HISTOLOGY OF THE CENTRAL NERVOUS SYSTEM OF THE SQUID, ILLEX ILLECEBROSUS ILLECEBROSUS (LESUEUR).

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HISTOLOGY OF THE CENTRAL NERVOUS SYSTEM OF THE SQUID, *Illex illecebrosus illecebrosus* (Lesueur).

by

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A thesis
submitted in partial fulfilment of the requirements
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ABSTRACT

This thesis describes the microscopic structure of the central nervous system of the squid Illex illecebrosus illecebrosus (Lesueur), and relates it to that of other cephalopods. Organs of secretion associated with the brain are also shown together with the results of histochemical tests used to detect the presence of carbohydrates, proteins lipids and nucleic acids.

The central nervous system of Illex is basically similar to the brains of other cephalopods. It consists of eight interconnected ganglia, some of which are divided into distinct lobes. The cerebral ganglion, situated above the esophagus, contains the association and higher motor centres of the brain, and its neurons are much smaller than the cells in the subesophageal masses. Directly below it and broadly connected to it by the anterior and posterior basal - subesophageal connectives on both sides of the esophagus lies the middle subesophageal mass which is the major source of nerves arising from the brain. The posterior subesophageal mass is separated from the middle mass by a curtain of connective tissue and has several divisions. Nerves from this ganglion innervate the mantle, fins, viscera and chromatophores. The peduncle and olfactory lobes are situated above and posterior to the optic tract. The anterior subesophageal mass, or brachial ganglion, which is separated from the central brain mass and

lies just posterior and ventral to the buccal mass, innervates primarily the arms. The superior and inferior buccal ganglia are located above the anterior subesophageal mass.

Small neurosecretory cells of two types occur in clusters within the tissue of the posterior subesophageal mass, adjacent to small blood sinuses. The components of both the optic glands and parolfactory vesicles were negative to neurosecretory stains. The colloid of the parolfactory vesicles was shown to be a mucoprotein or glycoprotein, containing little cysteine.

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ABBREVIATIONS USED ON FIGURES

AB - Anterior Basal lobe
ACh - Anterior Chromatophore lobe
afn - anterior funnel nerve
amc - anterior magnocellular commissure
AP - Anterior Pedal lobe
ASM - Anterior Subesophageal Mass
bm - buccal mass
brp - brachio-pedal connective
brvi - brachio-visceral connective
bus - buccal-subvertical connective
C - cartilage
Cbr - cerebro-brachial connective
Cg - Cerebral Ganglion
cpc - central pedal commissure
DB - Dorsal Basal lobe
E - Esophagus
F - Fin lobe
ga - giant axons
ian - inferior antorbital nerve
IB - Inferior Buccal Ganglion
ibr - interbrachial nerve
ibu - interbuccal connective
jgt - juxta-ganglionic tissue
LB - Lateral Basal lobe
lc - large cells
ln - labial nerves
Lin - lingual nerves
LP - Lateral Pedal lobe
M - Magnocellular lobe
MB - Medial Basal Lobe
mfn - median infundibular nerve
mn - mandibular nerve
mpa - medial pallial adductor nerve
MSM - Middle Subesophageal Mass
mxn - maxillary nerve
n - nerves
NSV - Neurosecretory System of the Vena Cava
O - Olfactory lobe
oc - ventral optic commissure
of - optic fibres
og - optic gland
Op - Optic lobe
P - Peduncle lobe
PB - Posterior Buccal lobe
PbC - Posterior Basal Complex
Pc - Precommissural lobe
pc - peduncle commissure

PCh - Posterior Chromatophore lobe
pl - posterior lobule
pn - pallial nerve
pon - posterior occulomotor nerve
PP - Posterior Pedal lobe
PSM - Posterior Subesophageal Mass
pv - parolfactory vesicles
rf - radial fibres
S - Subvertical lobe
san - superior antorbital nerve
SB - Superior Buccal ganglion
sbb - superior buccal-brachial connective
Sc - small cells
sc - statocyst cavity
SF - Superior Frontal lobe
sn - sympathetic nerve
Sp - spine
V - Vertical lobe
Vi - Visceral lobe
wb - white body

1
2
3
4
5
5 a
b
6 a
b
c
7 a
b
c
d
8

Numbers used to designate root bundles

Symbols used on table 2

+++ - strong reaction
++ - moderate reaction
+ - weak positive reaction
- - negative reaction
± - inconsistent areas which are both positive and negative

ACKNOWLEDGEMENTS

This research was conducted in the Department of Biology, Memorial University of Newfoundland, and I wish to acknowledge the aid given by the faculty and staff.

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The squid used were obtained through the kindness of Dr. F.A. Aldrich, Director of the Marine Sciences Research Laboratory of Memorial University.

INTRODUCTION

The squid Illex illecebrosus illecebrosus (Lesueur) is very important commercially to Newfoundland. Although not used for human consumption, it is the principal source of bait in the cod fishery.

The taxonomy of Illex has been discussed by Voss (1956) and Lu (1968). It is a coleoid cephalopod and a pelagic oegopsid squid of the Family Ommatostrephidae. The current belief is that there is one species, Illex illecebrosus, which exists in the form of three subspecies, inhabiting different areas. These are Illex illecebrosus illecebrosus (Lesueur) in the northwest Atlantic, Illex illecebrosus coindeti (Verany) in the eastern Atlantic, and Illex illecebrosus argentinus (de Castellanos) in the southwest Atlantic.

The systematics of the subspecies, and as a result, their geographical distributions are still not settled. Records from around Iceland, the Bay of Biscay, and northwest Africa are doubted by Clarke (1966) as on the eastern side there is much confusion with Illex illecebrosus coindeti. Illex illecebrosus illecebrosus is found predominantly in the western Atlantic between Massachusetts and Greenland, but has been reported as far south as the Gulf of Mexico. The situation was found to be very complex by Adam (1952), who analysed statistically the dimensions of the squid and found populations at

different stages of intermediacy between Illex illecebrosus and Illex coindeti, particularly in the Bristol Channel and the North Sea. In the text, references to I. illecebrosus and I. coindeti apply to the subspecies.

The purpose of this thesis is to describe the histology of the central nervous system, and to relate it to that of other cephalopods; to determine the presence of neurosecretory cells and to characterize their components histochemically.

Cephalopods have the most elaborate and complex nervous systems found in the invertebrates and are considered to be intelligent animals. There is little published on the nervous system of Illex and nothing has been done on the subspecies in North America. Richter (1913) described the anatomy of the nervous system of Illex and three other oegopsid squid and Thore (1939) discussed the histology of I. coindeti, interpreting it mostly in relation to Sepia and Octopus which have been studied intensively. Some of the earlier key works on the anatomy of cephalopod nervous systems are those by Chéron (1866), Owsjannikow and Kowalevsky (1867), Dietl (1876), Williams (1909), Hillig (1912) and Pfefferkorn (1915). The histology of the Octopus brain is well known because of recent work by Young (1960, 1962, 1963 - a and b, 1964 - a and b, 1965 - a, b and d), which has been extended into learning and discrimination experiments. Much of this information has been reviewed by Wells (1962, 1966) and

Young (1961, 1964a).

Gabe (1966) states that nothing in cephalopods has been ascertained to be neurosecretory. Scharrer (1937) surveyed the brains of two cephalopods, Octopus and Sepia, but failed to find any neurosecretory cells. Alexandrowicz (1964, 1965), however, described a distinctive tissue located at the posterior end of the posterior subesophageal mass in Eledone, Sepia and Octopus which he showed to be neurosecretory. He named it the "Neurosecretory System of the Vena Cava" (NSV system) but studied principally the nerves arising from it. Martin (1966) extended this to a description of the tissue and its cellular components in Illex coindeti and Ommatostrephes.

In cephalopods the organs originally thought to be neurosecretory were the epistellar body of octopods and the parolfactory vesicles of decapods. Young (1936) studied the epistellar bodies of octopods and the giant fibres in the stellate ganglion of decapods and suggested that the epistellar body was secretory and originated from the giant cell lobe of the stellate ganglion. Cazal and Bogoraze (1944, 1949) studied the histology more closely and accepted the neurosecretory interpretation. The parolfactory vesicles, discovered by Thore (1939) are very similar to the epistellar bodies (Bern and Hagadorn , 1965). They have been discussed by Haefelfinger

The terminology used is that of Boycott (1961), which is based mostly upon Dietl (1876) and Pfefferkorn (1915). It should be noted that the orientation used is the functional one and not the morphological.

(1954) who assumed that they are secretory and that the colloid may be mucoid. He cites Thore as supposing that it is in the category of proteins, but no histochemical work was done to verify this.

The optic gland has been described in Octopus by Cazal and Bogoraze (1943, 1949) as the peduncular gland, and briefly in Illex coindeti by Haefelfinger (1954) as the sub-peduncular body. Cazal and Bororaze assumed a neurosecretory role for the structure, but it was shown later that it is not nervous (Boycott and Young, 1956) and functions as an endocrine gland related to gonad development (Wells and Wells, 1959; Wells, 1960). The optic glands have been found in all cephalopods so far examined with the exception of Nautilus (Bern and Hagadorn, 1965); however, Young (1965c) has described some tissue in the brain of Nautilus which may correspond to the optic gland.

The contents of this thesis have been organized into four parts. The first briefly deals with the anatomy of the head region, the second with the histology of the central nervous system with the exception of the optic lobes, the third describes the secretory structures associated with the brain, and the fourth presents the results of the histochemical tests.

No physiological work could be carried out because of the inability to keep the animals alive in captivity.

MATERIALS AND METHODS

The tissues used in this study were collected between August and October, 1967 at Holyrood and Cuckold Cove and from formalin-preserved squid caught the previous summer. The specimens are listed in table 1. The squid were jigged using both the Japanese jigger and the Neyle-Soper single jigger. The brain capsule was removed immediately after capture on the fishing boat and fixed. After the tissue had gained suitable consistency, from two to three hours, it was removed, trimmed, and placed back in the fixative.

Several fixatives were used. The tissues intended for the stains on paraffin sections were fixed in Bouin, Zenker, Carnoy, 10% formol saline (Carleton and Drury, 1957) and Baker's formol calcium (Pantin, 1946). After fixation, the tissues were dehydrated in graded alcohol and treated according to Peterfi's celloidin-paraffin embedding method (Pantin, 1946). The blocks were sectioned on a standard rotary microtome at thicknesses between 7 - 12 μ. Serial sections were cut in all three planes: cross, sagittal and frontal. Numerous sections were made and mounted on slides with Tissue-tac slide adhesive, while many others were cut on the cryostat for studies on enzymes.

Routine Histological Methods

The methods chosen for the general staining of the

TABLE I

LIST OF SQUID SPECIMENS USED FOR HISTOLOGICAL STUDY

Specimen	Date of Capture	Mantle Length - mm	Sex	Fixative
1	Aug. 8, 1967	187	M	Formol saline
2	Aug. 8, 1967	207	M	Zenker
3	Aug. 8, 1967	195	M	Bouin
4	Aug. 8, 1967	218	M	Formol saline
5	Aug. 8, 1967	224	F	Bouin
6	Aug. 8, 1967	205	M	Bouin
7	Aug. 8, 1967	217	M	Baker's formol calcium
8	Aug. 8, 1967	218	M	Zenker
9	Aug. 8, 1967	211	F	Formol saline
10	Aug. 8, 1967	195	M	Carnoy
11	Aug. 8, 1967	205	M	Zenker
12	Oct. 6, 1967	245	M	Formol saline
13	Autumn, 1966	170	-	5-10 % Formalin in sea water(30%)
14	Autumn, 1966	178	-	5-10 % Formalin in sea water(30%)
15	Autumn, 1966	170-180	-	5-10 % Formalin in sea water(30%)
16	Autumn, 1966	170-180	-	5-10 % Formalin in sea water(30%)
17	Autumn, 1966	170-180	-	5-10 % Formalin in sea water(30%)

central nervous system were:

(1) Bielschowsky's silver method (Humason, 1967). The ammoniated silver solution used in the second impregnation was not diluted, but the length of the first impregnation was reduced. Many sections were gold-toned in 0.2% gold chloride and some were also counterstained in toluidine blue.

(2) Harris' Hematoxylin and Eosin (Carleton and Drury, 1957).

(3) Mallory's triple stain (Pantin, 1946).

(4) Masson's trichrome stain (Carleton and Drury, 1957).

(5) Fraenkel's Orcein Method (Lillie, 1954).

(6) Heidenhain's Iron Hematoxylin (Carleton and Drury, 1957).

Neurosecretion

Three methods were used for the detection of neurosecretion.

(1) Bargmann's modification of Gomori's chrom-alum hematoxylin, counterstained with phloxine (Pearse, 1960).

(2) The paraldehyde fuchsin method, modified by Cameron and Steele (1959), and counterstained by Halmi's stain (Halmi, 1952).

(3) Performic acid - Victoria blue method (PAVB) (Dogra and Tandan, 1964). This method is based on the oxidation of cysteine by performic acid and is also useful in the demonstration of sulphydryl groups. It was performed both on blocks of tissue and single sections. Adjacent unoxidized sections were stained as a control.

Carbohydrates

(1) The periodic acid - Schiff test (PAS) after the method of McManus was used (Pearse, 1960). This gives a positive

reaction for all carbohydrates with the exception of acid mucopolysaccharides. For controls, adjacent sections were treated with 1% malt diastase for 45 minutes to remove glycogen, and others were acetylated in a mixture of acetic anhydride and pyridine for 24 hours to block the PAS reaction by esterification of -OH and $-NH_2$ bonds (Lillie, 1954).

(2) Metachromasia. Two staining solutions of toluidine blue were used (Pearse, 1960): (a) 0.5% aqueous solution, (b) 0.1% solution in 30% ethanol. Solution (a), when left overnight, gave excellent staining of Nissl granules and nucleoli. Carbohydrates which exhibit metachromasia are those containing sulfate esters, acid mucopolysaccharides or hyaluronic acid.

(3) Alcian blue - Chlorantine fast red was used as a specific stain for acid mucopolysaccharides (Steedman, 1950).

Proteins and Nucleic Acids

The histochemistry of proteins is based principally upon the reactions given by specific groups of the amino acids, and because of this most amino acids cannot be identified.

