STRUCTURE AND DEVELOPMENT OF NERVE GANGLIA, PERIPHERAL NERVOUS SYSTEM AND SPECIAL SENSORY ORGANS IN HALIOTIS ASININA LINNAEUS

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This study was undertaken to investigate the histology, classes of cells in the nerve ganglia of adult *H. asinina* and their development, and the morphology and histology of the peripheral nervous system and the special sensory organs. The histology and the development of cerebral, pleuropedal and visceral ganglia during preadult stages of this abalone were studied by LM, using H&E, CH-P and PF stains. In late juvenile and adult ganglia, there are ten types of cells, consisting of three types of neurosecretory cells (NS_{1-3}), four types of neurons (NR_{1-4}), and three types of neuroglia (NG_{1-3}). These cells first appear in 1-month-old abalone in cerebral and pleuropedal ganglia, and their numbers and sizes increase with advancing ages. In the cerebral ganglia; NS, NR_1 are significantly increased in 5-and 10-month-old abalone, reaching maximum number in 12 months, and thereafter remaining constant. In the pleuropedal ganglia, NS and NR_1 are significantly increased in 4-and 7-month-old abalone, reaching maximum number in 11 months, and thereafter remaining constant. In the visceral ganglia, NS and NR_1 first appearing 2-month-old and significantly increase in 4-month-old abalone, reaching maximum number in 11-month-old abalone, and thereafter remaining constant. NR_2, NR_3, NR_4 and NG are present in all ganglia early in development from 1-month onwards, and their numbers increase rapidly with advancing age.

There are five groups of peripheral nerves from the central nervous system that supply the body, including the special sensory organs. The special sensory organs consist of a pair of cephalic tentacles, a pair of eyes, a pair of appendage tentacles, numerous epipodial tentacles and the osphradium. The cephalic and epipodial tentacles are similar in structure, but the latter are three times smaller and ten times shorter than cephalic tentacles. Using surface characteristics as observed by LM and SEM, each tentacle is divided into three parts: the basal part exhibits flat surface consisting of small folds and grooves; the middle part has short papillae; and the top part has very high papillae. In cross sections, there is a bundle of nerve which runs along the length of each tentacle, and its branches are distributed among the muscle, and the epithelium which is covered externally by microvilli. The epithelium of the tentacle's base is columnar type and its surface appears flat, with some areas exhibit small curve. At the middle part, the epithelial surface appears like hillocks, and at the top part the epithelium appears in cone-shaped structures. The hillocks of the middle part and the cones at the top are believed to be sensory papillae. In each papilla there are cilia extending out from the top, and there are three types of epithelial cells: the ciliated sensory cells, supporting cells and the goblet cells. The eye is an open vesicle which appears spherical in cross section, with its lens surrounded by the retina. The retina is composed of six layers: pigmented layer, pigmented cell layer, fibrous layer, receptor cell layer, loose connective tissue layer, and a layer of optic nerve, respectively. The receptor cells in the retina can be classified into three types; rc_1, rc_2 and rc_3. Appendage tentacle has a half-circle shape and is covered by numerous irregular folds and grooves. In cross sections, it reveals many muscle cells and accompanying nerve fibers that form the core materials surrounded by an epithelium. The epithelium is simple columnar that lies on a thick basement membrane. Three types of cells can be identified in the epithelium, *i.e.*, ciliated sensory cells, supporting cells and mucous secreting cells. The osphradium exhibits many tufts of rod-shaped cilia, paddle-like cilia, granules exocytosed from the pores on the surface of leaves and leaflets. In transverse sections, each of the leaf and leaflet can be divided into two areas: the basal area which consists mainly of two types of large goblet cells, and the apical area that contains a mixture of supporting cells, sensory cells and five types of mucous secreting cells. This study will apply to increase the number of abalone.

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การศึกษาลักษณะทางเนื้อเยื่อ การจำแนกชนิดลักษณะของเซลล์ในปมประสาทของหอยเป๋าฮื้อตัวเต็มวัย และการศึกษาการพัฒนาของปมประสาท cerebral, pleuropedal และvisceral โดยเทคนิคจุลทรรศน์ด้วยการย้อมสีพิเศษแสดงเซลล์ 10 ชนิด ได้แก่ เซลล์ประสาท 4 ชนิด เซลล์ประสาทผลิตฮอร์โมน 3 ชนิด และเซลล์พี่เลี้ยง 3 ชนิด เซลล์ทั้ง 10 ชนิดเริ่มปรากฏตั้งแต่เดือนแรกในปมประสาท cerebral และ pleuropedal และมีจำนวนมากขึ้นตามอายุ ในปมประสาท cerebral เซลล์ประสาทผลิตฮอร์โมนและเซลล์ประสาทชนิดที่ 1 มีจำนวนเพิ่มมากในเดือน 5 และ 10 จนมีจำนวนมากที่สุดและเริ่มคงที่ในเดือน 12 ในปมประสาท pleuropedal มีเซลล์ประสาทผลิตฮอร์โมนและเซลล์ประสาทชนิดที่ 1 เพิ่มมากในเดือน 4 และ 7 และเพิ่มขึ้นสูงสุดและมีจำนวนคงที่ในเดือน 11 ปมประสาท visceral มีเซลล์ประสาทผลิตฮอร์โมนและเซลล์ประสาทชนิดที่ 1 เริ่มปรากฏในเดือนที่ 2 และ เพิ่มมากในเดือนที่ 4 และเริ่มมีจำนวนคงที่ในเดือนที่ 11 เซลล์ประสาทชนิดที่ 2, 3, 4 และ เซลล์พี่เลี้ยงในปมประสาททุกชนิดเริ่มปรากฏตั้งแต่เดือนที่ 1 และเพิ่มจำนวนขึ้นตามอายุ

เส้นประสาทจากระบบประสาทส่วนกลางที่ไปเลี้ยงร่างกายและอวัยวะรับสัมผัสพิเศษแบ่งได้เป็น 5 กลุ่ม อวัยวะรับสัมผัสพิเศษประกอบด้วย cephalic tentacle 1 คู่, ตา 1 คู่, appendage tentacle 1 คู่, epipodial tentacle จำนวนมากและ osphradium. Cephalic และ epipodial tentacles มีโครงสร้างที่คล้ายคลึงกันแต่ cephalic tentacle มีขนาดใหญ่กว่าประมาณ 3 เท่า และยาวกว่าประมาณ 10 เท่า จากการศึกษาโดยใช้กล้องจุลทรรศน์และกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด พบว่า tentacle แต่ละเส้นแบ่งออกได้เป็น 3 ส่วนที่มีลักษณะแตกต่างกัน ส่วนโคนซึ่งมีผิวค่อนข้างแบนและเรียบประกอบด้วยสันและร่องสลับกัน ส่วนกลางซึ่งมี papillae ลักษณะคล้ายเนิน และส่วนยอดซึ่งมี papillae รูปกรวยยาว ในภาคตัดขวางมีมัดของเส้นประสาทอยู่ตรงกลางตลอดความยาวของ tentacle และมีแขนงกระจายอยู่ระหว่างกล้ามเนื้อซึ่งมีเยื่อบุผิวปกคลุมและมีไมโครวิลไลหุ้มอยู่รอบนอก เยื่อบุผิวของ tentacle ส่วนโคนเป็นชนิด columnar ส่วนผิวมีลักษณะค่อนข้างเรียบมีบางช่วงเป็นสันโค้งขนาดเล็ก ที่ส่วนกลางของหนวดเยื่อบุผิวปรากฏเป็นเนินขนาดเล็กและที่ส่วนยอดเยื่อบุผิวมีลักษณะคล้ายกรวย เนินขนาดเล็กของส่วนกลางและกรวยที่ส่วนยอดอาจจะเป็น sensory papillae แต่ละ papillae มี cilia ยื่นออกมาจากยอดและมีเซลล์ 3 ชนิด ได้แก่ sensory cell, supporting cell และ goblet cell ตาซึ่งมีลักษณะเป็นถุงกลมมีรูเปิด เลนส์อยู่ตรงกลางและถูกล้อมรอบด้วยจอตาซึ่งประกอบด้วย 6 ชั้น ได้แก่ pigmented layer, pigmented cell layer, fibrous layer, receptor cell layer, loose connective tissue layerและ optic nerve layer Receptor cell ในจอตาสามารถแบ่งออกได้เป็น 3 ชนิด Appendage tentacleมีรูปร่างเป็นแผ่นครึ่งวงกลม ส่วนผิวประกอบด้วยสันและร่องมากมาย ในภาคตัดขวางมีกล้ามเนื้อและเส้นประสาทรวมกันอยู่ตรงกลางและถูกล้อมรอบด้วยเยื่อบุผิวซึ่งเป็นชนิด simple columnar และอยู่บนเยื่อฐานที่หนา ในเยื่อบุผิวนี้มีเซลล์ 3 ชนิดเช่นกัน ได้แก่ sensory cell, supporting cell และ mucous secreting cell อวัยวะสุดท้ายได้แก่ osphradium ซึ่งมีกลุ่มของ cilia ซึ่งมีรูปร่างเหมือน rodและ paddle, มีรูเปิดจำนวนมากซึ่งเกิดจากการปล่อยแกรนูลออกมาอยู่บนผิวของ leaf และ leaflet ในภาคตัดขวาง leaf และ leaflet สามารถแบ่งออกได้เป็น 2 ส่วนได้แก่ส่วนฐานซึ่งประกอบด้วยเซลล์ 2 ชนิด สลับกันซึ่ง แกรนูลจะติดสีม่วงกับสีชมพู และ ส่วนยอดซึ่งมีsuppporting cell, sensory cell และ mucous cell 5 ชนิด

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

Figure **Page**

LIST OF ABBREVIATIONS

A°	=	angstrom
°C	=	degree celciate
CH-P	=	chrome-hematoxylin-phloxine
FSH	=	follicle stimulating hormone
g	=	gram
Gb	=	goblet cell
GH	=	growth hormone
H&E	=	Hematoxylin and Eosin
kV	=	kilovolt
LH	=	lutenizing hormone
ml	=	milliliter
mm	=	millimeter
NG	=	neuroglia
NR	=	neuron
NS	=	neurosecretory cell
Oc	=	oocyte
PAS	=	Periodic acid Schiff reagent
PF	=	paraldehyde-fuchsin
rc	=	receptor cell
μm	=	micrometer

CHAPTER I

INTRODUCTION

More than half of all molluskan species are gastropods. They range from primitive marine species to highly evolved terrestrial air-breathing snails and slugs, and are found in almost all terrestrial, freshwater and marine habitats. Modern classification divides them into three primary divisions, the subclass Prosobranchia, Opisthobranchia and Pulmonata (1).

Abalone are large herbivorous marine snails. There are almost one hundred different species, all belonging to the Genus Haliotis, Family Haliotidae, Superfamily Pleurotomariacea, Suborder Zygobranchia, Order Archeogastropoda, Subclass Prosobranchia, Class Gastropoda (2). Since ancient time, abalone are much sought after because of their decorative shells and food value. Presently, abalone are of considerable economic importance in fisheries and aquaculture, with relatively high commercial value both for domestic consumption and export (3,4,5). The commercial culturing and harvesting of abalone to satisfy these demands are mainly carried out in the United States, Japan, Mexico, Australia, New Zealand and South Africa. Almost all of these commercially cultured species are carried out in cool water of temperate region since most of them grow up to fairly large size.

In Thai water, abalone species are found along the coastline of Thai Gulf and Andaman Sea, usually in crevices on coral and rocky reefs, at the depth of 1 to 7 m (6,7,8,9). There are three species of abalone along the Thai coasts: *H. asinina*, *H. ovina* and *H. varia* (6,7,8). Among these species, *H. asinina*, which is sometimes called donkey's ear abalone, is considered to have the most economic potential, because of their relatively large size, maximum proportion of flesh and good taste. *H. asinina* is generally found off the eastern coast of the Gulf of Thailand around Chon-Buri, Rayong and Trad Provinces (9,10). The high demand for abalone has increased pressure on natural stocks, which needs to be maintained. The fisheries from natural habitat could not keep pace for increased demand. Recently, there have been a lot of interest in farming this abalone for commercial purpose, so research and development are being undertaken mostly on the most suitable aquaculture system and technique on getting maximum yield on fertilization and survival rate of larvae. Evidently, in 1991, the Coastal Development Center in Rayong Province has been successful in increasing the fecundity of *H. asinina* and the production of larvae by artificial fertilization (11). However, at present, there are very little studies on basic biology of reproduction of *H. asinina*, and still not enough information on endocrine function that controls reproduction and growth, which are related to the structure of the nervous system, especially with regard to the development of neurosecretory cells during the early stage of life.

Thus, the aim of the present study is to study the structure of the nervous system, its development, and the structure of special sensory organs, so that the information obtained could be applied to the understanding of their roles in

performing endocrine functions which regulate growth and reproduction, and the way that abalone may sense its environment, in finding food, and in performing its reproductive function. This knowledge will contribute to the improvement of the efficiency in aquaculture system of this species in Thailand.

CHAPTER II

OBJECTIVES

The objective of the present study are as follows:

1. To study the histology, classify and enumerate cells in the adult *H. asinina* nervous ganglia, which consists of cerebral, pleuropedal and visceral ganglia by light microscopic techniques, using paraffin methods. The data obtained will be used as the base lines for comparing with similar parameters during the development of the nervous system.

2. To study the growth and development of nerve ganglia of *H. asinina* which are cultured in controlled land-based aquaculture system for a period of one year.

3. To investigate the morphology and histology of the peripheral nervous system, especially with emphasis on those that innervate the special sensory organs, and the special sensory organs themselves by light microscopic, using paraffin, semithin methods, and scanning electron microscopic techniques.

CHAPTER III

LITERATURE REVIEW

The Nervous System of the Gastropods

Much studies have been devoted to understanding anatomy of the molluskan nervous system (14) such as *Bithynia tentaculata* (15), *Nordotis discus* (16), *Haliotis discus hannai* (17), *Achatina fulica* (18) which are essential to the understanding of the endocrine regulation of many important physiological functions, *i.e.*, heart function, metabolic function, hibernation, regeneration, sexual maturation, growth and reproduction. These latter functions are especially related to the economic success of abalone farming (19).

The nervous system of gastropods has been the subject of numerous investigations. The abalone is one of the most primitive gastropods in form and structure. The central nervous system of abalone is considered to be streptoneurous type, which is characterized by the marked absence of concentration of neuronal mass as it consists basically of several ganglia connected by nerve connectives and commissures (3,14). The ganglionic system consists of a pair of cerebral ganglia, a pleuropedal ganglion mass, a visceral ganglion, and several pairs of pedal-cord ganglia distributed along the pedal nerve cord (3,20). Each ganglion is composed of the outer cortex of neurons and glia cells surrounding a central neuropil which occupies the medulla region of the ganglion. It is formed by axonic and dendritic

processes, and tracts of axons entering and leaving the ganglion through the commissures and connectives of the peripheral nerves (21,22). In the ganglia, there are numerous types of cells. Bullock and Horridge (14) classified the neurons in the ganglia of mollusks on the basis of morphology (size and nuclear-cytoplasmic ratio of the cell body and perikaryon). The neurons in the ganglion of nervous system of the gastropod show similarities in structure. They are of the same shape but different sizes and contain nuclei of similar shape, except for the giant neurons which have different sizes and shapes in different species. The ganglion neurons are mostly unipolar (22,23). The neurons in the nervous system of *A. fulica* can be divided into five types according to their sizes: the globuli, small, medium, large and giant cells (24). By the use of multiple criteria (location of the cell in the ganglion, peripheral distribution of its efferent axon, its synaptic input, spontaneous activity, synaptic connections with other identifiable cells, and its appearance under the light and electron microscopes), ganglion cells were classified into large thirty cell types and eight clusters in the abdominal ganglion of *Aplysia californica* (25).

The neurosecretory cells in the gastropods have been investigated more intensively. Neurosecretory cells are found in large quantities and varieties in molluskan ganglia; at least ten different cell types have been reported in the ganglia of the central nervous system (26,27). The brain of *Helix aspersa* contains three types of neurosecretory cells (DGC, YGC and YC), classified on the basis of the reaction of the neurosecretory products with alcian blue/ alcian yellow technique (28). Yahata (16) and Hahn (17) reported that there were four types of neurons in the cerebral ganglia of *N. discus* and *H. discus hannai*, which were designated as types A, B, C and

D. Type A and B cells were believed to be neurosecretory cells. They are large and medium cells with light nuclei and contain neurosecretory granules in the cytoplasm. On the contrary, *B. tentaculata* has only one type of neurosecretory cell (S_1) in the cerebral ganglia (15), which is stained by phloxine. The cells are unipolar and their nuclei are usually indented on one side. Neurosecretory material accumulates in the periphery of the cytoplasm and the axon hillock (15). In the cerebral ganglion of *Lymnaea stagnalis* (29), two groups of neurosecretory cells have been described: Gomori-positive cells (light green cells and bright green cells) and Gomori-negative cells (caudo-dorsal cells and Sudan black B-positive cells). In pulmonate snail, *Australorbis glabratus*, each cerebral ganglion has a large group of neurosecretory cells which are called medio-dorsal body, which lies partly upon the intercerebral commissure. Furthermore, some special neurosecretory cells are located in the lateral lobe of this ganglion, of all basommatophoran snails (30). In *L. stagnalis*, three types of neurosecretory cells are present: light green cells, caudo-dorsal cells and bright green cells. These cells are located in different areas (26). Geraerts (31) studied the cerebral ganglia of *Bulinus truncatus*, which have five types of neurosecretory cells: light green cell, caudo-dorsal cell, left-lateral, medio-dorsal body and latero-dorsal body. Neurosecretory cells are found in the pleural ganglion of many gastropods such as *B. tentaculata* (15), *L. stagnalis* (26), *B. truncatus* (32) and *H. discus hannai* (33). Hahn described several cell types found in *H. discus hannai*, but only two cell types, #1-and #7-cells are thought to be neurosecretory cells (33). Wandelaar Bonga reported that there was only one type of neurosecretory cell (dark green cell) in the pleuropedal ganglion of *L. stagnalis* (26). Dark green cells are often found in the

pedal ganglia of *B. truncatus*. They are homologous to those of *L. stagnalis* (32). Lever (30) described only one type of neurosecretory cells in *A. glabratus*. In *Aplysia californica*, two classes of neurosecretory cells can be distinguished: the bag cells and the white cells (25). Five types of neurosecretory cells were distinguished in the parietal and visceral ganglia of *B. truncatus* (32). In contrast, *B. tentaculata* has only one type of neurosecretory cell (S_3) which have bipolar neuron and also stained by aldehyde fuchsin (15).

Neuroglia have been reported in *B. tentaculata* (15), *A. californica* (34); they contain ovoid nuclei and little perinuclear cytoplasm, and are interspersed among neurons and nerve fibers. Glial cells are distributed between the neurons and their sheath cells and along the outer surface of blood vessels (26,34,35). Their nuclei are round or oval (34). There are two types of glial cells in *L. stagnalis* (26). In *H. asinina* species collected from the Thai coast, Upatham et al. (20) studied the basic feature of cerebral ganglia and their histological characteristics and staining affinities, and were able to classify eight cell types: two types of neurosecretory cells, three types of neurons, and three types of neuroglia. The neurosecretory cells contain neurosecretory granules in the cytoplasm that are stained deep violet with paraldehyde-fuchsin, and they are present only in certain part of these ganglia's cortex. The three types of neurons are the most numerous cell (especially NR_2) types, and occur in all parts of the cortex. The neuroglia are small cells and contain spindle-shaped nuclei, and have the supporting roles very much like those in the nervous system of higher vertebrates (20). In studying the same species, Thongkukiatkul et al. (36) was able to classify cells in pleuropedal ganglion into ten types, which are similar

to those found in the pedal cord ganglia and visceral ganglia. These include three types of neurosecretory cells, four types of neurons, and three types of neuroglia. By utilizing TEM, Kruatrachue et al. also reported three types of neurons and three types of neuroglia in the cerebral and pleuropedal ganglia of *H. asinina* (37).

Development of Nervous System of the Gastropods

Although many fundamental knowledge on histological and physiological functions of the nervous system have been extensively studied in molluskan research, but little is known about neurogenesis in gastropods; this indicates major differences from other well studied invertebrates. In gastropods, the central ganglia arise by proliferation and later delamination and/or invagination of the ectoderm. Cell division continues in the peripheral proliferative zones throughout embryogenesis, and post mitotic cells then migrate inwardly to join the central ganglia which are formed nearby (38). Gangliogenesis in gastropods progresses in anterior to posterior direction with the cerebral ganglia developing first, followed by the pedal ganglia and then the more posterior ganglia of the abdominal loop (38,39).

In pulmonate snails, variation in morphology and lobelization of the ganglia is related to age and development (39). Roubos (40) studied the development of neuroendocrine centers of *L. stagnalis* and found that the dorsal bodies and light green cells are already present in snails of 1 mm in shell length, caudo-dorsal cells first appear in snails of 3 mm in shell length. The dorsal bodies and caudo-dorsal cell increase in number and size with increasing shell length, viz. caudo-dorsal cells from 6-8 cells per cluster in snails of 3 mm in shell length to 30 cells per cluster in adult snails. In addition, Dogterom (41) found that the intercerebral commissures of young

snails, which are in the male stage, contain only small quantities of ovulation hormone (CDCH). During the subsequent period of female maturation, the quantities of CDCH increase considerably up to a maximum. In *A. fulica*, the size of the ganglia: the cerebral ganglia, buccal ganglia, pleural ganglia, parietal ganglia, pedal ganglia, tentacular ganglia and a visceral ganglion, especially the number of nerve cells in the ganglia, increase with increasing age of snails. The prominent nerve cells in the ganglia are the large cells and the giant cells. The large cells have already occurred in all ganglia of the newly-hatched snails, while the giant cells first appear in the buccal ganglia, cerebral ganglia, pleural ganglia, parietal ganglia, pedal ganglia and a visceral ganglion in the snails at the age of 6,3,5,3,1 and 2 months, respectively. Similar to the other nerve cells, the large cells and the giant cells increase in size and number with increasing age of snail (42). In addition, during development of the nervous system, the neurosecretory cells as well as nerve cells markedly increase in size and number (14). The number and size of the neurosecretory cells in the cerebral ganglia of *A. fulica* first appear in 2-month-old snails. The number and size of neurosecretory cells increase with increasing age of snails, reaching a maximum in 8-month-old snails and thereafter remaining constant in 9-to 12-month-old snails (42).

According to Kerkut and Walker (39), the number of nerve cells in the brain of *L. stagnalis* with the weight of 14 and 16 gm. were about 13,000 and 16,000 cells, respectively. Lever et al. (30) investigated the location of neurosecretory cells in the nervous system of basommatophoran snails, *A. glabratus*. In the cerebral ganglia, the neurosecretory center at dorsal side of medio-dorsal groups, the number of cells was also variable, such as in the snails with shell diameters of 5.5-6.5, 10-11,15-18 and 20-

23 mm, there were 15, 28, 37 and 37 neurosecretory cells, respectively (30). In the left parietal ganglion, the number of neurosecretory cells at the right side was variable, such as 4, 7, 5 and 6 neurosecretory cells in the snails with shell diameters of 5.6-6.5, 10-11, 15-18 and 20-23 mm, respectively. The number of neurosecretory cells varied considerably; there were 21, 31, 31 and 39 neurosecretory cells in the snails with shell diameters of 5.5-6.5, 10-11, 15-18 and 20-23 mm, respectively. Coggeshall (34) studied the opisthobranch snails, *A. californica*, and found that during its maturation, the number of nerve cells in the ganglia increased by 40%, and that there was a greatest number of large neurosecretory cells in the fully-grown animals. In stylommatophoran snail, *Limax maximus*, the morphology of the dorsal body cells also changes during maturation (43). The neurosecretory cells are small and release little secretory product in the immature and early male-phase animals. In contrast, these cells become larger and release large amount of secretory product in the late female-phase animals (43). Smith (44) studied the changes of neurosecretory cells in the central nervous system in parallel with the maturation stage in *Arion ater*. The neurosecretory cells in the cerebral ganglia of *A. ater* could be divided into two groups: the posterior and the dorsal groups. During spermatogenesis, the snails with spermatocytes, mid-spermatids, late spermatids, early spermatozoa, mid-spermatozoa and late spermatozoa were shown to have a little secretion in the neurosecretory cells of the cerebral ganglia. The sign of most abundant secretion was present in the snails at the early spermatid stage. In addition, in the mid spermatozoa stage, when the first signs of maturation of the female part of the reproductive tract was observed, there was a large amount of neurosecretory material that appeared within the very small

cells that occurred in groups around the fibrous mass of the parietal ganglia. In addition, many small secretory cells occurred in the lateral cell groups of the pleural and around the pleuro-parietal and pleuro-pedal connections (44).

Special Sensory Organs of Gastropods

The nervous system of mollusk is primitive which consists of nerve ring around the esophagus; they have several ganglia located on this nerve ring, to form the central nervous system. The system of interconnecting nerve ganglia in abalone could be compared with the central nervous system of vertebrate from which peripheral nerves are send out to innervate various internal organs, pedal muscles and special sensory organs (3,45,46).

Gastropod mollusks have large numbers of receptor elements of all major modalities. Almost all the body surface and particularly the specialized regions of skin which form one or another types of sensory organs, are sensitive to various chemical and mechanical stimuli (47). Chemical and mechanical senses govern a wide range of behaviors in gastropods; examples are the identification of potential food sources, the initiation of feeding, detection of predators and noxious stimuli, the identification and location of conspecifics on aggregation and mating, and the synchronization of spawning (47,48,49). Apart form sensory cells in the skin of almost the entire body surface, gastropods have a number of sensory organs (47). The major chemosensory organs of gastropods have been identified; there are eyes, tentacles, rhinophores, osphradium and mantle papillae (45,48,49). All organs have common anatomical organization including bipolar primary sensory cells with cell bodies located subepithelially, and a distal dendrite extending to the free surface (47).

The sensory cell bears cilia or a combination of cilia and microvilli (47). These sensory organs are used by the abalone for identification of potential food sources (50), detection of predators and noxious stimuli (51,52), homing (53), the identification and location of mating partners, and the photoperiod which may affect the synchronization of spawning of male and female gamete cells (47,48,49,54).

The whole integument of abalone, including all the surface of the mantle with its glands, has general sense receptors due to the presence of neuro-epithelial cells located between the glandular and supporting epidermal cells. These sensory cells may be scattered, or collected into noticeable buds in regions with special tactile or chemical perception (3). Specialized neuro-epithelial cells are also aggregated into definitive sense organs, which are exceptionally profuse in *Haliotis tuberculata* (3); these sense organs are a pair of cephalic tentacles, epipodia, osphradium, a pair of eyes. Up to now, there have only been limited number of histological studies on special sense organs in mollusks in general and abalone in particular, except those classical studies of Croft (3) and Kohn (49).

Tentacles of Gastropods

Tentacles are the important sense organs of gastropod which contain both tactile and chemoreception cells in abundance (55,56). The number of tentacle varies among the subclasses, *i.e.*, prosobranchs have a single pair of cephalic tentacles, two pairs occur in higher pulmonates and many opisthobranchs; the anterior pair is cephalic tentacle and the posterior pair is rhinophores (45). Tentacles are not always restricted to the head; they may also be found on the foot or the mantle margin of archeogastropods. Tentacles are especially well developed around the margin of the

skirt like mantle of many limpets and abalone which are called epipodium tentacles. They sweep over the surrounding surfaces and may assist in the finding of food and suitable lodging (3,45). The tentacles are typically slender, cylindrical and pointed. Rhinophores, ranged from simple tapering rods to elaborate, are lamellae or tubercular organs and are usually capable of rapid retraction into protective pockets when they are touched (48).

Structure of the tentacles of gastropods has been examined histologically. The epithelium of tentacle of *Arion ater* has abundant free nerve endings (57) and has supporting cells, sensory dendrites, the processes of sheath cells and the ducts of dermal gland cells found on the superior and inferior tentacle tips. The wall of the tentacle is finely corrugated. On the tips, supporting cells and sensory dendrites, bears a brush border of unusual structure (58).

The cephalic tentacles are among the most important prosobranch sense organs, although little attention has been paid to their fine structure and function. They are richly endowed with sensory cells, which may be significantly different in each of the major groups of gastropods (48,59,60). The tentacle of *Pomatia elegans* consisting of specialized receptor zones, are concentrated at the tentacle tip, immediately beneath the sensory epithelium (61). The receptor cells were tall (about 50 μm) and cylindrical in shape, and the apical surface of most tentacles had a bundle of long, twisted cilia. Cephalic tentacles of *Haliotis* have a mixed sensory and motor nerves, which are centrally located and extends along the length of the tentacle. On the peripheral surface of the nerve, there are extensive sinuses that are located between muscle fibers, the latter being arranged longitudinally, obliquely and transversely.

External to the main mass of muscle fibers is a zone of the surface epithelium that is thrown into folds or papillae. The epithelium is of the cuboidal types, some may be ciliated (46,63). There are three cell types in the epithelium: sensory cells, supportive epithelial cells, and scattered mucous cells. The sensory cells are spindle-shaped with nuclei stained much more darkly than those of the supporting cells which are oval and more transparent (3,46).

The epipodium is a collarette arising from the dorsal part of the foot (3). They often have the same shape and structure as the cephalic tentacles (59). Croft (3) described that the epipodial tentacles have two types of epithelial cells: supporting cells and sensory cells.

Eyes of Gastropods

Eyes are characteristic of most gastropods, and are located at the diverse area in the cephalic region (45,48). The eyes of most gastropods would appear to detect only changes in general light intensity (45). However, more complex responses may be mediated via the eyes (48).

Previous anatomical works on the eye have shown that the most primitive eyes are found in patellogastropods, which have a simple pit containing photoreceptor and pigment cells lacking a lens (45,48,60). In most higher gastropods, Vetigastropoda, the eyes are narrowly open, but have a lens. At a slightly later stage (*Haliotis*, *Trochus*) the cup deepens, and its lumen is filled with a homogeneous material. Then the epidermis closes over the cup to form a cornea as in *Nerita*, *P. elegans* (61,62). The opisthobranch eye, which has been most intensively studied, is simple. It is a closed vesicle type as in *Aplysia*, *Navanax*, *Bulla* (64,65,66,67) or ovoid in shape as in

Hermissenda crassicornis (68), with a transparent lens, a large spheroidal homogeneous mass of finely granular material, nearly completely surrounded by the retina except for a small cornea. On the contrary, *Navanax* has a unique bi-lobed lens (67). In the higher gastropods, pulmonate eyes are reticular, the cornea is formed by flattened and transparent epidermis and the retina is composed of pigment and retinal cells (69,70).

The stratified retina consists of five layers: villous, pigmented support cells having pigment granules in their distal segments, somatic or nuclei of receptors (thousands of small receptor cells), neurons containing secretory granules, and glial cells (64,65,66). Histological studies of eye had shown that the eyes of *H. crassicornis* have three types of cells: non-pigmented sensory cells, pigmented supporting cell and small epithelial cells. The vesicle wall consists of a thin corneal section nearest the epidermal layer of the body and a thickened retina on the opposite side (48). The photoreceptor cells are high (50-60 μm) and cylindrical in shape, and their apical surfaces appear to bear microvilli. The receptor cells of *Navanax* are relatively large and there are relatively few of these cells in the eye. The receptor cells of the retina of *A. californica* are unusual in that they contain large number of vesicles (65). The cytoplasm of these cells, including the distal segments and neurites, is full of with 500A° clear vesicles which are often so densely packed that they assume a paracrystalline array when seen under electron microscope. In *Bulla*, the photoreceptors cell are elongated cells, approximately 20 by 90 μm (68). The retinal or receptor cells are sensory and give rise to nerve fibers, which penetrate the connective tissue of the eye capsule before becoming part of the optic nerve. There is

no optic ganglion (39,64,66,68,70). Beneath the retina, at the base of the nerve, there are small number of uni-, bi- and multipolar neurons of diameter 8-15 μm (61).

The eye of *Haliotis* spp. is cup-shaped. The cavity of the eye contains slender rod processes of the retinal cells and distal to these structures, a hyaline cuticular lens which in the living state is exposed to water (45). At the optical level, the retina consists of tall spindle shaped cells of two types (3). One of these, the sensory cell, interdigitates with other elongated cells containing numerous large pigment granules located in the distal one-third of the cytoplasm, followed by a basal zone containing the nucleus (3,47,68).

Osphradium of Gastropods

The position of the osphradium is constant in the main groups of prosobranch. It occurs in the cavity either on or near the gill (71,72,73,74). Osphradium is generally accepted to be a sense organ; it has two functions: chemoreceptor which involved in food location or detection of attractive substances (75,76,77,78,79,80), and a tactile organ concerned with estimating the amount of sediment carried into the mantle cavity, or assists the regulation of respiration by detecting changes in the pH of sea water (81,82).

In prosobranchs, morphology of the osphradia seems to be related to the habitat (terrestrial, freshwater, marine) and food habit (79,83). It varies from a simple, elongate ridge to a complex bipectinate or tripectinate structure. In algae and deposit-feeders such as *Littorina littorea*, it consists of a simple ridge. In predators like *Conus* spp., the osphradia are arranged into leaflets with varying degrees of mobility and size of receptor areas (79). In addition, the study on the surface of prosobranch *Bullia*

digitalis (73) and *Thais haemastoma canaliculata* (72) revealed that they are composed of many leaflets or lamellae, each of which is divided into two distinct regions by a shallow groove. The first region is the ciliated area which have either tufts or dense sheets. Another region is the smooth surface which has membrane-bound vesicles and numerous secretory cells.

