) and four species of stylommatophorans, viz. slugs *Arion hortensis* and *Derocheros reticulatum*, a land snail *Helix aspersa* (Wijdenes et.al., 1980), and a marine pulmonate, *Onchidium verruculatum* (Deshpande, 1980). The maps are primarily composed on the basis of staining results obtained with AB: AY staining technique for neurosecretion. Compared to the classical Gomori technique used in most other studies on neurosecretion in pulmonates a greater
differentiation of NSC was obtained through AB: AY. On the above mentioned basis 13 cells types were distinguished in *H. aspersa* 9 types in *D. reticulatum*, 11 types in *Arion hortensis* (Wijdenes *et al.*, 1980), while 6 types of NSC were observed in *O. Verruculatum* (Deshpande, 1980). In Basommatophora 7 types of NSC were observed in *L. stagnalis* (Wendelaar Bonga, 1970) and 11 types in *B. truncatus* (Boer *et al.*, 1980).

In recent years, the most appreciative work on neurosecretory phenomenon of the molluscs is carried out by using a new conventional staining techniques of Alcian blue /Alcian yellow (AB/AY) (Wendelaar Bonga, 1970). This technique is used to distinguish a great number of cell types on the basis of the different reactions of the neurosecretory material to these dyes; the colours varying from bright green via yellow green to yellow. The cell types identified in this way appears to have different types of elementary granules. Transport of these granules to neurohaemal areas and release phenomenon give further indication of their neurosecretory nature. Using AB/AY staining technique, seven types of neurosecretory cells (NSC) have been described in *Lymnaea stagnalis* (Boer *et al.*, 1977) ten types of NSC in *B. truncatus* and three types of NSC in *Helix aspersa*; (Kaikai and Kerkut, 1979), Pelluet and Lane (1961) described the relation
between neurosecretion and cell differentiation in the *Macrochlamys jucunda*.

Boer et al. (1977) while Comparing the neurosecretory system of the Stylommatophora with those of the Basommatophora in *L. Stagnalis* and *B. truncatus* respectively are of the opinion that in the main similarity in these appears to be the occurrence of groups of green staining cells in the dorsal and latero dorsal areas of cerebral ganglia. Further they opined that differences between the neurosecretory systems of species of the two pulmonate orders are: (1) NSC only occur in the buccal ganglia of the Stylommatophora, (2) a group of phloxinophilic cells occurring in the cerebral ganglia of the Basommatophora for *L. stagnalis* it has been established that they produce the ovulation hormone (Geraerts and Bohlken 1976) are not consistently present in the Stylommatophora (in a number of *Helix aspersa* phloxinophilic cells were, however, found in the cerebral ganglia), (3) the NSC of the ganglia of the visceral ring in Basommatophora generally occurs in distinct cell groups, in Stylommatophora they are scattered. In Basommatophora the neurohaemal areas of NSC are locked in the peripheries of commissures, connectives and nerves (Boer and Joosse, 1975). The location of neurohaemal areas in Stylommatophora is largely unknown. In a number of species, including *H.*
aspersa, axon tracts from the metacerebral cells have been followed into arteria cerebralis and into the intercerebral nerves which terminate in the connective tissue dorsal to the cerebral ganglia (Kuhlmann, 1963 and Nolte 1965, 1978).

Boer and Joosse (1975) have described the criteria for the identification of neurosecretory cells. According to above authors a neuron may be called a neurosecretory cell on the basis of (1) histochemical evidence suggesting protinacious nature of the secretory products as has been reported in the case of a number of pulmonates (Boer and Joosse 1975), (2) on the basis of ultrastructural observations of neurosecretory cells and neurosecretory granules, (3) on the basis of axonal pathways and neurohamal sites (where secretory terminals of neurosecretory cells release their secretory products). Thus a complete neurosecretory apparatus of gastropod in general comprises of a neurosecretory cell with neuronal and glandular morphological characters, neurosecretory material, and a release site for neurosecretory products and a target where it acts.