(1) Amino groups. The alloxan - Schiff method of Yasuma and Ichikawa (Pearse, 1960). The sections were treated with a 1% solution of alloxan in absolute ethanol for 24 hours at 37°C.

(2) Tyrosine. The Millon reaction after the modification of Baker (Pearse, 1960). Although Baker recommends celloidin

sections, paraffin sections were used here. The slides were covered with the Millon reagent and heated just to the boiling point. Here, the phenols form a complex with mercuric salts in the presence of nitrite in acid solutions.

(3) Indole groups. The DMAB-nitrite method (p-dimethylamino-benzaldehyde) (Barka and Anderson, 1965). This reaction, exhibited only by 3-indolyl derivatives, gives a blue colour to tryptophan-containing structures oxidized by sodium nitrite in concentrated hydrochloric acid.

(4) Nucleic acids. Einarson's gallocyanin method was performed according to Pearse (1960). Positive reaction was obtained at $_{p}$H 1.6 after staining sections for two days.

(5) DNA. Feulgen reaction (Pearse, 1960). The sections were hydrolyzed in 1N HCl at 60^{o}C and treated with the de Tomasi's Schiff reagent. The optimum time for tissues fixed in formol saline was 5 - 6 minutes.

Lipids

(1) Sudan black B in 70% alcohol (Pearse, 1960) was used on paraffin sections to detect lipids surviving dehydration and embedding.

(2) Copper phthalocyanin method (Pearse, 1960) on paraffin sections. This was previously thought to be specific for phospholipids, gangliosides and probably cerebrosides, but recent work has shown that it is not specific for either

choline-containing lipids or phospholipids, although it is still a useful stain for myelin.

Enzymes

Some tissues were fixed in formol saline at 4°C for 24 hours in a vacuum bottle and sectioned at 7 - 12 μ on a Lab-Tek cryostat at -30°C. Others were fixed in cold acetone dehydrated in acetone, and cleared in benzene in the refrigerator, and embedded in vacuo in Tissuemat at 56°C.

(1) Alkaline Phosphatase. For the demonstration of this enzyme Gomori's calcium - cobalt method was used (McManus and Mowry, 1965) with sodium β-glycerophosphate as the substrate, at $_{p}$H 9.0 - 9.2.

(2) Acid Phosphatase. Gomori's lead nitrate method (McManus and Mowry, 1965) was used with sodium β-glycerophosphate as substrate at $_{p}$H 5.2.

(3) 5-Nucleotidase. The method of McManus, Lupton and Harden was used with muscle adenylic acid as substrate at $_{p}$H 8.8 (McManus and Mowry, 1965).

(4) Non-specific Esterase. This group of enzymes was demonstrated by Gomori's α-naphthyl acetate method, with α-naphthyl acetate as the substrate and coupled with Fast Blue B at $_{p}$H 7.2 (McManus and Mowry, 1965).

ANATOMY OF THE HEAD REGION

The structure of the head region and the brain in Illex illecebrosus is shown in figures 1, 2, 3, and 4. The brain consists of four main ganglia: the cerebral ganglion and the anterior, middle and posterior subesophageal masses (Figure 1). The cerebral ganglion, which lies above the esophagus, is divided into five distinct areas. At the top the superior frontal lobe is anterior to the vertical lobe. These are the highest centres and are concerned with visual and tactile learning and memory in Octopus (Wells and Wells, 1957; Young, 1961). The third lobe, the posterior buccal, is located more anteriorly. This has been called the inferior frontal lobe by most authors (Hillig, 1912; Richter, 1913) but was recognized by Thore (1939) as the "pars posterior" of the buccal lobe. He correctly stated that decapods have no inferior frontal or subfrontal lobe. Immediately behind this is the anterior basal lobe, and posterior to this is the largest area of the cerebral ganglion, consisting of several not too well defined lobes, known collectively as the posterior basal complex or lobe. The posterior basal complex contains several areas differentiated primarily on the basis of function by Boycott (1961) in Sepia. These lobes are:1. subvertical, 2. precommissural, 3. dorsal basal, 4. medial basal, and 5. lateral basal. A sixth lobe, the interbasal, mentioned by Boycott (1961), could not be separated from the preceeding divisions in I. illecebrosus.

Below the esophagus there are three other separate ganglia: the brachial, pedal, and palliovisceral. Because of the apparent functional divisions in these ganglia in Sepia, it is preferable to name them the anterior, middle, and posterior subesophageal masses respectively after Boycott (1961).

The entire anterior subesophageal mass is the brachial ganglion. It is joined to the middle subesophageal mass by a large connective, which is not seen in Octopus and only barely visible in Sepia (Thore, 1939). Eight nerves arise from the anterior surface of this ganglion to innervate the eight arms of the squid. The mass is connected to the cerebral ganglion by a paired connective arising from its posterior dorsal end which passes lateral to the esophagus and enters the posterior buccal lobe anteriorly. It also has a paired connection with the superior buccal ganglion which arises from the posterior dorsal end of the mass and passes around the esophagus into the posterior dorsal end of the superior buccal.

Two pairs of nerves originating on the dorsal surface of the brachial ganglion and joining branches from the pedal lobe may correspond to the superior antorbital and interbrachial nerves. Another pair originating ventrally may be the inferior antorbital nerve described by Thore (1939) in Sepia and I. coindeti.

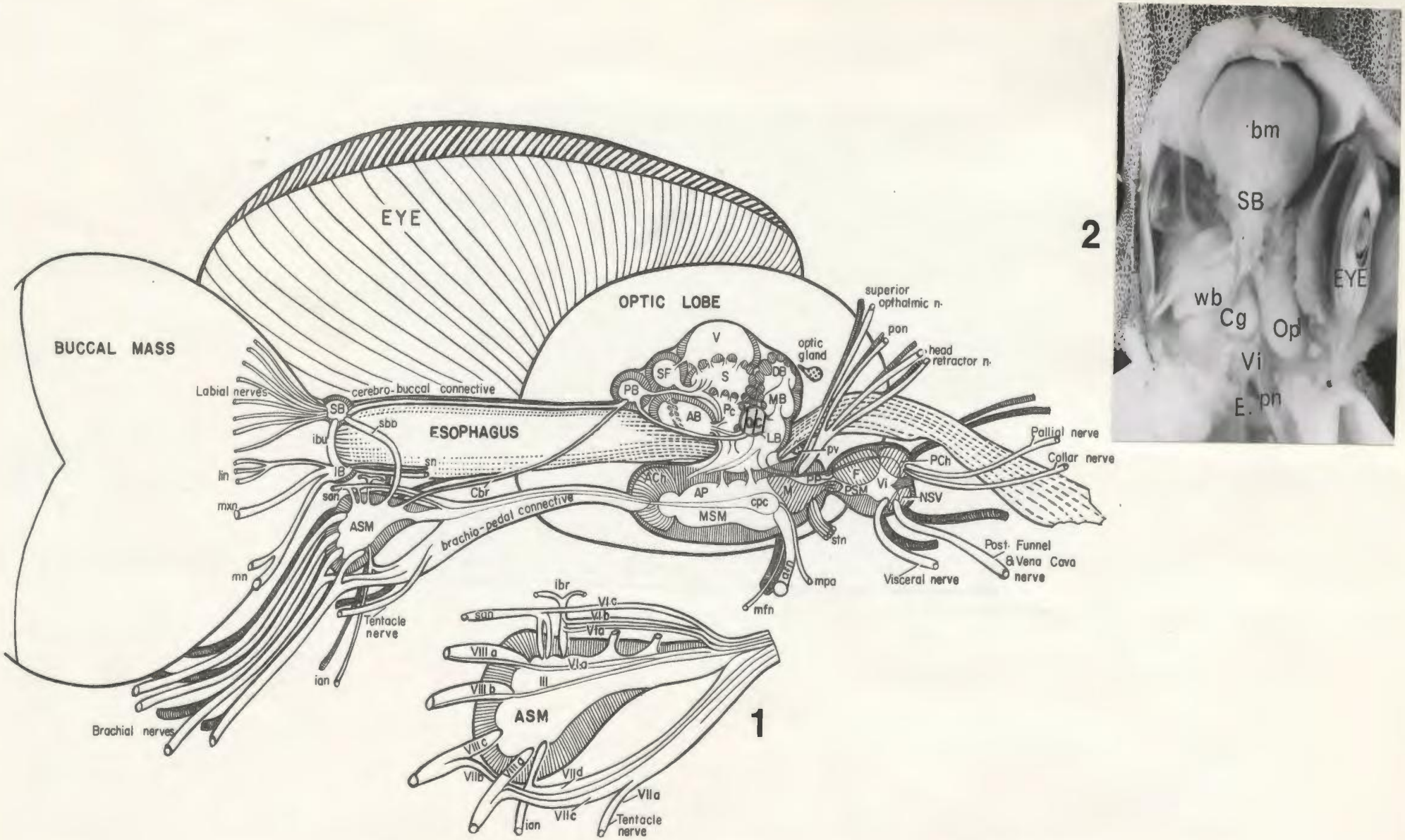


Figure 1. The central nervous system of Illex illecebrosus. The peduncle complex and lateral pedal lobe are not shown. Insert 1 - Anterior Subesophageal Mass. Insert 2 - Photograph of dorsal view of disected brain (photographed by J.W. Evans).

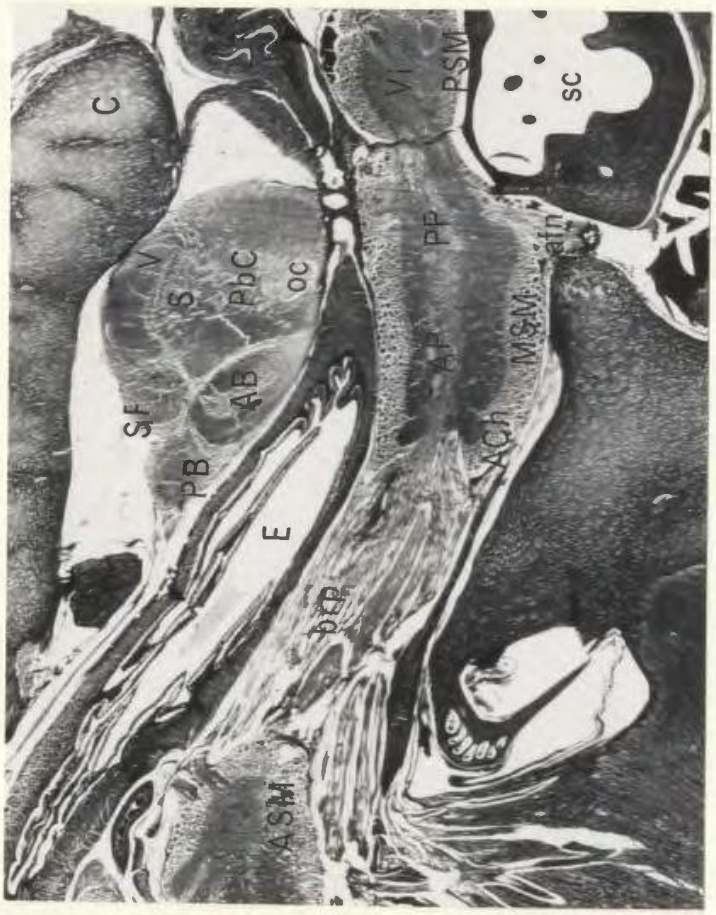


Figure 2. Central nervous system of Illex - median sagittal section; x4, Mallory triple, spec. 3.

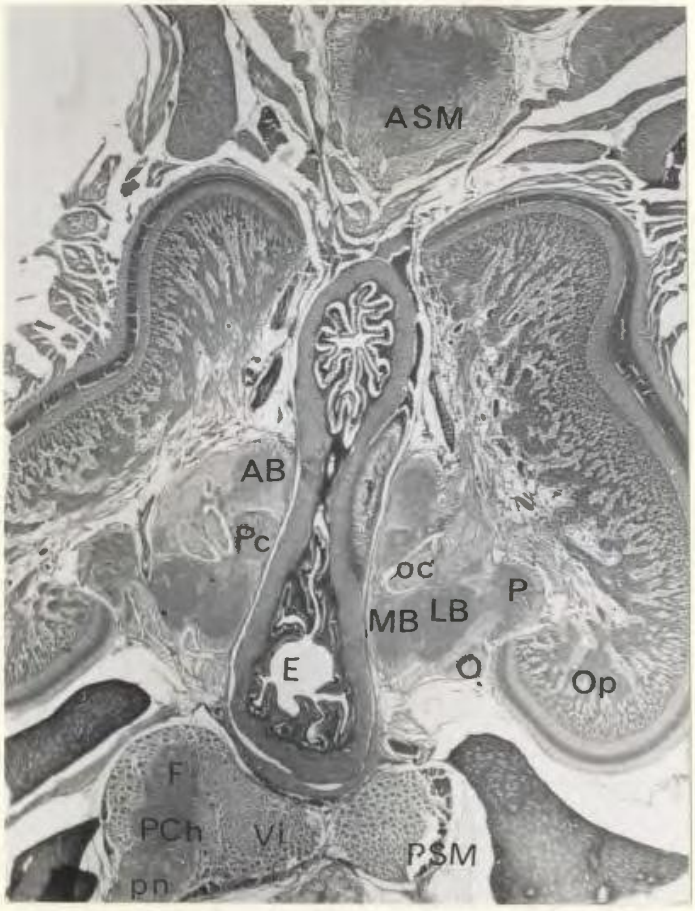


Figure 3. Central nervous system of Illex - frontal section at dorsal level of esophagus (the section is more ventral at left); x4, PAS, spec. 5.