The fine structures of osphradia in prosobranch have been investigated such as *B. digitalis* (73), *T. haemastoma canaliculata* (72), *Buccinum undatum* and *Conus* spp. (74) and many species by Haszprunar (71). In some study, it was found that the osphradium was clothed with a tall epithelium which had all of the following cell types: ciliated supporting cell, non-ciliated supporting cell, neurosensory cell, mucocyte, other gland cell, and pigment-containing cell. However, *B. digitalis* has only ciliated cell, tufted ciliated cell, supporting cell and mucous-secreting cell which lie on the basal lamina which separates them from the axial connective tissue (73). In addition, *T. haemastoma canaliculata* has pseudostratified epithelium which is separated from the central region of the lamella by basal lamina. Connective tissue, muscle fibers, nerves and blood spaces are present in the central region (72). Haszprunar studied the structure of osphradium in many species of prosobranch. They are resemble in most structure, but there are only few differences such as, *Patella* have free nerve endings, Trochoidea and Pleurotomarioidea have cilia bottles, and Neritoidea have pigment bodies. All of them have supporting cells, mucous cells, dark cells and sensory cells distributed in all area. In few species such as *B. undatum*, cell types are definitely zoned on the osphradium (71). There are central zone whose epithelium has sensory cells and the lateral zone of ciliated epithelium (73). Crisp

(74) found that in osphradia of *B. undatum* and *Conus* spp., there are three regions: the glandular region containing no receptor, the sensory region containing scattered ciliated and unciliated neurites arising from intraepithelial cell bodies, and the transitional region which lie between the glandular and sensory region. The last region contains a concentration of nerve endings terminating in the spaces at the base of the clefts of unciliated transitional cells. The neurites bear cilia of irregular fibril complement, and at least some of them originate from cell bodies in the sensory region (74).

In *Haliotis* spp., the osphradium consists of a long ridge of tissue showing groove and is innervated by the osphradial nerve (46). *Haliotis lemellosa* has five cell types in the sensory epithelium: mucous cells which are filled with different kinds of secretory granules and have elongated shape; supporting cells with microvilli, pigment granules and oval nuclei which are situated distally and possess electron-dense euchromatin; dark cells; sensory cells which possess thin process that reach the surface of the epithelium and bear one or two cilia, which can form paddle cilia; the last cell type is the ciliated bottle cell. All these cells lie on the basal lamina composed of two components. Both layers are interrupted by the associated nerves (71). Croft described three cell-types in the epithelium of osphradium in *H. tuberculata*: supporting cell, sensory cell, and small mucous cell sometimes protruding above the ciliated surface (3).

Gills of Gastropods

Gills are the principal organs of respiratory gas exchange in mollusks. They are positioned on the mantle cavity (3,84). The number of studies on gastropod gill

morphology is very limited; a few papers have been published on the structure of gills of pulmonate *Siphonaria capensis* (85), and of other seven species: caenogastropods (including *Planaxis sulcatus*, *Littoraria articulata*, *Bembicium auratum* and *Morula marginalba*), *Patelloida mimula*, *Nerita chameleon* and *Austrocochlea constricta* (84).

The gill structure of the investigated caenogastropods shows basic uniformity. The gill filaments are composed of a clearly defined ridge and an extended sheet of non-ciliated cells. The gill filaments of these species differ in the shape of the filaments (corrugated, triangular or rounded). The gills of *P. mimula* and *N. chameleon* are both triangularly shaped, but differ from those of caenogastropods by the presence of paddle cilia. The gills of *A. constricta* are characterized by blade shaped filaments covered with nodules and a striped pattern of ciliated cells. Each gill filament is covered with a single layered epithelium of either cuboidal (85) or columnar cells (3). However, there appears to be a difference in the thickness of the epithelial cells,which ranges from 4 μm for the cuboidal cells in the gill of *S. capensis* (85) to 14 μm for the columnar cells in the gills of the *A. constricta* (84). A hemocoelic space can be seen in the center of each filament (84,85). Croft found that the chitinous skeletal consists of V-shaped rod attached to one side of the epithelium as in caphalopoda (86).

The epithelium of the gill of *S. capensis* consists of three types of cell: non-ciliated cell, ciliated cell and secretory cell (85). In addition, *H. tuberculata*, studied by Croft showed the same results (3). From the investigation by Eertman (84), mucous-secreting cells were observed in all species except *P. sulcatus* and *P. mimula*. They were usually of the ordinary goblet type found in *S. capensis* and *H. tuberculata*.

However, in the *B. undatum*, the mucous cells were larger and were grouped in the anterior region of the filament.

CHAPTER IV

MATERIALS AND METHODS

Abalone were obtained from the Marine Biological Station, Chulalongkorn University, Angsila, Chonburi Province, Thailand. These animals were reared in a land-based aquaculture system by being placed in concrete tanks, which are well flushed with mechanically circulated water and air delivery system to maintain the stable controlled environment, and aerated with sea water. They are given appropriate algal food and supplemented with artificial food *ad libitum*, and kept under normal daylight cycle.

Samples of juvenile abalone from 1 month up to 16 months old, were collected at one month intervals and prepared for light microscopic observations by the paraffin method, semithin method, and prepared by conventional electron microscopic method for scanning electron microscopic observation.

1. Gross Anatomical Study

Abalone were anesthetized with 5% MgCl, after which their shells were removed. They were then placed on a layer of paraffin wax, and immersed in 70% alcohol. The dissections were made under an Olympus stereomicroscope, from which macrophotographs were taken and drawings of gross anatomy were performed.

2. Histological Preparation for Morphological Study of Adult Nerve Ganglia by Light Microscope

Abalone were anesthetized in 5% magnesium chloride ($MgCl_2$) for 30 minutes to 1 hour before they were cut into 3 pieces (anterior 1/3, middle 1/3 and posterior 1/3) and fixed in Bouin's solution in 0.14 M NaCl for 24 hours. Tissues were washed with 70% ethyl alcohol several times. Then, they were dehydrated through a graded series of ethanol (70%-100%) and cleared with dioxane twice, each step for 30 minutes. After dehydration, the tissues were infiltrated and embedded in parafin wax. Serial sections were cut at the thickness of 5 μm, and stained with Harris's hematoxylin and eosin, chrome-hematoxylin-phloxine, and paraldehyde-fuchsin. Neurons and cells in the cerebral, pluero-pedal, and visceral ganglia were observed and evaluated for their cell size and shape, nuclear size and shape, and staining affinities under an Olympus Vanox light microscope.

3. Protocol for Special Stainings

Two special staining techniques were used for identifying neurosecretory cells of the ganglia.

3.1) Chrome-hematoxylin-phloxine staining method

The technique followed the protocol of Gomori's chrome alum hematoxylin-phloxine method (12,13). Briefly, the ganglia were fixed in Bouin's solution and processed through paraffin and sectioned at 5 μm thickness. The sections were deparaffinized and dehydrated through a graded series of ethanol, treated for 5 minutes with a solution containing 0.3 % each of potassium permanganate and sulfuric acid, then decolorized with a 5% solution of sodium bisulfite, and washed in

running tap water. The sections were stained in the chromium-hematoxylin solution and examined periodically under microscopic viewing until the cells stood out as deep blue (after approximately 30 minutes). Subsequently, sections were differentiated in 1% (0.125 N) hydrochloric acid alcohol for about 30-60 seconds, and washed under tap water until they were clear blue. The sections were counterstained with 0.5% aqueous solution of phloxine B for 1 hour, then rinsed in distilled water. They were immersed in 5% phosphotungstic acid solution for 1 minute, and washed in tap water for 5 minutes until regaining their red color. Then they were dehydrated in a graded series of ethanol, cleared with several changes of xylene and mounted.

3.2) Paraldehyde-fuchsin staining method

The paraffin sections were deparaffinized, rehydrated and oxidized in Gomori's fluid (0.15 g of $KMnO_4$ in 50 ml of water containing 0.1 ml concentrated H_2SO_4) for 1 minute. All permanganate stain was removed by rinsing in 5% sodium bisulfite solution for 5 minutes and washed in tap water. Then they were stained for 30 minutes in paraldehyde-fuchsin solution (0.5 g of basic fuchsin, and 1 ml of paraldehyde in 100 ml 70% alcohol containing 1.5 ml concentrated HCl). The slides were quickly wiped and rinsed in 70% alcohol for 3 minutes. The sections were counsterstained in Halmi's mixture (0.2 g of light green yellow, 1 g of orange G, 0.5 g of chromotrope 2 R, 0.5 g of phosphotungstic acid and 1 ml of glacial acetic acid in 100 ml of distilled water) for 15 minutes. Then, the sections were differentiated in 95% alcohol, washed twice in absolute alcohol and xylene, and mounted. Examinations of the tissue sections were done under a bright field microscope.

4. Procedure for Studying the Development of Nerve Ganglia in Juvenile Abalone by Light Microscope

Abalone from 1 to 12 months old were collected, and the specimen preparation was performed similar to that described in part 1. The specimens were embedded in paraffin, and five-micron-thick sections were cut and deparaffinized, processed and stained in hematoxylin and eosin, or chrome-hematoxylin-phloxine, or paraldehyde-fuchsin. The sections were finally examined and photographed under an Olympus Vanox light microscope.

5. Procedure for Studying Special Sensory Organs by Light Microscope

Special sensory organs consisting of eyes, cephalic tentacles and epipodium tentacles were cut from adult abalone and prepared by paraffin method similar to those described in the part 1 and stained with H&E as mentioned previously.

For semithin technique, specimens were fixed in a solution of Karnovsky's fixative (2% paraformaldehyde and 4% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.8) at 4 °C, for overnight, and followed by washing in 0.1M sodium cacodylate buffer for removal of the fixative. The specimens were post-fixed in 1% osmium tetroxide in 0.1M sodium cacodylate buffer for 1 hour at 4°C. Then, they were dehydrated in a graded series of ethanol (50-100%) for 15 minutes each, cleared in two changes of propylene oxide (PO), infiltrated in a mixture of propylene oxide and Araldite 502 resin at the ratio of 3:1 for 1 hour, 2:1 for 2 hours and 1:2 for overnight. Then, they were embedded in pure Araldite 502 resin for at least 6 hours, and finally polymerized at 30°C, 45°C and 60°C for 24, 48 and 48 hours, respectively. Blocks of specimens were sectioned at 1 μm thickness by Porter Blum MT-2

ultramicrotome, and sections were stained with methylene blue and PAS-methylene blue.

6. Protocol for PAS-methylene blue staining

The semithin sections were incubated in 1% periodic acid solution for 5 minutes at 65°C and washed in distilled water. Then they were stained for 6 minutes at 65°C in Schiff's reagent. The sections were counterstained in 1% methylene blue (in 1%borax in distilled water) at 65 °C for 10 seconds. Finally, they were washed in distilled water, dried on a hot plate and mounted by using an epoxy glue. Examinations of the tissues were done under an Olympus light microscope.

7. Procedure for Studying Special Sensory Organs by Scanning Electron Microscope

Special sensory organs were cut and fixed in a solution of Karnovsky's fixative (2% paraformaldehyde and 4% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.8) at 4°C, for overnight, and followed by washing in 0.1M sodium cacodylate buffer for the removal of the fixative. The specimens were post-fixed in 1% osmium tetroxide in 0.1M sodium cacodylate buffer for 1 hour at 4°C. Then, they were dehydrated in a graded series of ethanol (50-100%) for 15 minutes each. After dehydration, they were dried in a Hitachi HCP-2 critical point drying machine, using liquid CO_2 as a transitional medium. They were then mounted on aluminum stubs and coated with platinum and palladium in an ion sputtering apparatus, E 5000. The specimens were examined by a Hitachi S-2500 scanning electron microscope with an accelerating voltage of 15 kV.

CHAPTER V

RESULTS

1. Gross Anatomy of *H. asinina*

The shell on top covers most of the abalone's body. It is roundish or ear-shaped and consists of whorls (Fig.1A). A row of respiratory pores is found on the left side of the shell. The more anterior the pore are located, the bigger they are in diameter, and those situated towards the posterior are usually blocked. The head of abalone points anteriorly, and the apex of the shell points posteriorly towards the right hand side. There are a pair of eyestalks, a pair of cephalic tentacles and a pair of appendage tentacles in the anterior surface of the head. A pair of cephalic tentacles is longest and stoutest with green color placed medially than a pair of eye, which seen superficially as a black spot at the tip of the pair of optic tubercles (Figs.1A,B). The dorsal part of cephalic tentacle is covered by a pair of appendage tentacles, which has a half-circle shaped. Around the body, the epipodium is a collarette arising from the dorsal part of the foot; it looks like the cephalic tentacle, but the size is smaller.

After removing the shell, the visceral organs are observed on the pedal muscle (Fig.1C). A bipectinate gill is located in the mantle cavity, which continutes into digestive tract that ends at the anus (Fig.1D). The osphradium is located dorsal to the gill and all of them are covered with mantle membrane.

2. Histology of the Nerve Ganglia

The central nervous system of *H.asinina* consists of three main ganglia: the cephalic cerebral, middle pleuropedal, and posterior visceral ganglia.

Cerebral ganglia

Cerebral ganglion has elongated sickle-shape with concave inner surface. The size is approximately 309x929 μm (from the abalone with shell length about 3.6 cm).The ganglion consists of the outer cortex and the inner medulla; the former contains neurons, neurosecretory cells and neuroglia, and bordered externally by the basement membrane (Fig.2A). It is surrounded by a loose connective tissue and capillaries. The dorsal and ventral parts of the cortex are relatively thick, and contain 4-5 layers of cells. In contrast, the lateral and medial parts of the cortex are thin, with the medial part thinner than lateral part and contains only 1-3 layers of cells. The medulla contains bundles of nerve fibers running in several directions.

Pleuropedal ganglion

Pleuropedal ganglion is the largest ganglion, which has an H shape and its size is about 0.60x2.53 mm (from the abalone with a shell length about 3.6 cm). The ganglion is composed of two parts: the outer cortex and inner medulla (Fig.2B). The cortex of the dorsal and lateral parts of the ganglion is relatively thick and contains 5-6 layers of cells which include neurons, neurosecretory cells and neuroglia. In contrast, the medial part of the cortex are thin and contain only 3-4 layers of cells. A loose connective tissue and capillaries are found surrounding the ganglion.

Visceral ganglion

Visceral ganglion is a single ganglion, located at the posterior end of the loop of the visceral cord. The ganglion is as small as 161x929 μm (from the abalone with a shell length of about 3.6 cm) and it appears like dumb-bell shape, with both sides of the ganglion forming bulb-like structures (Fig.2C). Most of the lateral and ventro-medial parts of the ganglion have thick cortex that contain 2-3 cell layers. In contrast, the remaining parts are relatively thin, and contain only a single layer of ganglionic cells. The visceral ganglion has fewer ganglion cells than other ganglia.

Classification of ganglion cells

The cells in all the ganglia mentioned above can be classified into ten types based on their histological characteristics and staining affinities to chrome-hematoxylin-phloxine and paraldehyde-fuchsin. There are three types of neurosecretory cells (NS_1, NS_2, NS_3), four types of neurons (NR_1, NR_2, NR_3,NR_4) and three types of neuroglia (NG_1, NG_2, NG_3) (Figs.2D and 3).

Type-1 neurosecretory cell (NS_1) These cells are the largest neurosecretory cells with the cell body having round or oval shape. They are about 10x20 μm in size (Fig. 3A). The nucleus is round, and contains mostly pale-stained euchromatin with only a thin rim of heterochromatin binding to the internal surface of the nuclear envelope. The nucleolus is small but very distinct. The cytoplasm is stained reddish pink with hematoxylin and eosin and violet with chrome-hematoxylin-phloxine (Fig.3C). There are numerous neurosecretory granules filling the entire cytoplasm which are stained purple with paraldehyde-fuchsin (Fig.3D).

Type-2 neurosecretory cell (NS_2) These cells are smaller than NS_1; they have round or oval cell body, and are about 10x12 μm in size (Fig.3A). The nucleus is round with most heterochromatin blocks attached to the periphery and some in the center, and together they resemble a clock-face pattern. The cytoplasm contains fewer neurosecretory granules than those of NS_1, and they are also stained purple with paraldehyde-fuchsin, while other cytoplasmic stainings are similar to those of NS_1.

Type-3 neurosecretory cell (NS_3) These cells are the smallest neurosecretory cells, with round cell body, whose size is about 8x10 μm (Fig.3A). The nucleus is round, located on one side of the cell, and contains a thick rim of heterochromatin attached to the periphery with lacey thick heterochromatin cords in the center. The cytoplasm contains abundant large granular materials, whose stainings are similar to those of NS_1 and NS_2.

Type-1 neuron (NR_1) These are the largest neurons, with oval or pyramidal shape. They are about 25x40 μm in size (Fig.2D). Their long axons extend inwardly into the medulla of the ganglion. The nucleus is round, and contains almost entirely euchromatin, with a large and eosinophilic nucleolus. The cytoplasm is stained homogeneously pink with hematoxylin and eosin and chrome-hematoxylin-phloxine, with no secretory granules that could be revealed by paraldehyde-fuchsin staining. NR_1 of the pleuropedal ganglion are larger and more numerous than those in the cerebral ganglia and visceral ganglion.

Type-2 neuron (NR_2) These cells are the most numerous among neuronal cells. They are concentrated mostly in the middle cell layer of the cortex. They have round to oval in shape, measured about 4x6 μm in size, and contain oval nuclei with patchy

heterochromatin (Fig.2D). The cytoplasm is extremely thin and shows no clear boundary.

Type-3 neuron (NR_3) These cells are slightly smaller than NR_2, but have similar shape, and are about 3x5 μm in size (Fig.2D). They occur in the innermost cell layer of the cortex. The nucleus is elliptical and contains completely dense heterochromatin. The cytoplasm is not clearly defined.

Type-4 neuron (NR_4) These cells occur in a small number. The nucleus is round or oval with a thick rim of heterochromatin attached to the periphery, while the central area is clear (Fig.3B). The nucleolus is round and very distinct. These cells are the fewest in numbers among neurons, and they are rarely observed in the cerebral ganglia.

Type-1 neuroglia (NG_1) These cells are scattered throughout the cortical region of the ganglion. They are small spindle-shaped cells about 3x6 μm in size and they contain similar shaped nuclei (Fig.2D). A thin rim of heterochromatin is attached to the inner surface of the nucleus membrane, while most of the remaining chromatin is euchromatic. There is a thin rim of cytoplasm around the nucleus.

Type-2 neuroglia (NG_2) The cell body and nuclear size of these cells are similar to those of NG_1, but they show completely dense chromatin (Figs.3A,B). These cells lie in a single row on the basement membrane.

Type-3 neuroglia (NG_3) They are the smallest cells with spindle-shaped nuclei that contain completely dense heterochromatin (Figs.2D,3A). These cells are scattered among nerve bundles of the medulla.

3. Development of the Nerve Ganglia

The shape, size, types of cells and their numbers in ganglia during various ages of developing abalones are summarized in Tables I, II, III.

Cerebral ganglia

Cerebral ganglia of the young abalone appear the age of 1 month. They have elongated bean shape whose size is approximately 121x471 μm (Fig.4A). Most of the ventral and dorsal parts of the ganglia have thick cortex that contains 3-4 cell layers, while the remaining parts are relatively thin, with the medial part contains only 0-1 cell layer (Fig.4B). NS cells first appear in 1-month-old abalone, and only NS_1 was found about 1-2 cells per section, and most of them are concentrated in the dorsal horn. Most types of neurons (NR_1, NR_2, NR_3, and NR_4) are present, but NR_2 are the most numerous, NR_3 and NR_4 are moderate in number, while the NR_1 are rarely found but where present are usually located in dorsal horn similar to NS cell. All types of NG are present but in a small number.

At 2-4 months, the ganglia appear similar to those in 1-month-old abalone (Fig.4A). The numbers of cell layers are increased with increasing ages (Fig.4B). The number of NS cell is increased to about 2-5 cells per section in 2-to 3-month-old and in 4-month-old abalone the number is about 10 cells per section; most of them are NS_1, while NS_2 was observed in 3-month-old and NS_3 in 4-month-old abalone. Most NS are concentrated in the dorso-lateral and dorso-medial, ventral and ventro-medial parts of the ganglia (Figs.4C,D). NR are similar in types and number as those in 1-month-old abalone. In addition, NR_1 are also found at the dorso-medial and ventral regions. NG are increased in number from one month onward.

At 5 months, the size of the ganglion is increased substantially to 202x632 μm (Fig.5A). The numbers of cell layers in the cortex region are significantly increased, especially in the ventral and dorsal parts (Fig.5B). The numbers of NS cells are significantly increased to about 20 cells per section, and all cell types are scattered in all parts of the ganglia, while most are still concentrated in dorsal and ventral horns (Figs.5C,D). NS cells are positively stained with paraldehyde-fuchsin and chrome-hematoxylin-phloxine. The number of NR is increased with age, and NR_1 are approximately 11-20 cells per section. They are present in dorsal and ventral areas. The numbers of NG are increased but not significantly.

From 6 to 9 months, the ganglia appear larger and more elongated than those in 5-month-old abalone, and they turn into sickle shapes. The cortex in all areas are thickened and the quantities and distribution of NS cells and NR are similar to those of 5-month-old abalone.

At 10 months, the cerebral ganglia are significantly increased in size to about 294x787 μm (Fig.6A). Other appearances are similar to those of 5-to 9-month-old abalone. But the number of NS cells and NR_1 are significantly increased (Figs.6C,D). NG is also increased with increasing age.

When abalones are 12 months old, their ganglia are fully developed and appear similar in all aspects to those of the adult abalone (Fig.7).

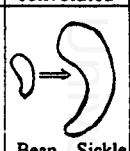
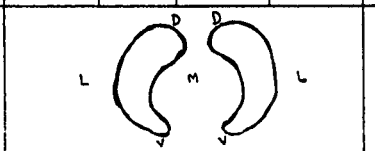
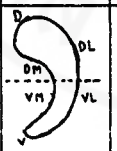
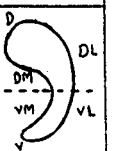
Month	Shape	Size (μm) WxL	Number of cell layers				Number of NS/ section	Location of NS	Relative number of NS			CH -P	PF	Relative number of NR				Location of NR_1
			Ventral (V)	Dorsal (D)	Medial (M)	Lateral (L)			NS_1	NS_2	NS_3			NR_1	NR_2	NR_3	NR_4	
1	Bean shape	121x471	4	3	0-1	2-3	1-2	D	+	-	-	-	-	+	++++	++	++	D
2	Bean shape	182x607	5	4	0-3	1-3	2-5	D,DL,DM	+	-	-	-	-	+	++++	++	++	D,DM
3	Bean shape	182x644	5	4	0-3	1-3	2-5	D,DL,DM, V,VM	+	+	-	-	-	+	++++	++	++	D,DM,V
4	Bean shape	174x628	6	7	0-3	2-4	10	D,DL,DM, V,VM	+	+	+	-	-	+	++++	+++	++	D,DM,V
5	Bean shape	203x632	6	7-8	0-3	1-4	20	in all areas	++	++	+	+	+	++	++++	+++	++	D,DM,DL V,VM,VL
6	Sickle shape	202x637	4-5	6-8	0-3	1-3	20	"	++	++	+	+	+	++	++++	+++	++	Same
7	Sickle shape	206x808	4-5	5-7	1-2	1-3	20	"	++	++	+	+	+	++	++++	+++	++	"
8	Sickle shape	273x735	4-5	5-7	0-3	1-5	20	"	++	++	+	+	+	++	++++	+++	++	"
9	Sickle shape	286x690	4-5	5-7	0-3	1-5	20	"	++	++	+	+	+	++	++++	+++	++	"
10	Sickle shape	294x787	4	6	0-3	1-4	30	"	+++	+++	++	+	+	+++	++++	++++	++	"
11	Sickle shape	377x810	4	5-7	1-3	1-4	30-40	"	+++	+++	++	+	+	+++	++++	++++	++	"
12	Highly elongated, convoluted	376x901	4-6	5-7	1-3	1-5	30-40	"	+++	+++	++	+	+	+++	++++	++++	++	"
	 Bean Sickle								- =0 cell/section + =1-5 cells/section ++ =6-10cells/section +++ =11-15cells/section ++++=>15cells/section			-=non-reactive +=reactive		- =0 cell/section + =1-10 cells/section ++ =11-20cells/section +++=21-30cells/section ++++=>30cells/section				

Table I Key Events During the Development of Cerebral ganglion in *Haliotis asinina*

Pleuropedal ganglia

Pleuropedal ganglia first appear in 1-month-old abalone (Fig.8A). It has butterfly shape and is about 189x418 μm in size. In the ventral and lateral parts of the ganglia, the cortex is thick and contains 2-5 cell layers (Fig.8B). While the remaining parts of cortex are relatively thin. At this age, the abalone rarely have NS cells, as only about 1-2 cells per section was observed, and they are confined to the dorsal-sulcus (Figs.8C,D). NS cells that are present are mostly NS_1 type. There are all types of NR, but a few NR_1 and NR_4 are present in the dorso-medial part. All types of NG cells are found.

At 2-3 months, the size of the ganglia is increased from 273x491 to 269x552 μm, but the shape is not altered. Numbers of cell layers in cortex, NS cells and NR cells appear to increase from 1-month-old, and most cells are distributed in other parts of the cortex. NR are found in the dorsal and dorso-lateral parts, while NS cells are found in the dorso-medial and lateral sulcus and most cells are positively stained with chrome-hematoxylin-phloxine.

At 4 months, the size of pleuropedal ganglia are significantly increased to approximately 337x556 μm (Fig.9A). The cortex becomes much thicker than in younger ages (Fig.9B). The numbers of NS cells increase to about 20 cells per section and a larger number are found at the dorso-lateral and ventro-lateral parts; and most of them are NS_1 (Fig.9C,D). These cells are positively stained with chrome-hematoxylin-phloxine (Figs.12A,B) and paraldehyde-fuchsin (Figs.12C,D). NR are increased in number with increasing age and are found in the ventro-medial, ventro-lateral and ventral sulci.

At 5-6 months, the ganglia have the same shape and size as those at 4 months. The numbers of NS and NR are similar to those in 4 months, but they are found in the other parts of ganglia as well. NS cells appear in the dorsal and ventral parts, while NR are found in all areas.

At 7 months, the ganglia have H-shape and their sizes are increased to about 544x1615 μm (Fig.10A). The numbers of cell layer are increased and NS cells are about 30-40 cells per section and are distributed in all areas (Fig.10B). These cells are mostly NS_1. NR are increased in number in comparison to earlier stages, especially NR_1 which is significantly increased when compared to those at 6 months (Figs.10C,D). From 8 to 10 months, pleuropedal ganglia are generally similar to those of 7-month-old abalone.

At 11 months, the ganglia are significantly increased in size with the ventral and dorsal horns elongated to about 589x2508 μm (Fig.11A). The numbers of cell layer in the cortex are increased from the younger ages (Fig.11B). The numbers of NS cells are about 60 cells per section. The numbers of NR are increased with increasing age, and distributed in all areas (Fig.11C,D). NG cells are also increased in number with increasing age.

At 12 months, the pleuropedal ganglia are fully developed and appear similar to those of the adult abalone.

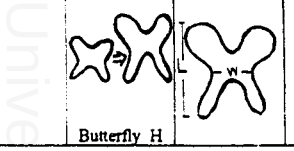
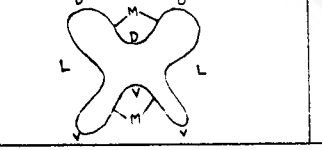
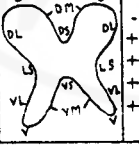
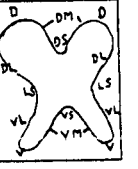
Month	Shape	Size (μm) WxL	Number of cell layers				Number of NS/ section	Location of NS	Relative number of NS			CH -P	PF	Relative number of NR				Location Of NR_1
			Ventral (V)	Dorsal (D)	Medial (M)	Lateral (L)			NS_1	NS_2	NS_3			NR_1	NR_2	NR_3	NR_4	
1	Butterfly shape	189x418	2-4	0-3	1-3	0-5	1-2	DS	+	-	-	-	-	+	+++	++	++	DM
2	Butterfly shape	273x497	2-4	1-5	1-2	1-5	2-3	DS,DM,LS	+	+	-	+	-	+	+++	++	++	DM
3	Butterfly shape	269x522	4	1-5	1-3	2-4	2-3	DS,DM,LS	+	+	-	+	-	+	+++	++	++	DM,DL,D
4	Butterfly shape	337x556	5	2-5	2-3	2-7	20	DS,DM,LS, DL,VL	++	+	+	+	+	++	+++	+++	++	DM,DL,D, VM,VL,VS
5	Butterfly shape	455x572	5	2-5	2-3	1-7	20	DS,DM,LS, DL,VL,V	++	++	+	+	+	++	++++	+++	++	Same
6	Butterfly shape	488x707	5	2-5	2-3	1-7	20	DS,DM,LS, DL,VL,V,D	++	++	+	+	+	++	++++	+++	++	"
7	H shape	544x1615	4-6	2-5	5	3-10	30-40	in all areas	+++	++	++	+	+	+++	++++	++++	++	"
8	H shape	546x1352	3-5	5	2-3	4-7	30-40	"	+++	++	++	+	+	++	++++	++++	++	"
9	H shape	572x1061	4-5	2-5	3-4	1-8	30-40	"	+++	+++	++	+	+	++	++++	++++	++	"
10	H shape	580x1300	4-5	2-5	3-4	2-7	30-50	"	++++	+++	++	+	+	+++	++++	++++	++	"
11	Highly elongated, enlarged	589x2508	5-6	4-6	5	3-10	60	"	++++	++++	+++	+	+	+++	++++	++++	+++	in all areas
12	H shape, similar	589x2543	5	5-6	3-4	7	60	"	++++	++++	+++	+	+	+++	++++	++++	+++	"
	 Butterfly H								- =0 cell/section + =1-5 cells/section ++ =6-10cells/section +++ =11-15cells/section ++++=>15cells/section			-=non-reactive +=reactive		- =0 cell/section + =1-10 cells/section ++ =11-20cells/section +++=21-30cells/section ++++=>30cells/section				

Table II Key Events During the Development of Pleuropedal ganglion in *Haliotis asinina*

Visceral ganglia

In 1-month-old abalone, the visceral ganglia are as small as 37x72 μm and they have bean shape (Fig.13A). The cortex has only one layer of cells. NS cells and NR_1 have not yet appeared at this month. In contrast, the remaining types of NR (NR_2,NR_3, NR_4) are present but still few in number. All types of NG are found (Figs.13B,C,D).

From 2 to 3 months, the ganglia change to dumbbell shape and its size is increased with increasing age. The cortex is thicker than that in 1-month-old abalone, especially on the lateral part. NS cells first appear at 2-month-old, and their number is about 1-2 cells per section. They are present in the left lateral, left latero-dorsal and left latero-ventral parts. There are all types of NR, but NR_1 are rarely found in left lateral and left latero-ventral parts.

At 4 months, the visceral ganglia are significantly increased in size to about 123x454 μm (Fig.14A). The numbers of cell layers in the cortex are increased (Fig.14B). The number of NS cells is increased more than at 3 months to about 10 cells per section and they are distributed in the right lateral part. NR cells are similar in number and their distribution is the same as those at 3 months (Figs.14C,D).

At 5 to 10 months, the ganglia are similar to those of 4 months in all aspects including size, shape, and thickness of the cortex. The numbers of NS and NR cells and the distribution are not changed.

At 11 months, the ganglion is increased in length, but still have similar width (Fig.15A). NS cells are increased in number, especially NS_1 which become distributed in all areas of the ganglia, but they are concentrated mostly in the left lateral part (Figs.15C,D).

At 12 months, the ganglia appear similar to those of the adult abalone.

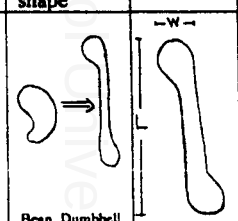
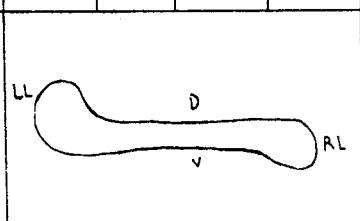
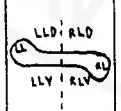
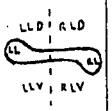
Month	Shape	Size (μm) WxL	Number of cell layers				Number of NS/ section	Location of NS	Relative number of NS			CH -P	PF	Relative number of NR				Location of NR_1
			Ventral (V)	Dorsal (D)	Medial (M)	Lateral (L)			NS_1	NS_2	NS_3			NR_1	NR_2	NR_3	NR_4	
1	Bean shape	37x72	1	0-1	1	1-2	-	-	-	-	-	-	-	-	++	++	+	-
2	Bean shape	48x74	1-2	1-2	1	1-3	1-2	LL	+	-	-	-	-	+	++	++	+	LL,LLV
3	Dumbbell shape	118x488	1-2	1-2	1	1-4	2-3	LL,LLV, LLD	+	+	-	-	-	+	++	++	+	LL,LLV
4	Dumbbell shape	123x494	1-2	1-2	1	2	10	LL,LLV, LLD	+	+	-	-	-	+	++	++	+	LL,LLV, LLD
5	Dumbbell shape	151x607	1-2	1-2	1	1-3	10	LL,LLV, LLD,RL	++	+	+	-	-	+	++	++	+	LL,LLV, LLD,RL
6	Dumbbell shape	155x923	1-3	1-2	1-2	1-3	10	same	++	+	+	+	+	++	++	++	+	Same
7	Dumbbell shape	168x640	1-3	1-2	1-2	1-3	10	"	++	+	+	+	+	++	++	++	+	"
8	Dumbbell shape	161x640	1-3	1-2	1	1-3	10	"	++	+	+	+	+	++	++	++	+	"
9	Dumbbell shape	160x700	1-3	1-2	1-2	1-3	10	in all areas	++	+	+	+	+	++	+++	++	+	"
11	Dumbbell shape	160x889	1-3	1-2	1-2	1-3	20	"	+++	+	+	+	+	++	++++	+++	++	in all areas
12	Dumbbell shape	165x939	1-3	1-2	1-2	1-3	20	"	+++	++	+	+	+	++	++++	+++	++	"
									- =0 cell/section + =1-5 cells/section ++ =6-10cells/section +++ =11-15cells/section ++++=>15cells/section			-=non-reactive +=reactive		- =0 cell/section + =1-10 cells/section ++ =11-20cells/section +++=21-30cells/section ++++=>30cells/section				

Table III Key Events During the Development of Visceral ganglion in *Haliotis asinina*

4. Peripheral nervous system

The nervous system of *H.asinina* consists of central and peripheral nervous systems like that in human. Central nervous system contains several ganglia; there are a pair of cerebral ganglia, buccal ganglion, pleuropedal ganglion, visceral ganglion and a pair of pedal cord ganglia. The central nervous system gives the peripheral nerves to the sensory organs. The peripheral nervous system consists of into 5 groups peripheral nerves.