A number of publications have appeared on the location of neurosecretory system and cells in Stylommatophora. According to Boer’s (1965) observations on the mesocerebral neurosecretory cells of the Stylommatophora are similar to the most of the neurons in other
pulmonates and are relatively large in size. Kuhlmann (1963) in addition to
the mesocerebral cells, reported two other groups of cells in the central and
dorsal parts of the mesocerebrum of the family Helicidae. Additionally,
neurosecretory cells have also been observed in other central ganglia of
Stylommatophora. Cook (1966) has reported Gomori positive and Gomori
negative cells and their release sites in the visceral ganglia of Succinea
putris.

In pulmonates, number of organs of endocrine origin have
been reported. In all investigated pulmonates, the medio-dorsal bodies are
mesodermal in origin attached to the medio-dorsal region of cerebral
ganglia have been reported as endocrine structures. The work of Geraerts
(1975) on L. Stagnalis and that of Wijdenes and Runham (1976) on
Agriolimax reticulatus have confirmed the endocrine role of the dorsal
bodies. Similarly presence of follicular gland (a remnant of distal part of
cerebral tube) as an endocrine gland have been reported to present in adult
basommatophorans and at least in embryonic stages in stylommatophorans

The right optic tentacle promotes morphogenesis of the male
tract and induces regression of the female tract. The effectiveness of the
tentacle increase during maturation of the male phase and declines during
the subsequent female phase. Pelluet and Lane (1961) suggested that a hormone from the optic tentacles acts on the ovotestis of *Arion* and *Limax* species, promoting spermatogenesis at the expense of oogenesis. Meenakshi and Scheer (1969) who demonstrated an inhibitory influence of the tentacle on the growth and synthetic activity of albumen gland of *Agriolimax columbianus*. In contrast Renzoni (1969) and Kuhlmann and Nolte (1967) were able to detect no effect of tentacle removal on egg production and ovotestis weight in *Vaginulus borellianus*, nor on spermatogenesis in *H. pomatia* respectively. Furthermore, the results of Gottfried and Dorfman (1969 and 1970) suggest that the tentacles exert an inhibitory influence on spermatogenesis; their removal provokes precocious testicular maturation in *A. californicus*.

The optic tentacles of land snails and slugs produce a hormone, the male tentacular factor (MTF) during the male phase. It stimulates sperm production, and inhibits the differentiation of the eggs and female accessory sex organs such as the albumen gland and egg laying. MTF is probably produced by the collar cells. The collar cells of the optic tentacles of stylommatophoran have been regarded as secretory neurons (Lane, 1964)/ or as special endocrine cells (Bierbauer and Vighteichmann, 1970). According to Lane (1964) each cell is bipolar in form, and gives rise
to one process directed to the epidermis and another which enters the
tentacular ganglion. However the hormonal role of optic tentacle is
controversial, not least because tentacle removal causes severe behavioral
disturbances, which may disrupt endocrine activity.

The information about the endocrine influence on the
spermatogenesis is fragmentary. In, *H. aspersa* the cerebral androgenic
factor not only favours differentiation of male cells but appears to be
essential throughout the spermatogenesis at list ‘in vitro’. Contrastingly,
all spermatogenic stages of *Arion ater* survived in a hormone devoid
medium (Badino, 1967). Sukumaran and Sriramulu (1977) have proposed a
tropic (hormonal) influence of optic tentacles on spermatogenesis in the
working on the aspect of neurosecretion have demonstrated various types
of neurosecretory cells in the central ganglia of the snail *H. aspersa*.

Literature regarding the annual cycle of neurosecretory cell
activity of gastropods are very less. Gabe (1954) reported annual cycle in
the secretory activity of neurosecretory cells in opisthobranchs and
pulmonates. Investigations were made on the seasonal variation in the
number of neurosecretory cells in the central nervous system of the
prosobranch, *Vivipara vivipara* ( Gorf, 1961,b ) and in the freshwater pulmonate, *H. tenue* ( Simpson et al., 1966 a,b ).

Very recently, studies on neurohormonal regulation of reproduction process in Stylommatophora were studied by Grygon *et. al.*, (1993), and showed that the effect of hormones and environmental factors on the gametogenesis process in the hermaphrodite gland Ohtake *et.al.* (1994) examined the dorsal bodies of giant African snail *Achatina fulica*, and suggested that each cell of the dorsal bodies is under the control of two axons from the cerebral ganglion: one that is stimulatory and another that may be inhibitory. Neuroseeretory activity in relation to reproduction was observed by Magre and Kulkarni (1993) in *Cerastus moussonianus*. During Seasonal cycle, changes in the number of neurosecretory cell types and their diameter were observed and they conclude that N.S. material is found to be accumulated during the growth of the gonads where as, it decreased during spent phase.