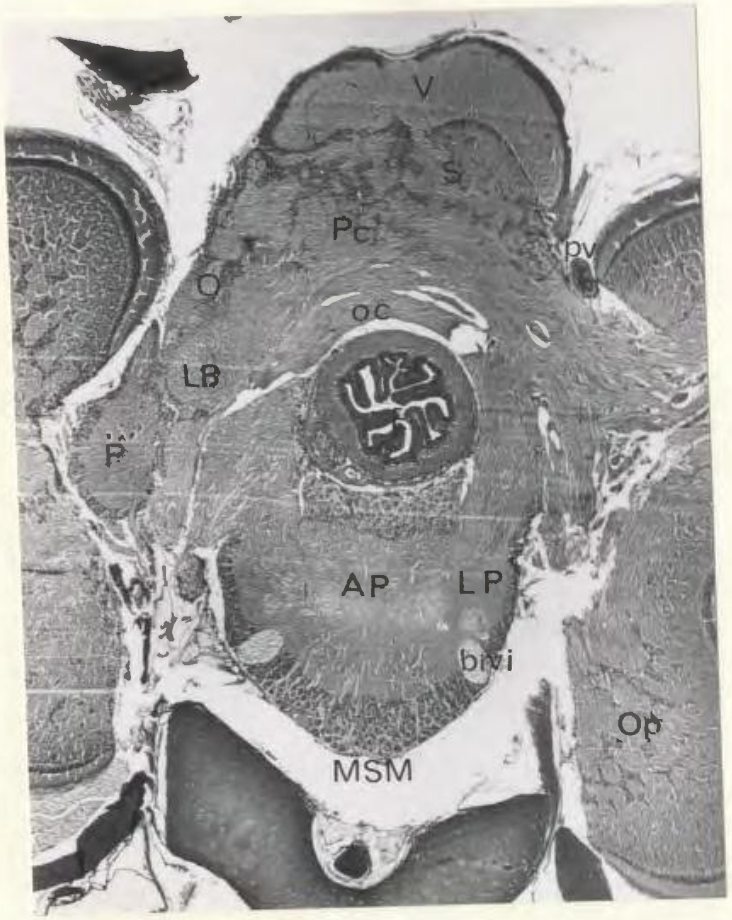


Figure 4. Central nervous system of Illex - cross section through level of the cerebral and pedal ganglia (the section is more posterior at left); x8, Mallory triple, spec. 12.

The middle subesophageal mass is divided into four regions: the anterior and posterior pedal lobes separated by the central pedal commissure, and two lateral pedal lobes. The paired anterior chromatophore lobe is situated anteriorly on either side of the anterior pedal lobe. Several nerves arise from the middle subesophageal mass. The single, broad brachio-pedal connective passes antero-ventrally to the region of the brachial ganglion, separates into twelve bundles, some of which are joined by fibres from the brachial ganglion and innervate the arms, tentacles, and other structures. The large paired anterior funnel nerves leave the lobe ventrally through an opening in the cartilage together with the single small median funnel nerve. The statocyst nerves arise more posteriorly. Another large paired bundle, which includes several nerves, leaves the mass dorso-posteriorly above and lateral to the posterior subesophageal mass.

The posterior subesophageal mass consists of the median visceral lobe, lateral to which are the accessory lobes of Thore (1939), each subdivided into the fin and posterior chromatophore lobes. Also located within this mass are the poorly differentiated vasomotor lobes. Several nerves leave this ganglion and pass posteriorly: the pallial nerves leave the dorso-posterior region and bundles containing the posterior funnel and vena cava nerves arise postero- laterally. The

visceral nerves emerge from the postero-ventral region.

Two additional ganglia are situated anteriorly (Figure 1): the superior buccal above the esophagus and the inferior buccal directly below it, at the level of the anterior subesophageal mass. The superior and inferior buccal ganglia are connected to each other by the paired interbuccal connective which encircles the esophagus. Numerous small labial nerves leave the anterior surface of the superior buccal ganglion. The lingual nerves stem from the central anterior surface of the inferior buccal ganglion, lateral to which arise two larger nerves, the maxillary and mandibular.

From a cross section (Figure 4) it is seen that the brain encircles the esophagus. The region lateral to the esophagus is the peduncle complex and portions of the posterior basal and magnocellular lobes, the latter containing the cell bodies of the first order giant axons. The cerebral ganglion is joined to the large optic lobes on either side by the optic tract. The eyes are situated a little anterior to the brain and numerous optic nerves pass from the back of the eye to the optic lobes.

The parolfactory vesicles exist as two pairs, the smaller pair dorsal to the optic tract and the larger pair posterior and more ventral to it. In fresh specimens they are easily seen because of their yellow to orange colour. The

optic glands are oval, paired, and situated posterior and lateral to the cerebral ganglion above the optic tract. They are also bright orange in colour.

The esophagus is a thick-walled tube of about 1.5 mm. in diameter beginning at the buccal cavity and extending posteriorly through the brain. It passes into the mantle cavity over the liver and then joins the stomach. Associated with it are two pairs of salivary glands. The anterior pair consists of two bulblike structures, attached to the buccal mass on top of and extending laterally to the esophagus. These glands secrete enzymes and mucus into the buccal cavity. The posterior pair is joined in the midline behind the posterior subesophageal mass of the brain and its duct courses alongside the esophagus through the central brain to join the buccal cavity.

Surrounding and protecting the brain is a cartilaginous cranium. The cranial cartilage is not as well developed in Illex as it is in octopods (Thore, 1939) and this may be due to the decentralization and subsequent elongation exhibited by the squid brain. The cartilage has several openings, two of which are lateral and receive the optic tracts. The optic lobes are not enclosed by cartilage. The cartilage is also open anteriorly and posteriorly. At the anterior end the brachial and the two buccal ganglia lie outside the cartilage. At the posterior end the cartilage extends a little beyond the posterior subesophageal

mass, which is apparently not the case in Illex coindeti, where part of this mass is unprotected by cranium (Thore, 1939). Another opening in the cartilage is situated in the ventral portion, below the middle subesophageal mass, and accommodates the large anterior funnel nerves. Just posterior to this the statocyst is embedded in a large cavity within the cranium under the visceral and posterior pedal lobes.

Between the cartilage and the brain there is a space filled by a large white organ of jelly-like consistency called the white body. This surrounds the optic peduncle dorsally and laterally and must be removed before the dorsal aspect of the brain can be viewed. The white body is believed to function as a hemopoietic organ (Messenger, 1967).

HISTOLOGY OF THE CENTRAL NERVOUS SYSTEM

Cerebral Ganglion

This is the major supraesophageal ganglion of the brain and contains the sensory and higher motor centres. Each lobe comprising the cerebral ganglion will be discussed in turn.

Vertical Lobe

The vertical lobe is situated dorsally and resembles a cap fitting over and enclosing several divisions of the posterior basal complex (Figures 1 and 2). It is unpaired, bilaterally symmetrical and not divided into gyri. This lobe, as well as the entire cerebral ganglion, is covered by a thin layer of collagenous connective tissue. It is supplied by many blood vessels which can be seen clearly in the cell layer and neuropile.

Cell layer

The vertical lobe is easily distinguished from the adjacent lobes by the characteristics of its cell layer, which is most developed at its anterior and posterior ends and laterally. The ventral surface also has many neurons, separating this from the subvertical lobe, but neurons are absent from the dorsal median area (Figure 5). Two types of cells were found in the rind. The majority are small and round (5 to 6μ, Figure 6) with a single slender axon divided into branches, and

the cell body taken up by the nucleus. The only other neurons smaller than these are those in the lateral regions of the superior frontal lobe and the tiny amacrines of the optic lobes, but these are more basophilic than the small neurons of the vertical lobe. The large neurons (up to 12 μ) are pear-shaped, have more cytoplasm, a thicker axon and are usually found at the periphery of the neuropile. Their axons pass vertically into the neuropile. Cell islands, containing both types of cells (pear-shaped neurons up to 18 μ) as well as multipolar neurons, are found in the neuropile. The orderly arrangement of many cell islands, spaced 60 - 100 μ from the cell rind, may have an important functional significance.

Neuropile

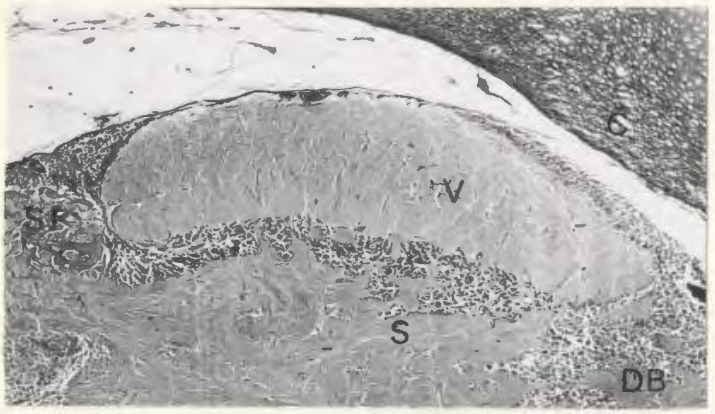
The neuropile of the vertical lobe has a characteristic structure and consists of two parts (Figure 7). The fibres of the outer layer are parallel to the surface of the lobe, whereas in the much larger inner layer the neuropile is a tangled mass. These two regions are separated by the row of cell islands mentioned previously.

Because the fibres are small, their histological differentiation was difficult. The axons form bundles which pass through the parallel neuropile into the inner neuropile. Under high power the inner neuropile is seen as a delicate irregular meshwork. Spindle-shaped bipolar neurons present

Figure 5. Vertical lobe; sag., x21, Mallory triple, spec. 3.

Figure 6. Neurons of vertical lobe; frontal, x200, H and E, spec. 5.

Figure 7. Connection between vertical and superior frontal lobes; frontal, x 35, Fraenkel's orcein, spec. 5.



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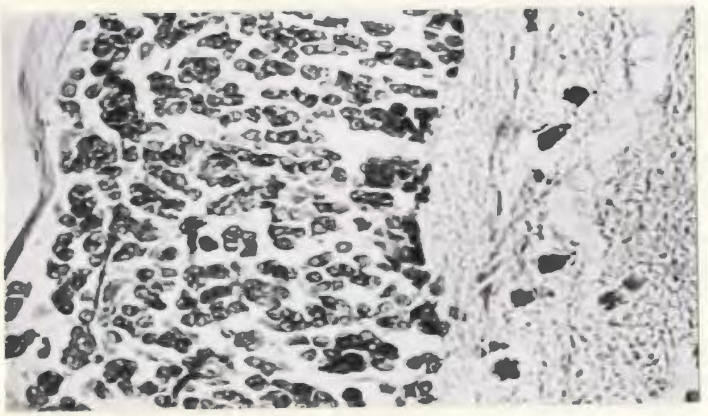

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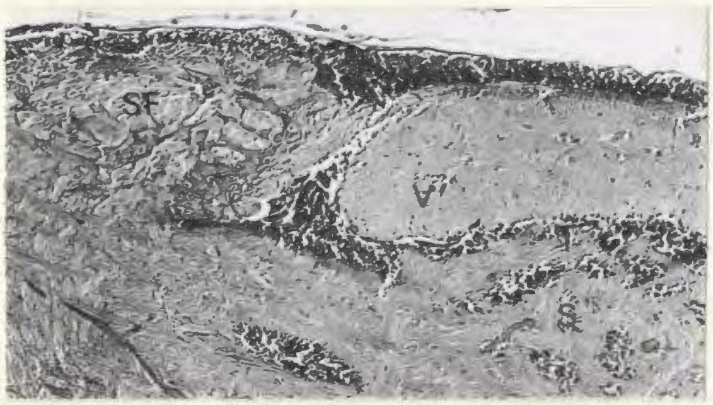

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in the neuropile have their axons oriented dorsally and ventrally, facing the cell rind and the subvertical lobe respectively. The fibres from the cell rind probably anastomose with the multipolar cells in the neuropile, the incoming fibres and each other. The multipolar neurons may also synapse with fibres from, and send fibres to the subvertical lobe.

Some axons leave the cell rind and enter the parallel neuropile. In frontal sections (Figure 7), the parallel neuropile proceeds anteriorly where it almost unites in the midline above the superior frontal-subvertical tract, and throughout the entire area bundles of nerve fibres either enter or leave the superior frontal lobe. Posteriorly a similar situation exists: the fibres proceed laterally, then turn to the inner neuropile, and some may also pass into the subvertical lobe.

Connections

The connections of the vertical lobe are restricted to the higher centres of the brain. It gives rise to no nerves, and its cells, neuropile and connections confer to it an association function.

(1) Connection with superior frontal lobe (Figure 7). The fibres of this paired tract, which stems from the parallel neuropile directed antero-posteriorly, converge at the junction of the vertical and superior frontal lobes where exchange takes

place. The efferent fibres are medial to the afferent.
(2) Connections with subvertical lobe. Numerous tracts of varying sizes and shapes extend through the cell layer separating the vertical and subvertical lobes. Both afferents and efferents are present.

Superior Frontal Lobe

The position of this unpaired and bilaterally symmetrical lobe can be seen in figures 1 and 2. It can be readily distinguished from other lobes because of its characteristic neuropile. The lobe is basically spherical but tapers out laterally where the optic fibres enter. In its central anterior portion there is a division based mainly on the size and shape of the constituent neurons and is surrounded dorsally, ventrally and laterally by the greater part of the lobe. In frontal sections the cell layer of the lateral walls folds in and thus helps to distinguish the medial region.

Cell layer

This is well developed in the anterior region, is thinnest ventrally and almost absent posteriorly. Two types of neurons exist, localized in two distinct regions (Figure 8). The larger neurons of the anterior region are less basophilic with cell diameters up to 10 μ and a nuclear diameter of 4 - 6μ. They are somewhat pear-shaped and have fairly thick tapering axons gathered into bundles. The smaller cells of the lateral region have a nuclear diameter of about 4 μ and a cell diameter

of 4 - 5 μ. They are round and contain little cytoplasm, resembling the amacrines of the optic lobes. Many have a single process which usually separates into two divisions turning in opposite directions. Others have one longer axon with several collaterals.

There are no cell islands in the superior frontal lobe, but some single cells lie in the neuropile just outside the cell rind anteriorly and posteriorly. These include triangular tripolars, spindle-shaped bipolars, pear-shaped monopolars, and neuroglia.

Neuropile

Its central nerve bundles are arranged in a distinctive radial pattern (Figure 9). Many fibres pass along the periphery of the lobe in a circular manner, particularly in the medial part. The thickness of the circular region is not greater than that of the cell rind. Some of the circular fibres originate in the vertical lobe and may be joined by axons from the smaller cells of the superior frontal lobe or send fibres which synapse with the smaller cells. Some circular fibres appear to pass to the subvertical lobe by way of the smaller lateral connectives and also to the vertical lobe.