1. Nerves from the cerebral ganglia, which send many branches to supply many organs.

1.1 Tentacular nerves arise from the ventral edge of the ganglion, enter into the center of the tentacle. Their branches supply the sensory structures of the tentacle.

1.2 The optic nerves are located lateral to the tentacular nerve; they innervate optic tubercle, eye, muscle and epithelium of the eye.

1.3 Appendage tentacular nerve, innervates appendage tentacles.

1.4 Epipodium tentacular nerves from cerebral ganglia, cerebro-pedal and cerebro-pleural connectives send branches to supply the epipodium tentacles in the cephalic part.

2. Buccal nerves from buccal ganglion, innervate buccal mass, ventral esophagus and radular, and the organs near the ganglion.

3. Nerves from visceral ganglion give many branches to the visceral organs: intestine, digestive glands, gonad, and osphradium nerves to the osphradium.

4. Epipodium nerves from pleuropedal ganglion give branches to supply the epipodium tentacle.

5. Nerves from pedal cord ganglia supply foot muscle, and epipodium nerves from the ganglia supply the epipodium tentacles.

5. Special Sensory Organs

5.1 Cephalic tentacle

Cephalic tentacle is round and tapered from the base to the top. From the surface, it is divided into three parts; 1/10 basal, 1/10 middle and 8/10 top.

At the basal part, the surface is rather flat. It consists of many folds and grooves (Figs.17A,B). Many papillae are located on the fold (Fig.17C). On the top of the papillae, cilia are arranged as a circular fringe like a sweep's brush. On the middle part of the tentacle, the folds are absent (Figs.17A,C). The papillae are distributed separately and are higher than those at the base. They appear like hillock covered with microvilli and the cilia encircling at the middle top (Fig.17D). On the top part, the papillae are much higher than those on the middle part (Fig.18A). They appear as a slender truncated cone, covered with microvilli (Figs.18B,C). The papillae project perpendicular to the tentacle, and on the top there are also tuft of cilia (Fig.18D).

In paraffin sections, the cephalic tentacle can also be divided into 3 parts (Fig.19A). In the axis of the basal part, there is a bundle of tentacular nerve which run along the length of tentacle and their nerve fibers are distributed among groups of longitudinal muscle (Figs.19C,D). The simple columnar epithelium surrounds the muscle of the tentacle, it slicks but in some area has a small prominence (Fig.20A). The middle part of the tentacle shows structures similar to those on the basal part, but the epithelium has stalks called papillae (Figs.20C,D). The papillae have a hillock shape, they have sensory cells, supporting cells and goblet cells lying on the basement

membrane. At the base, fibers of the tentacular nerve terminate on the epithelial cell. The whole surface of the epithelium is covered with brush border and has many cilia on the top of the papillae. In contrast, the top part has a tentacular nerve bundle and nerve branches, muscle and epithelium like those of the basal and middle parts, but the epithelium shows long papillae that look like spines (Fig.21A). Each papilla has cone shape, is covered with brush border, and has cilia on the top (Figs.21C,D). The surface epithelium has numerous brown pigments. The epithelial cells of these papillae look like those in basal and middle parts.

The cells in the epithelium can be classified into three types based on their histological characteristics and staining affinities.

1. Sensory cell (se). These cells are small with the cell body having round or oval shape (Figs.20A,D). The nucleus is oval and contains mostly dark-stained chromatin (Figs.20D,21B). The cytoplasm is stained blue with methylene blue and pinkish purple with PAS-methylene blue (Figs.20B,21D).

2. Supporting epithelial cell (su). These cells are larger than the sensory cell. They have round or oval cell body (Figs.20C,D). The nucleus is round or oval, and contains euchromatin with a few block of heterochromatin (Figs.21B,C). The cytoplasm is light blue after staining with methylene blue and pinkish with PAS-methylene blue (Figs.20B,21D).

3. Goblet cell (Gb). These cells are the mucous cell; the nucleus is small oval with euchromatin (Fig.21B). The cytoplasm is large and stained light blue with methylene blue and pinkish with PAS-methylene blue (Figs.20B,21D).

5.2 Eyes

The eyes are on the optic tentacle which has muscles around them and is surrounded by an epithelium (Fig.22B). The optic nerves, which supply the eyes and these tentacle come from the cerebral ganglia. They extend to the base of the eye and are divided into branches covering the eye; the external surface is covered with capillaries (Fig.22A). The isolated eye is an open vesicle type and appears spherical in shape; it has a large lens surrounded by the retina organization (Fig.22B). The retina is composed of 6 layers: pigmented layer, pigmented cell layer, fibrous layer, receptor cell layer, loose connective layer and layer of optic nerve, respectively (Figs.22D,23A,B). Towards the opening of the optic cup, all layers of the retina are gradually reduced in thickness, and the pigmented cell layer is prominent (Fig.22C).

The receptor cells can be classified into three types based on their histological characteristics.

1. Receptor cell type 1 (rc_1). These cells are small with the cell body having elongated shape (Fig.23D). The nucleus is round, and contains mostly pale-stained euchromatin with some blocks of heterochromatin scattered centrally. The cytoplasm is stained transparent blue with methylene blue.

2. Receptor cell type 2 (rc_2). These cells are slightly smaller than the type 1 cell. They have elongated cell body (Fig.23D). The nucleus is oval with small heterochromatin blocks in the center. The cytoplasm is stained intensely blue.

3. Receptor cell type 3 (rc_3). These cells have approximately the same size as the type 2 cell, with elongated body. The nucleus is oval, and contains densely stained chromatin (Fig.23D). The thin cytoplasm is stained more densely when compared to those of the former two cell types.

Pigmented cells are relatively small with elongated shape (Fig.23A). They contain a long nucleus with mostly euchromatin and have some nucleoli. Black-brown pigment granules are located in their distal half segments that appear as a dark layer next to the lens (Fig.22D).

5.3 Appendage tentacle

H.asinina has a pair of appendage tentacles located medial to the optic tentacles and cover the base of the dorsal side of the cephalic tentacles (Fig.24A). Each appendage tentacle has a half circle shape and is covered by numerous irregular folds and grooves (Figs.24B,C). The folds are covered with numerous microvilli (Fig.24D).

Transverse sections through an appendage tentacle reveal many muscles and accompanying nerve fibers that form the core materials surrounded by an epithelium (Figs.25A,B). The epithelium is simple columnar and lies on a thick basement membrane (Figs.25C,D). Three types of cells can be identified in the epithelium. The first cell type is sensory cell; it has oval body with a round or oval nucleus containing euchromatin with a few block of heterochromatin in the center. These cells are densely-stained with methylene blue and PAS-methylene blue (Figs.25C,D). The second type of cell is supporting cell. It has an oval shape; the nucleus contains euchromatin with a few block of heterochromatin in the middle. This cell type is more lightly stained with methylene blue. The last cell type is mucous-secreting cell; it has a typical goblet cell appearance. The cell is small has oval shape, and is positively stained with PAS (Fig.25D). All area of appendage tentacle is covered with brush border.

5.4 Epipodium tentacle

The structures of the countless epipodium tentacles are the same as that already described for the cephalic tentacles, but they are about three times smaller and ten times shorter than the cephalic tentacle. It is divided into three parts: 1/5 basal, 1/5 middle and 3/5 top (Fig.26A). The surface at the basal part has many folds and grooves (Fig.26B). On the folds, there are many short papillae. The middle top of some papillae have a circle of cilia (Fig.26A). In contrast, at the middle part there are no grooves and folds. The papillae are larger and higher than those in the base; they appear like a hillock with a circle of cilia on the top. The last part, the top, has many high papillae which are slender in shape (Fig.26D). The papillae appear like a truncated cone with a circle of cilia in the middle top. Most surfaces of the papillae are covered with microvilli (Figs.26E).

In paraffin sections, the epipodium tentacles resemble the cephalic tentacles. There is a bundle of epipodium tentacle nerve in the center and their branches are distributed among the muscle bundles (Figs.27A,B). The surface epithelium is covered by brush border (Fig.27). The epithelium of the base is flat with some area having a small curve (Figs.28A,B). The middle part has surface that appears like hillocks (Fig.28C). In contrast, the epithelium of the remaining top part is quite different; it has many cone-shaped structures (Figs.29A,B). The hillocks in the middle part (Fig.28C) and the cones in the top part (Figs.29B,C,D) are the same structure, which are believed to be sensory papillae. It has cilia penetrating at the top through the brush border that covers them (Fig.29C).. In the papillae, epithelial cells are classified into three cell types based on their characteristics and staining affinities.

1. Sensory cell (se). These cells are small with the cell body having tall columnar shape (Figs.28A,29C). The nucleus is oval, and contains mostly euchromatin with a thin rim of heterochromatin (Fig.28C). The cytoplasm shows the blue staining with methylene blue and pinkish purple with PAS-methylene blue (Figs.28B,D,29D).

2. Supporting cell (su). These cells are larger than the sensory cell, with columnar cell body (Figs.28A,29C). The nucleus is round or oval, and contains mostly pale-stained euchromatin with prominent nucleoli (Fig.29C). The cytoplasm shows pale staining with methylene blue and pinkish color with PAS-methylene blue (Figs.29C,D).

3. Goblet cell (Gb). They are mucous-secreting cell which have small oval shape nuclei (Fig.28A). The apical cytoplasm is large, and shows intense staining with methylene blue and pinkish color with PAS-methylene blue (Figs.28B,C).

5.5 Gills and Osphradium

5.5.1 Gills

H.asinina has two bipectinate gills, positioned slightly left of the center in the mantle cavity and pointing anteriorly with apices to the right side (Fig.1D). The gill is attached to the mantle by a thin membrane (Fig.30A). There are equal filaments on both sides. Towards the anterior and posterior ends of the gill, the filaments gradually decrease in length (Fig.30B). All filaments lie parallel to each other. They are delicate pleats with blunt free tips and are corrugated in the middle (Figs.30B,31A).

Transverse sections through gill filaments reveal a single layered epithelium (Figs.30C,D). Each filament is supported axially by a thin collagenous connective

tissue, enclosing the hemocoelic space which contain hemocytes (Fig.30C). In the efferent side, there is a V-shaped opaque rods or chitinous skeletal rods (Figs.30C,31B). The rods serve for attachment of muscles that bring about considerable movement of the plates. There is columnar epithelium that varies much in thickness in different parts of the filments.

Cells in the filament are tall columnar mixed with ciliated and secretory cells, but in the central region of each plate the cells are cuboid and have no cilia (Figs.30C,31D). The columnar cell has an oval nucleus with mostly euchromatin and a few nucleoli. There are numerous cilia on the blunt tip of efferent side and the both side of hemocoelic space, but the cilia on the tip are much shorter (Fig.30C). Longitudinal muscle bands are seen on the wall of the efferent sinus. They are attached to the curved part of the V-shaped skeletal rod, but do not penetrate into the filament. Five types of cells can be identified in the efferent epithelium:

1. Cuboidal cells with round nuclei are observed on the lateral side of the skeletal rod (Fig.31B).

2. Tall columnar cells with cilia are seen in the terminal epithelium (Fig.31B).

3. Goblet cells with large basophillic granules and small nuclei. They are also found on the lateral side of the rod (Fig.31B).

4. Goblet cells with small metachromatic granules. They are less numerous than type-3 cells and are found only at the tip of the efferent end (Fig.31B).

5. Small columnar cells with dense granules and microvilli (Fig.31B).

The afferent epithelium of the gills consists of three types of cells.

1. Cuboidal cells with round nuclei are seen on the lateral epithelium. They have euchromatic nuclei with distinct nucleoli (Fig.31D).

2. Tall columnar cells with round nuclei are found in the terminal epithelium. They possess oval nuclei with a few nucleoli (Fig.31D).

3. Goblet cells with numerous granules in the apical cytoplasm. These cells have densely stained nuclei with some blocks of heterochromatin. Their granules are intensely stained with methylene blue (Fig.31D).

5.5.2 Osphradium

The osphradium of *H.asinina* is located on the dorsal surface and runs parallel to the gill. It occurs as yellow-brown ridge with one side having a thick mantle. It consist of many axis from which 8-11 leaves arise (Figs.32A,B). The leaves decline from the axis to the lower level and branch into 2-3 terminals. Many leaflets apart from both sides of each leaf (Figs.32A,C). The osphradium exhibits many tufts of rod-shaped cilia, paddle-like cilia, granules and their exocytosed pores on the surface of major and minor folds, which are called leaves and leaflets. (Figs.32D,E,F,G). In contrast, the junction of axis and leaf has a few ciliary tufts, round granules and their exocytosed pores on the fold. There are deep grooves between the flat folds (Fig.32H).

In the transverse section, the osphradia are present on the dorsal side and are located between both sides of gill (Fig.30A). Both lateral sides of osphradia are connected to the gill by the mantle membrane. The osphradia appear like folds; the major fold is described as leaf and the minor fold as leaflets. Each leaf and leaflet can be divided into two areas: the basal area which consists mainly of large goblet cells, and the apical area that contains a mixture of supporting cells, sensory cells and mucous-secreting cells (Figs.33C,D). In the basal area, there are alternation of two

types of cells whose large mucin granules were stained either purple or pink (Fig.35D). All of cells and granules are in the outer region of the epithelium, which lies on the connective tissue that contain the nerve fibers that converge to form nerve tract within the leaf and leaflet (Figs.33C,D). The cells found in the osphradia are supporting cells, sensory cells and mucous cells.

1. Supporting cell (su). These cells vary in shape, which can be triangular, oval and spindle (Figs.34B,D,35B,C). The nucleus is oval, and contains mostly pale-stained euchromatin (Fig.34B). The upper part of these cells is wide and shows many long cilia (Figs.34B,D).

2. Sensory cell (se). These cells are elongated in shape, and contain euchromatic oval nuclei with many nucleoli (Figs.34B,C). The upper part of the cell is narrow and reaches the surface of the epithelium; it bears some cilia.

3. Mucous cells. They are filled with many secretoy granules and have oval shape similar to that of the goblet cell.

Type-1 mucous cell. The nucleus is round, and located near the basement membrane. The cytoplasm has numerous round light pink granules (Fig.35B).

Type-2 mucous cell. The nucleus is flattened with euchromatin and nucleoli. The cytoplasm contains numerous blue granules (Fig.35B).

Type-3 mucous cell. This cell is small in size. The cytoplasm is filled with many round granules, most of which are positively stained with PAS (Fig.35B).

Type-4 mucous cell. This cell is larger than type-3 cell. The nucleus is small and has oval shape. The secretory granules vary in sizes and staining intensity, some granules are larger and densely stained with methylene blue (Fig.35B).

Type-5 mucous cell. This cell is the largest and shows many large granules in the apical cytoplasm (Fig.35C). The granules are heterogeneous in staining property; some granules are stained with methylene blue, others are metachromatically stained and show pink color.

Figure 1 Gross anatomy of *H. asinina*

A) Photograph showing the external features of *H. asinina* on the dorsal side with shell. At-appendage tentacle, Ep-epipodium tentacle, Ey-eye, pe-pedal muscle, sh-shell, Te-cephalic tentacle

B) Photograph showing the external features of *H. asinina* on the lateral side with shell. Pedal muscle (Pe) is also shown.

C) Photograph showing the internal structures of *H. asinina* after removal of the shell on the dorsal side. ad-adductor muscle, he-head, mt-mantle, Os-osphradium

D) Photograph showing the internal structures of *H. asinina* after removal of shell, the mantle was dissected to show gill (gi) and osphradium (Os). di-digestive gland, go-gonad, in-intestine, rt-rectum

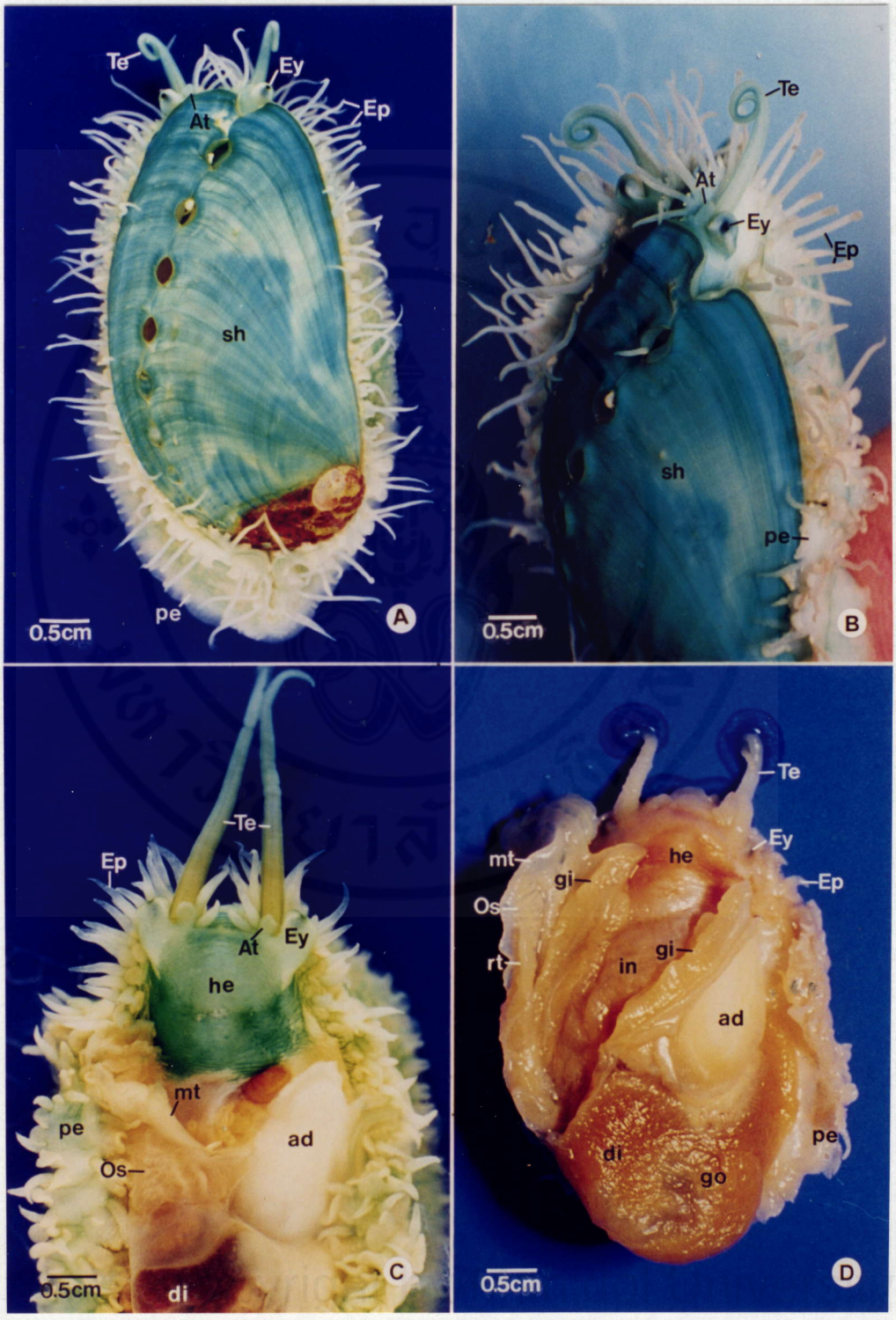

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Figure 2 Paraffin sections of the ganglia of *H. asinina* stained with H&E

A) A low-power micrograph of cross section of the cerebral ganglion, showing thick cell layers on the ventral (V) and dorsal (D) sides. Ca-capillary, Co-cortex, L-lateral, M-medial, Me-medulla, Mu-muscle

B) A low-power micrograph of cross section of the pleuropedal ganglion showing thick cell layers on the dorsal and lateral sides.

C) A low-power micrograph of cross section of the visceral ganglion showing thick cell layers on the ventral side.

D) A high-power micrograph of the pleuropedal ganglion showing various types of nerve cells in the cortex region. NG_1-Type 1 neuroglia, NG_3-Type 3 neuroglia, NR_1-Type 1 neuron, NR_2-Type 2 neuron, NR_3-Type 3 neuron, NS_3-Type 3 neurosecretory cell

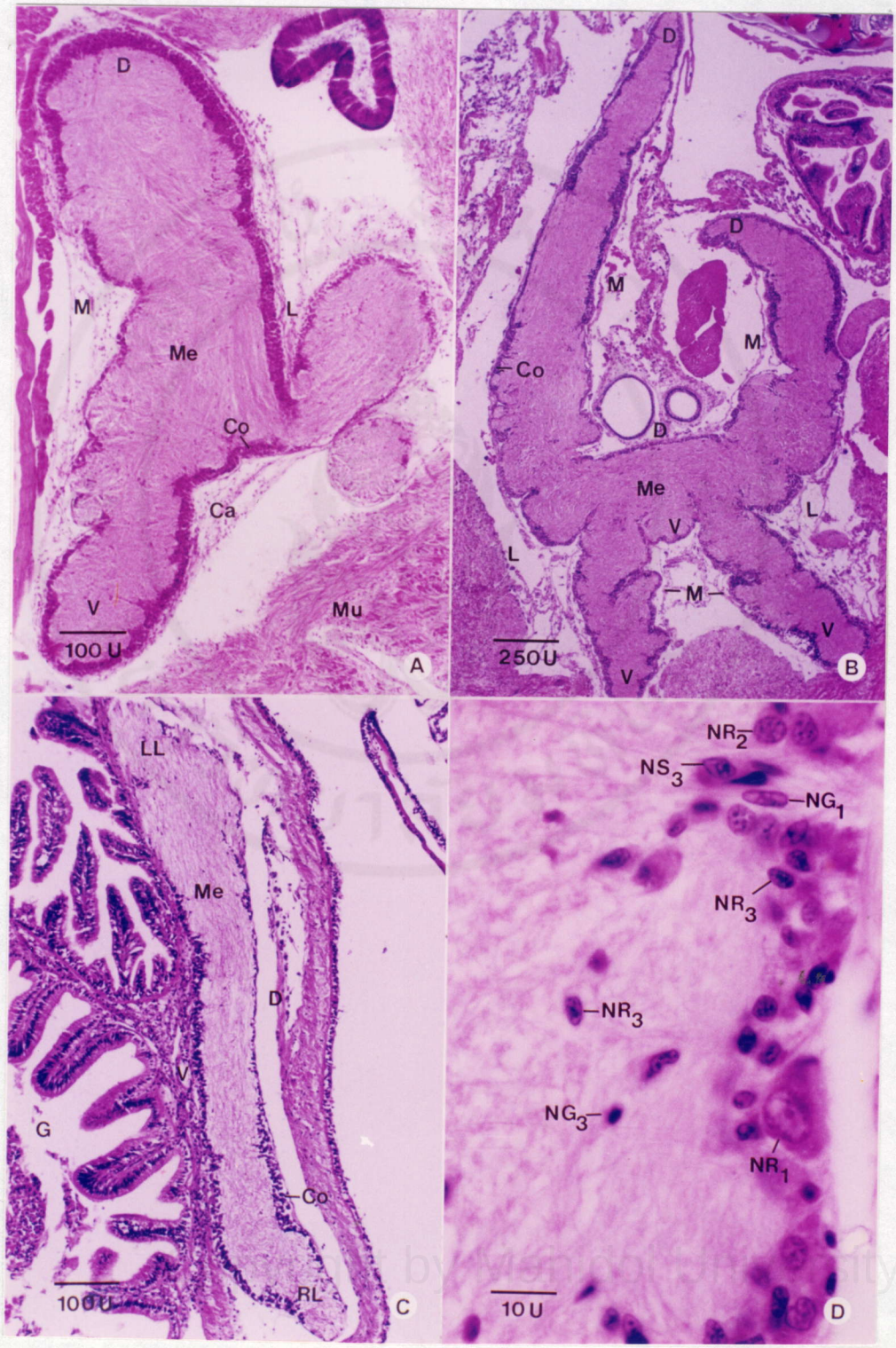
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Figure 3 Paraffin sections of the pleuropedal ganglia of *H. asinina*

A,B) High-power micrographs showing various types of nerve cells in the cortex region of the ganglion stained with H&E. NG_2-Type 2 neuroglia, NG_3-Type 3 neuroglia, NR_4-Type 4 neuron, NS_1-Type 1 neurosecretory cell, NS_2-Type 2 neurosecretory cell, NS_3-Type 3 neurosecretory cell

C) A high-power micrograph showing various types of nerve cells in the cortex region of the ganglion stained with CH-P. Notice that neurosecretory cells (NS) are stained violet. NR-neuron, NS_1-Type 1 neurosecretory cell

D) A high-power micrograph showing various types of nerve cells in the cortex region of the ganglion stained with PF. Notice that the granules of neurosecretory cells are stained purple. NS-neurosecretory cell, NS_1-Type 1 neurosecretory cell, NR-neuron

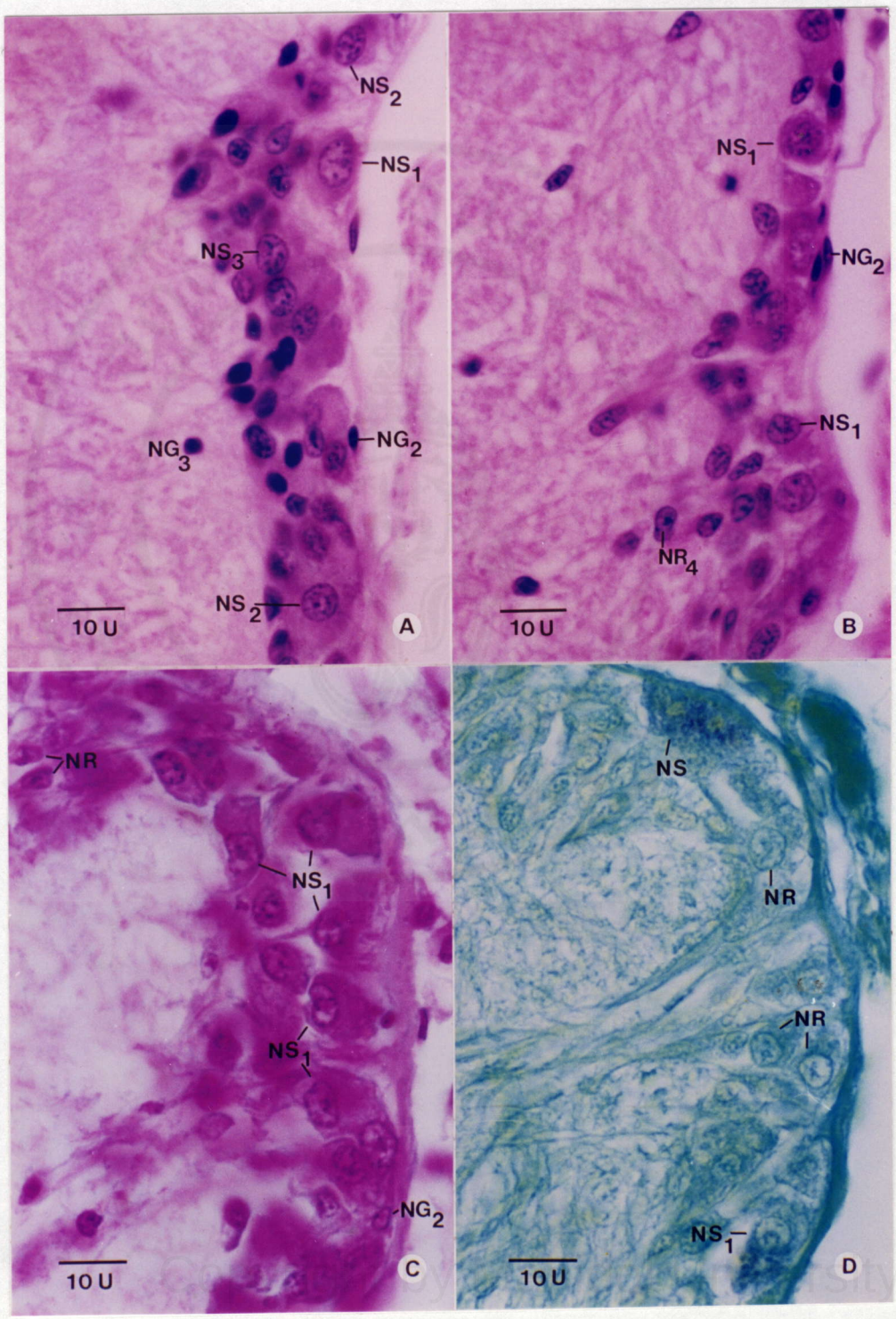
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Figure 4 Paraffin sections of a cross section of the cerebral ganglion of a 4-month-old abalone stained with H&E.

A) A low-power micrograph of the cerebral ganglion which has a bean shape and is surrounded by connective tissue. Orientation of the ganglion is indicated as dorsal (D), ventral (V), lateral (L) and medial (M) sides.

B) A medium-power micrograph of the dorsal part of the ganglion showing cell layers of the cortex. NR_1-Type 1 neuron, NR_2-Type 2 neuron, NS-neurosecretory cell, NG_3-Type 3 neuroglia,

C,D) Higher magnifications showing various types of neurons (NR), neurosecretory cell (NS) and neuroglia (NG) in the cortex.

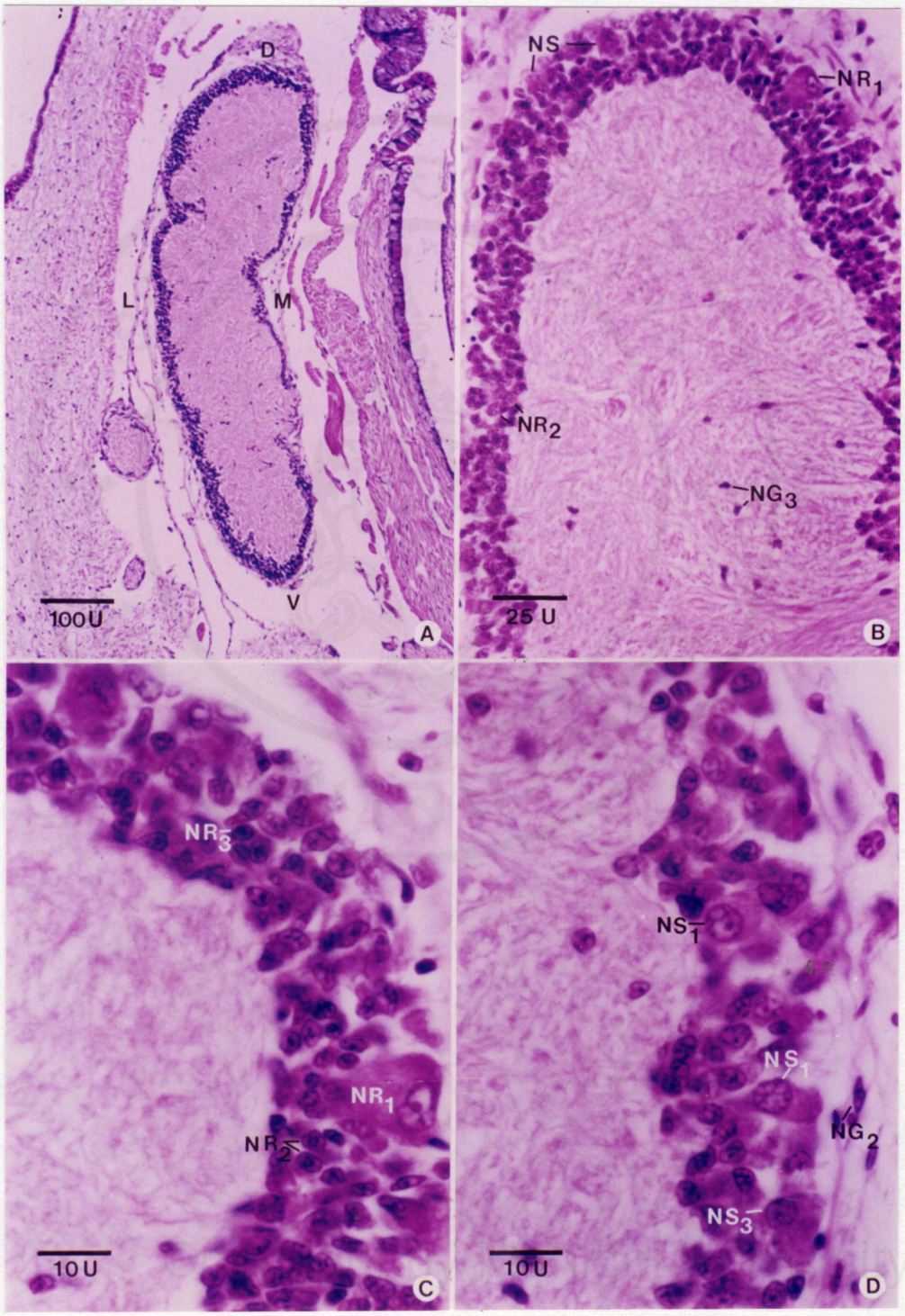
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Figure 5 Paraffin sections of a cross section of the cerebral ganglion of a 5-month-old abalone stained with H&E.