With this brief summary of the neurosecretory phenomenon in the phylum mollusca, the present chapter is framed to study in detail some aspect of neurosecretory system of the stylommatophoran land snail, *Macrochlamys petrosa*. The present investigation was undertaken-

i) to describe morphology of central nervous system.
ii) to study histology of different ganglia and associated endocrine glands.

iii) to see the possible role of the cerebral neurosecretory cells activity and dorsal body cell activity with respect to the reproductive cycle.

iv) to observe optic tentacular neurosecretion and its involvement in regulation of reproduction.

3.2 MATERIAL AND METHODS:

*Macrochlamys petrosa* were collected from the different localities in and around the city, Aurangabad, Maharashtra, India, during different seasons from 1999 to 2000. Normal sized, (1.5-1.7 cm shell diameter) healthy animals were dissected and their control nervous system and optic tentacles were removed. In order to study morphology of central nervous system, entire intact central ganglia in the form of circum-esophageal ring, was removed and was preserved in either 70% alcohol or 4% aqueous formalin solution observations were made under binocular dissecting microscope or using magnifying lens for histomorphological studies. The tissues were fixed in Bouins fluid (aqueous) and/or Stieve’s
sublimate for 24 hours. The tissues which were fixed in Stieve’s fixative were post, treated with iodine alcohol in order to remove mercury from the tissues. After fixation tissues were subjected to normal dehydration, process. Later on cleared in xylol and embedded in 56 to 58c melting point paraffin ware. Serial sections were cut at 4 to 5 μ in thickness and were stained with following conventional differential staining methods for neurosecretion.

(1) Mallory’s Triple stain (Mallory, 1944).
(2) Paraldehyde fuchsine (PAF) (Ewen, 1962).

**Morphology of central nervous system.**

In order to study, morphological features of neuroendocrine system, of M. petrosa, the entire nervous system (central ganglia) along with buccal ganglia were dissected out from the snail body. The freshly dissected central ganglia were taken out and transferred to cavity slide, with few drops of Molluscan Ringer (physiological saline) solution. Morphological features of each ganglion with nerve innervations were noted down, while observing the system (circumoesophageal ring) under binocular dissecting microscope.

**Histology of Central nervous system:**
Microscopical observation were made to identify different types of neurosecretory cells in every ganglion of the central nervous system cell and nuclear diameters of the identified neurosecretory cells were measured using ocular micrometer.

**Seasonal changes in neurosecretory activity:**

Changes in neurosecretory cell activity in cerebral ganglion of *M. petrosa* during different seasons of the year was recorded by the criteria as described by Boer and Joosse (1975) (morphometrical karyometric changes and changes in staining intensity within cell perikaryon). The changes in different neurosecretory cells within cerebral ganglion together with dorsal body cells activity during different reproductive activity periods was observed and their probable role in the regulation of reproduction if any was tried to correlate with the monthly changes in gonad cytology.

**Histomorphology of optic tentacle:**

Optic tentacles after getting processed for neurosecretory studies, observations were made under microscope, serial sections after getting stained with conventional staining methods mentioned earlier.

Statistical methods:
Wherever is possible different statistical methods were applied to calculate mean cell-nuclear diameters with standard deviation of the data collected.

3.3 OBSERVATIONS AND RESULTS:

3.3.1 Morphology of central Nervous system:

The neurosecretory cells are present in all central ganglia of circum-oesophageal ring. The circum oesophageal ring is located just beneath at the buccal mass surrounding the anterior oesophageal part of the oesophagus. When observed carefully, it is consisted of two rings. (1) Dorsal to the oesophagus supra oesophageal ring having made up of paired buccal and cerebral ganglia. (2) Ventrally sub oesophageal ring having made up of paired pedal, pleural, parietal ganglia and single visceral ganglion.

In all, the entire circumoesophageal ring is having total 11 central ganglia. (plate 13-Fir A,B).