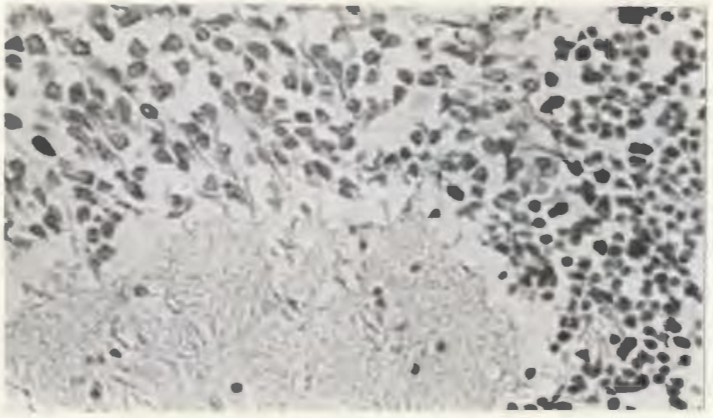
The radial fibres make up the main part of the neuropile in the rest of the lobe; they are also present in the circular region, but in smaller bundles than the circular fibres

Figure 8. Both types of neurons of the superior frontal lobe; frontal, x200, H and E, spec. 5.

Figure 9. Superior frontal lobe showing radial fibres and connection to subvertical lobe; sag., x35, Mallory triple, spec. 3.

Figure 10. Lateral region of superior frontal lobe showing the fibres from the optic lobe; cross, x60, Mallory triple, spec. 15.

Figure 11. Connection between superior frontal and posterior buccal lobes; sag., x60, Bielschowsky, spec. 16.



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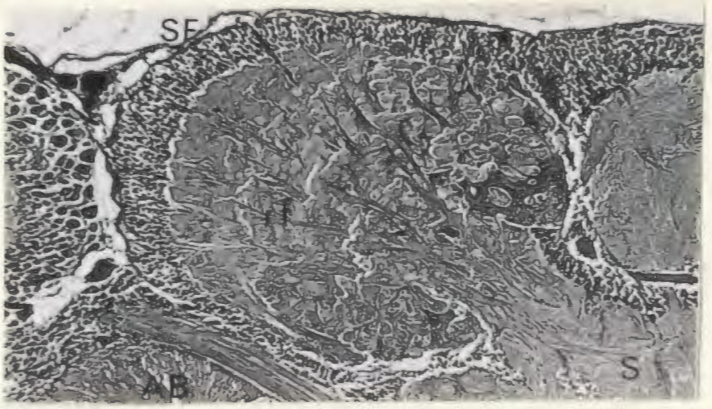

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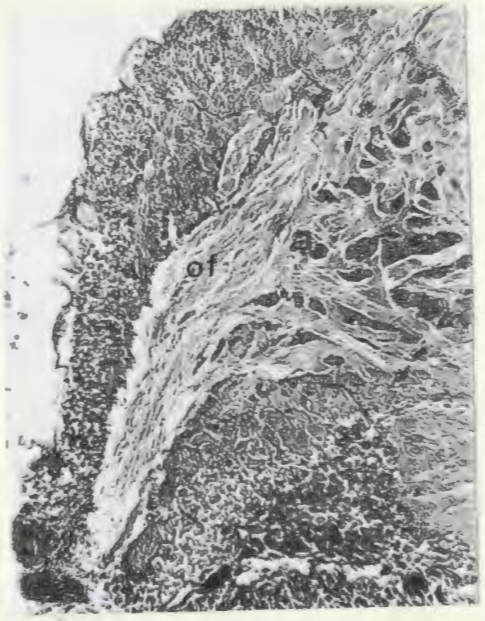

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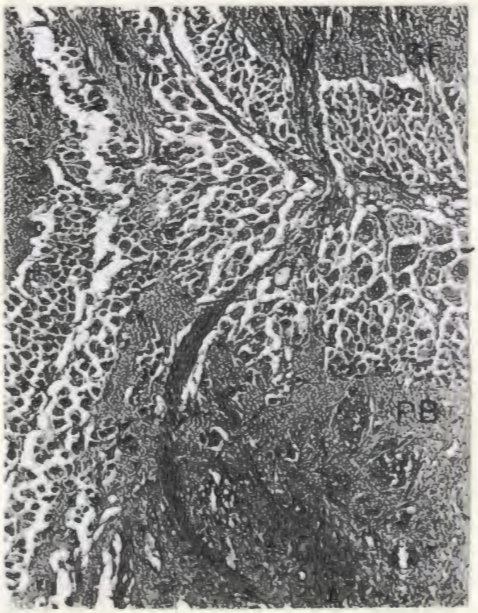

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and therefore are not as prominent. These stem entirely from the larger cells of the anterior surface, whose processes gather into bundles and proceed through the circular layer to converge into larger bundles. Throughout their course they come in contact with fibres from the optic lobe, which intersperse between the radial bundles and probably synapse with them. (Figure 10). The superior frontal lobe may relay information from the optic lobe to the vertical lobe. In frontal sections the bundles of optic fibres enter the lateral areas at all levels and proceed transversely between the radial fibres. The radial fibres enter and leave the vertical lobe lateral and medial to the optic branches.

Connections

The superior frontal lobe has few connections, and gives off no nerves.

(1) Connection with vertical lobe. The paired efferent tract arises from the radial fibres, passes lateral to the large superior frontal subvertical tracts and enters the parallel neuropile of the vertical lobe. Afferents enter the superior frontal lobe lateral to the efferent tracts.

(2) Connection with subvertical lobe. There are three pairs of these in Illex. The largest pair, which is also the largest tract of the lobe, arises from the radial fibres and is subdivided by a wall of cells situated in the anterior part of the subvertical lobe (Figure 7); each half is about 300 - 350 μ in

diameter. The other two much smaller pairs are situated ventro-laterally. The more medial of these is larger. Both originate from the lateral region of the circular fibres and their axons appear to stem from the smaller cells.

(3) Connection with optic lobe. This large paired tract enters the superior frontal laterally and ventrally, and proceeds upwards giving off many bundles which pass through the lobe.

(4) Connections with posterior buccal lobe (Figure 11). Two small tracts were observed on the left side and one on the right. The larger paired connective is about 60 by 30 μ while the smaller one is only 20 μ in diameter. The fibres enter the latero-ventral part of the superior frontal lobe where they diverge and some appear to contact the multipolar cells of the neuropile.

Subvertical Lobe

The subvertical lobe occupies the dorsal part of the posterior basal complex under the vertical lobe (Figure 12). The lobes of the posterior basal complex are not as distinct from each other as are the other lobes of the cerebral ganglion and in many cases share neuropiles making histological differentiation difficult. Ventrally its neuropile is continuous with that of the precommissural lobe. Its common border with the dorsal basal lobe is complicated by the many cell islands in this region so the division must be an arbitrary one: it is

taken as the straight row of cell islands extending from the posterior wall of the cerebral ganglion (Figure 16). At the anterior end there are two small protrusions which extend from the posterior buccal tract into the subvertical lobe. In frontal and sagittal sections the lobe appears oval-shaped. It is unpaired and bilaterally symmetrical; it is partially divided by a tongue of cells extending ventrally from the dorsal cell rind in the anterior region.

Cell layer

The lobe has a well developed cell rind in several places, especially in the two anterior protrusions. The cell layer on the ventral side invaginates and partly separates these protrusions. Posteriorly the invaginated cell layer proceeds more dorsally and breaks off from the ventral region. Dorsally the cell layer is continuous with that of the vertical lobe. The cell islands are larger and contain more cells than those of the vertical lobe, and are symmetrically arranged bilaterally.

The largest cells were in the 25 - 35 μ range with a round nucleus of 8 - 12 μ in diameter containing 1 or 2 nucleoli. These large cells are on the outside of the dorsal cell rind next to the cells of the vertical lobe, but their axons pass into the subvertical. On the inner side of the rind there are numerous cells with large nuclei of 5 - 6 μ. In the cell islands some irregular and bipolar neurons are

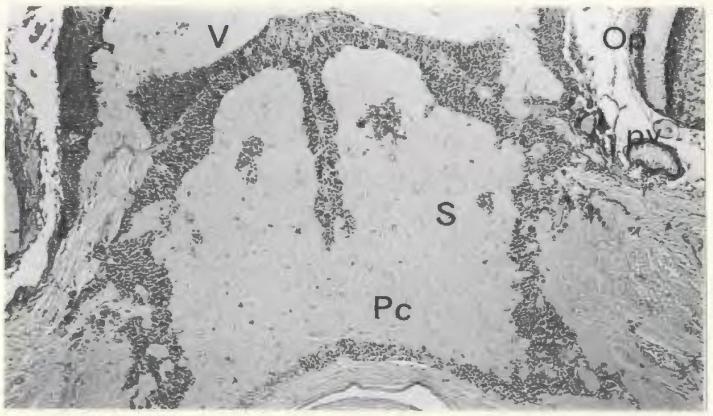


Figure 12. Subvertical lobe; frontal, x21, H and E, spec. 14.

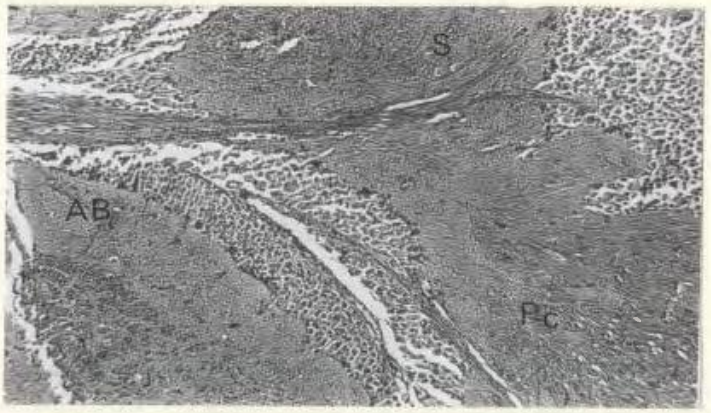


Figure 13. Pathway to subvertical and precommissural lobes from posterior buccal lobe; sag., x60, Bielschowsky, spec. 16.

present, as well as some oval neurons with one thick axon, ranging in size from 7 - 12 μ with a nucleus of 5 - 6 μ.

Neuropile

Numerous fibres pass around the cell islands in all directions but many proceed in an antero-posterior direction. Their features will be discussed below.

Connections

(1) Connections with the vertical lobe (see page 26). After entering the subvertical lobe the fibres separate and weave in all directions around the cell islands in the upper part of the lobe. Most of the fibres here are afferent.

(2) Connections with superior frontal lobe (see page 29). The fibres from the medial tract weave around the cell islands dorsally and ventrally and many reach the posterior end of the lobe while some also appear to enter the cell islands. The fibres of the two lateral pairs spread out at the anterior and are lost in the maze of fibres. It is assumed that all of these tracts are afferent.

(3) Connection with posterior buccal lobe. A paired tract (Figures 13, 22), appearing as two oval pathways, each measuring 120 by 200 μ, enters the subvertical lobe anteriorly into the two anterior protrusions. The fibres proceed posteriorly and dorsally through the neuropile and some pass directly to the cell islands (Figure 13). Many fibres in this connective arise in the brachial ganglion and pass through the posterior

buccal lobe.

(4) Connection with precommissural lobe. Their neuropiles are continuous ventrally (Figure 13) and an extensive exchange of fibres, probably mutual, appears to take place.

(5) Connection with dorsal basal lobe. Their neuropiles are continuous dorsally, between the cell islands (Figure 2).

(6) Connection with optic lobe. The fibres in the tract between these two lobes are efferent (Bullock, 1965). The fibres arise over a wide area in the dorsal region of the lobe and converge ventrally. They probably pass lateral to the precommissural lobe and posterior to the fibres entering the superior frontal lobe.

It is possible that connections exist with the medial basal lobe but this could not be ascertained because of the difficulty in determining the limits of the subvertical and precommissural lobes anteriorly and of the dorsal and medial basal lobes posteriorly.

Precommissural Lobe

The position and shape of this lobe can be seen in figures 1 and 2. It derives its name from its location in front of the ventral optic commissure. It is not large and measures 0.5 mm long and 1.5 mm wide. It is embedded in the cerebral ganglion with its ventral surface directly above and partly lateral to the esophagus. In frontal sections it is oval and indented in the middle. Ventrally it divides,

extending in two halves around the esophagus.

Cell layer

The lobe has an anterior cell wall of about 60 - 80 μ which is separated from the anterior basal lobe. Posteriorly it has a thin irregular cell rind below the level of the ventral optic commissure. The cell types of both layers are similar. The neurons are mostly pear-shaped monopolars whose axons taper into the neuropile, usually in bundles. There are round monopolar cells, similar to those seen in the superior frontal lobe, measuring 4 - 6 μ and also some multipolars. The largest cells observed measured 25 μ, with a nuclear diameter of up to 11 μ. The other cells ranged between these extremes. The smallest cells are much more basophilic than the larger ones. There are no cell islands in the neuropile, only single cells many of which are neuroglia.

Neuropile

The fibres travel in a dorso-ventral direction and also laterally. A thin pathway of fibres penetrates the depth of the lobe near the anterior wall and the bundles of fibres from the anterior surface run posteriorly through it. The cells on the posterior wall send axons anteriorly, also in bundles. In the lower region of the lobe fibres proceed laterally across the lobe and ventrally along the walls of the esophagus enroute to the subesophageal centres.

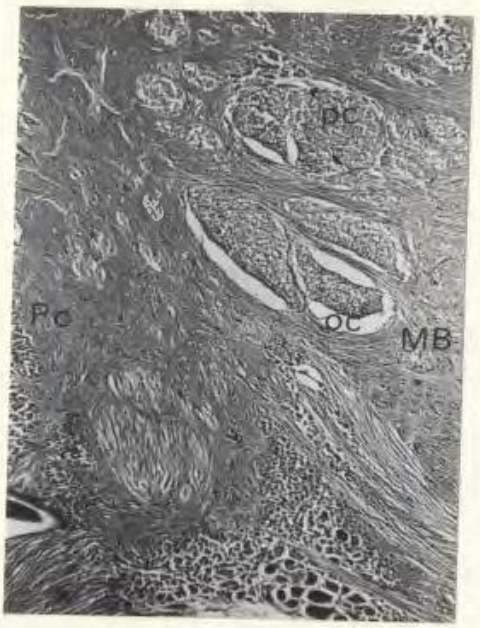


Figure 14. Connections between precommissural and medial basal lobes; sag., x28, Mallory triple, spec. 3.