A) A low-power micrograph of the cerebral ganglion which has a bean shape. D-dorsal, L-lateral, M-medial, V-ventral

B) A medium-power micrograph of the dorsal part of the ganglion showing cell layers of the cortex. NG_3-Type 3 neuroglia, NR-neuron, NS-neurosecretory cell

C,D) High-power micrographs showing three types of neurosecretory cells ($NS_{1,2,3}$) in the cerebral ganglion. NR_2-Type 2 neuron

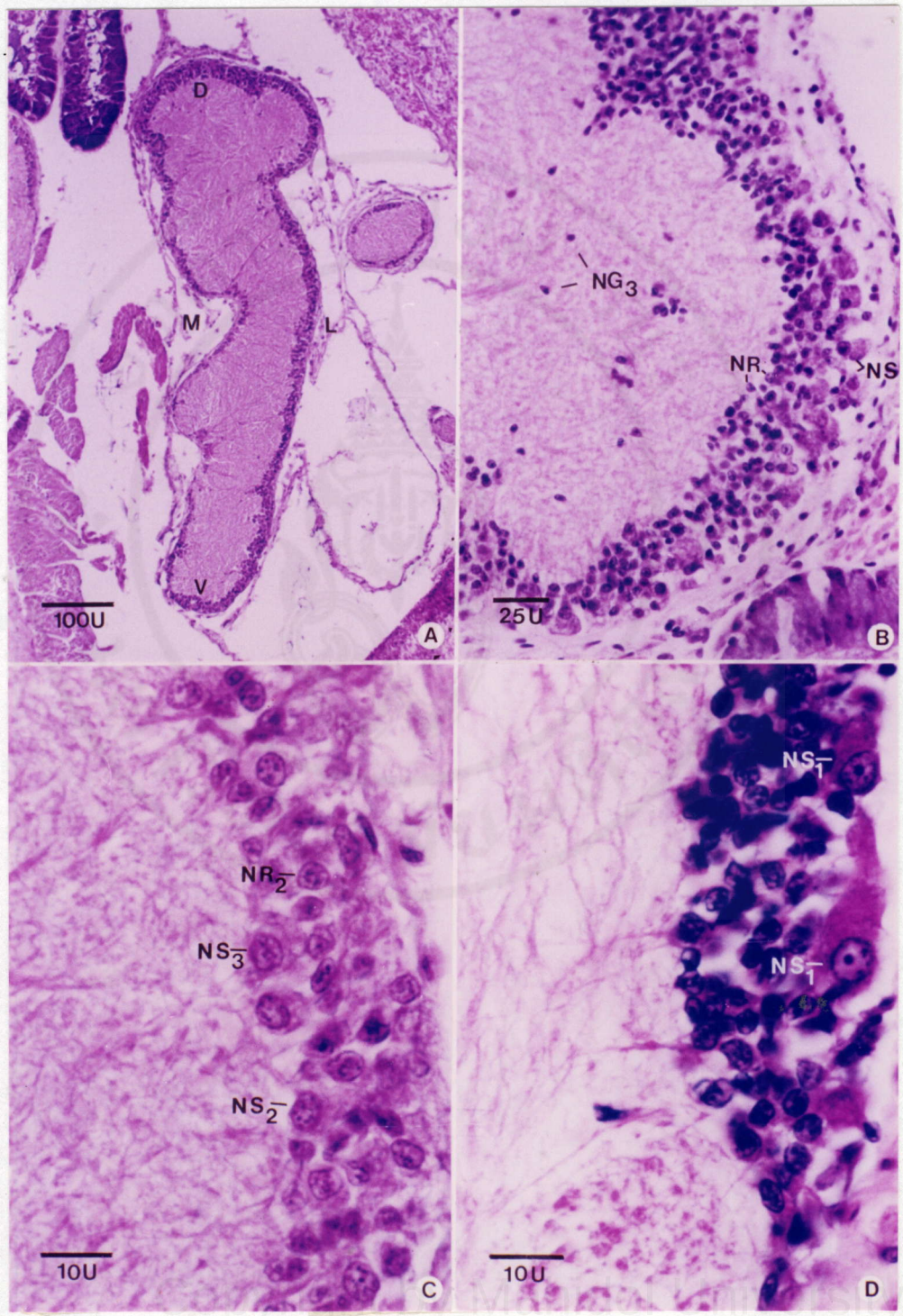
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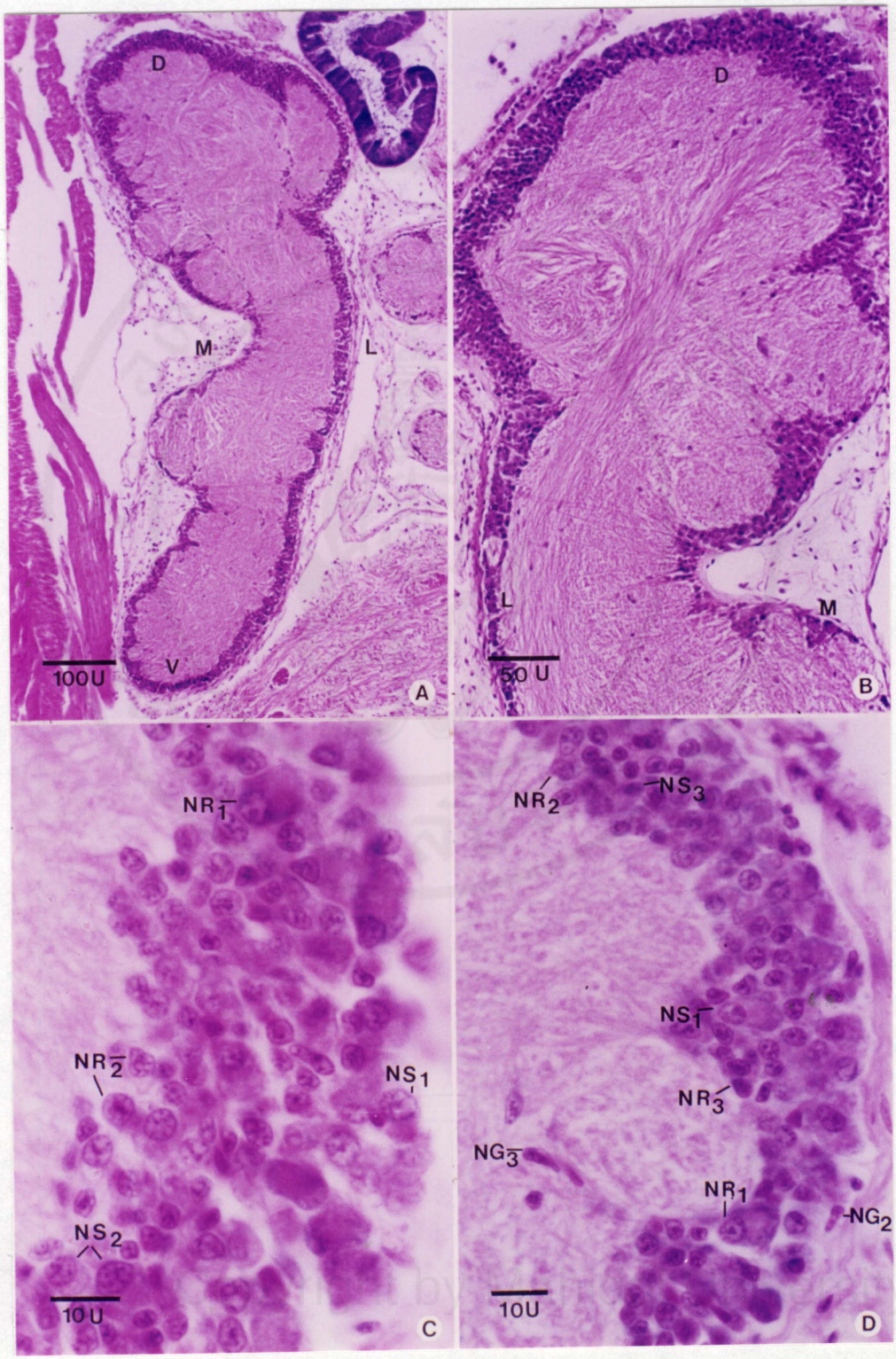
Figure 6 Paraffin sections of a cross section of the cerebral ganglion of a 10-month-old abalone stained with H&E.

A) A low-power micrograph of the cerebral ganglion which has sickle shape. D-dorsal, L-lateral, M-medial, V-ventral

B) A medium-power micrograph of the dorsal part of the cerebral ganglion showing cell layers of the cortex and bundle of nerve fibers in the medulla

C) A high-power micrograph of the cerebral ganglion showing various types of cells in the cortex region; neuron type 1 and 2 (NR_1, NR_2) and neurosecretory cell type1 and 2 (NS_1, NS_2).

D) Another view of the cerebral ganglion showing three types of neurons, type 1 and type 3 neurosecretory cells, type 2 and type 3 neuroglia. Notice the type 3 neuroglia (NG_3) is located in the medulla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NS1
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NG3
NR1
NG2
10U
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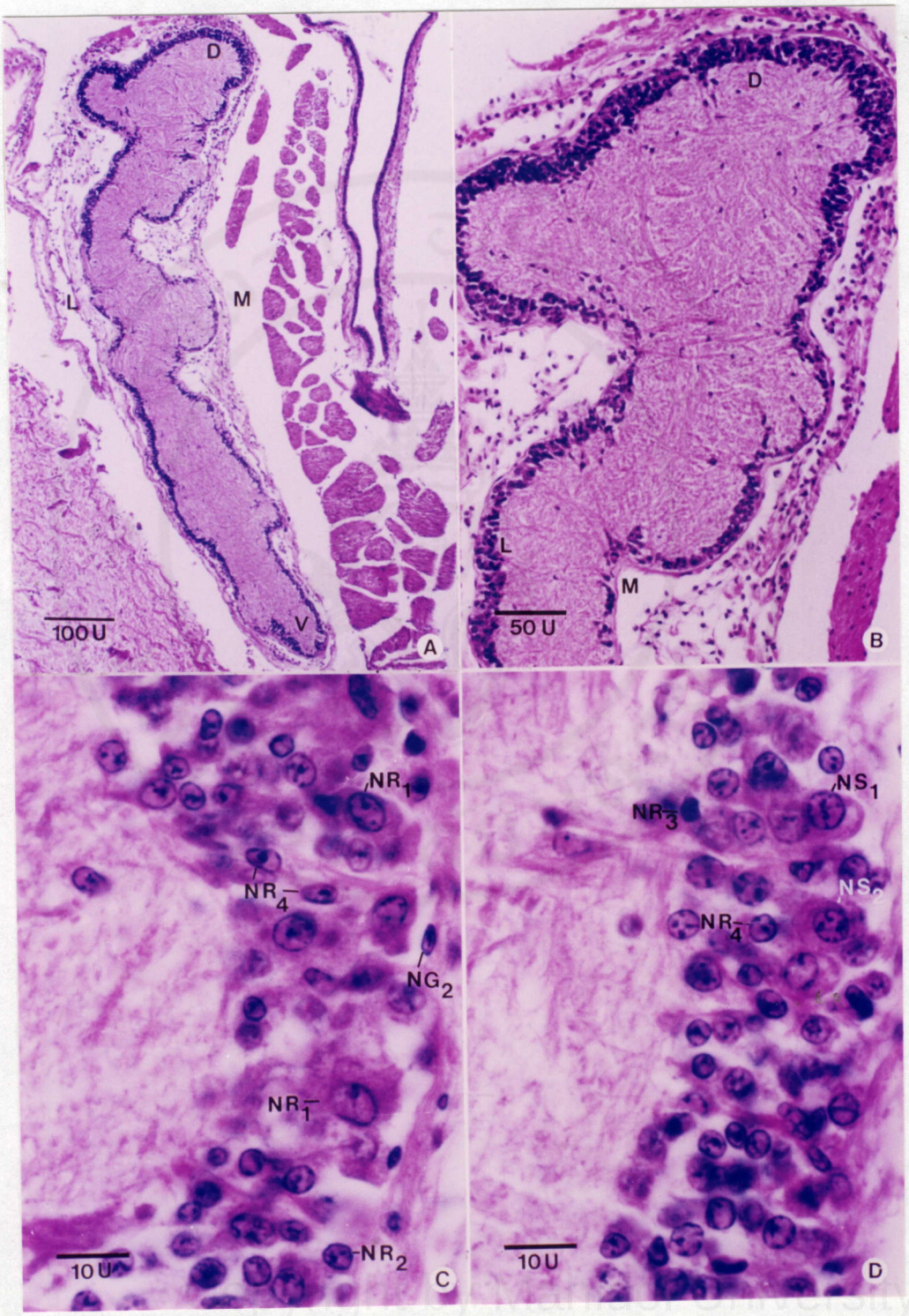
Figure 7 Paraffin sections of a cross section of the cerebral ganglion of a 12-month-old abalone stained with H&E.

A) A low-power micrograph of the cerebral ganglion which is more convoluted and elongated D-dorsal, L-lateral, M-medial, V-ventral

B) A medium-power micrograph of the dorsal part of the cerebral ganglion showing cell layers of the cortex. The nerve fibers in the medulla run in many different directions.

C,D) Higher magnifications showing all types of cells in the cerebral ganglion. NG_2-Type 2 neuroglia, NR_1-Type 1 neuron, NR_2-Type 2 neuron, NR_3-Type 3 neuron, NR_4-Type 4 neuron, NS_1-Type 1 neurosecretory cell, NS_2-Type 2 neurosecretory cell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NR3
NR4
10 U
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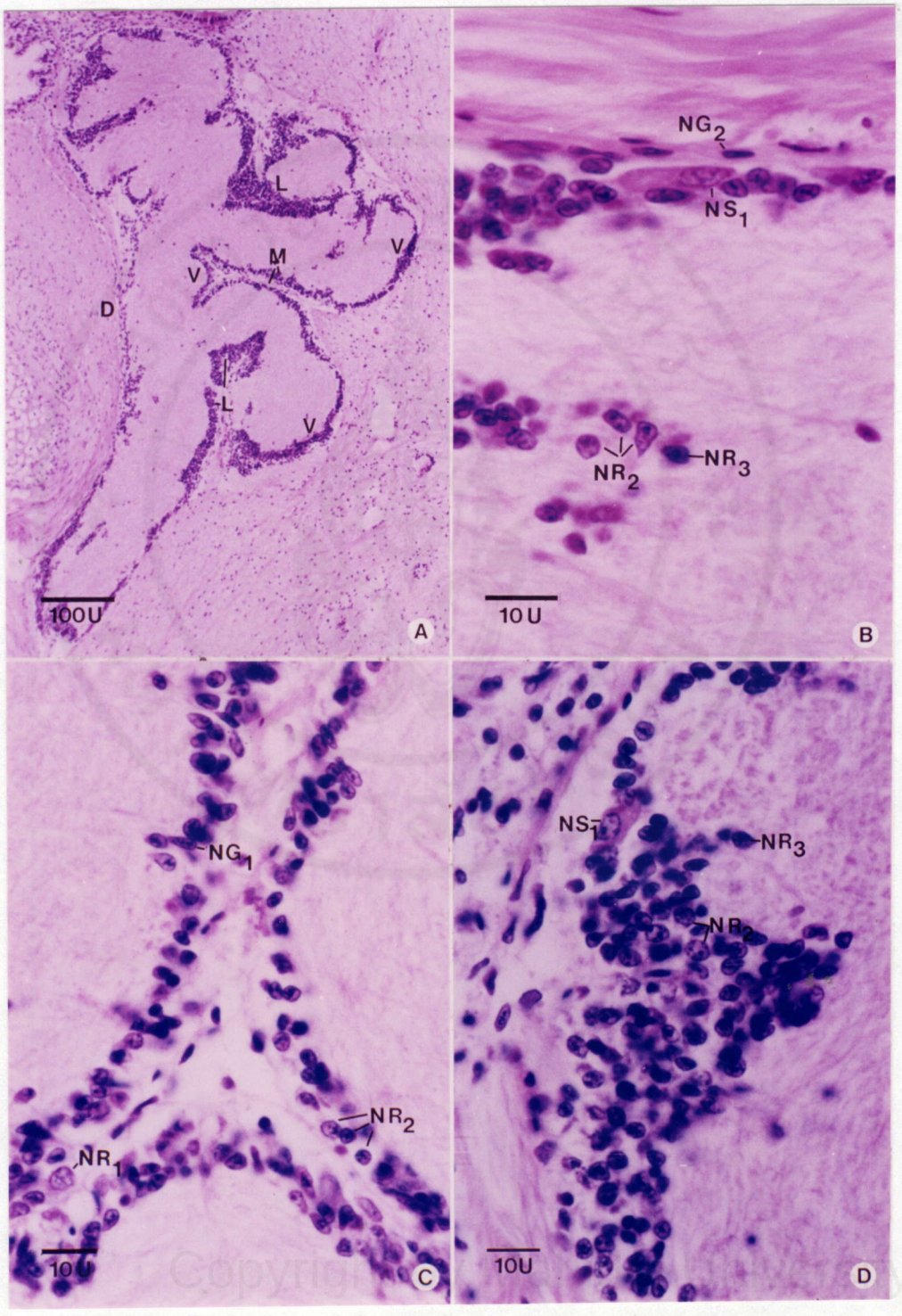
Figure 8 Paraffin sections of a cross section of the pleuropedal ganglion of a 1-month-old abalone stained with H&E.

A) A low-power micrograph of the pleuropedal ganglion which has a butterfly shape and is surrounded by connective tissue. Orientation of the ganglion is indicated as dorsal (D), ventral (V), lateral (L) and medial (M) sides.

B,C,D) High-power micrographs showing all types of cells in the pleuropedal ganglion. Notice the presence of only the first type of neurosecretory cell (NS_1) at this age. NG_1-Type 1 neuroglia, NG_2-Type 2 neuroglia, NR_1-Type 1 neuron, NR_2-Type 2 neuron, NR_3-Type 3 neuron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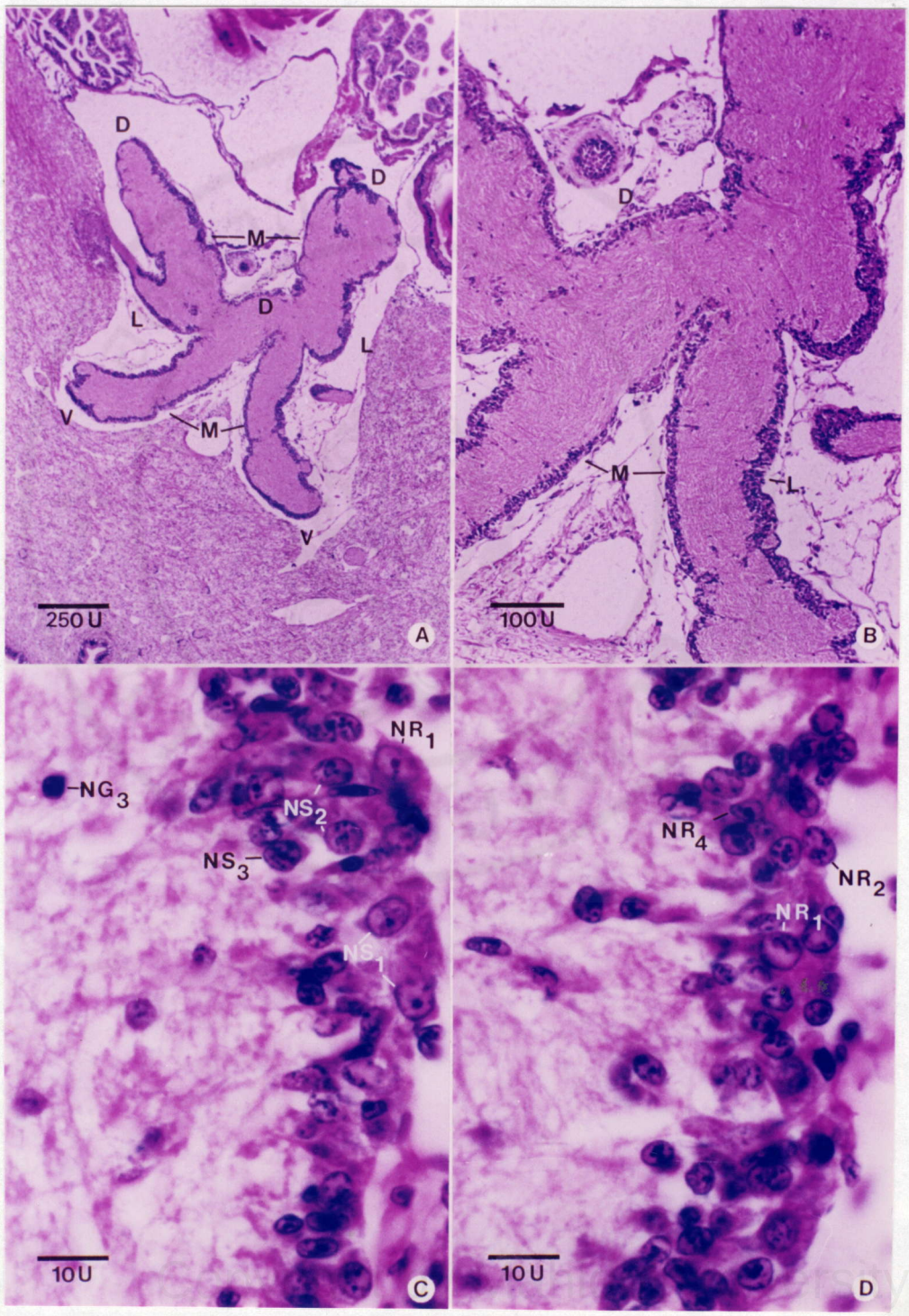
D

Figure 9 Paraffin sections of a cross section of the pleuropedal ganglion of a 4-month-old abalone stained with H&E.

A) A low-power micrograph of the pleuropedal ganglion which is larger in size and still has a butterfly shape. D-dorsal, L-lateral, M-medial, V-ventral

B) A medium-power micrograph showing cell layers of the cortex. Nerve fibers occupy most part of the medulla.

C,D) Higher magnifications showing neuroglia type 3 (NG_3), neuron type 1, 2and 4 (NR_1, NR_2, NR_4) and neurosecretory cell type 1, (NS_1) in the cortex. Type 2 and type 3 neurosecretory cells (NS_2, NS_3) are also present in the cortex at this age.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D

Figure 10 Paraffin sections of a cross section of the pleuropedal ganglion of a 7-month-old abalone stained with H&E.

A) A low-power micrograph of the pleuropedal ganglion which has a H shape. D-dorsal, L-lateral, M-medial, V-ventral

B) A medium-power micrograph of the ganglion showing cell layers of the cortex. The statocysts (s) are also observed in this area

C,D) High-power micrographs showing all types of cells in the cortex of the pleuropedal ganglion. NG_2-Type 2 neuroglia, NR_1-Type 1 neuron, NR_2-Type 2 neuron, NR_3-Type 3 neuron, NS_1-Type 1 neurosecretory cell, NS_2-Type 2 neurosecretory cell

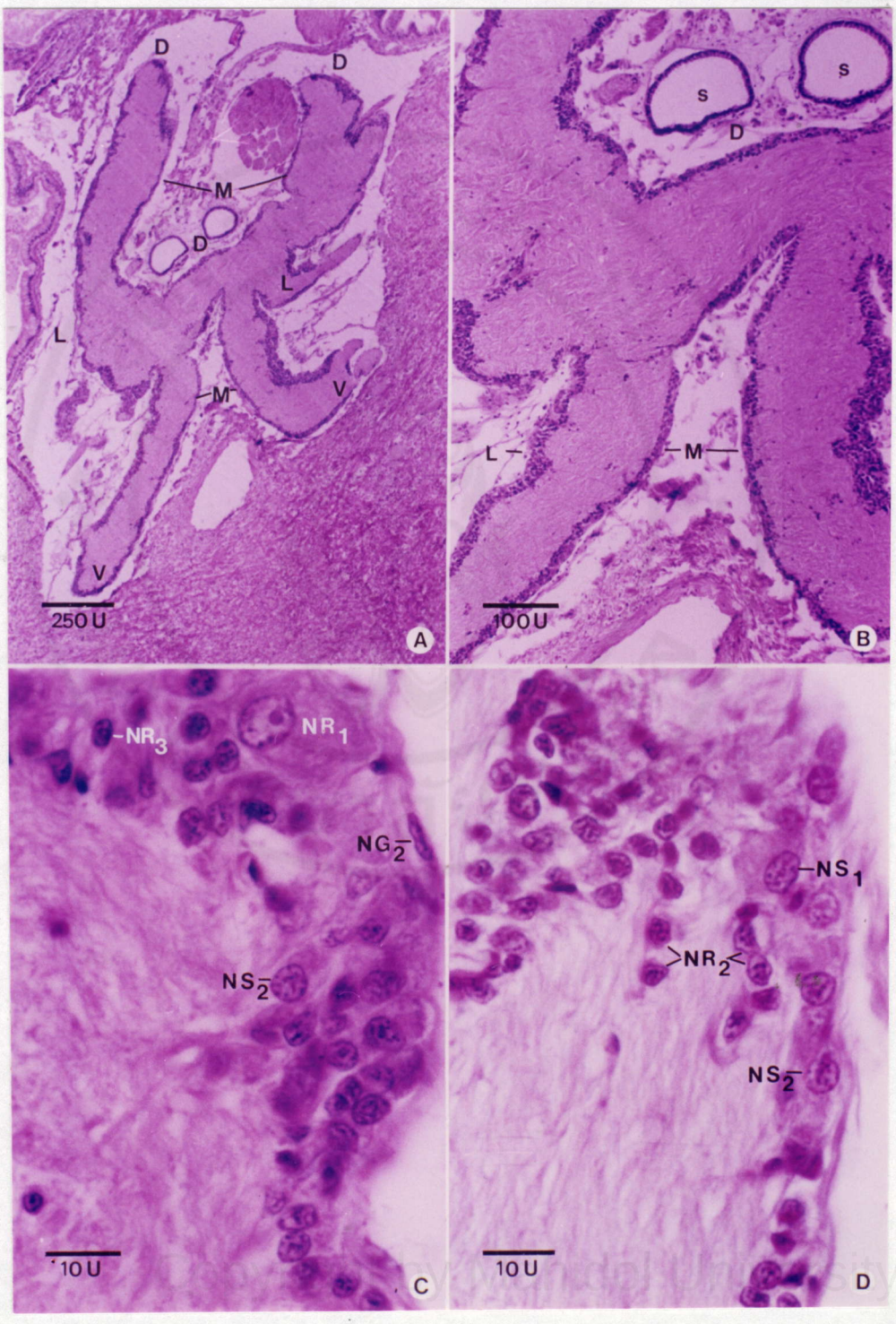

D
D
M
D
L
L
M
V
V
250 U
A
S
S
D
L
M
100 U
B
NR3
NR1
NG2
NS2
10 U
C
NS1
NR2
NS2
10 U
D

Figure 11 Paraffin sections of a cross section of the pleuropedal ganglion of an 11-month-old abalone stained with H&E.

A) A low-power micrograph of the pleuropedal ganglion which is larger and still has a H shape. D-dorsal, L-lateral, M-medial, V-ventral

B) A medium-power micrograph of the ganglion showing cell layers of the cortex and nerve fibers in the medulla.

C,D) High-power micrographs showing neuroglia type 1 (NG_1), neuron type 1,2 and 3 (NR_1, NR_2, NR_3) and neurosecretory cell type 1 and 2 (NS_1, NS_2) in the cortex.

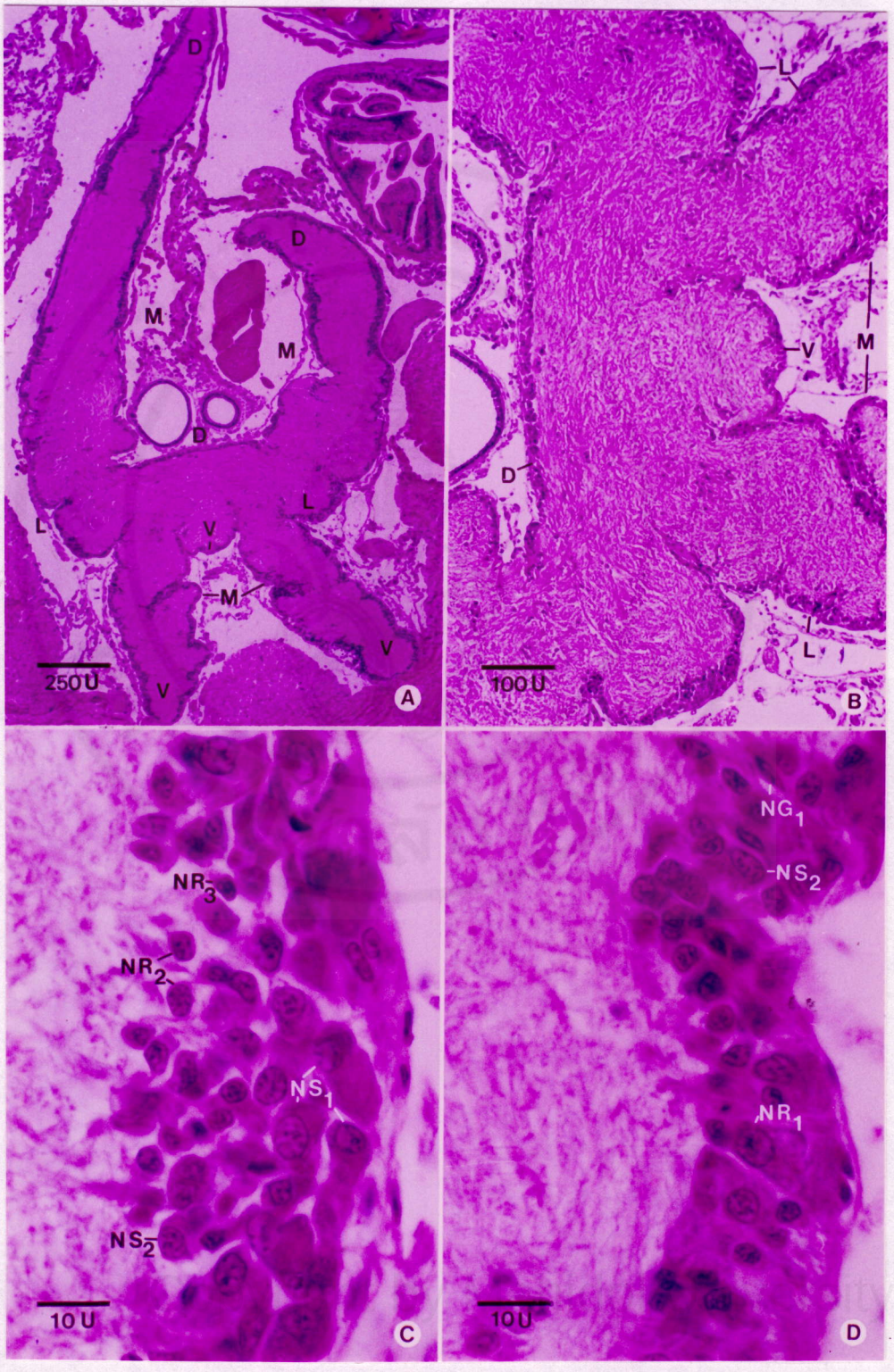

D
D
M
M
D
L
L
V
M
V
V
250 U
A
L
V
M
D
L
100 U
B
NR3
NR2
NS1
NS2
10 U
C
NG1
NS2
NR1
10 U
D

Figure 12 Paraffin sections of a cross section of the pleuropedal ganglion of 4-month-old abalone

A,B) High-power micrographs of the ganglion stained with chrome-hematoxylin-phloxine showing various types of nerves cells in the cortex. Notice that the cytoplasm of the type 1 and type 2 neurosecretory cell (NS_1, NS_2) is stained violet. NR_1-Type 1 neuron, NR_2-Type 2 neuron, NR_4-Type 4 neuron

C,D) High-power micrographs of the ganglion stained with paraldehyde fuchsin. Notice that the granules of neurosecretory cell (NS) are stained purple.

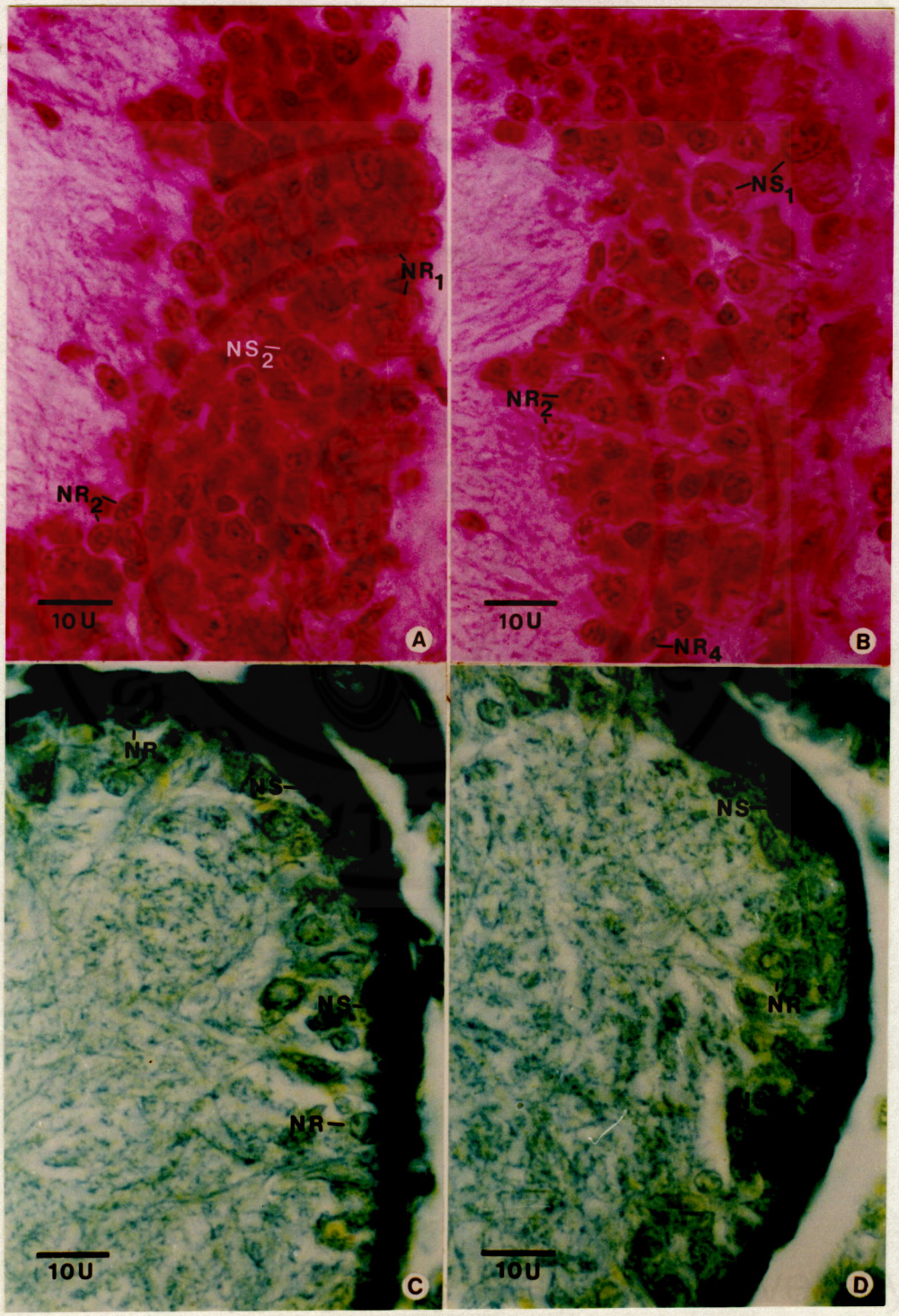

NR1
NS2
NR2
10U
A
NS1
NR2
NR4
10U
B
NR
NS
NS
NR
10U
C
NS
NR
10U
D

Figure 13 Paraffin sections of a cross section of the visceral ganglion of a 1-month-old abalone stained with H&E.