Supra-oesophageal ring:

Supra-oesophageal ring is composed of two paired, buccal cerebral ganglia. The buccal ganglia are very small ova in shape and located at the base of buccal mass. These are not placed faraway from each other. The buccal commissure joins the two ganglia. There major nerves are given off
from each ganglion at they interior side and innervates various parts of the buccal mass. At the posterior region each ganglion remains attached to cerebral ganglia by two stout cerebro-buccal connectives, which give rise branches.

Cerebral ganglia are oblong in shape, located overlapping to pedal ganglia. These ganglia remain attached with pedal on either side of each ganglia by two stout cerebropedal connective. In the posterior region at the commissure part two cerebral ganglia are held together by thick nervous tissue, called perineurium. At the anterior region, from procerebrum part of cerebral ganglion various nerves are given off such as tentacular, optic, median lip, oral lobe small tentacles and dorsal and lateral sides of buccal cavity. The cerebral commissure is not distinct, due to thick nervous covering. Associated with this region are two stout elevations of mass of tissue motired, are called as medio-dorsal bodies (Lever, 1957).

**Sub-oesophagcal ring :**

The second half of the circum-oesophagcal ring is referred as sub-oesophagcal ring situated ventral to the oesophagus and remains attached to the supra-oesophagcal ring by cerebropedal and cerebropleural connections. Compared with other ganglia, pedal ganglia are larger in size. Two pedal ganglia are repeated at anterior and posterior regions. Pedal ganglia are
broader at posterior conical apical shape at anterior region two nerves are
given from antero-lateral and three nerves are given off from postero-
lateral, region of each pedal ganglion. A single stout nerve innervates two
foot region is given off from the posterior part of the pedal ganglion. A
small nerve from statocyst, is given off and joins to cerebral ganglion.
Pedal ganglion remains attached to pleural ganglion in the posterior region
by pleuro-pedal connectives on either side of the ring. A statocyst is
present in the postero-dorsal part of the pedal ganglion. Pleural ganglion of
either side is of same size and nerves are not given off from pleural
ganglia. Pleural ganglia are attached to parietal ganglia on both sides of the
ring by pleuro-parietal connectives. Right pleural ganglion is larger than
left one. Left parietal ganglion is similar is size of pleural ganglion from
the posterior region of each parietal ganglion, nerves are given off.
Unpaired visceral ganglion remains attached to left and right parietal
ganglia central in position in the posterior part of, the sub-oesophageal
ring. Visceral ganglion is larger in size, except pedal ganglion. Nerves are
given off from posterior region of visceral ganglion, which innervates to
various visceral organs of the snail (see figure).

3.3.2 Histology of Central Nervous system:

Cerebral Ganglion:
Longitudinal sagital section of cerebral ganglion of *M. petrosa* shows there distinct groups of neurosecretory cells, topographically (1) medio-dorsal cells (MDC), (2) caudo-dorsal cells (CDC) and (3) Laterodorsal cells. Neurosecretory cells from medio-dorsal group are large in size compared with other groups of the cerebral ganglion. From the point of cytomorphological characteristic features, each groups is having two distinct types, type ‘A’ and type ‘B’ neurosecretory cells. The characteristic features of these cell types are shown in table 5.

Neurosecretory cells type ‘A’ are large sized, with oval to oblong in shape of the cells body. Their cell and nuclear diameters measure 75 to 83 u and 62 to 70 u respectively. Nucleus is polymorphic, round, oblong, oval or some times kidney shaped (see plate 15 fig. A) ‘A’ type of Neurosecretory cells of MDC. Group is largest in size compared to the cells from other two groups. The cytoplasm material is purple in colour when stained with PAF stain. The number of these cells is less in each group compared with other type ‘B’ neurosecretory cells. The cell perikaryon stains deep blue in colour when stained with Mallory’s Tripple stain.

Neurosecretory cell type ‘B’ from each group of NSC from cerebral ganglion are small in size and more in number compared with type ‘A’ neurosecretory cells in all the three groups. These cells are with
rounded cell body with polymorphic nucleus similar to that of ‘A’ cells. Their cell and nuclear diameters measure 42 to 55u and 30 to 37u respectively. Neurosecretory material is in the form of flakes and is violet in colour with PAT stain. When stained with Mallory’s Tripple stain, their cytoplasmic inclusions stain reddish in, their colour. These cells send their axonal tracts right angles to neuropilar region of each ganglion.