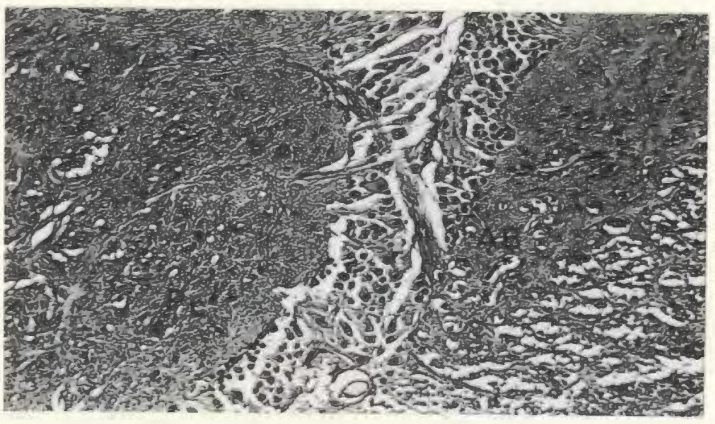


Figure 15. One of the connections between precommissural and anterior basal lobes; sag., x80, Bielschowsky, spec. 16.

Connections

(1) Connections with subvertical lobe, (see page 34).

(2) Connections with medial basal lobe. Fibres are exchanged between these two lobes in several places. The largest pathway is found laterally under the ventral optic commissure (Figure 14). Bundles from the precommissural lobe pass above and through the bundles of the optic and peduncle commissures and divide around the laterally directed optic fibres in the medial basal lobe.

(3) Connection with posterior buccal lobe. This paired tract is a branch of the posterior buccal-subvertical tract. The fibres instead of continuing posteriorly into the subvertical lobe turn ventrally and pass along the anterior periphery of the neuropile in the precommissural lobe (Figure 13). Some of these fibres may originate in the brachial ganglion.

(4) Connections with anterior basal lobe. These tracts are usually paired (not more than four pairs) and small. Most of the fibres appear to be efferent and leave the anterior surface of the precommissural lobe laterally (Figure 15).

(5) Connection with optic lobe. The paired bundle originates in the ventral portion of the optic lobe below the esophagus and enters the precommissural lobe latero-ventrally. Here they separate into three or four large bundles and several smaller ones on each side, and proceed laterally across the midline.

(6) Connection with lateral pedal lobe. A large pair extends laterally along the esophagus to the lateral pedal lobes. It is the major efferent pathway of the lobe, but afferents may be present as well.

Dorsal Basal Lobe

The position and shape of this lobe can be seen in figures 1, 2, and 16. Its junction with medial basal lobe, which lies ventral to it is not distinct. The division was made arbitrarily at the most ventral cluster of cell islands just above the level of the ventral optic commissure. Across from this, along a straight line, the cell rind varies in thickness. Dorso-laterally the lobe communicates with the olfactory lobe.

Cell layer

Posteriorly and laterally the lobe is covered by a thick cell rind relatively uniform at 300 μ on its posterior surface but a little less laterally. A prominent feature of the lobe is its several large cell islands located in the upper posterior region, although not confined there. There are no large neurons in this lobe. In the cell islands the largest measure 30 μ with a nucleus of 13 μ; others are in the 15 μ range with a nuclear diameter of 6 - 7 μ, and the smallest are 4 μ in cell diameter. Both monopolar and multipolar cells are present. In the cell rind the largest neuron was 20 μ, with a nuclear diameter of 8 μ and the smallest had a nuclear

diameter of 4 μ. The medium-sized pear-shaped cells in the centre of the rind send axons into the neuropile in large bundles.

Neuropile

The large bundles from the posterior cell wall pass through the neuropile anteriorly along an irregular course and some continue ventrally into the medial basal lobe near the ventral optic commissure. Many fibres also cross the lobe laterally in small or large bundles and in sagittal sections the axons of the cell islands weave around these fibres. The neuropile resembles that of the subverticallobe, but most of its fibres run anteriorly or laterally.

Connections

(1) Optic gland nerve. This small nerve is the only one originating from this lobe. Its fibres pass along the side of the olfactory lobe, surrounded by much connective tissue.
(2) Connection with optic lobe. The axons of this tract pass through the dorsal basal and cross in the midline. They separate into many small tracts which extend posteriorly and anteriorly around rows of cell islands mainly in the central or lower part of the lobe. The bundles from the posterior cell rind interdigitate with them anteriorly.
(3) Connection with olfactory lobe. A bundle of fibres from the posterior region of the olfactory lobe continues into the dorsal basal lobe where it divides, sending one branch

along the side of the lobe, while the other passes laterally and is crossed by bundles from the posterior surface. Dorsal to this their neuropiles are continuous. In Octopus (Messenger, 1967) exchange occurs both ways,and afferents and efferents are believed to be present in Illex.

(4) Connection with the subvertical lobe (see page 34).

(5) Connection with medial basal lobe. Their neuropiles are continuous over a wide area ventrally and a considerable exchange of fibres appears to take place (Figure 16).

(6) Connection with peduncle lobe. Their neuropiles are continuous over a narrow area. It is difficult to determine the extent of this connection because of the lack of differentiation exhibited by the lobes in this region.

(7) Connection with magnocellular lobe. A tract to the magnocellular lobe may exist. It was not detected in frontal or cross sections, but in sagittal sections a connection may be present laterally, complicated by the lack of definition of the lobes.

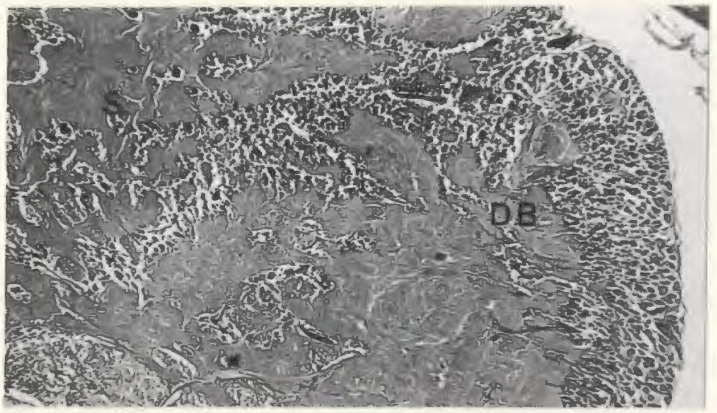
Medial Basal Lobe

This is located in the ventral and posterior end of the posterior basal complex. It is difficult to distinguish from the lateral basal, dorsal basal and the lateral pedal lobes, all of which have confluent neuropiles with the medial basal.

Figure 16. Dorsal basal lobe; sag., x28, Mallory triple, spec. 3.

Figure 17. Cells of medial basal lobe; frontal, x80, Fraenkel's orcein, spec. 5.

Figure 18. Lateral basal and part of the medial basal lobe; frontal, x28, Mallory triple, spec. 5.



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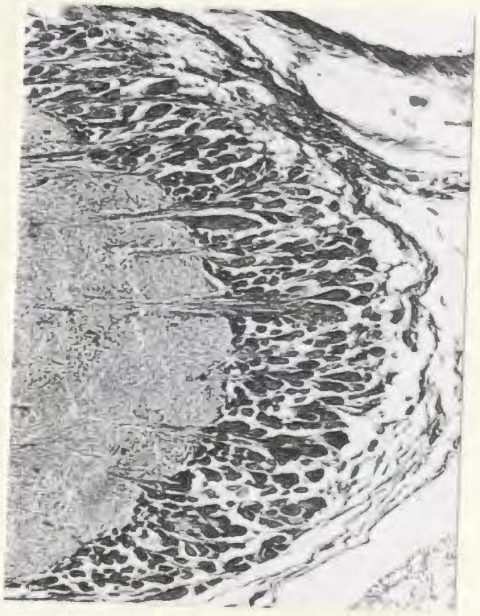

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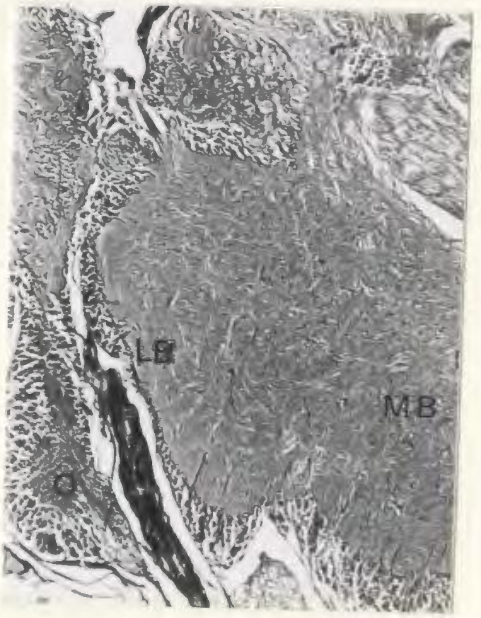

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Cell layer

Posteriorly the lobe is covered by a thick cell rind which is continuous dorsally with that of the dorsal basal lobe. The lobe is indented medially at the level of the esophagus. The cell rind varies between 175 - 250 μ and is thickest at its posterior extremity. The range in the size of neurons is greater than in the dorsal basal lobe. The largest cell measured was 55 μ in diameter although the majority of the larger cells did not exceed 25 μ. Close to the neuropile the cells range from 5 to 15 μ, their nuclei from 4 to 7 μ. Most cells are pear-shaped monopolars and send their axons into the neuropile in large bundles (Figure 17). There are also single cells scattered at random throughout the neuropile, most of which are neuroglia.

Neuropile

The structure observed here is similar to that of the dorsal basal lobe. The axons from the cell rind pass anteriorly in bundles and many fibres continue laterally especially in the medial ventral region above the esophagus, but beyond this no clear differentiation could be made. Many of the fibres in the centre of the lobe are efferents enroute to the subesophageal centres.

Connections

(1) Connections with precommissural lobe (see also page 37). Fibres passing above the ventral optic commissure could be followed nearly to the posterior cell wall and are believed to

be efferents.

(2) Connection with dorsal basal lobe (see page 40).

(3) Connection with lateral basal lobe. Their neuropiles are continuous and difficult to separate laterally (Figure 18). Considerable exchange appears to take place.

(4) Connection with optic lobe. This large paired tract enters the medial basal at its most anterior point under the peduncle commissure, separating into smaller branches, some of which enter the lateral neuropile, but the majority appear to continue along the centre through the thickness of the lobe. This is particularly noticeable in the lower region above the esophagus where the efferents from the medial basal leave for the pedal lobes and the optic fibres probably anastomose with them.

(5) Connection with peduncle lobe. This paired tract originates in the centre of the peduncle lobe and enters the medial basal where the latter merges with the lateral basal and continues posteriorly in the lobe.

(6) Connection with lateral pedal lobe. This large paired pathway passes laterally along the walls of the esophagus. The tract to the lateral pedal lobe is more anterior and medial than that to the posterior pedal. Efferents may also be present.

(7) Connection with posterior pedal lobe. This paired connective adjoins the tract to the lateral pedal lobe in the region of the connective to the magnocellular lobe and also stems from lateral fibres.

(8) Connection with brachial ganglion. This is part of the brachio-pedal connective which courses through the anterior and lateral pedal lobes in a well defined bundle before turning dorsally into the posterior basal complex. The fate of the fibres in the medial basal lobe could not be determined.
(9) Connection with magnocellular lobe. This is also paired, with one branch on each side of the esophagus, leading to the dorsal part of the magnocellular lobe where its fibres disperse. It is medial and ventral to the lateral basal-posterior chromatophore connective and is larger.

Lateral Basal Lobe

The position and shape of this lobe can be seen in figures 3 and 18. Its lateral surface is flanked by the peduncle complex. The lobe is paired and each part extends along the esophagus ventro-laterally on the outside of the cerebral ganglion.

Cell layer

This exists only on the lateral and dorsal side. The cell layer ranges from 70 - 175 μ and is thickest at the postero-ventral corner. Its appearance is identical with that of the medial basal lobe although it contains fewer large cells; the largest observed was 36 μ. There are also some cells, mostly neuroglia, scattered throughout the neuropile as in the medial basal lobe.

Neuropile

The neuropile of this lobe is a continuation of that in the medial basal lobe laterally with some differences. In the dorsal area the fibres are more tightly packed and fewer run in a dorso-ventral direction. Posteriorly the axons enter the neuropile in bundles and can be traced for a considerable distance in the ventral half as they show up more clearly than in the other lobes of the posterior basal complex.

Connections

(1) Connection with optic lobe. Many fibres enter the basal lobes from the optic and most appear to pass to the medial and anterior basal lobes. Associated with these are fibres to the lateral basal but these are quickly lost in the maze.

(2).Connection with peduncle lobe. This paired tract originates from two areas in the peduncle lobe. The fibres enter the anterior region of the lateral basal where they become lost. Many fibres from the posterior wall of the lobe enter the anterior part and may anastomose with them.

(3) Connection with medial basal lobe (see page 43).

(4) Connection with posterior chromatophore lobe. It is difficult to find the exact origin of this connective which starts in the medial area of the lobe and passes posteriorly above the magnocellular lobe (see page 97).

(5) Connection with anterior chromatophore lobe. This connective

arises from the medial area of the lobe and appears to gather fibres from a wide region. It continues anteriorly through the cell layer of the lateral pedal lobe and into the postero-lateral region of the dorsal anterior chromatophore lobe, where its fibres disperse through the lobe. It is not known if a separate branch serves the ventral half of the anterior chromatophore lobe, or if these fibres spread to that region.

Anterior Basal Lobe

This lobe is situated below the superior frontal and anterior to the precommissural lobe (Figures 1 and 2) and has a complex structure. Its specialized features are best observed in frontal sections which show it to be rectilinear in shape, but wider posteriorly (Figures 20 and 21). Two rows of cell islands extend from a single row on the dorsal surface, then turn posteriorly conforming to the shape of the lobe (Figure 19).

Cell layer

This is best developed in the anterior part of the lobe reaching 150 - 250 μ in thickness. The largest neurons are found in the anterior region ventrally (Figure 21). Cells up to 35 μ with a nucleus of 10 - 12 μ were seen but the majority are between 10 and 25 μ in diameter. The cells are pear-shaped with a round or slightly oval nucleus and send large axons into the neuropile in bundles. Laterally in the anterior half of the lobe the cells are round, about 5 - 7 μ in diameter, and have

little cytoplasm but as this layer continues ventrally, larger cells between 10 and 20 μ are seen. Posteriorly the cell layer is separated from that of the precommissural lobe, but resembles it in cell types and thickness. Many single cells of varied sizes, mostly neuroglia, are scattered throughout the neuropile, but multipolar triangular cells are also observed. The small round cells have one thin axon which gives rise to several collaterals. The cells in the two rows of cell islands are small, increase in size from dorsal to ventral, and resemble those of the lateral surface in size and shape.