A) A low-power micrograph of the visceral ganglion which has a bean shape and is surrounded by connective tissue. Orientation of the ganglion is indicated as left lateral (LL), medial (M), right lateral (RL) and ventral (V). Gu-gut

B,C,D) High-power micrographs showing the cells which are found in the visceral ganglion at this age. Notice that there is only a single layer of cells in the ganglion. NG_1-Type 1 neuroglia, NG_2-Type 2 neuroglia, NG_3-Type 3 neuroglia, NR_2-Type 2 neuron, NR_3-Type 3 neuron, NR_4-Type 4 neuron

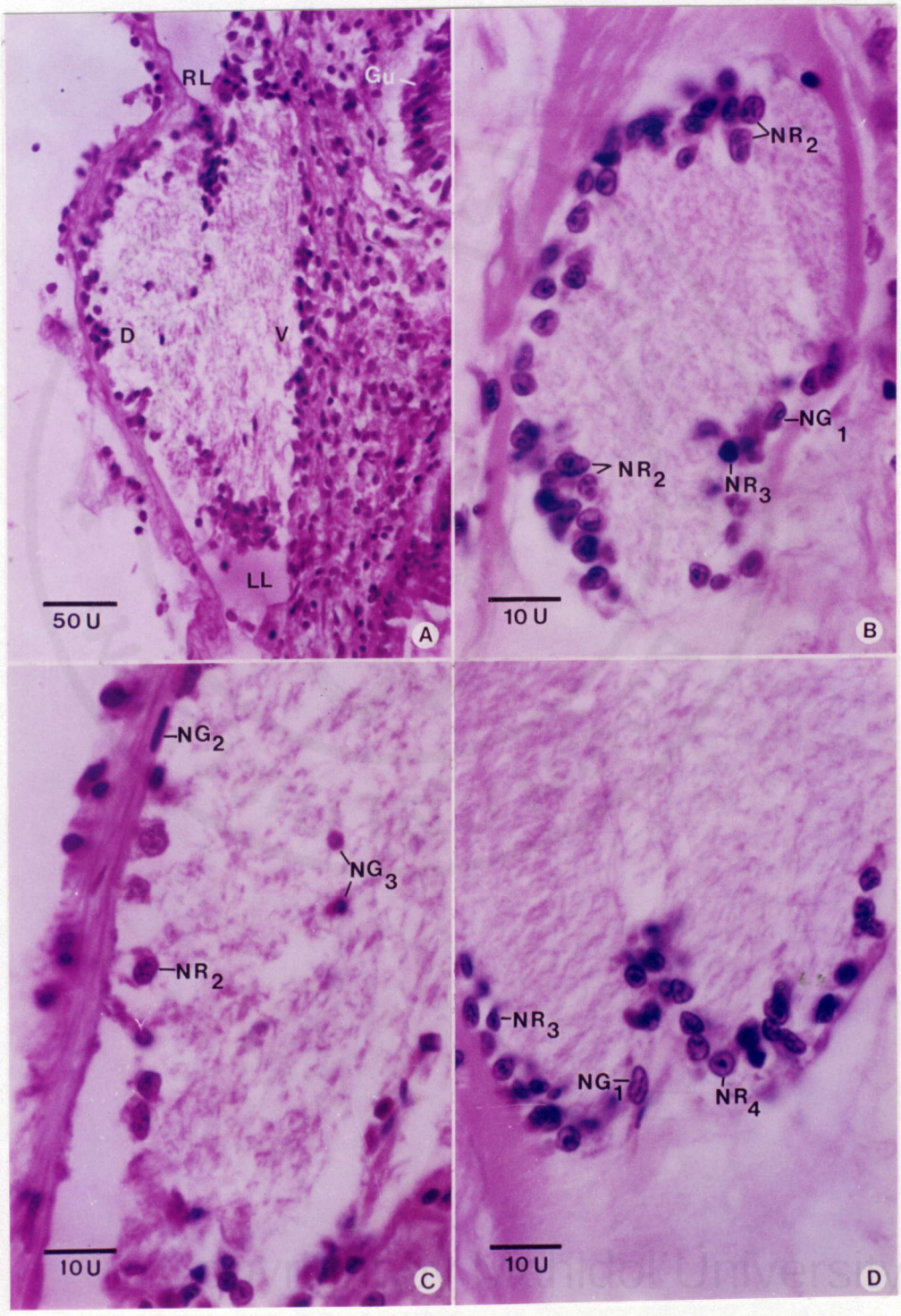

RL
Gu
D
V
LL
50 U
A
NR2
NG1
NR2
NR3
10 U
B
NG2
NG3
NR2
10 U
C
NR3
NG1
NR4
10 U
D

Figure 14 Paraffin sections of a cross section of the visceral ganglion of a 6-month-old abalone stained with H&E.

A) A low-power micrograph of the visceral ganglion which has a dumbbell shape. D-dorsal, LL-left lateral, M-medial, RL-right lateral, V-ventral, Gu-gut

B) A medium-power micrograph of the left lateral part of the ganglion showing two cell layers of the cortex.

C,D) Higher magnifications showing the cells which are found in the visceral ganglion. Notice that Type 1 neuron (NR_1), Type 1 and 2 neurosecretory cell (NS_1, NS_2) appear at this age. NG_3-Type 3 neuroglia, NR_2-Type 2 neuron, NR_3-Type 3 neuron

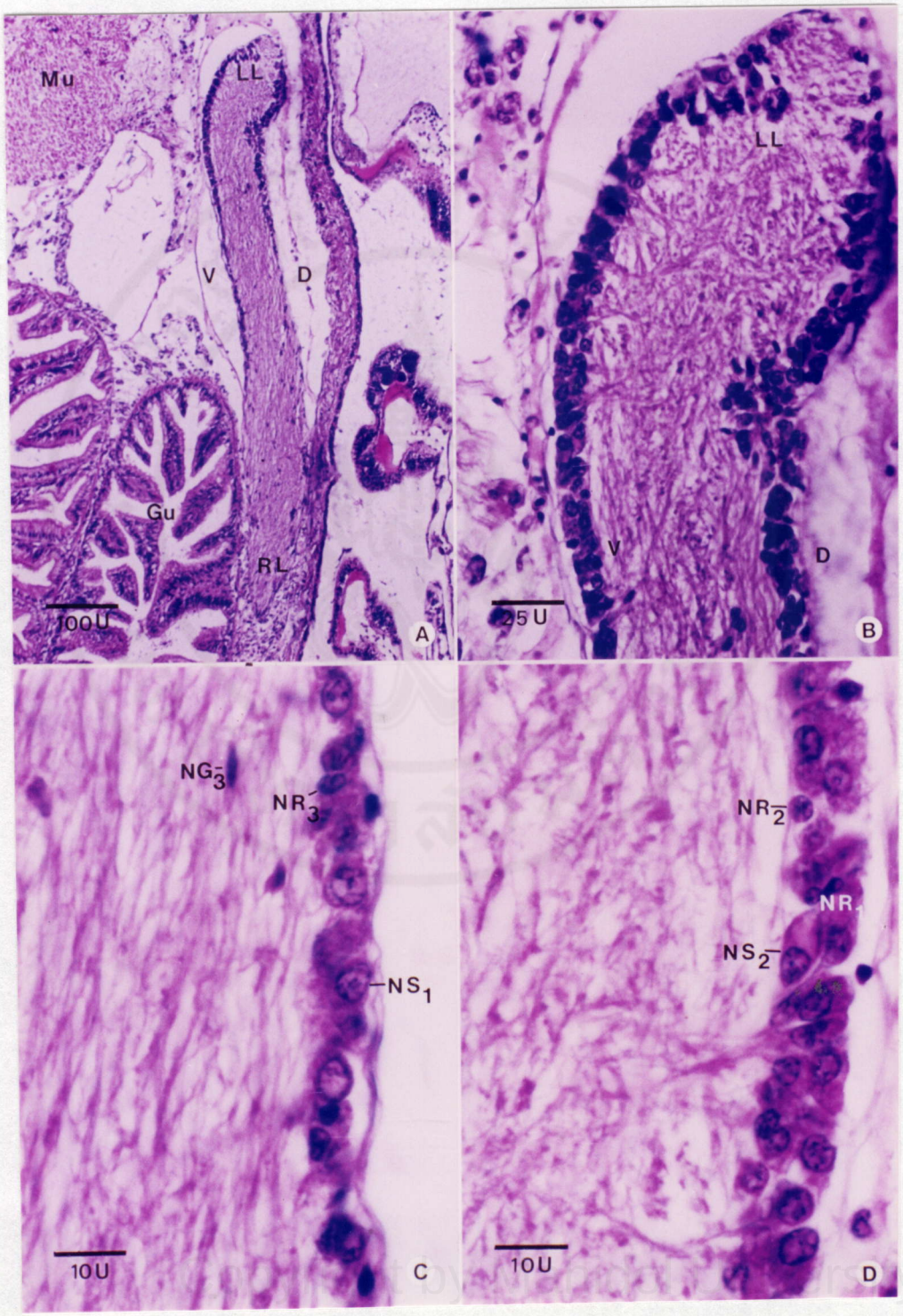
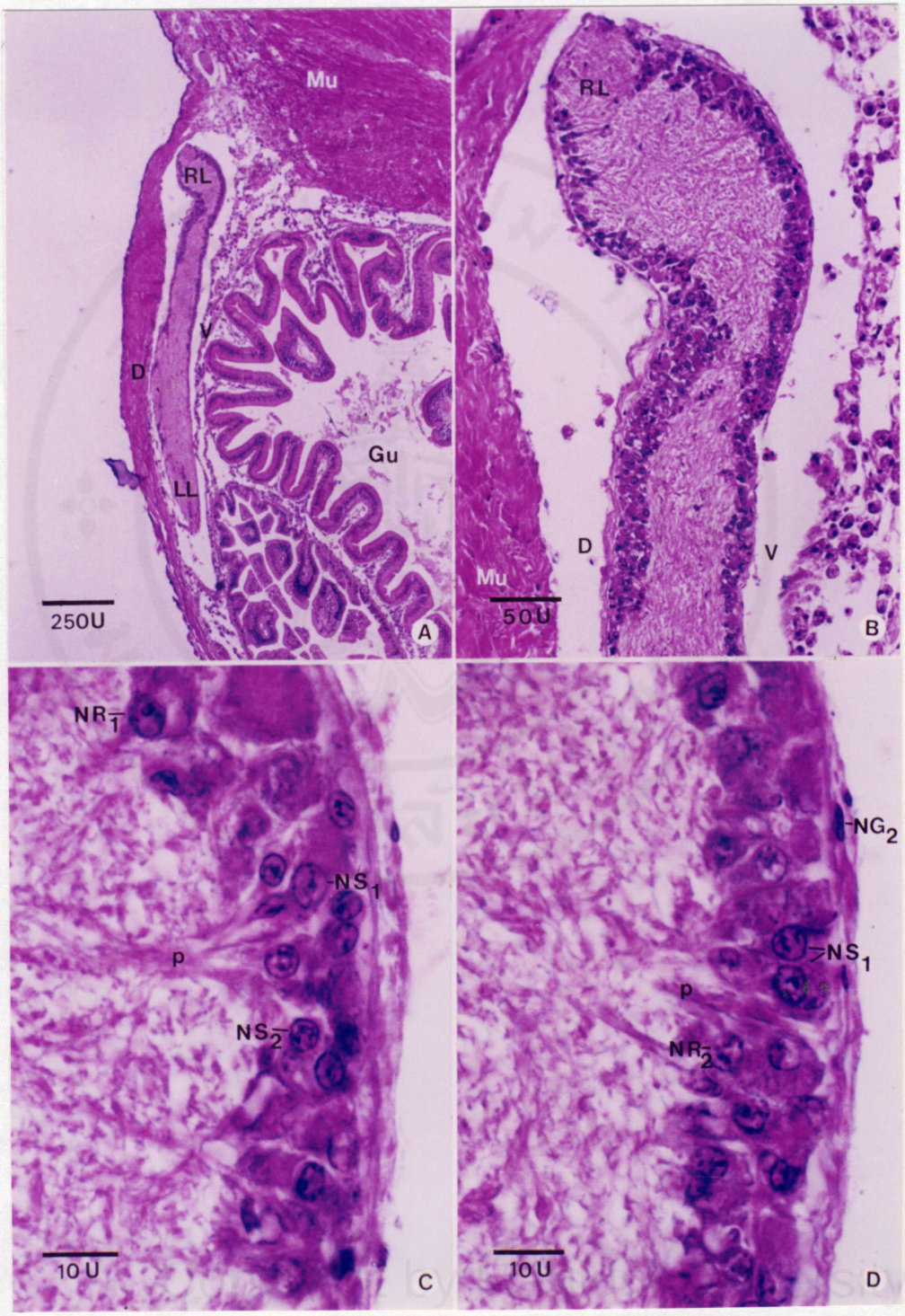

Mu
LL
V
D
Gu
RL
100U
A
LL
V
D
25U
B
NG3
NR3
NS1
10U
C
NR2
NR1
NS2
10U
D

Figure 15 Paraffin sections of a cross section of the visceral ganglion of an 11-month-old abalone stained with H&E.

A) A low-power micrograph of the visceral ganglion which is elongated and still has a dumbbell shape. D-dorsal, LL-left lateral, M-medial, RL-right lateral, V-ventral, Gu-gut, Mu-pedal muscle

B) A medium-power micrograph of the right lateral part of the ganglion showing three cell layers of the cortex.

C,D) High-power micrographs showing all types of cells in the ganglion: neuroglia type 2 (NG_2), neuron type 1 and 2 (NR_1, NR_2) and neurosecretory cell type 1 and 2 (NS_1, NS_2). Notice that the processes (p) of the neurons project into the medulla.


Mu
RL
V
D
LL
Gu
250U
A
RL
D
V
Mu
50U
B
NR1
NS1
p
NS2
10U
C
NG2
NS1
p
NR2
10U
D

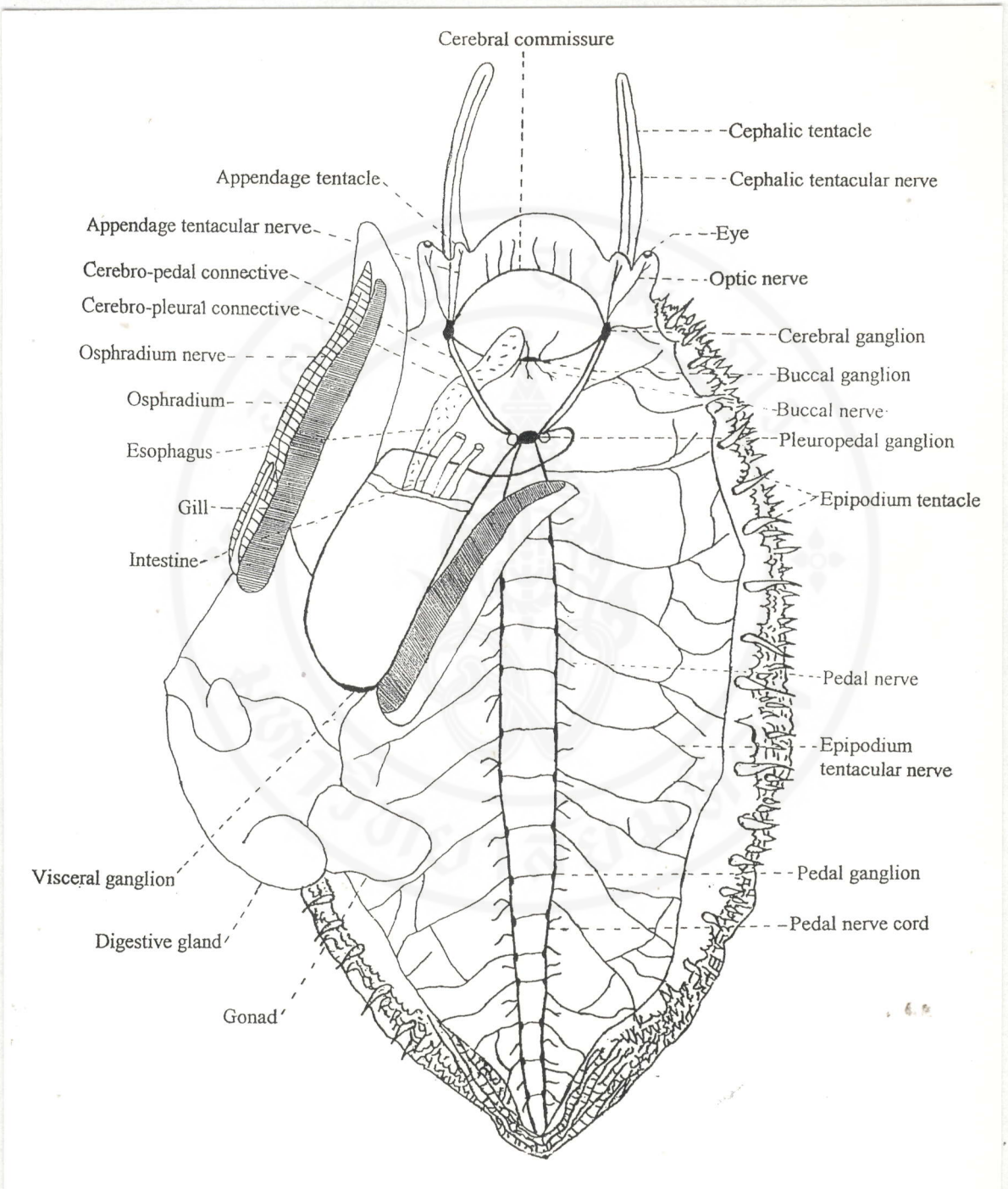


Figure 16 Diagramatic drawing of the gross anatomy of the adult abalone showing the ganglia and their peripheral nerves. [modified from Crofts (3)]

Figure 17 SEM micrographs of the cephalic tentacle of an adult abalone

A) A low-power micrograph of the basal (1) and middle (2) parts of the tentacle.

B) A medium-power micrograph of the base part showing the surface which has groove (g) and fold (f) containing short papillae (pa) on them.

C) A medium-power micrograph of the middle part showing the surface papillae (pa). Notice that the papillae are higher than those on the basal part.

D) A high-power micrograph of a papilla of the middle part showing a tuft of cilia (C) on the top.

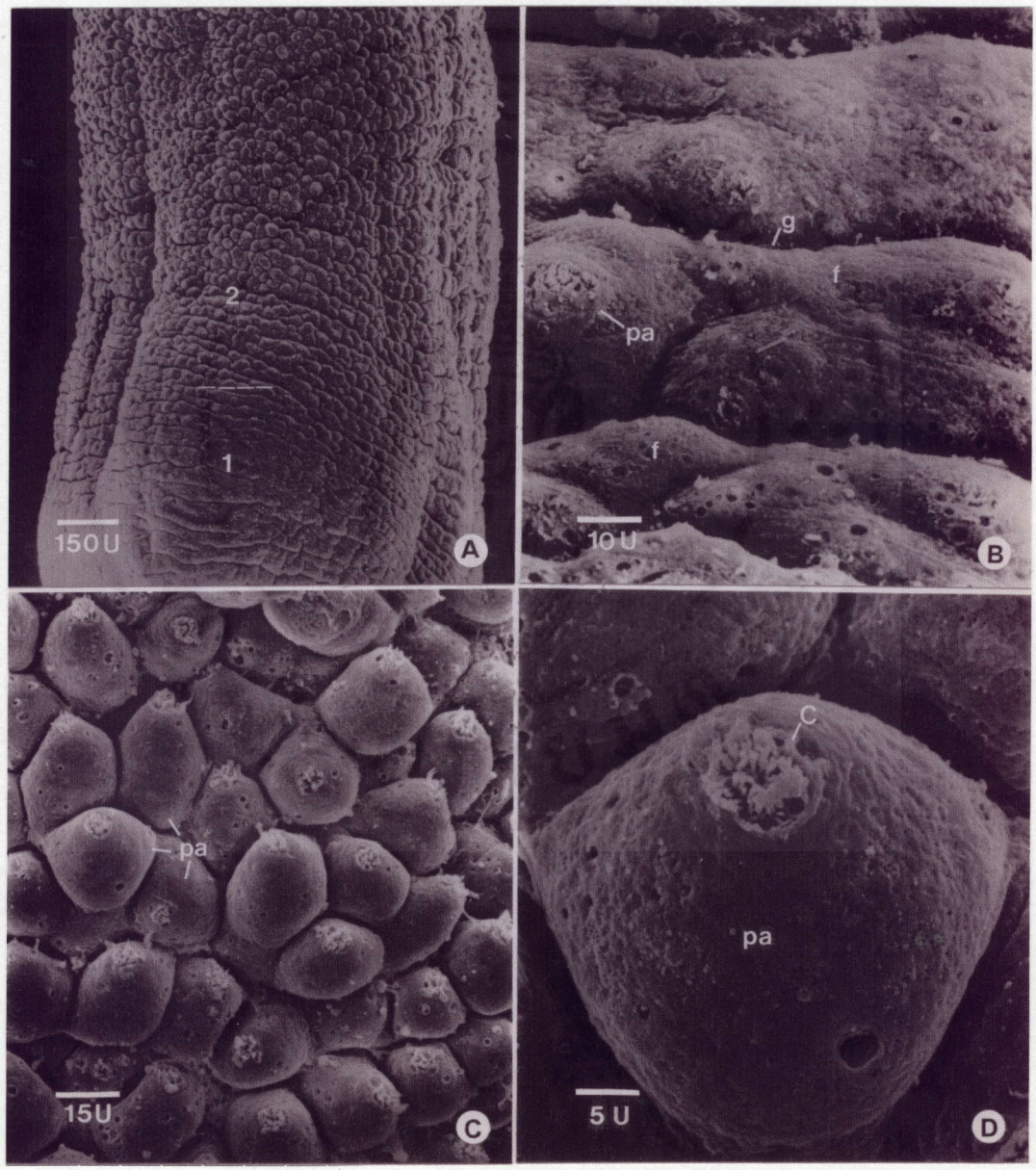


Figure 18 SEM micrographs of the apical part of a cephalic tentacle

A) A low-power micrograph of the tentacle showing numerous surface papillae.

B) A medium-power micrograph of the papillae (pa), all of which have a cone shape.

C) A high-power micrograph of the papillae (pa) showing many cilia on the top part. The surface of a papilla is enlarged in the inset.

D) A high-power micrograph of the top part of a papilla showing a circle of cilia (C).

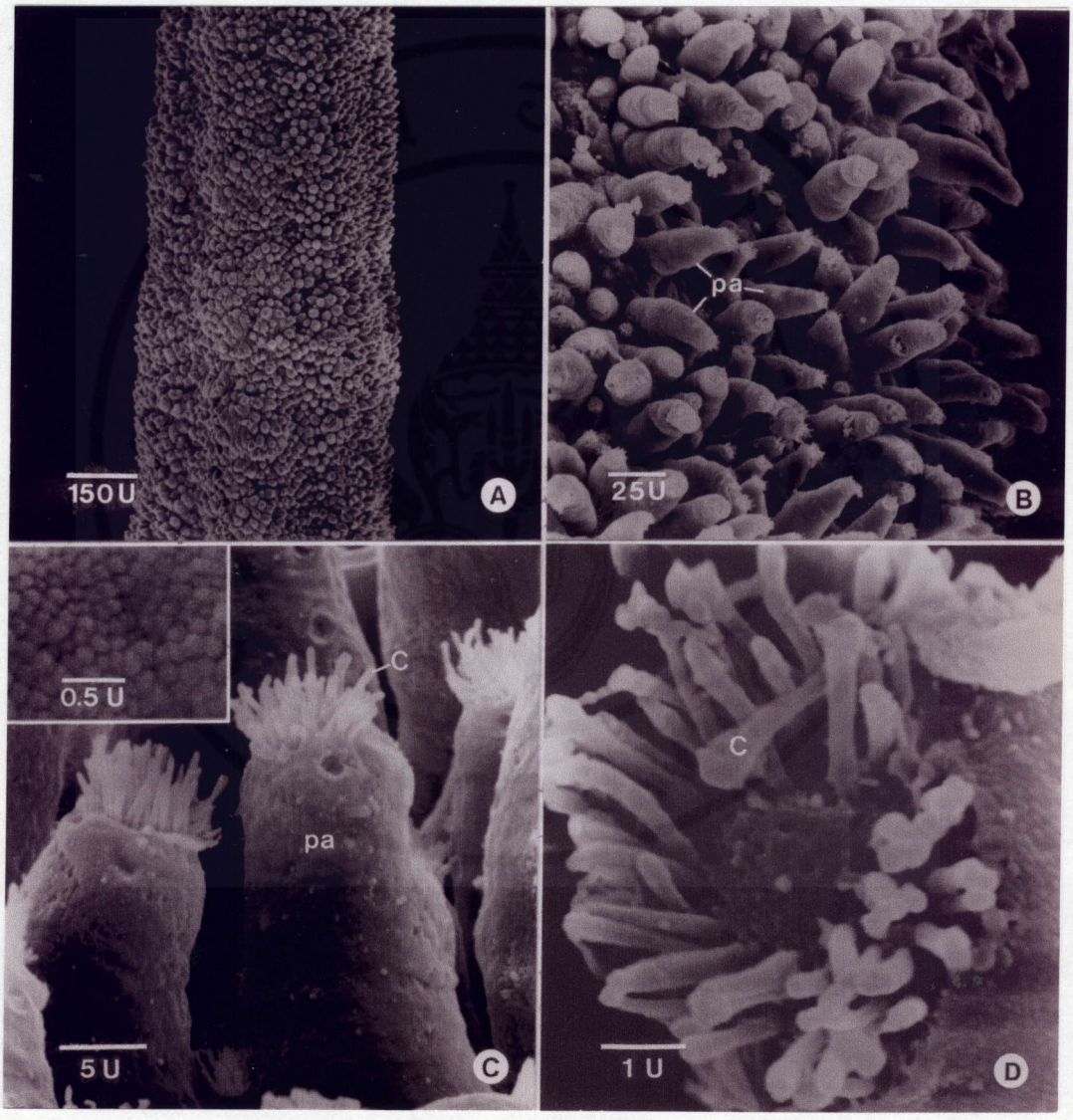


Figure 19 Paraffin sections of the cephalic tentacle stained with H&E

A) A low-power micrograph of a longitudinal section of the cephalic tentacle which can be divided into 3 parts: basal (1), middle (2) and top (3) parts. Notice the presence of cephalic tentacular nerve bundle (Teb) in the axis of the tentacle.

B) A medium-power micrograph of a cross section of the basal part of the cephalic tentacle showing a cephalic tentacular nerve bundle (Teb) in the axis. The tentacle is surrounded by an epithelium (E).

C,D) Higher magnifications of the tentacular nerve bundle (Teb) in Fig.B showing that their nerves (Ten) are distributed among muscle fasciculi (Mu).

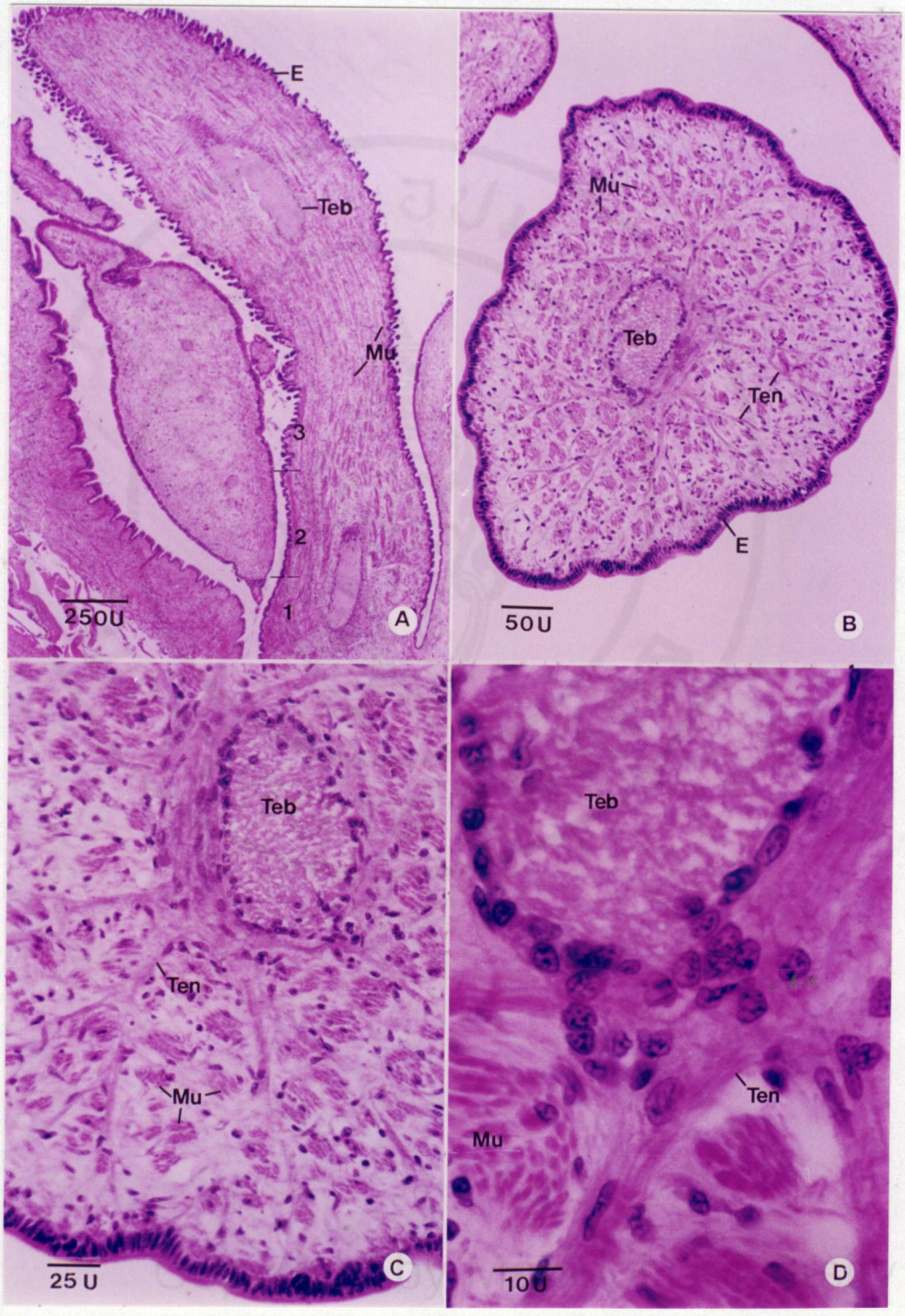

E
Teb
Mu
3
2
1
250U
A
Mu
1
Teb
Ten
E
50U
B
Teb
Ten
Mu
25 U
C
Teb
Ten
Mu
10U
D

Figure 20 Semithin sections of the cephalic tentacles

A) A high-power of light micrograph of the basal part of the cephalic tentacle stained with methylene blue showing the epithelium with the brush border (bb). The epithelium consists of sensory cell (se), supporting cell (su) and goblet cell (Gb). Muscle fasciculi (Mu) are seen in the connective tissue underlying the epithelium.

B) An adjacent area of the basal part of the cephalic tentacle stained with PAS methylene blue showing many goblet cells (Gb) which are positively stained with PAS.

C,D) High-power of light micrographs of the middle part of the cephalic tentacle stained with methylene blue showing the tentacular nerve branches (Ten) projecting to the base of papilla. Notice the presence of a group of cilia on the top of each papilla.