**Medio-dorsal bodies:**

Associated with the cerebral commissure, this bulbous, rounded bodies are present. These are called as medio-dorsal bodies. (M.D.B.). MDB are loosely attached with the cerebral commissure and are non-neural endocrine glands are secretory in nature. The secretory cells of MDB are simple, epithelial type with thin cytoplasmic region. Two different zones can be identified Histologically, outer cellular area and inner cell processes are of the M.D.B.( see plate 16).

**Buccal ganglia:**

Longitudinal sagittal section of buccal ganglion shows, presence of neurosecretory cells throughout entire peripheral region of each ganglion. Two types of cells can be described as mentioned earlier, central core part of each ganglion is referred as neuropile (see plate12 figA).

**Histology of Sub-oesophageal ring:**
Supra-oesophagal ring is composed of paired pedal. Pleural, parietal and single visceral ganglion (see plate 13 fig.A). Sagittal longitudinal section passing through each ganglion, show, presence of neurosecretory neurons throughout peripheral regions of each type of ganglion. Neurosecretory cells are more numerous in pedal and visceral ganglion. The neurosecretory cells types of sub-oesophaeal ring, ganglia can be categorized into two types as described for cerebral ganglion neurosecretory cell type ‘A’ and ‘B’. The cytoarchitectural features of these cell type are similar as described earlier.

Neurosecretory cell type ‘A’ are more numerous in anterior region of each pedal ganglion. In the posterior region type ‘B’ cells are more numerous. (see plate 13 fig. C). At the posterior extremity of each ganglion, there is presence of statocyst dorsally. Neurosecretory cells and pleural ganglion are less in number and are of same type since the right parietal ganglion is larger in size, the number of neurosecretory cells in this ganglion are more than left one. Type ‘A’ cells are less numerous in left parietal ganglion. Both the, cell types of cells are present in visceral ganglion of *M. petrosa*.

**Neurohaemal Areas:**
Peripheral part of the cerebral commissure at the juncture of medio dorsal body shows similar type of stain like neurosecretory material, may probably the storage site of neurosecretory material released from medio dorsal cells of cerebral ganglion. The other such type of areas are noticed in the peripheral regions of medium lip nerve of cerebral and visceral nerve indicative of neurohaemal areas of these centre.

3.3.3 Optic tentacular Neurosecretion:

The histological picture of optic tentacle of M. petrosa, shows presence of an eye or optic bulb located at the distal end of the tentacle just below the dermomuscular sheath. There is presence of distinct bulbous tentacular ganglion. The dermomuscular sheath consists of outer epithelial layer and inner to this is muscular wall. The epithelial layer is covered externally by cuticle. From the tentacular ganglion originates tentacular nerve which is stout and connects to the procerebrum part of the cerebral ganglion parallel to this is a minute nerve, originates from the eye, called as optic nerve also joins the cerebral ganglion (see plate18fig.A).

From the tentacular ganglion, there gives rise finger like nerve processes at its lip. Surrounding to these, nerve, processes tentacular ganglion are collar cells. These cells are large and secretory in nature. These collar cells differ from ordinary neurons in size, but also in their
cytoplasmic inclusions since, these cells are glandular in nature regarded as neurosecretory neurons. Depending upon their cytomorphological features, collar cells can be divided into three categories: Lateral processed ‘A’ and ‘B’ cells which are round to oval in shape with distinct nuclei and ganglion cells embedded within the wall.

3.3.4 Cerebral neurosecretory cell activity:

Topographically, the cerebral ganglion of *M. petrosa* is having three distinct groups of neurosecretory cells. Viz. mediodorsal cells, latero-dorsal cells and caudo-dorsal cells present in the posterior caudal region of the ganglion. (see plate 12 fig. B). Associated with cerebral ganglia are non-nervous, epithelioid endocrine glands are called as mediodorsal bodies, referred as because of their medial in position over the cerebral commissure.

All the three types of cerebral neurosecretory cells along with dorsal body cells show cyclical changes in their synthesis and secretory activity have some correlations with reproductive activity of the snail, *M. petrosa*. MDC and LDC have similar pattern of their neurosecretory material synthesis, accumulation and release patterns. These cells show maximum neurosecretory material synthesis and release during the months June and July which is the pre reproductive period of the snail (see plate 17.) In these
months in the hermaphrodite gonad, there is increased gametogenic activity such as gonial cells are proliferated actively within the ovotesticular follicles. During these months gonadal follicles are filled with earlier phases of oogenesis and spermatogenesis is almost completed.