Neuropile

In sagittal sections numerous fibres, particularly optic, pass laterally in the lobe and are arranged in two rows. Both lie in the anterior with the posterior row in front of the first row of cell islands, and the anterior just outside the anterior intrusion of cells. The cells on the anterior surface send axons beyond the lateral bundles and some could be followed to the second row of fibres; these fibres then turn ventrolaterally and leave the lobe. More ventrally some cells send axons under the first row of fibres where they join a pathway originating higher in the lobe, and then continue along the antero-ventral periphery of the neuropile. In the posterior dorsal end no clear pattern could be seen. In other planes the majority of the fibres also are directed laterally. The anterior row of fibres, probably originating in the optic lobes, enters laterally and proceeds across the midline and out of the lobe.

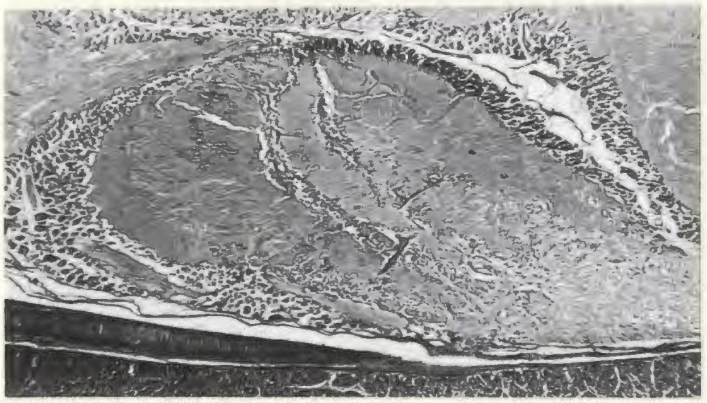
Figure 19. Anterior basal lobe showing the two rows of cell islands; sag., x28, Mallory triple, spec. 3.

Figure 20. Anterior basal lobe (dorsal region); frontal, x 28, Fraenkel's orcein, spec. 5.

Figure 21. Anterior basal lobe (ventral region); frontal, x 21, Heidenhain's Iron-Hemotoxylin, spec. 5.

Figure 22. Anterior basal lobe showing optic fibres passing through lobe; cross, x21, Mallory triple, spec. 5.

Figure 23. Connection between anterior basal and anterior and lateral pedal lobes; cross, x35, H and E, spec. 15.



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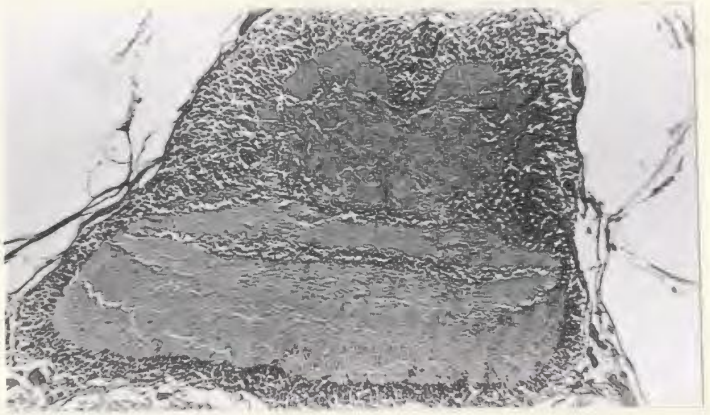

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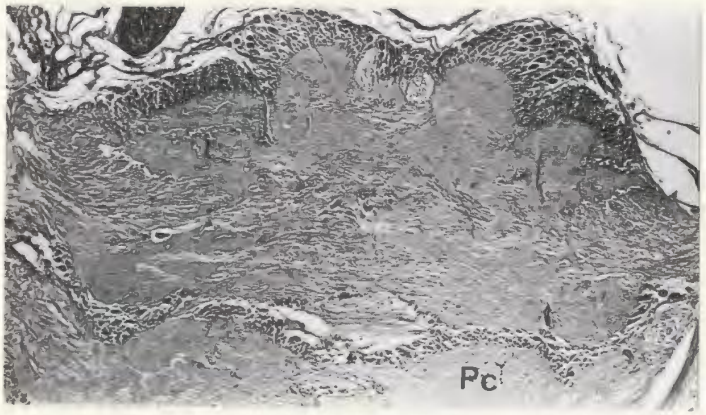

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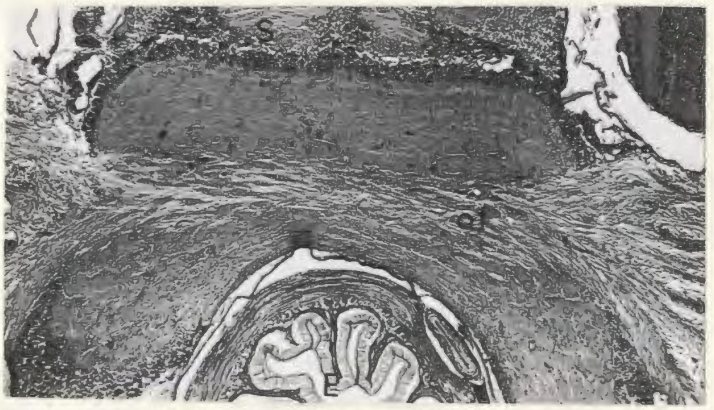

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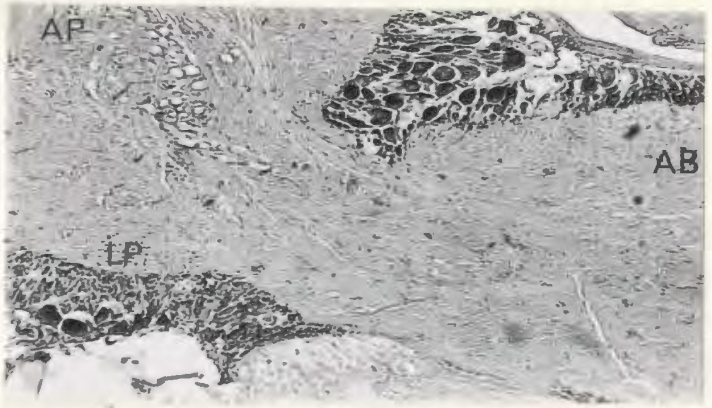

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Connections

(1) Connections with optic lobe (Figure 22). The exchange between these two lobes occurs laterally. There are two main paired branches, one anterior and the other posterior. The anterior pair courses along the lateral anterior wall, but its ultimate destination could not be determined. Axons from the large neurons on the anterior surface cross in the midline and then pass to the optic lobes.

(2) Connections with precommissural lobe (see page 37). These tracts enter postero-laterally and divide in the neuropile behind the optic fibres.

(3) Connection with lateral and anterior pedal lobes. This tract proceeds laterally in the postero-ventral part of the lobe across the midline in front of the precommissural-lateral pedal tract and joins it (Figure 23). After circling the esophagus the tract either continues ventrally into the lateral pedal lobe or turns into the anterior pedal lobe. This second pathway is better defined and circumflects other pathways in the lobe. The anterior pedal connective is more medial and the fibres of the lateral pedal connective more posterior. It is assumed there are afferents as well as efferents in these pathways.

Posterior Buccal Lobe

The posterior buccal is situated at the anterior end of the cerebral ganglion (Figures 1 and 2). It is spherical and

measures about 1 mm in diameter (Figures 24 and 25). The lobe appears unpaired and bilaterally symmetrical.

Cell layer

The cell rind is thickest dorsally (175 μ) and at the posterior ventral corner (250 μ), and thinnest laterally (100 μ). Its neurons are the largest in the cerebral ganglion with a cell diameter of up to 50 μ and a nuclear diameter of 18 μ. The nuclei may be round or oval and contain one nucleolus measuring 2 - 4 μ. The largest cells are located at the periphery of the cell rind. As they approach the interior they gradually get smaller until in the innermost layer they measure 5 - 7 μ. The large cells have single axons which either form small bundles or proceed singly between the small cells into the neuropile. Bundles of axons are not a feature of the lobe. The ventral surface has more small cells than the other regions. Among the smaller neurons two types of axons are present. Some are very thin and barely visible, while others have larger axons tapering out from the cell body. Many other small cells may be neuroglia. Some small cell islands are present in the neuropile.

Neuropile

Several pathways lead into, out of, and through the posterior buccal lobe. These can be followed but not as clearly as those of the subesophageal centres. Numerous small bundles pass in the lobe arranged in no apparent order, surrounding the fibres which enter or pass through the lobe. The majority

course in an antero-posterior direction but pass laterally as well, especially in the dorsal region.

Connections

(1) Connection with the superior buccal ganglion. This slender paired tract, corresponding to the cerebro-buccal connective, is flattened dorso-ventrally above the esophagus, but becomes circular as it approaches the posterior buccal lobe, which it enters in its dorso-anterior region. From here the fibres converge to the centre of the lobe and in the neuropile anastomose with bundles which surround them. Axons probably pass both ways in this tract.

(2) Connection with brachial ganglion. This loops around the esophagus and enters the posterior buccal lobe antero-ventrally, but a little more laterally than the preceeding connective. Many of the axons do not end here but pass through to the subvertical and precommissural lobes (Figure 24). Efferent fibres may be present.

(3) Connection with subvertical lobe (see page 33). Afferents may be present here as well.

(4) Connection with precommissural lobe (see page 37). Some afferents may be present.

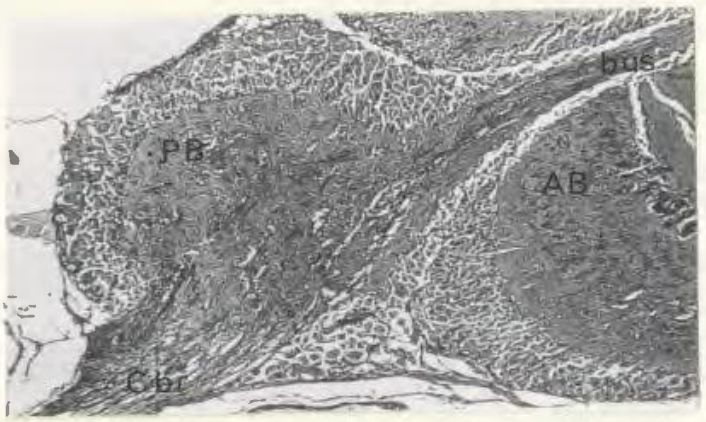
(5) Connection with superior frontal lobe (see page 30).

(6) The other major connective of this lobe is paired and leaves the lobe postero-ventrally to enter the cell layer of the anterior basal lobe (Figures 21, 26). Here the connectives are

Figure 24. Posterior buccal lobe showing connections from brachial and to subvertical lobe; sag., x35, Bielschowsky, spec. 16.

Figure 25. Posterior buccal lobe; cross, x28, Mallory triple, spec. 12.

Figure 26. Posterior buccal lobe showing connective passing through the cell layer of the anterior basal lobe and connection to subvertical; sag., x60, Bielschowsky, spec. 16.



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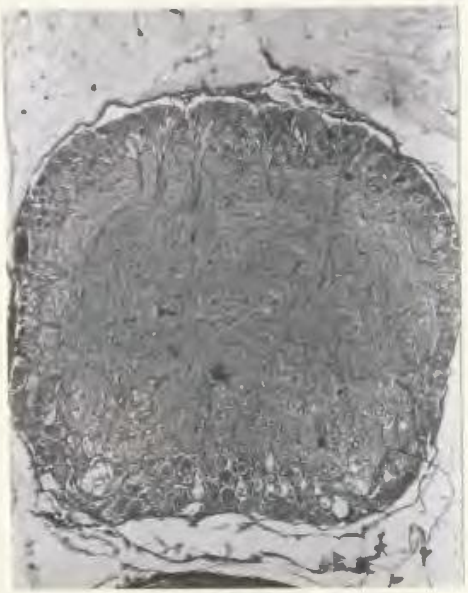

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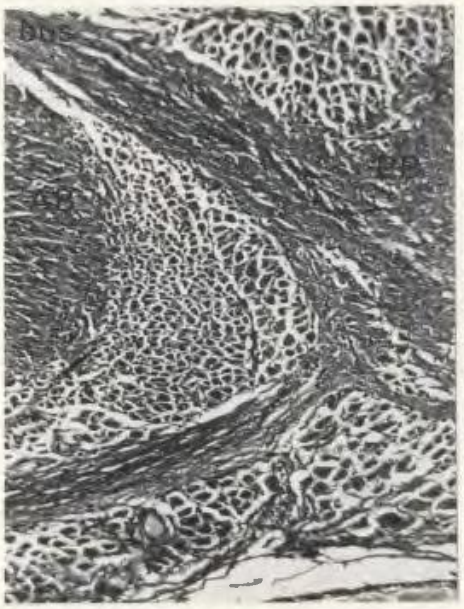

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close together, oval-shaped and measure 140 by 90 μ in diameter. After passing through the anterior basal lobe and turning posteriorly under the cerebral ganglion they were traced only to the level of the ventral optic commissure. It appears that it contains a considerable number of efferent fibres and that it may also receive fibres from the brachio-posterior buccal connective.

Superior Buccal Ganglion

This ganglion is not a part of the central brain mass. It is dorsal to the esophagus and extends around it laterally; its position in relation to the rest of the brain can be seen in figure 1. It is flattened dorso-ventrally and on this axis is only 250 - 350 μ thick. The ganglion appears unpaired but is bilaterally symmetrical, although Richter (1913) reported that it is paired in Illex coindeti. The ganglion is not subdivided into lobes and is surrounded by a thin covering of connective tissue.