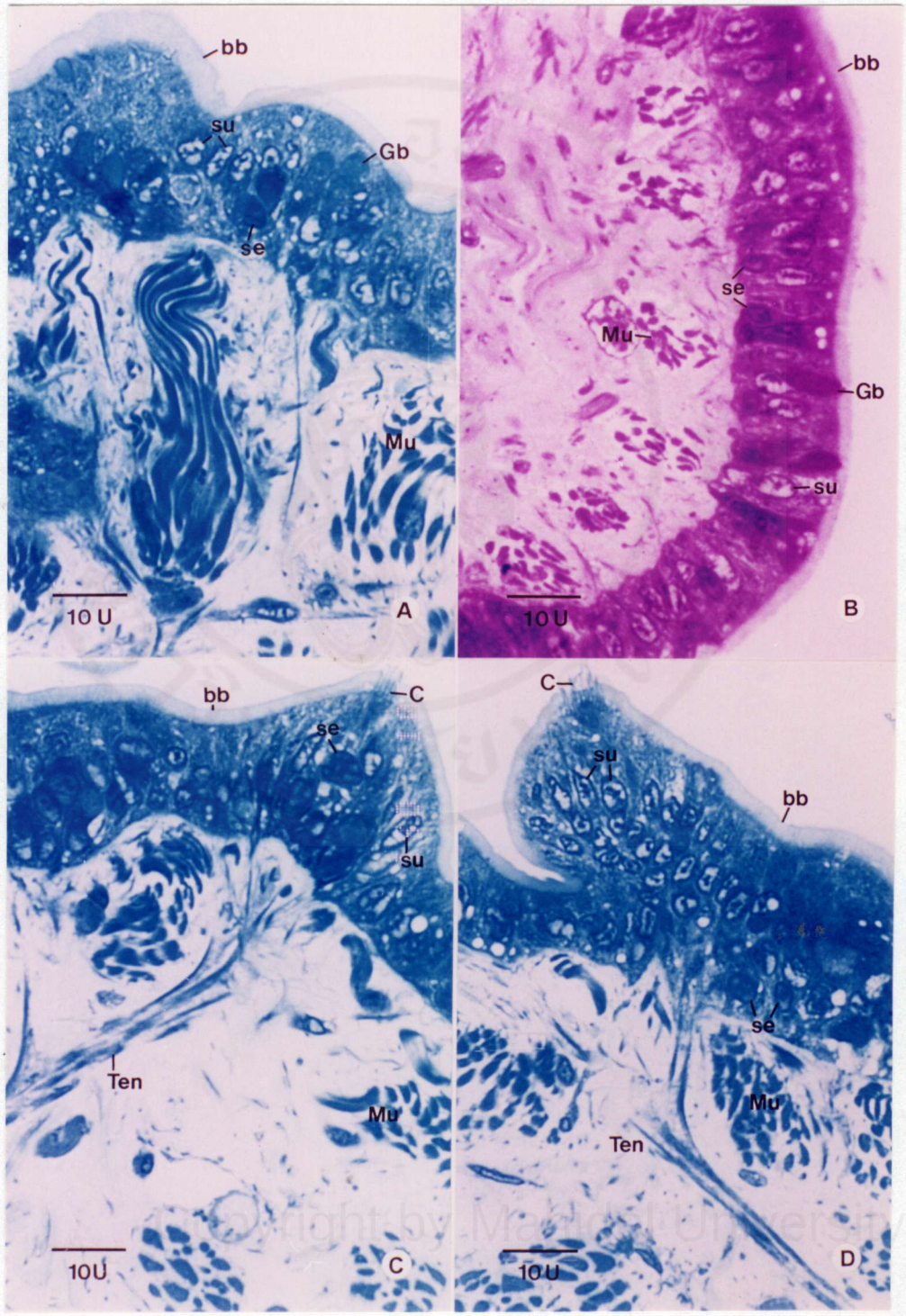


Figure 21 Cross sections of the top part of the cephalic tentacle

A) A low-power micrograph of the cross section of the top part of the cephalic tentacle stained with H&E showing the cephalic tentacular nerve bundle (Teb) in the axis of the tentacle. The surface of the tentacle shows numerous papillae (pa). Mu-muscle, Ten-cephalic tentacular nerve branch

B,C) Plastic sections of the top part of the cephalic tentacle stained with methylene blue showing 3 types of cells in the epithelium : sensory cell (se), supporting cell (su) and goblet cell (Gb). A group of cilia (C) is present on the top of the papillae which are more slender and longer than those on the middle part of the tentacle.

D) An adjacent area of the tentacle stained with PAS-methylene blue showing many goblet cells (Gb) whose cytoplasm is stained with PAS. The brush border (bb) appears on the surface of the epithelium.

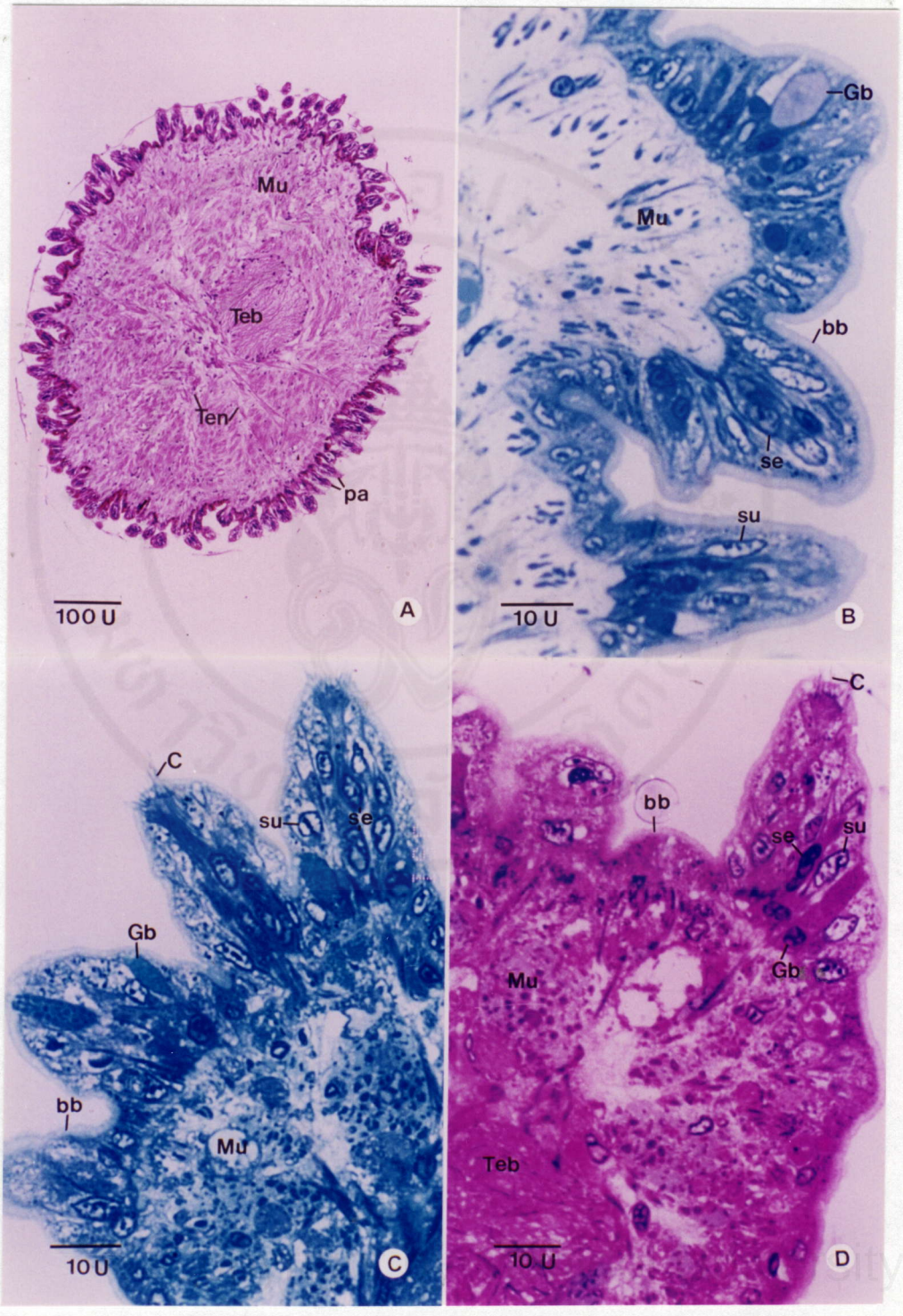

Mu
Teb
Ten
pa
100 U
A
Gb
Mu
bb
se
su
10 U
B
C
su
se
Gb
bb
Mu
10 U
C
C
bb
se
su
Gb
Mu
Teb
10 U
D

Figure 22 Paraffin sections of the eye of *H.asinina* stained with H&E

A) A low-power micrograph of a longitudinal section of an eye showing numerous nuclei of the receptor cells in the retina. Notice that the retina is surrounded by the optic nerve (Opn).

B) A low-power micrograph of a cross section of an eye showing the lens (L) that is enclosed by the retina (Re). An asterisk indicates the opening pore of the eye. ca-capillary

C) Higher magnification of the outer part of the eye showing the epithelium of the optic tentacle. The arrows point to the transitional area where the retina is thinner and has fewer cell layers. p-pigmented layer, pc-pigmented cell layer, rc-receptor cell layer

D) An adjacent inner part of the eye showing 6 layers of the retina: pigmented layer (1), pigmented cell layer (2), fibrous layer (3), receptor cell layer (4), loose connective layer (5),and optic nerve layer (6).

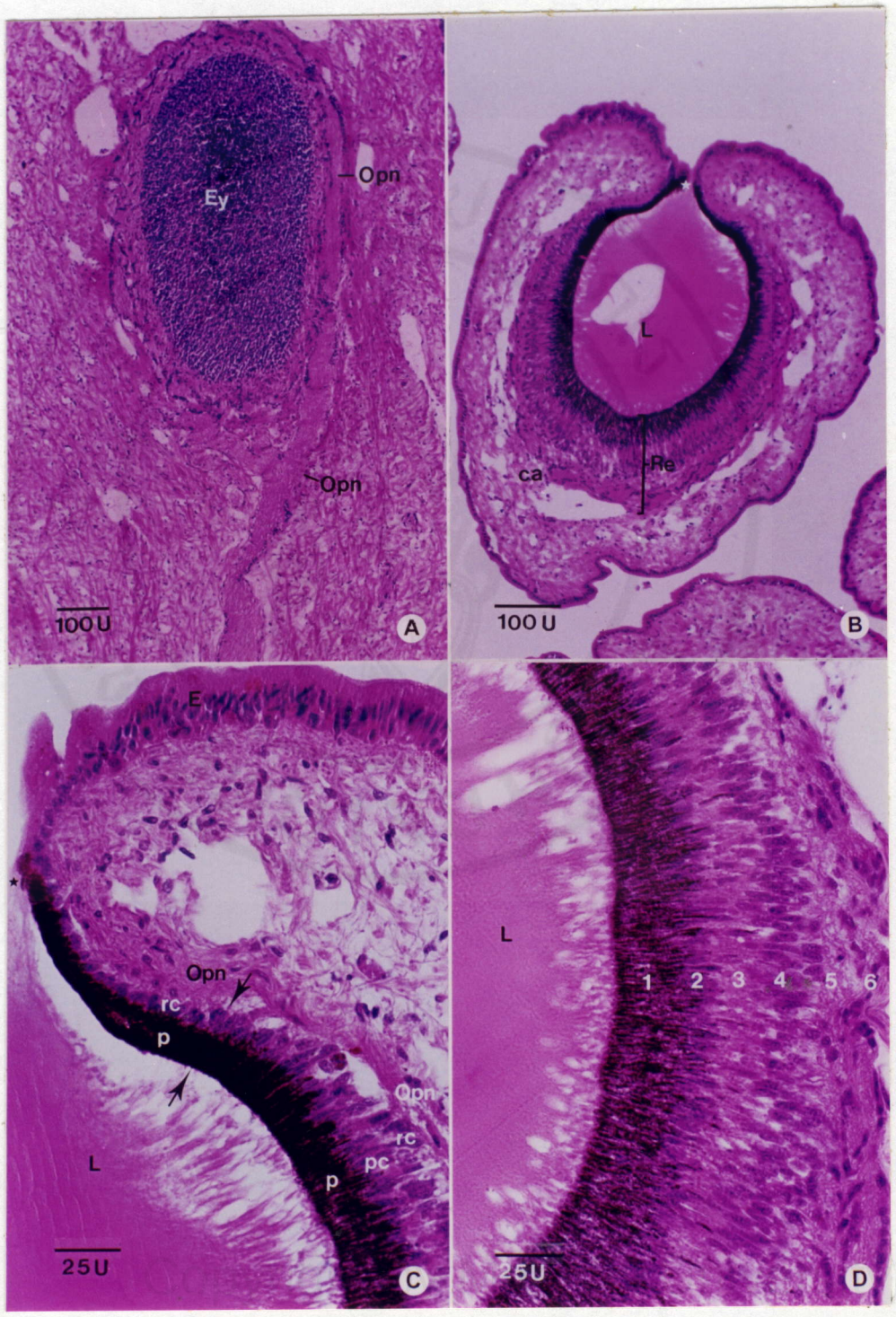


Figure 23 Cross sections of the inner part of the eye

A) A high-power micrograph of the retina stained with H&E showing 6 layers: pigmented layer (p), pigmented cell layer (pc), fibrous layer (f), receptor cell layer (rc), loose connective tissue layer (l) and optic nerve layer (Opn).

B) A plastic section of the retina stained with methylene blue showing 6 layers: pigmented layer (p), pigmented cell layer (pc), fibrous layer (f), receptor cell layer (rc), loose connective tissue layer (l) and optic nerve layer (Opn).

C) A higher magnification of the first and second layers of the retina showing pigmented granule (p), pigmented cell (pc) and receptor cell (rc).

D) An adjacent area of the fourth to sixth layers of the retina showing 3 types of receptor cells: type 1 (rc_1), type 2 (rc_2) and type 3 (rc_3).

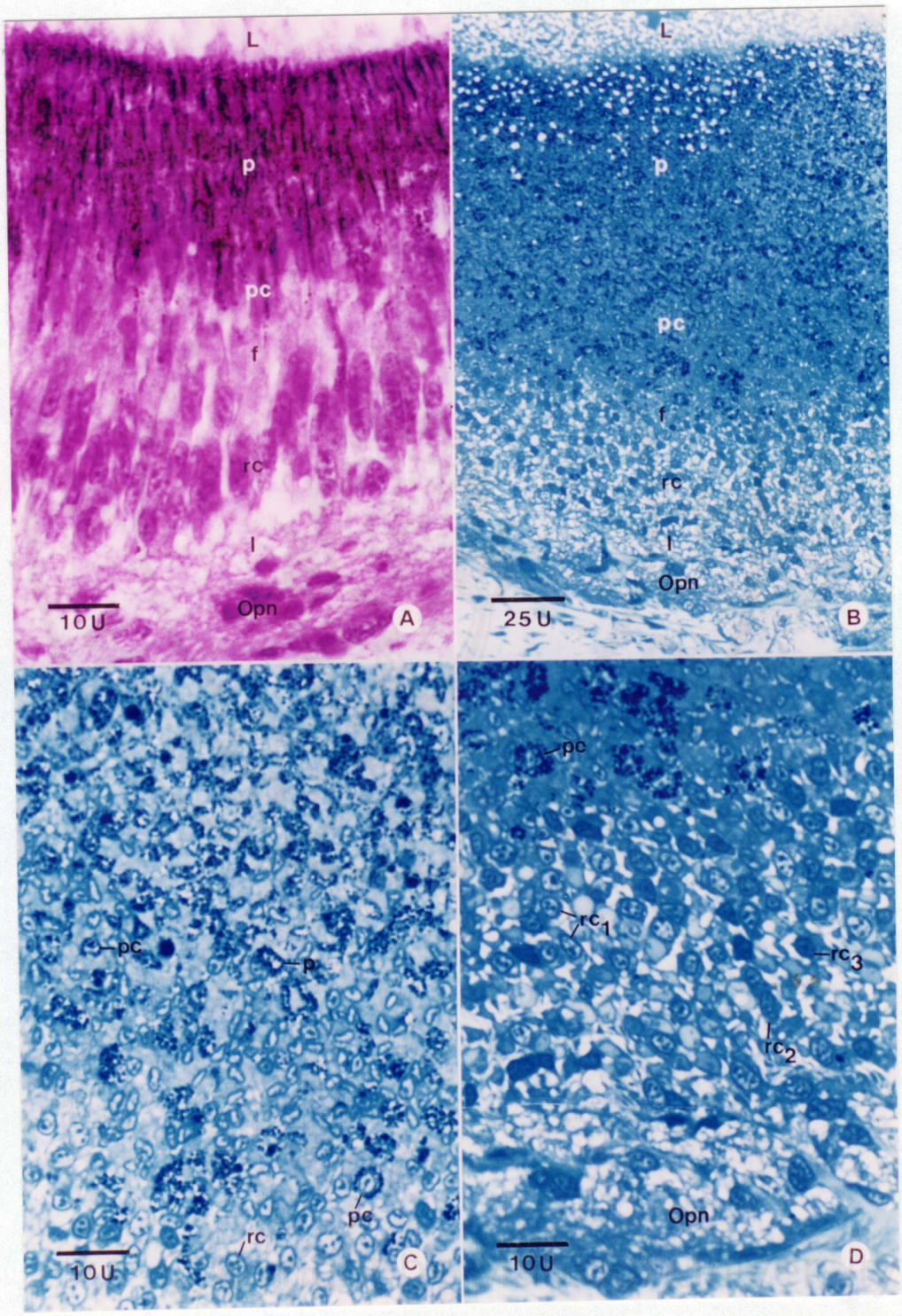

L
p
pc
f
rc
l
Opn
10 U
A
L
p
pc
f
rc
l
Opn
25 U
B
pc
p
pc
rc
C
pc
rc1
rc3
rc2
Opn
10 U
D

Figure 24 SEM micrographs of an appendage tentacle

A) A low-power micrograph showing the location of appendage tentacle (At), eye (Ey) and cephalic tentacle (Te).

B,C) Higher magnifications of the appendage tentacle showing many grooves (g) and folds (f).

D) A high-power micrograph of the fold showing numerous microvilli.

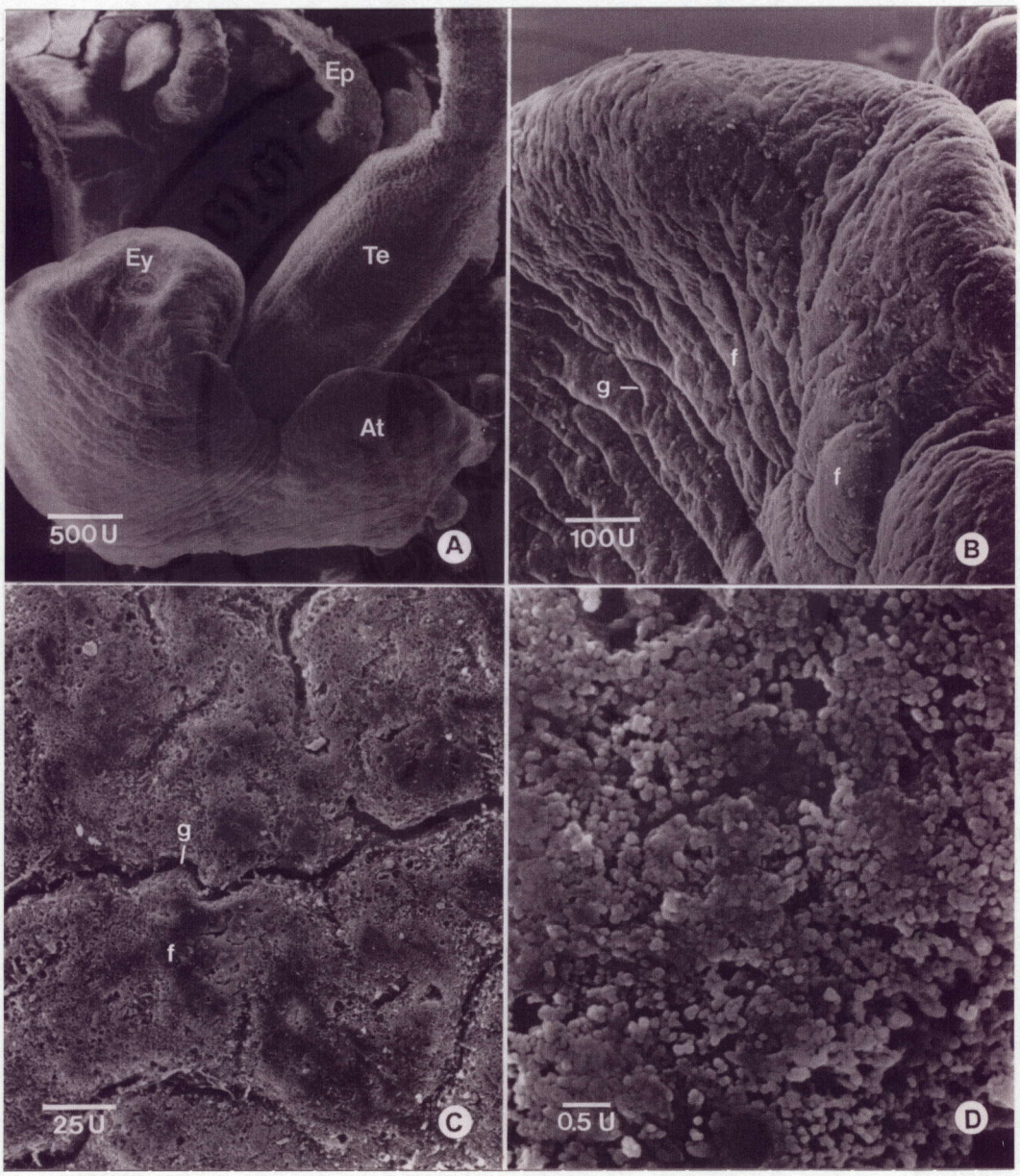


Figure 25 Cross sections of the appendage tentacle

A) A survey micrograph of the cephalic portion of *H. asinina* stained with H&E showing the cross section of appendage tentacle (At), eye (Ey) and cephalic tentacle (Te).

B) A cross section of the appendage tentacle stained with H&E showing corrugated surface epithelium (E).

C) A plastic section of the surface of an appendage tentacle stained with methylene blue showing simple columnar epithelium that lies on a thick basement membrane. The epithelium consists of sensory cell (se), supporting cell (su), and goblet cell (Gb). Many muscle fibers (Mu) are seen in the connective tissue.

D) A similar area of the appendage tentacle stained with PAS-methylene blue showing that the goblet cells (Gb) are positively stained with PAS. Notice the presence of nerve fibers (n) projecting onto the epithelium.

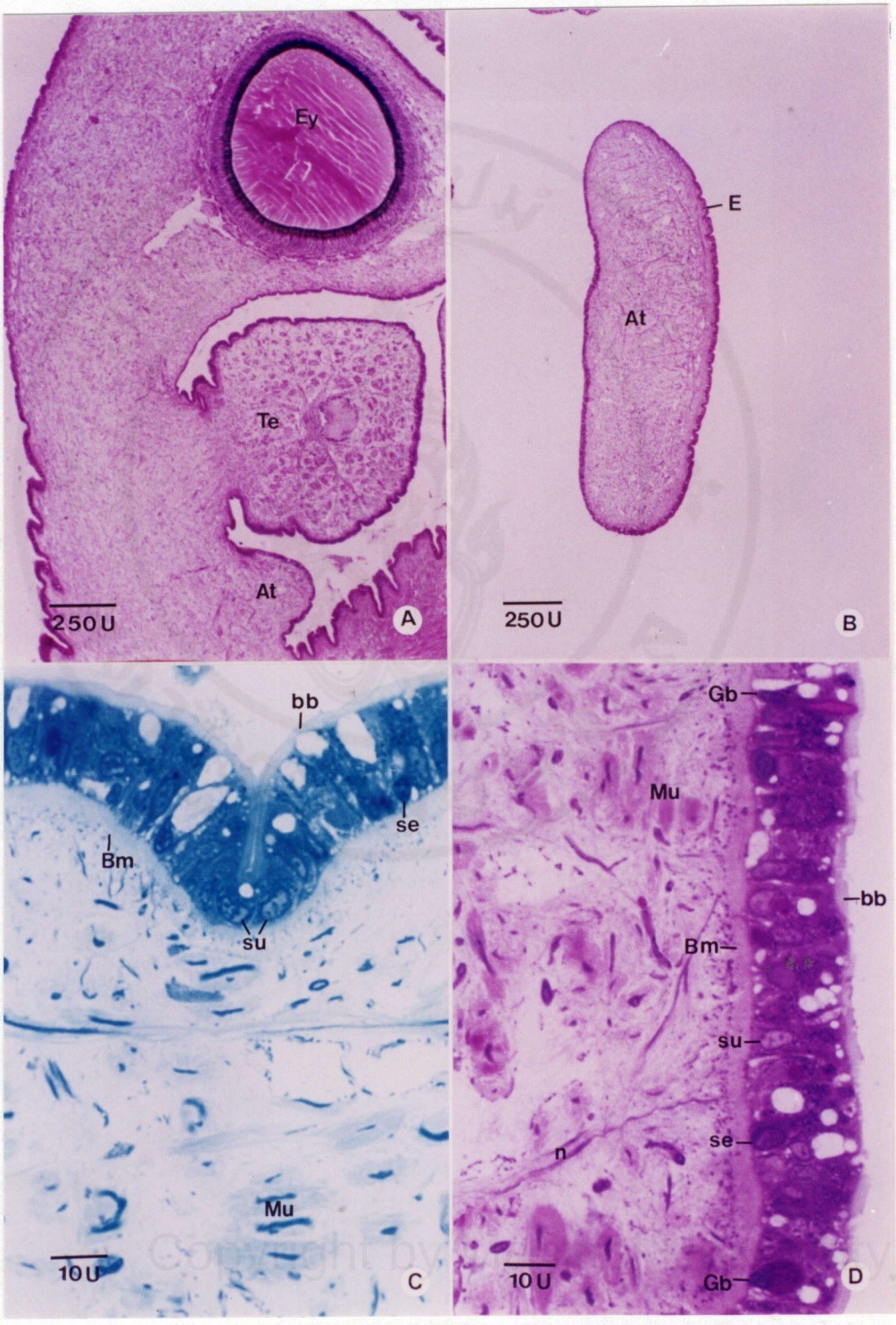

Ey
Te
At
250 U
A
E
At
250 U
B
bb
se
Bm
su
Mu
10 U
C
Gb
Mu
bb
Bm
su
se
n
10 U
Gb
D

Figure 26 SEM micrographs of an epipodium tentacle

A) A low-power micrograph showing the cone shape and the thorny surface of an epipodium tentacle. The tentacle is divided into 3 distinct area: base (1), middle (2), top (3) parts.

B) A medium-power micrograph of the basal part showing grooves (g) located between folds (f) which contain many short papillae (pa).

C) A medium-power micrograph of the middle part showing a group of papillae (pa) with a tuft of cilia (C) on the top part.

D) A medium-power micrograph of the top part showing many tall papillae (pa) with many long cilia (C).

E) A high-power micrograph of the surface of a papilla showing numerous microvilli.

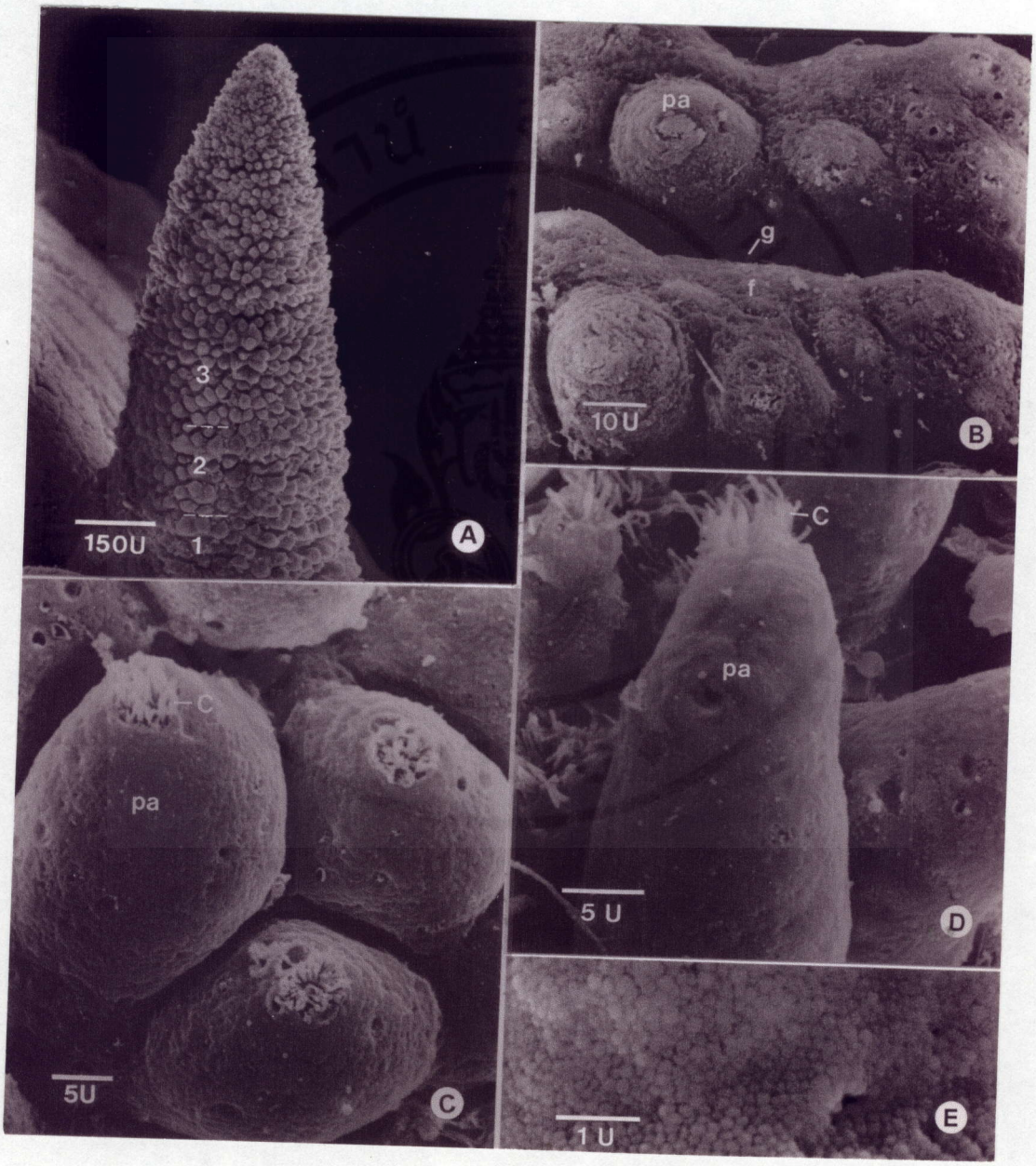


Figure 27 Paraffin sections of an epipodium tentacle stained with H&E

A) A low-power micrograph of the cross section of the base part of an epipodium tentacle (Ep) showing surface epithelium (E) and a nerve bundle (Epb) in the axis.

B) A medium-power micrograph showing the epipodium tentacular nerve bundle (Epb) and their nerve branches (Epn) distributed between the muscle bundles (Mu).

C) A high-power micrograph showing the epipodium tentacular nerve bundle (Epb), their nerve branches (Epn) and muscle fiber (Mu).

D) A high-power micrograph of the surface of an epipodium tentacle showing the epithelium which consists of goblet (Gb), sensory cell (se) and supporting cell (su).

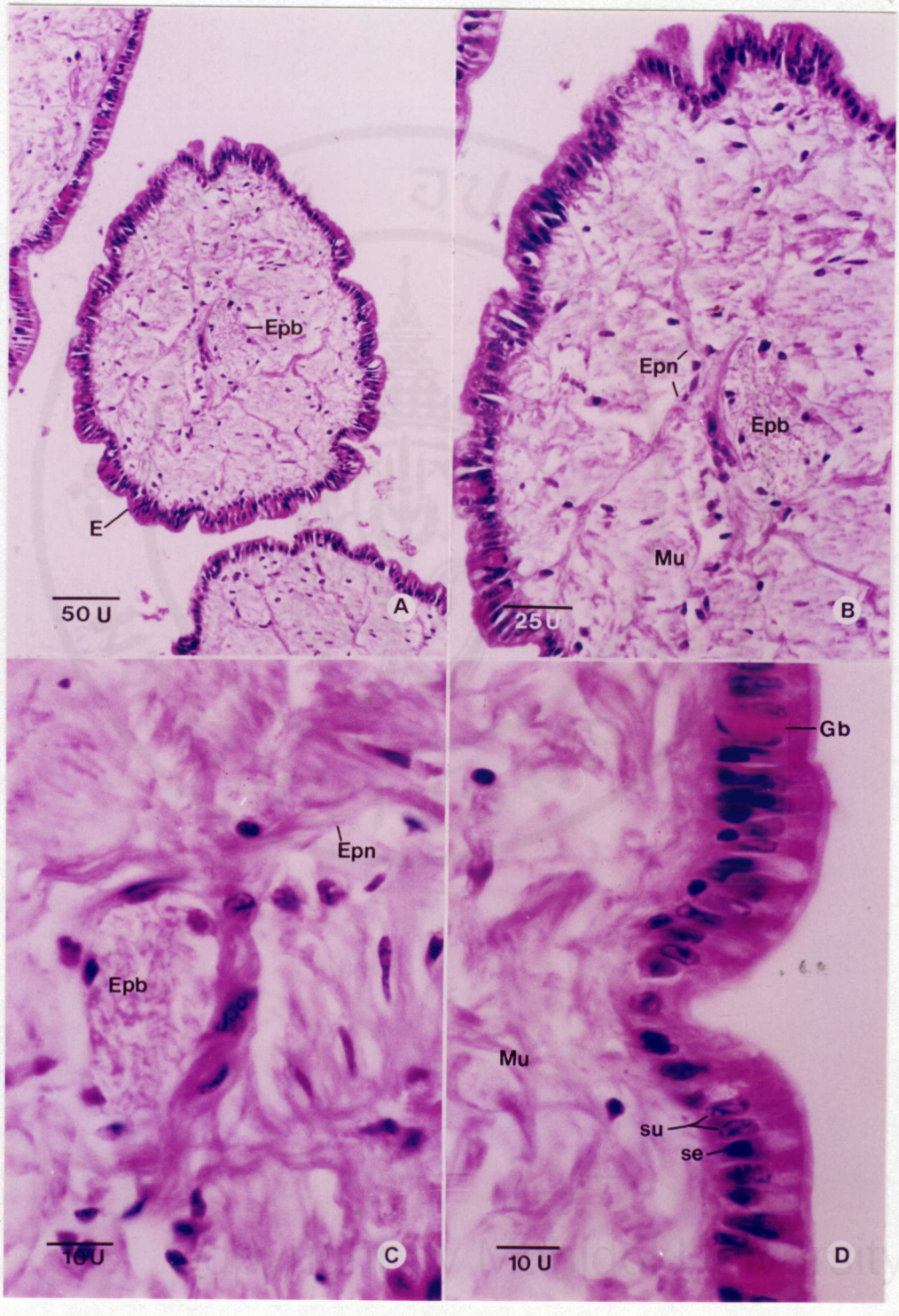

Epb
E
50 U
A
Epn
Epb
Mu
25 U
B
Epn
Epb
10 U
C
Gb
Mu
su
se
10 U
D

Figure 28 Plastic sections of epipodium tentacles

A) A high-power photomicrograph of the interpapillary area of the base part of an epipodium tentacle stained with methylene blue showing surface epithelium with brush border (bb). The epithelium consists of goblet (Gb), muscle (Mu), sensory cell (se) and supporting epithelium cell (su). The underlying connective tissue contains muscle bundle (Mu) and nerve bundle (Epn).