During peak breeding period (August-October) the process of Gametogenesis is simultaneous with vitellogenic and release of egg maximal in these months. The third group of N.S. cells (CDC) is more actively involved in synthesis and secretion of neurosecretory material, reveals possibility of their involment in spawning. The ovotesticular follicles are having, large number of vitellogenic ova during this period.

The secretory activity of medio-dorsal cells is also increased during vitellogenic phase of the gonadal maturation. The secretory cells of dorsal bodies are distinct with stainable material within their cytoplasm during peak breeding of the snail. At the juncture of M.D.B and axonal terminals of M.D. cells, in the region of cerebral commissure, there is accumulation of N.S. material indicates medio- dorsal body activity under the cerebral M.D. cells during vitellogenic.

3.3.5 Optic tentacular neurosecretory cell activity:

Optic tentacles of *M. petrosa* is having a distinct tentacular ganglion which joins the procerebrum part of cerebral ganglion with tentacular
nerve. Surrounding this ganglion is numerous neurosecretory collar cells’ are the only major secretory cells involved in the formation of optic tentacular hormones. The secretory cells of optic tentacle also show same specific patterns in their secretory activity during annual course of reproductive cycle of the snail. Since, optic tentacles bear optic bulb, at then lip having functional eye. The information about the photoperiodic rhythms is being transmitted in the form of photic cues /stimuli to the brain, through hormones. The secretory cells of optic tentacles are maximally active during late summer month i.e. May and early June. This is the period, during which peak spermatogonia activity takes place in the hermaphrodite gonad of these land snails. The process of spermatogenesis or male phase may be under the environmental photoperiodic control. May and June are the months with maximum photoperiodic day length. The photoperiodic information’s is transmitted trough optic tentacles via their hormones to cerebral neurosecretory cells particularly, latero-dorsal cells are more active during these months, are of male phase maturation of hermaphrodite gonad of M. petrosa. Optic tentacular cells show ceased secretory activity during post reproductive period ( Oct. to November) where the Spermatogenic activity within ovotestis is practically stopped. (see plate 18 ).
3.4 DISCUSSION:

The tendency of aggregation of central ganglia amongst molluscs is more in pulmonets. This may be due to the phenomenon of torsion in Mollusca. Because of this fact, in pulmonates, all central ganglia are present in the form of condensed mass of ring (circum-esophageal). According to Bergmann (1930), the central chain of ganglia can be categorized into eight different types of which Veronicaellid type of nervous system is unique one. The arrangement of central ganglia in the form of circumoesophageal ring, in *M. petrosa* is of same type. Because all five type of different ganglia are fused to form a ring.

The cerebral ganglia of stylommatophoran snail *Macrochlamys petrosa* show two distinct lobes, each lobe is having three distinct region (i) procerebrum (ii) mesocerebrum and (iii) metacerebrum. These three region have been identified and described in other stylommatophoran slugs, like *Arion rufus* (Van Mol, 1967; Kerkut and Walker, 1975) and in *Onchidum verruculatum* (Deshpande, 1980). The presence of these divisions seems to
be ubiquitous in the order Pulmonata, however their position seems to vary according to the phylogenetic status of the animal.