Cell Layer

The cell rind is thin, but thicker on the dorsal than on the ventral side (Figure 27). On the dorsal surface it has three thickenings, one medial and two lateral, measuring 80 μ, separated by two shallow depressions of 40 μ. The ventral rind is usually about 30 - 40 μ thick. The motor neurons at the periphery are large and become progressively smaller inwardly, giving the ganglion the appearance of a typical motor lobe. In places where the cell rind is very thin there may be a single large motor neuron, with two or three very small neurons at the junction of the neuropile and cell rind. The greatest proportion

of the large cells is at the posterior end where the cell rind is thickest. The neurons have a diameter of 40 - 60 μ and a nuclear diameter of up to 20 μ. Their nuclei are round and contain one or two nucleoli. These cells send large axons into the neuropile, singly or in small bundles. The smallest cells on the inside of the cell rind have a diameter of 5 - 7 μ. Some are oval, like those on the anterior surface of the superior frontal lobe, while others have several processes. Some of them are probably neuroglia.

Neuropile

The neuropile of the superior buccal ganglion has no recognizable or unique features. Many small bundles travel laterally across the midline. Other tracts, resulting mostly from nerves or connectives, also proceed in an antero-posterior direction. The fibres of the labial nerves and interbuccal connective are confined to the anterior half of the lobe, while those of the brachial and cerebral connectives are in the posterior end.

Connections

(1) Labial nerves (Figure 28). At least 20 nerves leave the ganglion anteriorly and form a semi-circle around the esophagus. They are all surrounded by a continuous sheet of collagenous connective tissue. The axons of these nerves were traced into the centre of the ganglion. Some axons were also seen leaving the cells on the anterior surface of the ganglion and passing into the labial nerves. Both afferents and efferents are present.

(2) Interbuccal connective (Figure 29). This arises in the

Figure 27. Superior buccal ganglion; cross, x21, H and E, spec. 15.

Figure 28. Anterior part of superior buccal ganglion showing labial nerves; sag., x100, Bielschowsky, spec. 16.

Figure 29. Interbuccal connective; cross, x35, PAS, spec. 15.

Figure 30. Esophagus, with associated nerves; cross, x21, PAS, spec. 12.

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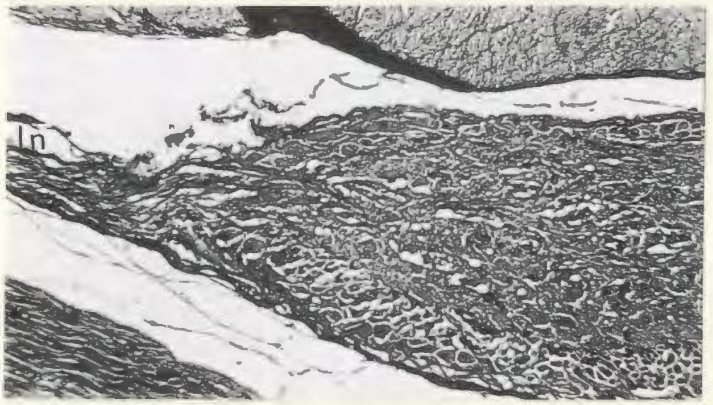

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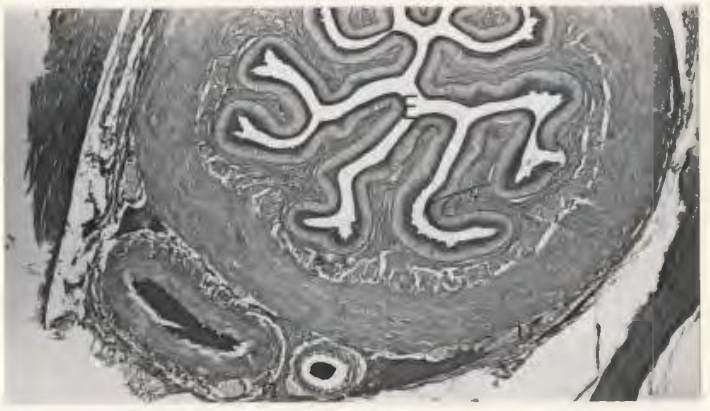

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antero-lateral region, and proceeds ventro-laterally around the esophagus into the inferior buccal ganglion. Axons could be followed for a considerable distance within the ganglion.
(3) Connection with brachial ganglion (see page 65). Each branch of this large paired connective leaves the posterior dorsal end of the brachial ganglion to join the postero-lateral face of the superior buccal. The axons proceed in a postero-anterior direction in the ventral portion of the neuropile, and for a short distance together with the superior buccal - posterior buccal connective.
(4) Connection with posterior buccal lobe (see page 51). This paired connective is much smaller than the brachial - superior buccal connective and enters the ganglion a little more medially and dorsally.
(5) Nerves passing posteriorly along the wall of the esophagus (Figure 30) may correspond to those described in Octopus which arise from the superior buccal ganglion (Young, 1965a) but these were not followed to their origins or terminations.

Inferior Buccal Ganglion

This ganglion is also separated from the central brain mass and lies anterior to it directly below the esophagus under the superior buccal ganglion, to which it is joined by the inter-buccal connectives around the esophagus (Figure 1). In Illex it is considerably larger than the superior buccal ganglion.

Its maximum dimension is along the antero-posterior axis, as in the superior buccal, but its shape is different; it is thicker dorso-ventrally and does not extend as far laterally. The lobe is about 600 μ thick with a crevasse in the ventral cell layer (Figure 31) about 125 μ deep, occupied by the posterior salivary gland duct.

Associated with this ganglion is a structure identified in Octopus as the juxta-ganglionic tissue (Cazal and Bogoraze, 1944; Young, 1965a), which covers its surface posteriorly, laterally and around nerve roots. The two are separated by a thick layer of connective tissue in places (Figure 32), but some cells also lie inside this layer. This tissue has no distinct neuropile but pathways of nerve fibres penetrate between the cells.

Cell layer

It is not thick and in places difficult to separate from the juxta-ganglionic region. Dorsally its thickness is uniform at 100 - 125 μ and ventrally it varies between 50 and 200 μ. The cells are large at the periphery and are smaller near the neuropile, particularly on the dorsal surface, varying in size from 5 - 60 μ but averaging 20 - 30 μ. The larger cells with nuclei of up to 20 μ are located mainly in the anterior, but the medium-sized neurons (10 - 20 μ) found in the basal lobes are rare here. There are some multipolar cells in the neuropile. Small bundles of fibres pass to or from the neuropile separating the cells of the rind. A large blood vessel passes through the

posterior part of this region (Figure 33), giving off branches particularly in the juxta-ganglionic tissue.

The cells of the juxta-ganglionic tissue are usually small bipolars and tripolars (5 - 10 μ), and their cytoplasm is more basophilic than of those in the ganglion. Their axons penetrate between the cells, which are also surrounded by irregular bundles of nerve fibres.

Neuropile

The neuropile of the ganglion contains some nerve bundles which correspond to the roots of its nerves and connectives. In sagittal sections most of the fibres are directed antero-posteriorly.

Nerves and Connections

The nomenclature followed here is that of Richter (1913).

(1) Lingual nerves. These arise from the central anterior region where the lobe is indented from a single bundle which separates into five nerves. Their fibres are of the anterior cells and pass along the periphery of the neuropile, while others come from the centre of the lobe and may be directly from the interbuccal connective. The nerves continue between the two anterior salivary glands. One probably innervates this gland; the others were not followed to their destination.

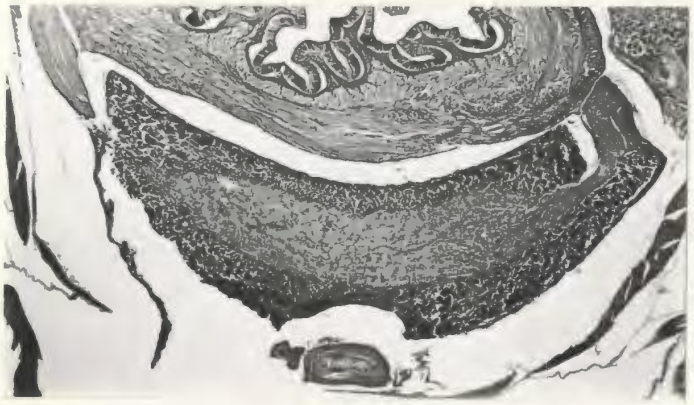
(2) The two mandibular nerves arise anteriorly lateral to the lingual nerves. Each was followed dorsally and laterally to the anterior end of the salivary gland on each side where it branches.

Figure 31. Inferior buccal ganglion showing indentation in ventral cell layer; cross, x21, PAS, spec. 15.

Figure 32. Inferior buccal ganglion: posterior region showing part of juxta-ganglion tissue, sag., x60, Mallory triple, spec. 3.

Figure 33. Juxta-ganglionic tissue; frontal, x60, Heidenhain's Iron-Hematoxylin, spec. 5.

Figure 34. Inferior buccal ganglion: anterior region showing roots for maxillary nerve; sag., x60, Gomori's chrom-hematoxylin, spec. 3.



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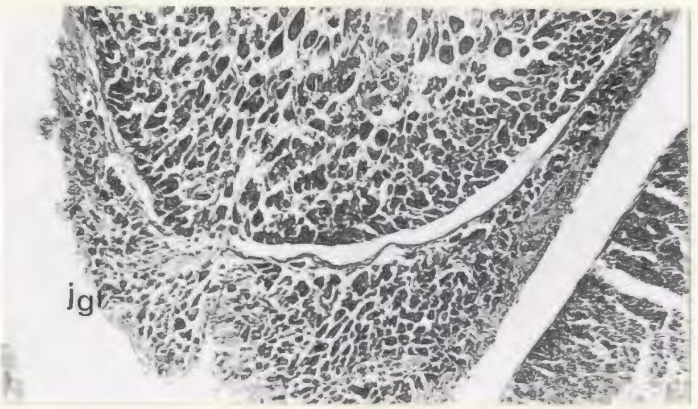

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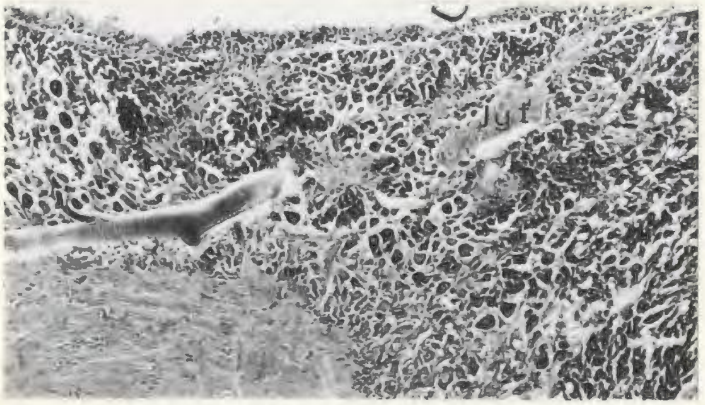

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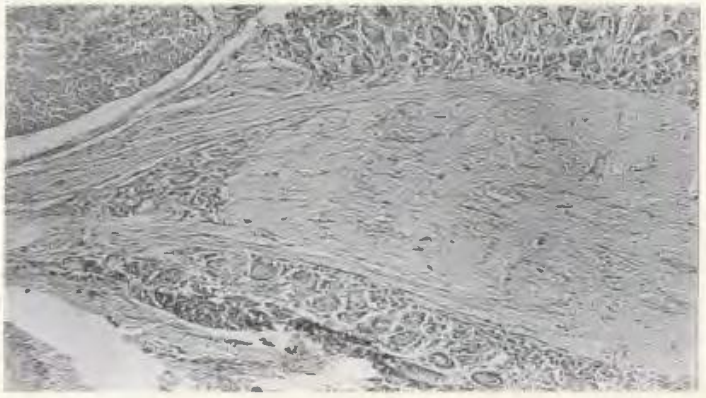

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(3) The maxillary nerve is also anterior and paired, but dorsal and somewhat lateral to the mandibular nerve. It remains on the ventral side of the buccal mass, whose musculature is penetrated by its branches.

(4) The interbuccal connective (see also page 54). The two branches from the superior buccal entering the ganglion in its posterior dorsal region are its only connection with the brain. The axons then continue anteriorly where some appear in the centre of the neuropile, while others pass into the maxillary nerve.

(5) Sympathetic nerve (Figure 39). This nerve arises from the posterior border of the ganglion, a little left of the midline, and passes posteriorly. Upon leaving the cell layer it is divided into two unequal branches which leave the ganglion together. The smaller, more dorsal branch is about 40 μ in diameter, while the larger is oval and measures 175 by 75 μ. Many of the fibres in this nerve are derived from the anterior neuropile which passes posteriorly through the ganglion. Their axons innervate the muscles of the esophagus and crop, and afferents as well as efferents are present (Young, 1965a).

Anterior Subesophageal Mass (Brachial Ganglion)

It lies anterior to the central brain ganglia and directly under the inferior buccal ganglion (Figures 1 and 2). The structural pattern of the ganglion can be best seen in cross sections (Figures 36, 37, 38, 39 and 40) which show it to be

unpaired and bilaterally symmetrical. In Sepia it is an intermediate motor centre (Boycott, 1961). It contains numerous well defined pathways and root bundles, some of which pass directly through it. Its shape and relative size in sagittal sections can be seen in figures 2 and 35. It is joined to the middle subesophageal mass by the large brachio-pedal connective.

Cell Layer

The cell layer is bilaterally symmetrical, does not form a complete covering around the lobe, and its extent is principally determined by the root bundles and pathways present. The neurons are large and stratified typically with large cells at the periphery and smaller ones inside. They range from 5 to 70 μ in diameter, although a few over 100 μ were seen, with nuclei up to 30 μ. There is little difference in the composition of the cell layer in the anterior and posterior regions. There are no large cell islands in the neuropile but numerous single cells are present.

Neuropile and Root Bundles

The neuropile is composed primarily of root bundles, particularly in the peripheral areas, and pathways which pass through the lobe (Figures 36, 37, 38, 39, 40 and 41). Axons from the larger cells of the rind penetrate between and within the root bundles. It is assumed that these are the primary source of nerves which leave the ganglion. In the centre the fibres are generally smaller and the neuropile is more tightly packed, probably because the axons originate from

the smaller cells of the lobe or from the single cells in the neuropile of which many are neurons, and anastomose with the axons entering the lobe.

The roots will be numbered to facilitate identification. Roots 1, 2 and 3 pass into the ventral region of the ganglion and the paired roots, 4 and 5, through the neuropile dorsally (Figure 40). Root 6 is paired and consists of three branches which continue above the brachial ganglion (Figures 39, 40) while root 7 is also paired and divided into four branches which pass along the ventral surface of the ganglion (Figures 39, 40). Root 8 also consists of four pairs of bundles, but originates within the neuropile of the brachial ganglion (Figures 37, 38).