B) Similar area of the base part of the epipodium tentacle stained with PAS-methylene blue. The epithelium shows many PAS-positive goblet cells.

C) A high-power micrograph of the middle part of an epipodium tentacle stained with methylene blue showing a part of the papilla whose epithelium contains similar cells as shown in Fig.A.

D) A high-power micrograph showing epithelium of the middle part of an epipodium tentacle stained with PAS-methylene blue showing similar epithelial cells as shown in Fig.B.

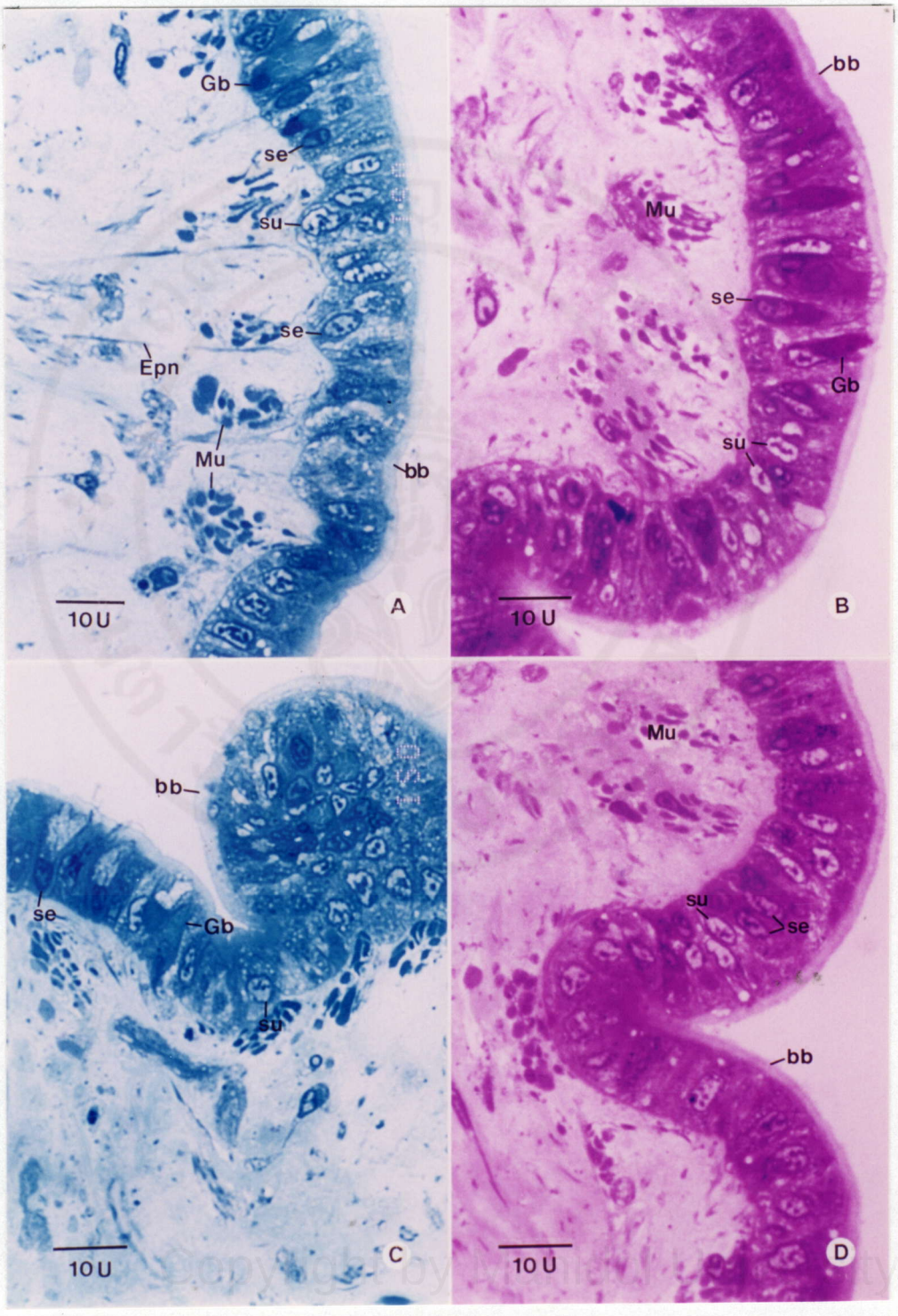

Gb
se
su
se
Epn
Mu
bb
10 U
A
bb
Mu
se
Gb
su
10 U
B
bb
se
Gb
su
10 U
C
Mu
su
se
bb
10 U
D

Figure 29 Cross sections of the top part of the epipodium tentacle

A) A low-power micrograph of the top part of epipodium tentacle stained with H&E showing many surface papillae (pa). Epb- epipodium tentacular nerve bundle, and Epn- epipodium tentacular nerve branch

B) A medium-power micrograph showing numerous brown pigments in the surface epithelium.

C) A high-power micrograph of the papillae showing sensory cell (se) with cilia (C), supporting cell (su) and goblet cell (Gb). The epithelium is covered with brush border (bb).

D) A high-power micrograph of the papillae of epipodium tentacle stained with PAS-methylene blue showing three types of cells in the epithelium. The brush border (bb) of the epithelium is not densely stained with PAS.

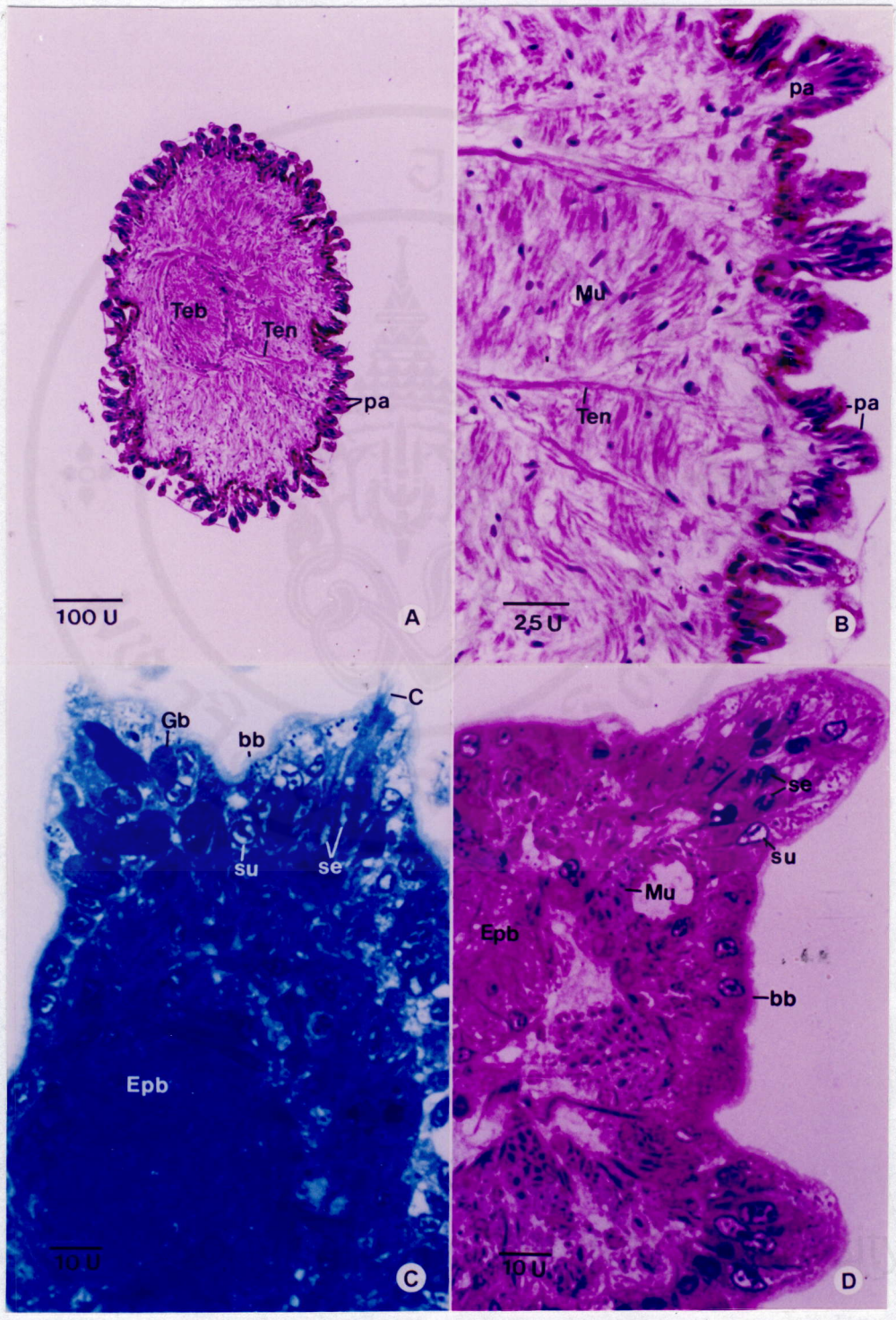

Teb
Ten
pa
100 U
A
pa
Mu
Ten
pa
25 U
B
C
Gb
bb
su
se
Epb
10 U
C
se
su
Mu
Epb
bb
10 U
D

Figure 30 Paraffin sections of the gill of *H.asinina*

A) A survey photomicrograph showing the location of gill (gi) and osphradia (Os) which are connected by the mantle (mt). D-dorsal, rt-rectum, V-ventral

B) A medium-power micrograph of the gill showing many filaments (fi) located parallel to each other. af-afferent side, ef-efferent side

C) A high-power micrograph of the efferent side of the gill showing hemocoelomic spaces (hs) containing many hemocytes (hc). ro-rod, Mu-muscle, C-short cilia, LC-long cilia

D) A high-power micrograph of the afferent side of the gill showing hemolymph in the hemocoelomic space of each filament. hc-hemocyte

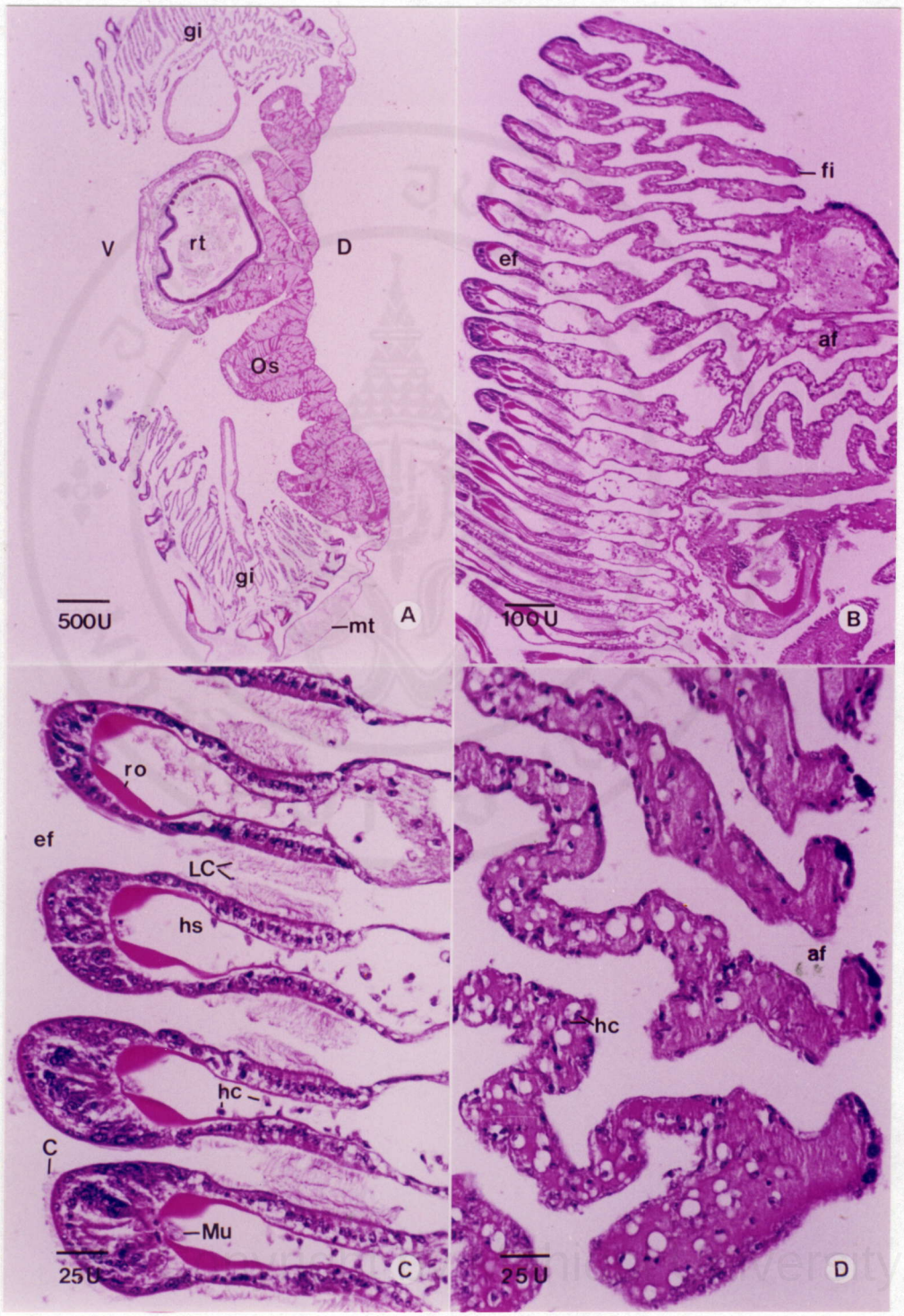

gi
V
rt
D
Os
gi
500U
mt
A
fi
ef
af
100U
B
ro
ef
LC
hs
hc
C
Mu
25U
C
af
hc
25U
D

Figure 31 Plastic sections of the gill of *H. asinina* stained with methylene blue

A) A low-power micrograph of the filaments showing hemocoelic space (hs). af-afferent side, ef-efferent side

B) An enlargement from the efferent end of the filament in Fig.A showing five types of cells (1-5) in the terminal epithelium which possess both microvilli and short cilia (C). A bundle of muscle (Mu) is attached to the V-shaped skeletal rods (ro).

C) An area adjacent to the lower part of Fig.B showing many high columnar cells with long cilia (LC). The hemocoelic space (hs) is enclosed by endothelial cells (en).

D) An enlargement from the afferent end of the filament in Fig.A showing three types of cells in the epithelium (1-3). A hemocyte (hc) is seen in the hemocoelic space (hs).

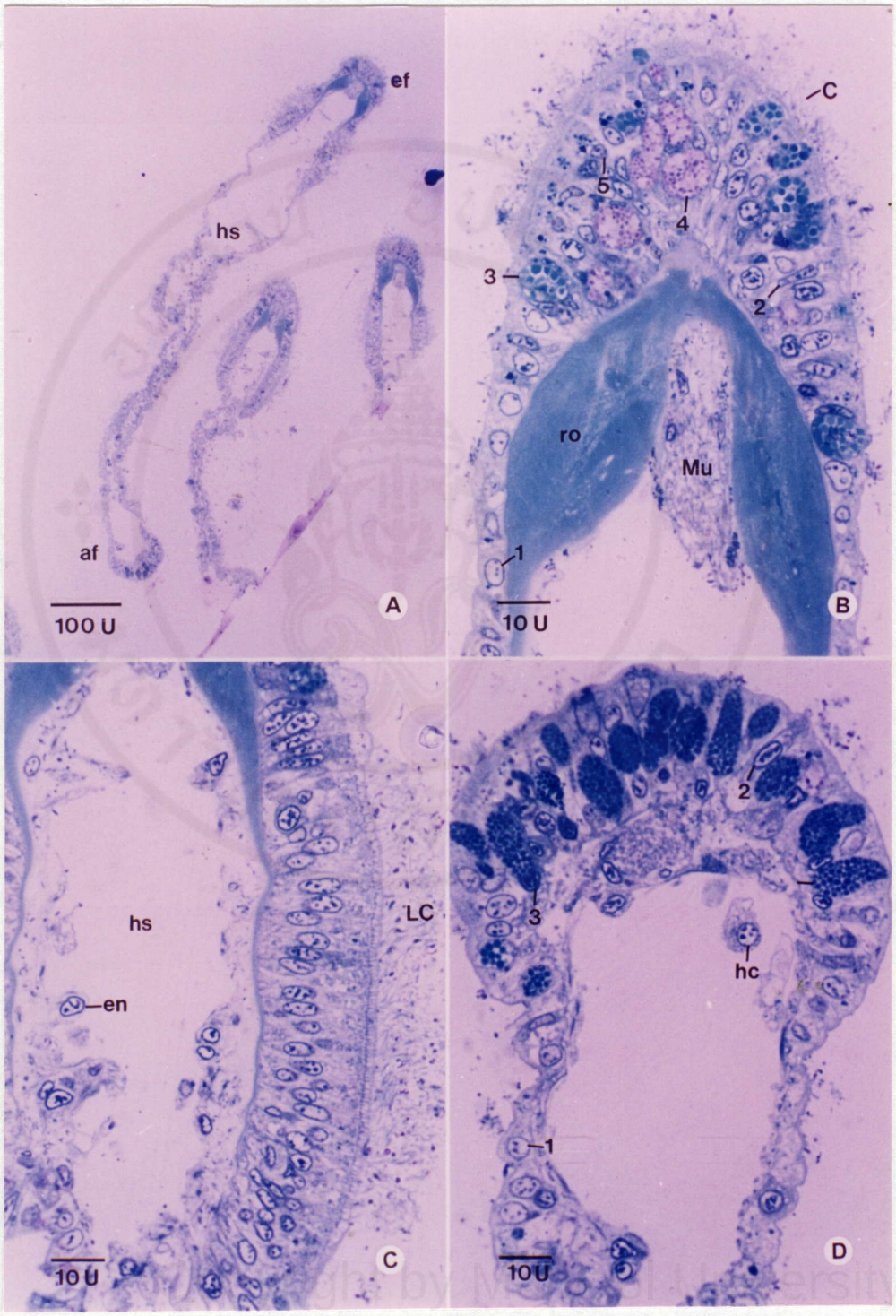

ef
hs
af
100 U
A
C
5
4
3
2
ro
Mu
1
10 U
B
hs
LC
en
10 U
C
2
3
hc
1
10 U
D

Figure 32 SEM micrographs of an osphradium

A) A low-power micrograph showing the leaf (le) which arises from the axis (ax), and leaflets (lf) which are branched from both sides of the leaf.

B) A medium-power micrograph of the junction between the axis (ax) and leaf (le).

C) An adjacent area showing the leaf (le) and leaflet (lf) at the same magnification.

D) A high-power micrograph of a part of the leaflet showing many ciliary tufts (Ct) that are enlarged in the inset. Exocytosed pore (p) and excretory granules (gr) are also shown.

E) A nearby area at the same magnification showing many paddle-like cilia that are magnified in the rectangle.

H) Higher magnification of a part of the axis showing ciliary tuft (Ct). Exocytosed pores (p) and excretory granules (gr) are seen on the tegumental fold. g-groove

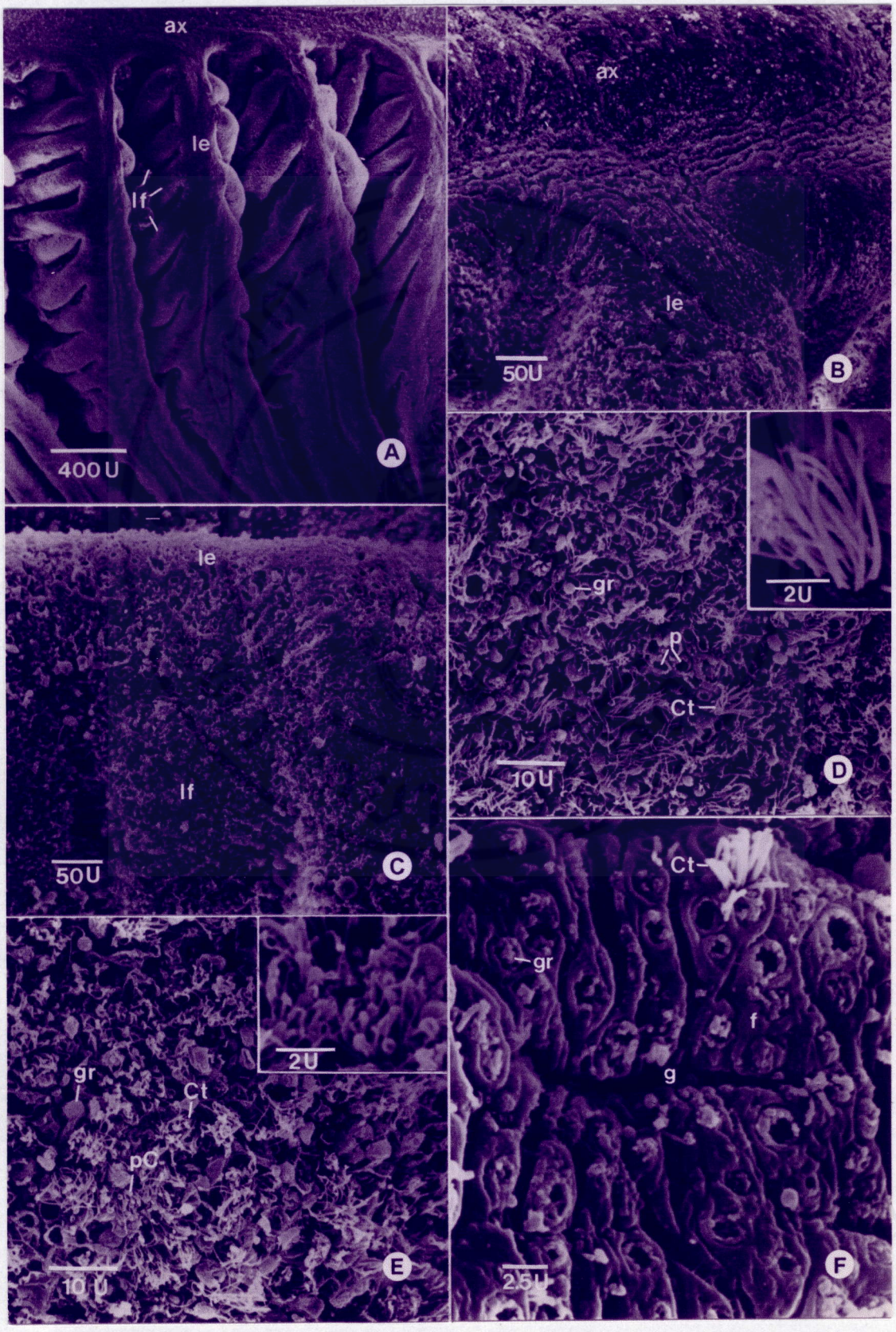

ax
le
lf
400 U
A
ax
le
50U
B
gr
p
Ct
10U
D
2U
le
lf
50U
C
gr
Ct
pO
10 U
E
2U
Ct
gr
f
g
25U
F

Figure 33 Paraffin sections of an osphradium stained with H&E

A) A survey micrograph of the cross section of an osphradium showing many leaves (le) and leaflets (lf). The osphradium is connected to the mantle (mt).

B) A medium-power micrograph showing the junction between the mantle and osphradium.

C) A high-power micrograph of leaflet (lf) showing many epithelial cells and nerve fibers (nf) in the core.

D) A high-power micrograph of the leaf showing epithelial cells lying on the connective tissue which contains nerve fiber (nf).

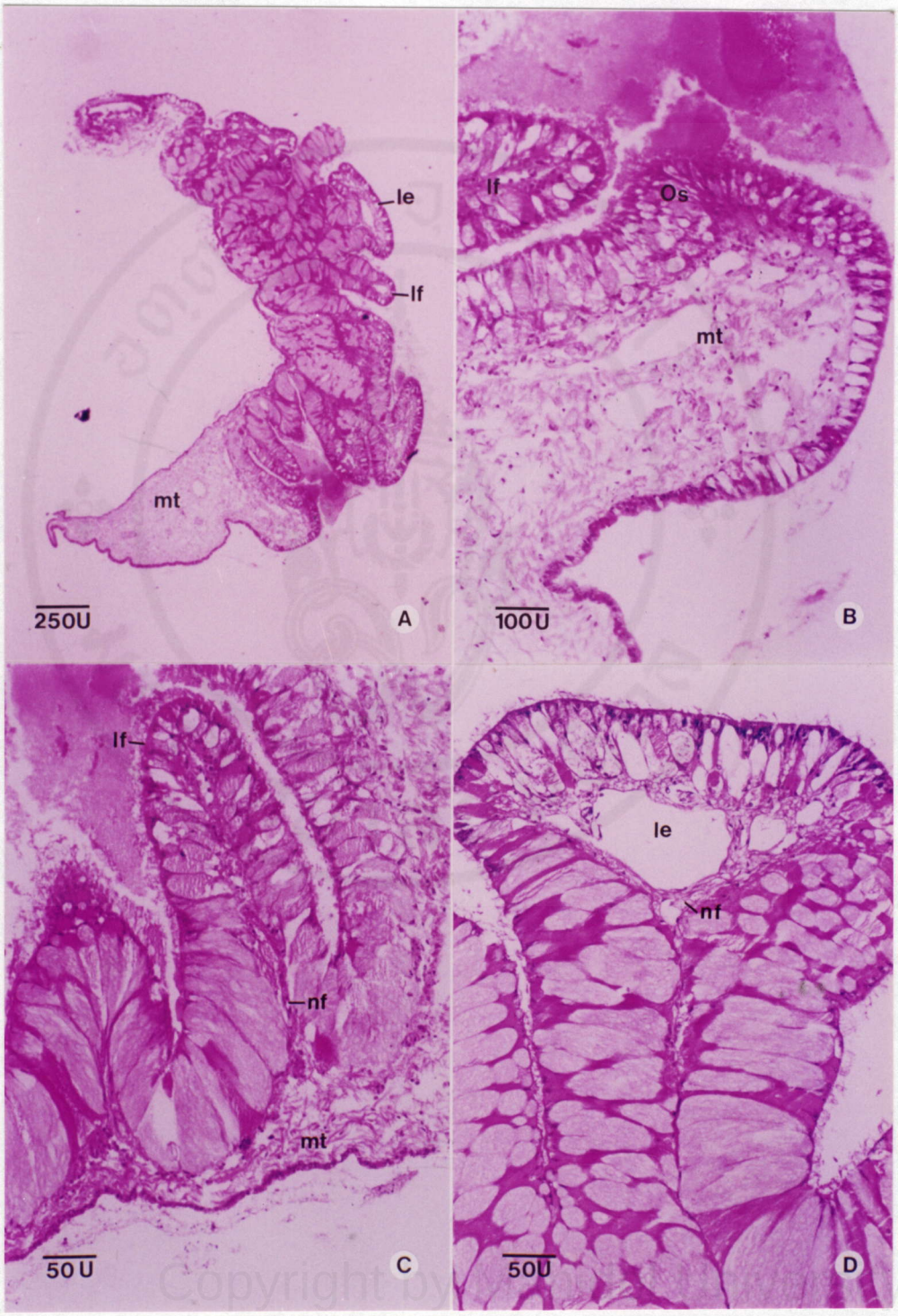


Figure 34 Semithin sections of the leaf of an osphradium stained with methylene blue

A) A medium-power micrograph showing the epithelium of the leaf and nerve fiber (nf) in the connective tissue.

B,C) High-power micrographs of the apical part of the leaf showing the sensory cell (se) and supporting cell (su) in the epithelium which lie on connective tissue containing nerve fiber (nf).

D) An adjacent area in the middle part of the leaf showing many mucous cells in the epithelium. The large mucous cells possess many large mucous granules some of which show metachromatic staining with methylene blue. Many granules (★) are seen released from the apical part of epithelium.

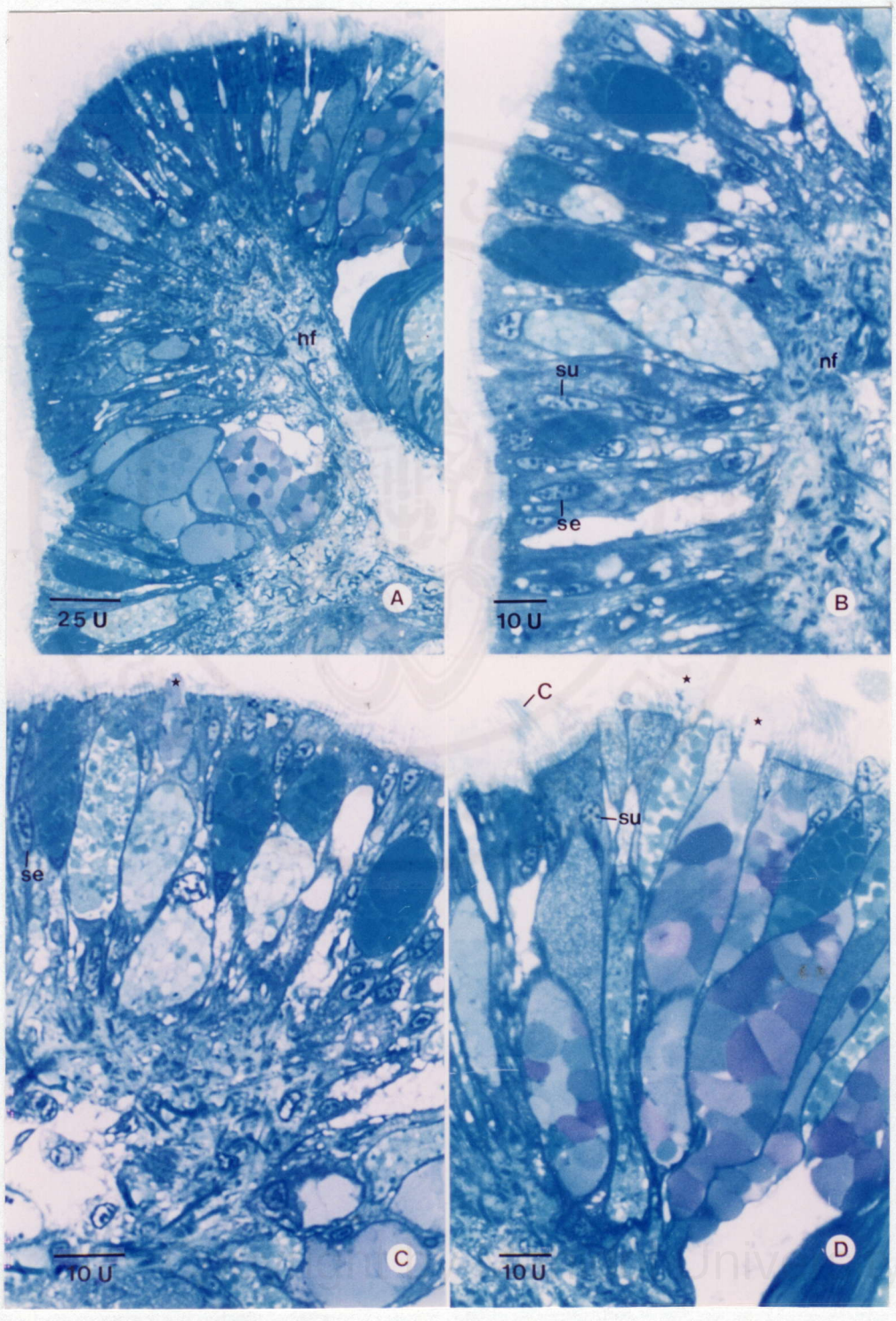
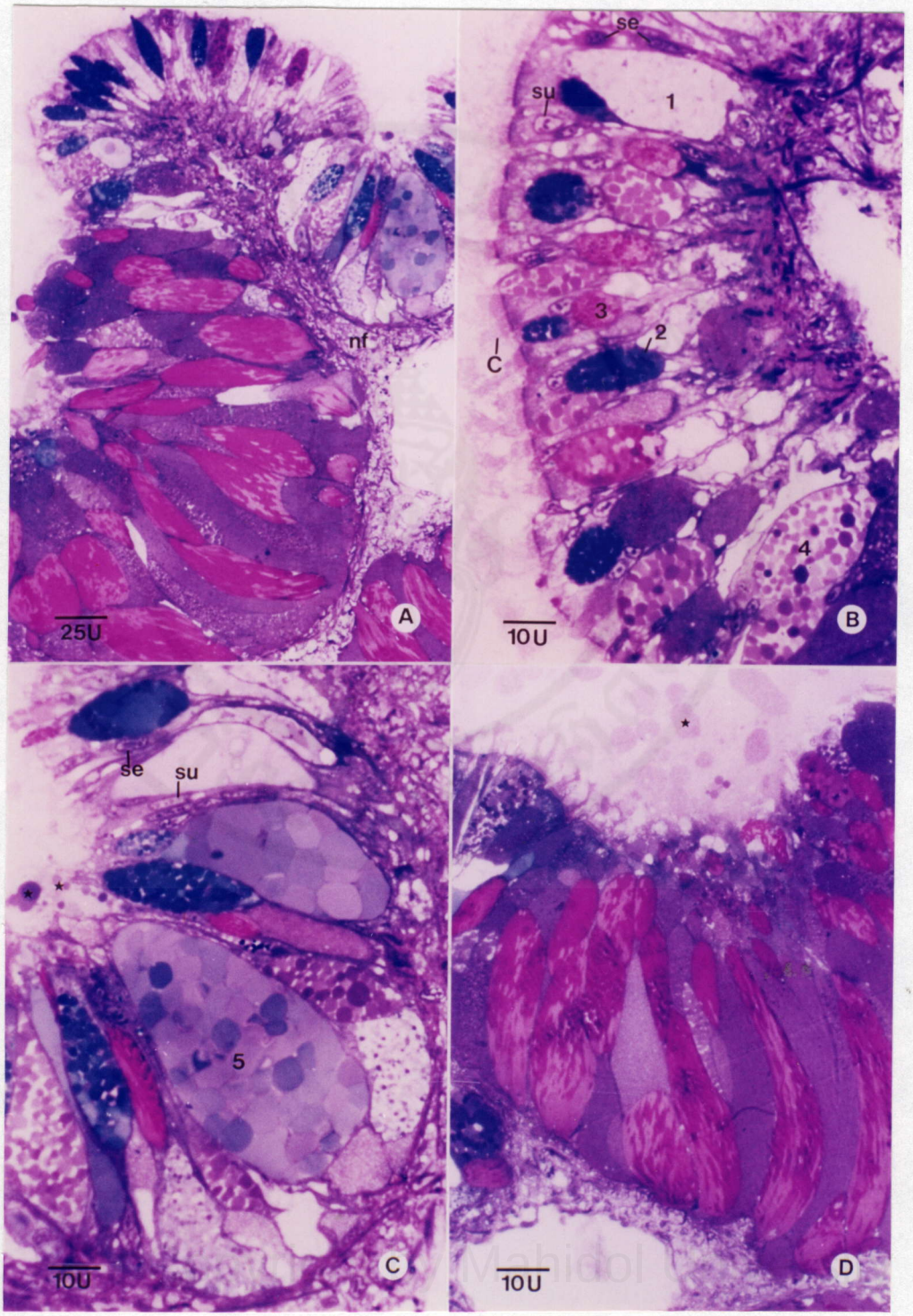

nf
25 U
A
su
nf
se
10 U
B
se
10 U
C
C
su
10 U
D

Figure 35 Semithin cross sections of the leaf of an osphradium stained with PAS-methylene blue

A) A medium-power micrograph of a leaf of an osphradium showing epithelium and connective tissue core.