Numerous studies of the central nervous system since that of Scharrer (scharrer and scharrer, 1937) have resulted in the description of cells of possible neurosecretory function in all major molluscan groups (Gabe, 1966 and Durchon, 1967). The phenomena of neurosecretion has been studied in a number of pulmonates (Boer and Joosse, 1975), mapping of neurosecretory cells in detail are available for only two species of basommatophorans VIZ. *L. staganalis* (Wendelaar Bonga, 1970) and *Bulinus truncatus* (Boer et al, 1971) and four species of stylommatophoran Viz. slugs, *Arion hortensis* and *Deroceros reticulatum* a snail *Helix aspersa* (Wijdenes et al. 1980). And a marine slug (Pulmonata) *Onchidium verruculatum* (Deshpande, 1980). The neurosecretory cell mapping primary composed on the basis of staining results obtained by AB/AY staining techniques for neurosecretion. Compared to the classical Gomori technique used in most other studies on neurosecretion in pulmonates, a greater differentiation of N.S.C. was obtained through AB/AY staining method with this method various neurosecretory cell type can be recognized within the class of ‘Gomori Positive’ cells. N.S. cells take up these stains and stains different shades of green and yellow colour
combinations, depending upon the chemical composition of their contents (Minnen et al., 1980). On the basis of differential staining affinity, 13 cell types were distinguished in *Helix aspersa* 9 types in *Deroceros reticulatum*, 11 in types in *Arion hortensis* (Wijdenes, et al., 1980). While 6 types of N.S.C. were noted in *Onchidium verruculatum* (Deshpande, 1980). Amongst the Basommatophora, 7 types of NSC cells were observed in *L. stagnalis* (Wendelaar Bonga) and 11 types in *Bulinus truncatus* (Boer et al., 1980) including buccal ganglia. In M. petrosa all central ganglia show presence of paraldehyde fuchsin positive neurosecretory cells. In the present investigation AB/AY staining method is not being used to stain the neurosecretory cells. The neurosecretory cells of all the central ganglia stain purple to violet in colour to their cytoplasmic inclusions suggestive of proteinaceous nature of neurosecretory material rich in cysteine and cystaine amino acids. Depending upon location of neurosecretory cells within cerebral ganglia of the snail, three distinct groups of cells were identified by paraldehyde fuchsin staining method (see results). Though the neurosecretory cells can be classified on the basis of staining affinity, colour reaction with AB/AY and through their location with convention neurosecretory stains, however, their real status can only be assessed through two ways (1) through ultrastructural microscopic studies wherein
quantitative variations in the secretory granules can be carried out and (2) through classical cauterization and reimplantation techniques popular in endocrinological studies to assess their endocrinological status. Different cell types of qualifying above test are that of *L. stagnalis* VIZ. caudo-dorsal cells (CDC) and light green cells (LGC) of the cerebral ganglia (Geraerts, 1975 and Geraerts and Bohlken, 1976). These two cell types, can very well be given the status of systems’ as they have all the three essential components to fulfill the requirement of a ‘system’ i.e. neuro-endocrine organ, neurohaemal areas and target organs. These systems can thus serve as model system for further endocrinological studies such as neuroethology, neuropharmacology etc. The neuroendocrine aspect is of great importance as the pulmonate nervous system has several advantages such as giant size of neurons compared to the rest of the animal kingdom, simplicity of structure and complexity of function and easy availability (Joosse et al., 1982).

The present investigation on neuroendocrinological aspect of *M. petrosa* cerebral ganglion, fulfills the criteria of neuroendocrine organ. When stained with paraldehydefuchsin stain all the three different groups pick up the stain and show characteristic features of glandular cells. Their cytoplasmic inclusions show synthesis and storage of secretory material.
Their axons traverse towards central neuropile area, where their axon terminals are in close contact with blood vessels, enables them to direct release of secretory material within circulating body fluid. Neurosecretory material is detected in their axons, suggestive axonal transports of materials. Similar type of colour reaction is also observed in the neuropilar region of the ganglion, may also act as possible neurohaemal organ.

Bern (1962) laid down certain principles which provide criteria for assessment of neurosecretory status of the animal concerned. He suggested that (1) neurons are possibly neurosecretory on the basis of presence of chromalumhaematoxylin phloxin (CHP) and paraldehyde fuchsin (AF) positive inclusions in their perikaryon, (2) they are probably neurosecretory on the basis of histophysiological evidence (3) on the basis of the provision of experimental evidence involving the clear cut demonstration that the elements concerned are the source of vascular hormones.

The endocrine significance has been imputed to a number of structures of glandular appearance ‘Dorsal bodies’ (Joosse, 1972) or equivalent structures (Organes juxta ganglionaire medio-dorsal bodies) have been reported in representatives of all gastropod molluscs. (Joosse and Geraerts, 1983). In the present land snail, *M. petrosa*, there presence of paired such dorsal bodies associated with cerebral commissure of the snail
deeply embedded in the connective tissue and can not be easily observed externally. Kuhlman (1966) reported, medio-dorsal bodies in stylommatophoran snails and slugs. They are less discrete in form and are embedded in the connective tissue which surrounded the cerebral ganglia. In these snails dorsal bodies are separated from cerebral ganglia by perineural tissue. M.D. Bodies Histologically shows, epitheloid secretory cells from outer side and cell processes towards the attachment portion to commissure. These cell processes may end in close association with new secretory axons coming from medio-dorsal cell group which are adjacent to dorsal bodies. Similar type of observations has been made in various pulmonate species. Dorsal bodies are separated from the cerebral ganglia by the perineurium and are said to lack vascularization and innervation (Boer, et al., 1968 and Simpson, 1969), although small numbers of neusosecretory fibres may penetrate into the connective tissue separating the gland cell clusters (Wndelaar Bonga, 1970a) and other data suggest that gland cell processes may penetrate into the nervous system (Gubicza and Rozsa, 1971).

The optic tentacles of land snails and slugs produce a hormone, the made tentacular factor (MTF) during the male phase. The collar cells’ of the optic tentacles of stylommatophorans have been regarded as secretory
neurons in *Helix aspersa* (Lane 1964) or special endocrine cells (Bierbuer and Vigh Tiechmann, 1970) According to Lane each cell is bipolar in form and gives rise to one process directed to epidermis and another enters the tentacular ganglion. Similar types of collar cells are present surrounding to tentacular ganglion of *M. petrosa*. However, recent studies show that the collar cells and nearly lateral processed cells contain secretory droplets 2-3 μ size. The lateral processed cells are large in size and possess secretory droplets in their cytoplasm.

Observations relating to endocrine mechanism controlling the reproductive biology of mesogastropod molluscs show reasonable harmony, but the mechanisms differ markedly from those of the archaeogastropods. Genital tracts of the protandric hermaphrodites *Calyptraea sinenisis* and *Crepidula fornicala* exhibit great stability when maintained in an hormonal environment (Streiff, 1970 a,b). The optic tentacle promotes morphogenesis of the male tract and includes regression of the female tract. The effectiveness of the tentacular increases during maturation of the male phase and decline during the subsequent female phase maturation. The optic tentacular cells are more active during summer months i.e. May and June suggestive of their involvement in regulation of spermatogenic activity during male phase maturation of *M. petrosa*. 
There are conflicting and apparently non convincing observations relating to the control of Gametogenesis in pulmonates. Pelluet and Lane suggested that a hormone from the optic tentacles acts on the ovotestis of *Arion* and *Limax* species, promoting spermatogenesis at the expense of oogenesis. A second hormone originating from the cerebral ganglion stimulated egg production. So that control of gonads depends on the balance of the two types of principles. Removal of optic tentacles in *M. petrosa* caused induced maturation of ova within hermaphrodite follicles suggests that tentaclectomy cause withdrawal of male phase inducing factor.

Using in vitro methods, Guyard (1967,70) and Gomot (1970) have shown that the ovotestis of *H. aspersa* is controlled by two hormones originating from the cerebral ganglia. The ganglia secrete one hormone during the early stages of testicular development which promotes spermatogenesis but inhibits ovarian differentiation. Another hormone is produced by the ganglia during the female dominated later stages of development, and induces rapid degeneration of male elements and differentiation of young oocytes. In the present snail the male phase maturation stats during late summer month’s and continues up to arrival of monsoons i.e. up to June. During this period tentacular as well as, lateral
neurosecretory cells in cerebral ganglia are more active, may these principles are involved in male phase maturation of *M. petrosa*. Then there starts peak reproductive period of snail, during which vitellogenic activity is found enhanced in the months August-September. Tentacular secretory activity is ceased with instruction of medio-dorsal cell activity in association with medio-dorsal cell group within cerebral ganglia. This is the period of female phase, which is under the control of dorsal body cells regulate vitellogenic process within developing ova, under the influence of medio-dorsal cells, regulates the maturation of ova.

During this season a third group of neurosecretory cells caudo-dorsal cells are involved in the secretion and releases of neurosecretory material coincides the spawning/egg production and release in *M. petrosa*. Gomot and Gugard,(1964) specified that the hormones are necessary, not only for the proliferation and differentiation of male and female gametocytes but also for the further development of primary spermatocytes (involving meiosis) and for further oocytes growth and release by the snail. *H. aspersa*. Similar type of hormonal mechanism may be involved in the regulation gametogenic process of *M. petrosa* during annual course of reproductive cycle.