B,C) High-power micrographs of the apical part of the leaf showing the supporting cell (su), sensory cell (se) and 5 types of mucous cells: Type-1 cell (1), Type-2 cell (2), Type-3 cell (3), Type-4 cell (4), Type-5 cell (5). Some granules are released (★) from the type-4 cells.

D) A high-power micrograph of the basal part of the leaf showing alternated location of two cell types; the first cell-type is mucous cell which shows positive staining with PAS, the second cell type is stained with methylene blue. Many heterogeneous granules (★) are seen released from the apical part of the epithelium.


se
su
1
3
2
C
4
nf
25U
A
10U
B
se
su
5
10U
C
10U
D

CHAPTER VI

DISCUSSION

Histology of Nerve Ganglia in Adult *H. asinina*

There have been a number of studies on the histology of the nervous system of gastropods because of the benefit that could be gained from applying the knowledge of this system, especially the development of the nerve ganglia, to increase the yield in aquaculture systems. In the present study, the cerebral, pleuropedal and visceral ganglia of *H. asinina* were investigated. All these ganglia are different in shape and size, but they have similar histological features. These ganglia consisted of two regions; the outer cortex and inner medulla. The outer cortex, contains many ganglion or nerve cells as found in other gastropods (26,28,31), thus it is comparable to the gray matter in the brain of vertebrates. The medulla consists of nerve fibers, which are connected to other ganglia and organs of the body.

In the previous studies of the histology of nerve ganglia in gastropod, there were multiple criteria to classify the ganglion cells, *i.e.*, size, shape, nuclear characteristics, distribution and staining affinity. Andrew (15) classified four types of cells in the nervous system of *B. tentaculata* and suggested that the cerebral ganglia had only one type of neurosecretory cell (S_1). Yahata and Hahn also described four types of ganglion cells in the cerebral ganglia *H. discus hannai* (16,17); however, there were two types of neurosecretory cells in these ganglia called Type A and B cells. On the other hand, Upatham et al. described eight types of cells in these ganglia of *H. asinina*,

and there were two types of neurosecretory cell (NS_1 and NS_2) which contained neurosecretory granules that were stained deep violet with PF and purple with CH-P (20). In our study of *H. asinina*, we have used the following characteristics for the classification of the cell types: 1) the appearance of the chromatin in the nucleus, 2) cell size, 3) cell shape and 4) staining affinity of the cytoplasm with CH-P and PF. By using these rather stringent morphological criteria, we have identified ten types of cells: three types of NS cells, four types of NR and three types of NG. On the basis of the similarities in size, shape, nuclear characteristics, distribution, and staining affinity, the NS_1 and NS_2 in this study correspond to type A and B cells, respectively, as reported by Hahn (16) and Yahata (17), and correspond to NS_1 and NS_2 as reported by Upatham (20).

It was shown that the pleural and pedal ganglia of *A. glabratus* lack NS cell (30). On the contrary, Wandelaar Bonga reported that there was only one type of NS cell (DGC) in the pleuro-pedal ganglion of *L. stagnalis* (26); and the pleural ganglion of *B. tentaculata* also has one type of NS cell which is stained intensely by aldehyde fuchsin (15). On the other hand, Hahn described several cell types in the pleural-pedal ganglion in *H. discus hannai*, but only two cell types were thought to be NS cells (17). On the other extreme, Thongkukiatkul et al. (36) suggested that there were three types of NS cells in *H. asinina*: NS_1, NS_2 and NS_3. Based on the similarities in size, shape and staining affinity, the NS cell type 1(NS_1), 2(NS_2) and 3(NS_3) in the present study should correspond to NS_1, NS_2 and NS_3 as reported by Thongkukiatkul (36).

Up to now, most studies have not definitely classified various cells in the visceral ganglia in gastropod, they only suggest broadly that there are three types of NS cell in the ganglia of *L. stagnalis* (26). However, only one type of neurosecretory cell was

found in the visceral ganglion of *A. glabratus* (30) and *B. tentaculata* (15). Thongkukiatkul et al. was able to classify NS cells in the visceral ganglion of *H.asinina* into three types: NS_1, NS_2 and NS_3 with similar appearances to those present in the cerebral and pleuropedal ganglia (36). In the present study, we were also able to identify three types of NS cells in the visceral ganglion.

In our study, the NS cells in *H. asinina* are distributed in all areas of the cortex in the cerebral, pleuropedal and visceral ganglia. It was found that the release of neurosecretory materials occurred inside the perineurium surrounding the ganglia (23). The hormones must diffuse through the perineurium and the connective tissue before entering the hemolymph (23,40). In *H. asinina* we have observed small dilated hemolymph vessels surrounding each ganglion, and they could act as the receiving sites for the released hormones. In contrast, in the higher gastropods such as *L. stagnalis* (26,29), *H. aspersa* (28), *A. glabratus* (30), the neurosecretory cells of the same type are often located together in a specific area of the ganglion with the smaller cells located near the neuropil and the larger ones at the periphery near the hemolymph vessels (23). These are specialized neurohemal regions of large surface area for release of secretory granules that are widely distributed along the periphery of nerves, connectives and commissures such as medial lip nerves and intercerebral commissure which have the axon terminals from light-green cell and cordo-dorsal cell, respectively (26,29). Similarly, annelids, cruataceans and insects also have special neurohemal organs for the release of neurohormones into the hemolymph (87) such as protocerebrum which is the principal endocrine centers of insects (45). When compared to the vertebrate, this region is comparable to the pituitary gland which

produces several hormones that have important functions in the regulation of metabolism, growth and reproduction.

Bullock and Horridge was the first to classify the neurons in the ganglia of gastropods on the basis of morphology (14); and in general neurons in the nervous ganglia of various gastropods show similarity in structure; they are giant neurons, ordinary neurons and globuli cells (14,15,22,23,24,25). In abalone spp., two types of neurons (Type C and D cells) were described in *N. discus* and *H. discus hannai* (16,17). On the other hand, Upatham et al. suggested that there are three types of neurons in the cerebral ganglia of *H. asinina* (20), while in the pleuropedal and visceral ganglia, there are four types of neurons (36). On the basis of size, shape of cells, and their nuclear characteristics, the present study shows that there are four types of neuron: NR_1, NR_2, NR_3 and NR_4 in the cerebral, pleuropedal and visceral ganglia of *H. asinina*, which is similar to the classification reported by Kruatrachue et al. (36). NR_1 is the largest neuron; and these cells are pyramidal in shape with a round nucleus and possess characteristics similar to typical motor neurons of vertebrates such as ventral motor neurons of the spinal cord and Purkinje cells in the cerebellum. Consequently, they may be involved in controlling and co-ordinating motor activities. It is noticeable that the NR_1 are more abundant in pleuro-pedal ganglion than in cerebral ganglia. Other types of neurons (NR_2, NR_3, NR_4) may belong to the same group which appear like ordinary neuron and globuli cells in other species (14,15,16,17). It is possible that these neurons are association neurons.

Up to now most studies of the nervous system of the gastropod have not focused on the neuroglia cell. Most studies only suggest broadly that there are one type of neuroglia in *B. tentaculata* (15) and two types in *L. stagnalis* (26). In recent study,

Upatham et al. reported three types of neuroglia in the cerebral ganglia of *H. asinina* (20). This finding was supported by Kruatrachue et al. (36) who also reported three types of neuroglia in pleuropedal ganglion and visceral ganglion. The present study also demonstrates the presence of three types of neuroglia in the cerebral, pleuropedal and visceral ganglia which have similar characteristics to the NG_{1-3} described by Upatham (20) and Kruatrachue (36). Glial cells observed in the present study are interspersed amongst neurons and along the outer surface of blood vessels and their sheath cells, respectively. Based on their position, NG_1 are probably the general glial cells of the cortex. They have spindle nuclei with mostly euchromatin which is similar to astrocyte in the central nervous system of vertebrate. Since NG_2, lie in a single row on the basement membrane which separated the ganglion from the capillaries, they may be comparable to a part of the blood-brain-barrier in the vertebrate. NG_3 which are distributed in the neuropil of medulla region, may be comparable to oligodendroglia which are involved in the synthesis of the myelin-like structure surrounding the nerve fibers in the neuropil.

Development of Nerve Ganglia, Neurosecretory Cells and Neurons

There have been several studies on the postembryonic development of the nervous system of gastropods. Bullock and Horridge showed that the nerve cells and neurosecretory cells of mollusc increased in size and number during development of the nervous system (14). The neuroendocrine cells of *L.stagnalis* also increase in number and size with increasing shell length (40). In addition, Lever showed the same result in the cerebral and parietal ganglia of *A.glabratus* (30). Similarly, Kruatrachue et al. reported that the number and size of neurosecretory cells in the cerebral ganglia

of *A.fulica* increased with increasing age (42); and similar trend was observed in *H. asinina* in the present study. Furthermore, our histological study indicated that the cerebral, pleuropedal and visceral ganglia first appeared in 1-month-old abalone. There are changes in the size and morphology of these ganglia, especially the number of neurons and neurosecretory cells in all ganglia markedly increase with the increasing age of abalone.

There have been many reports on the functions of neurosecretory cells in the cerebral ganglia of snails. Some snails have a factor that stimulates growth rate (88) and shell regeneration (89). Thongkukiatkul et al. reported that neurosecretory cells in the cerebral ganglia of *H. asinina* were stained with anti-human GH and anti-human insulin (90). In the present study, it has been observed that the neurosecretory cells in the cerebral ganglia first appear in 1-month-old abalone and increase with increasing age. A large number of neurosecretory cells are found in the 5-and 10-month-old abalone which are assumed to be juvenile and pre adult stage. They reach a maximum number and appear adult like in 12-month-old abalone which may reach the adult stage. It seems, therefore, that the increase in number of NS cells is correlated with the increase in growth.

Similar studies in other species were later performed by many investigators and suggested that the number and staining property of neuro-endocrine cell in pleuropedal and visceral ganglia were related with the gonadal stage or maturation (34,41,43,44). In our study, it has been observed that the neurosecretory cells in the pleuropedal and visceral ganglia first appear in 1-month old-abalone. The number of neurosecretory cells in the pleuropedal ganglia are significantly increased in the 4-and 7-month-old, while those in visceral ganglia were increased in 4-month-old abalone.

Then they reach a maximum number like in the adult in 11-month-old abalone. The development of these neurosecretory cells might be correlated to the development of the reproductive organs. From the study in adult abalone spp., Yahata and Hahn found that neurosecretory cells in cerebral ganglia showed seasonal changes in the staining intensity of paraldehyde-fuchsin. Hence, the rise and fall of the neurosecretory material coincided with gonadal maturation (16,17). Thongkukiatkul also reported that neurosecretory cells in the pleuropedal and visceral ganglia were stained with anti-human LH, while anti-human FSH were stained in pleuropedal ganglion only (90). It was reported that early spermatocytes and spermatids appear at four months, and early oocytes (OC_{1-2}) at six to seven months (91), while fully mature spermatozoa appeared in the gonads as early as 6 to 7 months. At this age, there are already a large number of neurosecretory cells in the visceral and pleuropedal ganglia of the abalone. Moreover, Sobhon et al. showed that a large number of mature oocytes of the reproductive cycle of abalone occurred at 10 to 11 months (91), the age at which the neurosecretory cells in pleuropedal and visceral ganglia reached a maximum number and appeared adult-like. Our observations supported by Yahata who demonstrated that the pleuropedal and visceral ganglia might produce and release factors which could induce spawning (92)

From the previous discussion, the giant neurons or NR_1 is believed to be similar to motor neurons in the ventral horn of spinal cord of the vertebrates. The numbers of these cells increase following the development of the ganglia. In the cerebral ganglia, they are significantly increased in number in 5-and 10-month-old abalone which are assumed to be in the juvenile and pre-adult stage. They reach a maximum number and appear adult-like in 12-month-old abalone which is considered to reach the adult

stage. NR_1 first appears in the dorsal horn of the cerebral and pleuropedal ganglia, where they are regularly found in certain positions *i.e.*, dorsal and ventral horn. Our study was supported by Dorsett who reported the location of the giant neurons in the brain of nudibranch *Tritonia hombergii* (93). Thus, NR_1 may proliferate relatively later than the other types of neurons (NR_2, NR_3, NR_4) which are present in abundance at very early age. This may be related to the more active movement by muscular activity as the abalone become older.

Special Sensory Organs

Tentacles

Our observations reveal that although the external appearance of the special sensory organs are similar to those of other species of prosobranch mollusks (62), there are fundamental differences in the histological structure. As in other prosobranch mollusk, the cephalic tentacles of *H.asinina* consisted of quite large, round cephalic tentacle which are the outgrowths from the anterior end of the head (60,61,62). In cross sections the basic structure of the cephalic tentacle is similar to that of other species of *Haliotis* (3,46,62), in having the tentacular nerve bundle in the middle of groups of muscle fibers which are covered by the epithelium. The observations of Wright (58) on *Arion ater* and Rogers (47) on *Helix*, lead to the conclusion that there are two cell types located on the lobules below the epithelium. On the other hand, Matera and Davis found that the tentacles of *Pleurobranchaea californica* had ciliated receptors laid in the epithelium formed by columnar supportive cells (94). These receptor cells do not have distal dendrites, but rather bear the cilia on the cell body itself (94). In the present study, the simple columnar

epithelium of cephalic tentacles of *H. asinina* have three types of cells: sensory cell, supporting cell and mucous cell. This is in agreement with studies by Croft who reported similar finding in the cephalic tentacle of *H. tuberculata* (3). The receptor cells of their tentacular organs were not subepithelial, as in pulmonate mollusc (58), but intraepithelial like in *P. elegans* (61). In the present study, the sensory cells in *H. asinina* have oval shape and contain oval nucleus with mostly densely-stained chromatin, which are similar to those of *H. tuberculata* studied by Croft (3). These sensory cells are widely distributed between supporting cells in the epithelium in the basal part of the cephalic tentacle, but in the middle and top parts, they are concentrated in the center of each papilla. Below the papillae, there are nerve branches from the bundle of the tentacular nerve that supply the sensory cells. This situation is similar to the nerve bundle of *P. elegans* which send their sensory dendrites to the epithelium of the tentacle tip (61). At the apical surface of most tentacles, there is a bundle of long, twisted cilia (61). Our study shows that at the top of the papillae of the cephalic tentacle, there is a circle of cilia.

The cephalic tentacle contains sensory cells in their ciliated epithelium very much like in the olfactory epithelium or taste buds of vertebrates. Thus, it is comparable to the olfactory organs and taste buds of the vertebrates. This is supported by many investigators who show that, in addition to tactile reception the cephalic tentacle are also the chemical receptor because they can respond to the chemical irritation, odor, and food (50,51,52,53,54,55). During these stimulations, the mucous cells may be stimulated to secrete mucous to lubricate and protect the epithelium.

In the SEM study, the surface of cephalic tentacle exhibits many papillae similar to those in Vetigastropoda and Seguenzioidea (59). Our study shows that the papillae

are present in a small number in the base part and they become more numerous in the middle and top parts, with the latter bearing smaller and taller papillae. These results are in accordance with the sectional LM study, which reveals that the epithelium of the base is flat, while the middle part has the stalk-liked, and the top part has long spine-liked papillae. Hence, there could be the correlation of the abundance of receptor cells on various regions of the tentacle with the sensitivity of various part of them. The tentacle tips are presumbly the most sensitive to touch and other stimuli such as pressure, temperature, and chemical (57,58,61).

The epipodial tentacles are smaller and shorter than the cephalic tentacles. They are numerous and located around the foot muscle. The epipodial tentacles have basic structure similar to that of the cephalic tentacles(3,60). However, the muscles in the epipodial tentacles are smaller and do not appear in regularly-arranged groups as these in the cephalic tentacles. The cephalic tentacles are used to explore the environment and seek out food while avoiding danger, so they need more muscles to stretch and retract for the long distance. On the other hand, the epipodial tentacles have more function in support of the cephalic tentacles in finding food and receiving chemical stimuli (3). Hence, they have smaller size and shorter length than the cephalic tentacles, and only project out for a short distance around abalone body; and they do not need strong muscle as in cephalic tentacles. It was also reported that the epithelium of epipodial tentacle of *H. tuberculata* has two types of cells: supporting and sensory cells (3); while in the present study, there are three types of epithelium cells in *H. asinina* epipodia. The first two types are similar to those in the *H. tuberculata* found by Croft (3) and the last type is the mucous cell.

The pulmonate mollusk have two pairs of tentacles: superior and inferior tentacles or anterior and posterior tentacles which are called cephalic tentacle and rhinophore (48). Rhinophores have shapes ranging from simple tapering rods to elaborated lamellae or tubercular organs (48). In the prosobranch mollusk, there is one pair of cephalic tentacles, and they do not have rhinophore (62). In the present study, we found a pair of small tentacles, which have a half-circle shape, located on the dorsal surface of the base of the cephalic tentacle, which are called appendage tentacles. It is possible that the appendage tentacles are the homologue of the rhinophore tentacles found in pulmonate, but they are rather small and have rudimentary shape as compared to pulmonate rhinophore. Similar to other tentacles in this study, the appendage tentacle has columnar epithelium containing sensory, supporting and mucous cells, but they are much less numerous. In the middle part of the appendage tentacle, there are also muscles and nerve fibers. The function of the appendage tentacles is not clear. They may help to support the cephalic tentacles and receive chemical stimuli, eventhough they may be rudimentary in structure.

Eyes

The eyes of *H. asinina* were located, as in other prosobranch mollusks, at the anterior of the head (45,46,61). They are cup-shaped similar to those of other *Haliotis* spp. (46). In the present study, it was found that the eyes of *H. asinina* contain a lens surrounded by retina, consisting of receptor cells and pigmented cells. Our observations are similar to the findings by Jacklet (64), Zaitseva (61) and Croft (3) who reported similar structures in *A. californica*, *P. elegans* and *H. tuberculata*, respectively. It was found that the photoreceptor cells of *P. elegans* (61) and of *H.*

tuberculata (3) were tall and cylindrical in shape (61), and likewise the receptor cells in the eyes of *H. asinina* are also tall and have spindle shape. Croft (3) found only one type of receptor cells in *H. tuberculata*, in contrast to Eakin who reported five types of receptor cells in the eye of *H. crassisornis* (68). The eyes of many primitive gastropods have receptor cells containing vesicles about 500A° in diameter in the cytoplasm (65,95) and there are numerous dense granules in the neuronal processes of the retina, which appear to be typical neurosecretory granules (65,95). In the present study, the eyes of *H. asinina* as studied by light microscopy did not reveal any vesicles or granules in the cytoplasm of the receptor cells. However, we can classify receptor cells into three types by the pattern of chromatin condensation in the nucleus and staining affinity of the cytoplasm. The fourth cell type in the retina of *H. asinina* is the pigmented cells, which have elongated shape, with the nucleus in the proximal region of the cytoplasm while the distal region containing many pigmented granules. This general structure structure is in agreement with the report by Croft who observed similar pigmented cells in *H. tuberculata* (3).

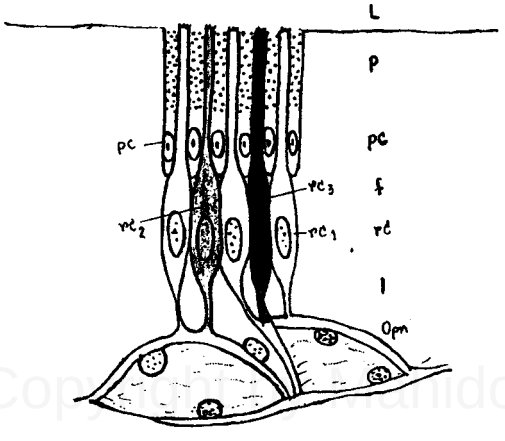


Diagram of the retina of *H. asinina*

The structure of retina of *H. asinina* is summarized in the diagram above: the receptor cells interdigitate with the pigmented cells, and the distal end of the receptor

cells may have many microvilli. In *H. aspersa* (94), *P. elegans* (61) and the nudibranch *Hermissenda crassicornis* (68), the structure of the receptors are basically similar to *H. asinina*. The inner photoreceptor surface has a tuft of microvilli; and the opposite end of the cell is the origin of the axon. In all gastropod eyes, the microvilli of the cells are adjacent to the lens (61,65,68,95). It is possible that the distal end of the receptor cell is stimulated by light and the cells give excitatory, depolarizing responses to light, and send impulses along the optic nerve fiber to the cerebral ganglia (64). The receptor cells in *H. asinina* send processes, to the fiber plexus of optic nerve beneath the receptor cell layer, which also contains many supporting cells. This is in agreement with the studies of Jacklet who found that in *A. californica*, the thin optic nerve arises at the back of the eye, which also contains other nonreceptor elements and their processes (64,65). Furthermore, the photoreceptor cells and pigmented granules are located adjacent to the lens. It is possible that the pigmented cells may act as a light filter for receptor cells, so that the receptor cells receive an appropriate amount of light that stimulates the nerve signal that could be sent to the cerebral ganglia.

Osphradium

It was found that the osphradium of *Conus striatus* is wedge shaped consisting of the central and lateral zones, but that of *N. lapillus* has central axis with low rounded leaflet on both sides (79). On the other hand, the osphradium of *T. haemastoma canaliculata* is composed of 150-200 lamellae; each of which is divided into two distinct regions by a groove situated parallel to the dorsal edge of the organ (72). On the other extreme, the osphradium of *Crepidula* appears monopectinate with the right

side having stubby leaflet (79). In the present study, the osphradium of *H. asinina* has a major axis where leaves arise and many leaflets extend from both sides of each leaf. The surface of the leaf and leaflet exhibits many rod-shaped and paddle-like cilia arranged in tufts. It was reported that chemical synapses in the osphradial epithelium of *Bullia digitalis* were associated with tuft ciliated cells (73). The gross morphology of these tufted cells is similar to that of the tufted ciliated cells observed in this study. It is possible that the arrangement of the osphradium of *H. asinina* into major and minor folds or leaf and leaflet may help to increase the area of sensory reception (3). Moreover, the surface of osphradium of *H. asinina* has many granules from their pores which are similar to those of *T. haemastoma canaliculata* (72). However, at the junction of the axis and leaf, there are fewer number of ciliary tufts and exocytosed granules than on the surface of leaf and leaflet.

Crisp (74) divided the osphradial leaflet of some prosobranch into 3 regions: sensory, glandular and transitional regions. The glandular region contains two types of mucous cells, while the sensory region consists of scattered ciliated sensory cell and unciliated cell. The transitional region is located between the glandular and sensory regions. In the present study, we divide the osphradial leaf and leaflet into 2 areas: apical and basal areas. The apical area contains a mixture of supporting cells, sensory cells and mucous-secreting cells. In the basal area, there are alternation of 2 types of cells which have large mucin granules. These two areas correspond to the sensory and glandular regions in the other species of prosobranch as described by Crisp (74). All of these cells are located outside the basement membrane, which is similar to the location of epithelial cells in the peripheral region of osphradium in *Conus flavidus*

(96). In the inner region of the basement membrane, there are connective tissues that contain nerve fibers.

It was found that the sensory cells of *C. flavidus* osphradium were approximately 40μm in height, and they bear hairy projections about 15μm long (97). On the other hand, the sensory cells in the osphradium of *H. tuberculata* had elongated shaped (3), which are similar to the sensory cells observed in *H. asinina*. The upper part of the cells reaches the surface of the epithelium and bears some cilia. Based on their characteristic, the sensory cells correspond to the Si2 cells reported by Welsch and Storch in *B. undatum* (98). These cells are scattered in all apical areas of leaves and leaflets where they could be easily stimulated.

In earlier studies of osphradium in molluscs, there were no reports on the heterogeneity of mucous-secreting cells (74,96,97). Most investigators just reported that the osphradium had only one type of mucous cell scattered among the sensory and supporting cells, and sometimes they protruded above the ciliated surface (3,73,96). However, Crisp (74) found that there were two types of mucous cells in the glandular region of the osphradium of some prosobranch. The first cell type contained electron-lucent granules that were stained metachromatically with toluidine blue and the second cell type was a goblet cell containing membrane-bound, round, and more electron-opaque granules (74). In the present study, using staining with PAS-methylene blue, two types of mucous cells could be revealed in the basal area, with large mucin granules appearing either purple or pink. In contrast, there are five types of mucous cells with different staining pattern in the apical area.

It is impossible to implicate the functions of mucous cells on the basis of the structure alone. However, from the previous studies, it was suggested that the

osphradium is an organ of chemo- and tactile receptors concerning with sensing the amount of sediment carried into the mantle cavity (75,76,77); and that it can also assist the regulation of respiration by detecting changes in the pH of sea water (81,82). Based on the function of these organs, the role of each type of mucous cells will be described below. It has been argued that the osphradium may act as a turbidity receptor, and protective organs for the gill. It is likely that, when foreign irritating particles from turbid water are attached to the surface of the osphradium, the mucous cell may release mucus to bind foreign particles which will be brushed away by the ciliary action of the epithelial cells. The mucous cells that perform this function may be type-2 mucous cell because they have similar characteristics to the type of mucous cells observed in the gill epithelium. Croft found that irritating oils introduced into the entrance of the respiratory chamber seemed to be perceived at once by the osphradium, and the shell closed down abruptly (3), and at once a large amount of mucus was released from the mucous cell (3). In similar experiment, Alexander introduced milk into the respiratory chamber, and obtained similar responses (97). Furthermore, some mucous cells may release mucin into the sea water perhaps to clear away the offending substance, as well as to adjust pH of sea water to be suitable for respiration. The mucous cells used in this protective action may belong to two types, *i.e.*, the acid and basic mucous cells. It is possible that these two cell types correspond to the type-4 mucous cell and type-1 or 3 mucous cell. The remaining mucous cells may act as general mucous cell which produce mucin to lubricate the organ and protect the epithelium.

Gills

Light microscopic observation shows that the internal structure of the gill and the gill filaments of *H. asinina* have a standard internal architecture similar to the gills of other mollusks. The gills are bipectinate, with individual filaments that show basic similarities. All filaments are positioned parallel to each other and are linked by a common base, through which the hemolymph is directed and distributed to the individual filaments (84). Each filament is corrugated in the middle part, which encloses the hemocoelic space, thus it may be a modification to enlarge the total gill surface area and improve respiratory gas exchange (3,84). The epithelium of the gills in *H. asinina* is a columnar or cuboidal simple epithelium, which presumably help to enhance a rapid gas exchange. This feature has been found in most species of mollusks studied so far (3,84,85). The gill filaments possess areas of ciliated cells alternating with areas of non-ciliated cells. In our study, the efferent margin of the filament consists of ciliated cells, and both sides of the hemocoelic space in the efferent side are covered by ciliated cells with longer cilia (3). Yonge has demonstrated in *P. vulgata*, that the movement of cilia creates a water current in the opposite direction to the hemolymph flow through the gill filaments, thus establishing a counter current mechanism for respiratory gas exchange (99). He has also suggested that the ciliated cells of the gill of *Siphonaria* aid in forming small currents across the lamellae, the major water current being produced by cilia of the dorsal and ventral sides (99). On the other hand, ciliated cells are thought to be used for moving mucus. Ciliary movement also takes part in distributing mucous secretions, that serve to capture foreign particles and remove them from the gills (100). The dorsal and ventral ciliary movement carry particles either to the tips of the filament or to the axis of the

gill. The foreign particles may be ejected from the tips or carried anteriorly, and is attached to mucus provided by mucous cell (84,85). Hence, the ciliated cells lining the gills observed in the present study may play both roles in creating water current and removing irritating particles.

Nuwayhid et al. did not find any mucous cells in the gills of *P. vulgata* (100), whereas *S. capensis* has a large number of these cells (85), and *A. constricta* has two types of goblet mucous cells (84). The present study revealed three types of mucous cells on the gill of *H. asinina*. All of them are of the ordinary goblet type and are grouped in the efferent and afferent sides of the filament. The mucous cells are found either totally enclosed by other cells or bordering the filament surface. It is unclear whether the secretory products are secreted externally or into the hemolymph or both. It is well established that gill is an organ of gas exchange comparable to the lung in the vertebrate. The areas of the gas exchange in *H. asinina* are on both sides of the gill filaments which are comparable to the alveoli in the lung of the vertebrate. Hence, it was suggested that some mucous cells may function in gill cleaning or removing dirt in co-ordination with muscle contraction and ciliary movement (99). The chitinous skeletal or rods found in the efferent side (3,86) may serve for attachment of muscles that bring about considerable movement of the gill filaments. In *S. capensis*, it was found that the muscle fibers are located at intervals on hemocoelic surface (85). On the contrary, the present study reveals that the muscle fibers are attached to the inner surface of the rod. The function of this muscle may be the same as described above.

CHAPTER VI

CONCLUSIONS

The results in the present study can be concluded as follows:

1. The central nervous system of *H. asinina* consists of three main ganglia: the cerebral, pleuropedal and visceral ganglia. Each of these ganglia possesses ten types of cells based on their characteristics and staining affinities with H&E, chrome-hematoxylin-phloxine and paradehyde-fuchsin. There are three types of neurosecretory cells (NS_1, NS_2, NS_3), four types of neurons (NR_1, NR_2, NR_3, NR_4) and three types of neuroglia (NG_1, NG_2, NG_3). The nuclei of the neurosecretory cells contain varying degree of heterochromatin, ranging from almost euchromatin in NS_1 to clumps of heterochromatin in NS_2 and thick cords of heterochromatin in NS_3. Their cytoplasm contains different types of neurosecretory granules. The pattern of nuclei of NR_1, NR_2 and NR_3 is similar to that of NS_1, NS_2, NS_3, respectively. However, their cytoplasm is not stained positively with chrome-hematoxylin-phloxine and paraldehyde-fuchsin. NG_1, NG_2 and NG_3 have small spindle-shaped nucleus with thick cords of heterochromatin, but their locations are different.

2. The development of cells and the ganglia during various ages of developing abalones are studied. In the cerebral ganglia, NS and NR_1 are significantly increased in number in 5-and 10-month-old abalone, reaching a maximum number in 12 months, and thereafter remaining constant. In the pleuropedal ganglia, NS and NR_1 are significantly increased in number in 4-and 7-month-old abalone, reaching a

maximum number in 11 months, and thereafter remaining constant. In the visceral ganglia, NS and NR_1 first appear in 2-month-old abalone and are significantly increased in number at 4 months, reaching a maximum number in 11 months, and thereafter remaining constant. NR_2, NR_3, NR_4 and NG are present in all ganglia early in development from 1 month onwards, and their numbers increase rapidly with age.

3. The central nervous system gives nerve branches to innervate the body and special sensory organs as peripheral nerves that can be divided into 5 groups. The special sensory organs consist of a pair of cephalic tentacles, a pair of eyes, a pair of appendage tentacles, numerous epipodial tentacles, and osphradium.

The cephalic tentacles and epipodial tentacles are similar in structure, but the latter are about three times smaller and ten times shorter than the cephalic tentacles. The surface of both tentacles can be divided into three parts: the basal part exhibits flat surface consisting of small folds and grooves, the middle part has short hillock-shape papillae, and the top part has very high cone-shape papillae. In cross sections, there is a bundle of nerve which runs along the length of each tentacle, and its branches are distributed among the muscles. The epithelium of both tentacles is covered externally by the brush border. The epithelium of the tentacle's base is columnar type and its surface appears flat, with some areas exhibiting small curve. At the middle part, the epithelial surface appears like hillocks, and at the top part it appears as cone-shaped structures. The hillocks of the middle part and the cones at the top are believed to be sensory papillae. In each papilla, there are cilia extending out from the top, and there are three types of epithelial cells: the ciliated sensory cells, supporting cells and goblet cells. The sensory cells may be innervated by branches from the midline nerve bundle.

The eye is an open vesicle which appears spherical in cross section, with its large lens surrounded by the retina. The retina is composed of six layers: pigmented layer, pigmented cell layer, fibrous layer, receptor cell layer, loose connective tissue layer, and a layer of optic nerve, consecutively. The receptor cells in the retina can be classified into three types: rc_1, rc_2 and rc_3.

The appendage tentacle has a half-circle shape and is covered by numerous irregular folds and grooves. In cross sections, it reveals many muscle cells and accompanying nerve fibers that form the core materials surrounded by an epithelium. The epithelium is simple columnar that lies on a thick basement membrane. Three types of cells can be identified in the epithelium, *i.e.*, ciliated sensory cells, supporting cells and mucous-secreting cells.

The osphradium exhibits many tufts of rod-shaped cilia, paddle-like cilia, and granules exocytosed from pores on the surface of major and minor folds, which are called leafs and leaflets. In transverse sections, each of the leaf and leaflet can be divided into two areas: the basal area that consists mainly of two types of large goblet cells, and the apical area that contains a mixture of supporting cells, sensory cells and five types of mucous-secreting cells.

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