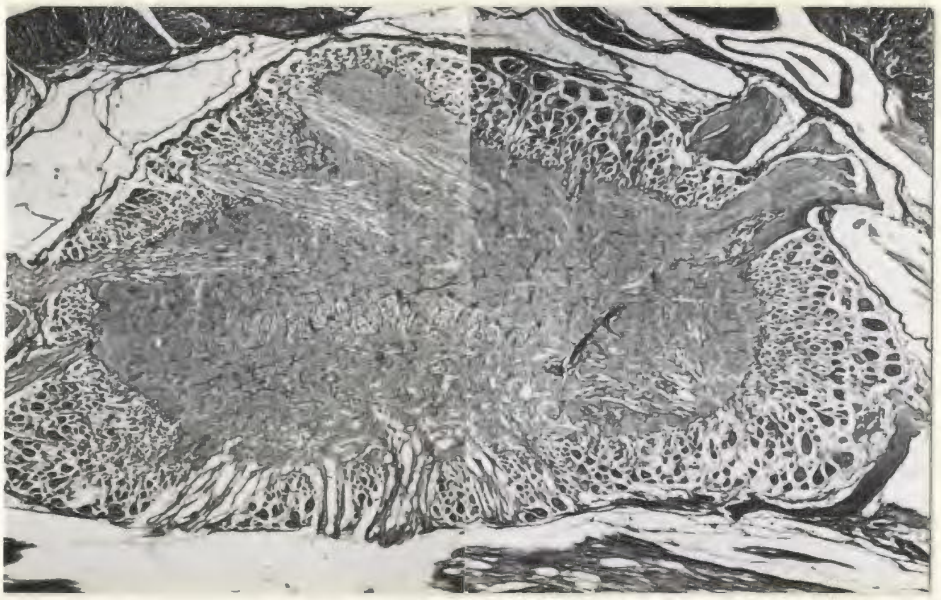
Root 1 (Figures 40, 41) is oval, unpaired, 180 by 475 μ and does not extend far into the ganglion. It divides into two or three smaller bundles: the dorsal one mixes with fibres from the lateral bundles, while the ventral one remains distinct for a longer distance. In this area there is much intermingling of root bundles. Further posteriorly the ventral part of the root becomes enclosed by the lateral bundles and lies in the centre of the brachio-pedal connective. This is the only bundle that can be traced back to the anterior pedal lobe.

Root 2 is paired, larger, and more round than root 1 (Figure 40). It measures 350 by 475 μ and is separated from root 1 by a layer of cells. Further anteriorly its roots are not distinct where it merges with the neuropile and the fibres of root 1, although smaller portions extend beyond it. The origin of these two roots is basically in the same area.

Figure 35. Brachial ganglion; sag., x21, Mallory triple, spec. 3.

Figure 36. Brachial ganglion: anterior region showing root bundles giving rise to the brachial nerves; cross, x21, Mallory triple, spec. 15.

Figure 37. Brachial ganglion, a little more posteriorly; cross, x21, Mallory triple,spec. 15.



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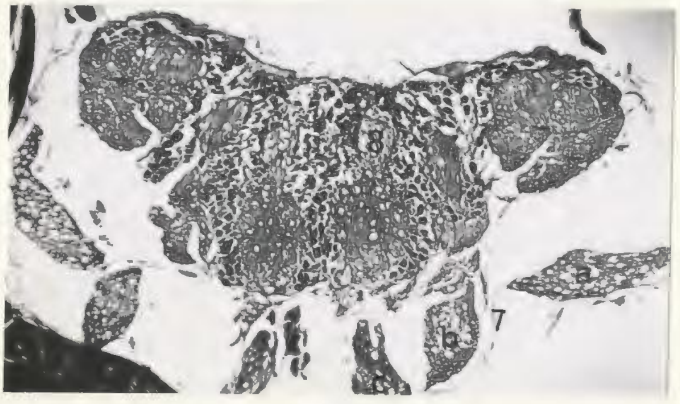

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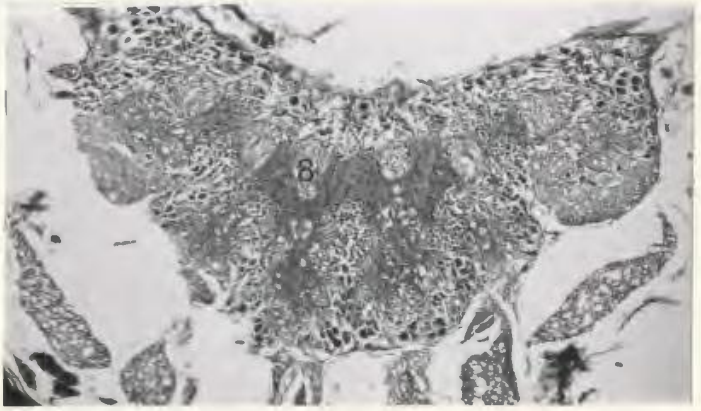

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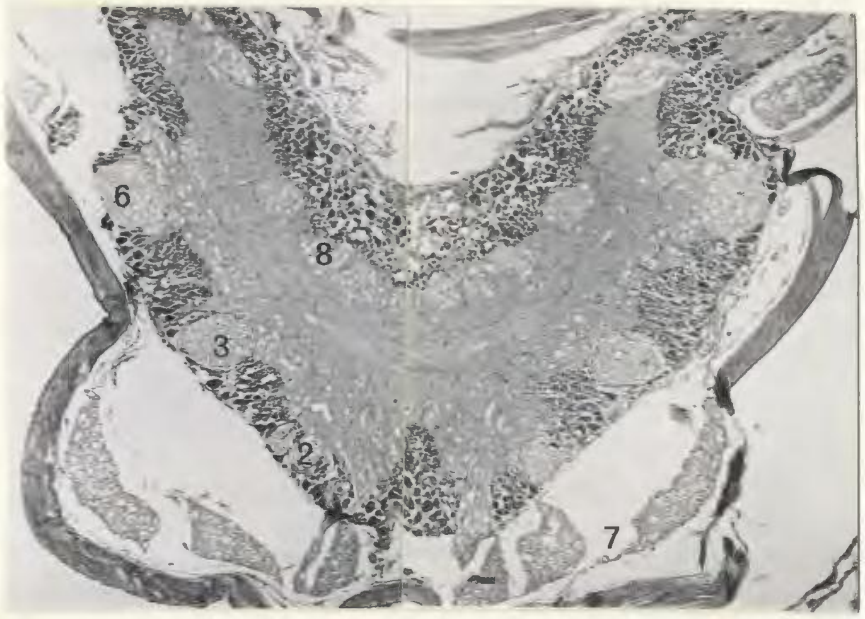
Figure 38. Brachial ganglion showing root bundle 8 in dorsal part of ganglion; cross, x21, H and E, spec. 15.

Figure 39. Brachial ganglion; cross, x21, Masson's trichrome, spec. 15.

Figure 40. Brachial ganglion in posterior region showing root buncles; cross, x21, PAS, spec. 15.

Figure 41. Brachio-pedal connective, directly posterior to brachial ganglion; cross, x21, Mallory triple, spec. 15.



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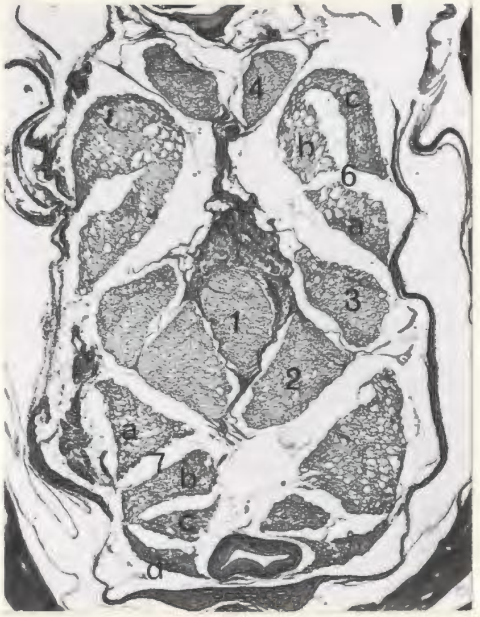

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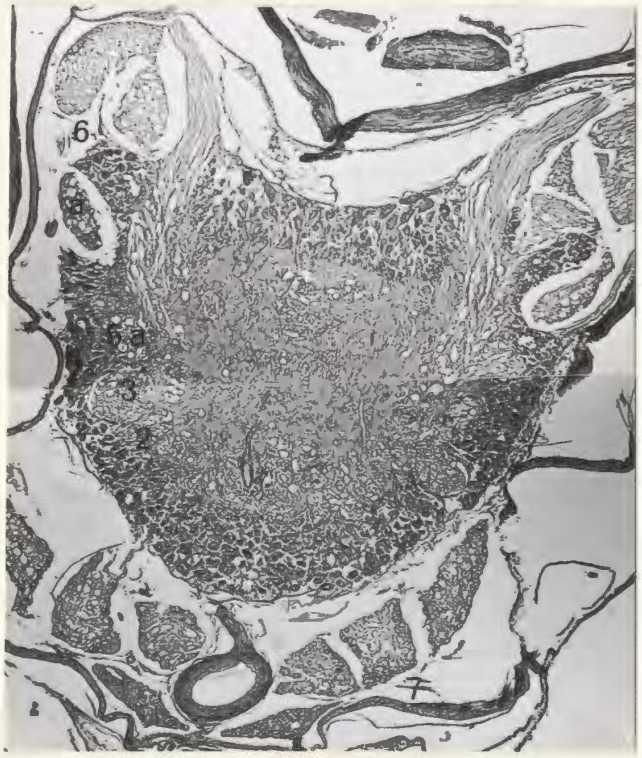

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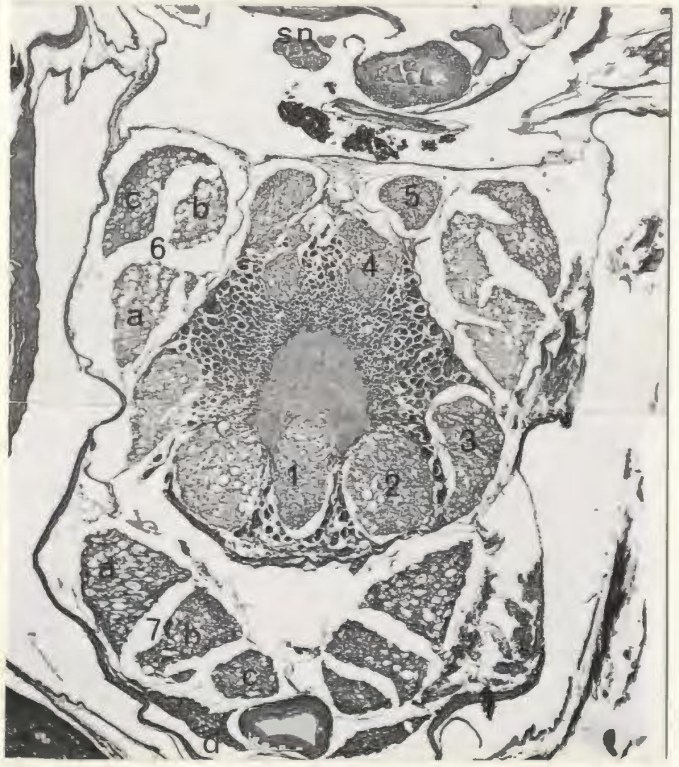

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Root 3 covers the dorsal half of root 1 in the brachio-pedal connective, and is the same size as root 2 (Figure 40). As it moves dorso-anteriorly its dorsal half becomes part of the neuropile in the vicinity of the origin of roots 1 and 2. The ventral half separates into two or three distinct bundles which pass along the periphery of the neuropile where they gather more fibres and continue through the lobe anteriorly forming part of the second pair of brachial nerves (Figure 37).

Root bundle 4 on the dorsal surface is covered by cells laterally and has an irregular shape (Figure 40). The tract slips under root 5 and forms the dorsal part of the brachio-pedal connective. It is smaller and more oval than the previous three roots and its origin within the ganglion is more posterior.

Root 5 is dorsal to root 4 and is the superior buccal-brachial connective (see page 57). It emerges from the cell layer above root 4 and continues above it to the posterior extremity of the brachial ganglion, where it turns sharply anteriorly.

Root 6 is paired and more massive than roots 1, 2 and 3. After passing to the posterior end of the lobe, it divides into three well-defined bundles termed a, b, and c on each side. These originate within the middle subesophageal mass and most do not enter the brachial ganglion, but continue above it (Figures 39, 40) Root 6a, the most ventral of these, separates into two parts. The ventral part measures 120 by 150 μ and enters the brachial ganglion where it separates into two branches which are later reunited, and with fibres from the brachial ganglion forms the

first pair of brachial nerves. The second part is larger, measuring 120 by 250 μ, fits in a groove on the surface of the ganglion (Figure 39), turns laterally but receives no fibres from the ganglion. Root 6b is dorsal to root 6a and medial to root 6c through two thirds of the length of the ganglion, then receives fibres from the brachial ganglion, and turning in an antero-dorsal direction extends lateral to the esophagus. Its destination was not determined. This nerve may correspond to the large upper pair of interbrachial nerves which receives fibres from both the pedal and brachial ganglion in Illex coindeti (Thore, 1939). Root 6c also remains outside the lobe. It does not extend as far anteriorly as a or b, but is joined by a nerve of the brachial ganglion whose fibres originate along the entire side of the ganglion (Figure 39) and turns sharply dorsally around the esophagus where it may unite with a second nerve on the dorsal surface in this region. All three may form the paired superior antorbital nerve described by Thore (1939) in I. coindeti.

Roots 7a, b, c, and d, starting from the most lateral pair, arise from the middle subesophageal mass and pass ventral to the brachial ganglion (Figures 37,38,39,40). The axons in root 7d are smaller than in the other three. Anterior to the superior antorbital nerve roots, tract 7d receives a branch from the ventral surface of the brachial ganglion (Figure 1), then passes ventrally into the musculature. This would then correspond

to Thore's inferior antorbital nerve (1939). Pair 7a proceeds anteriorly where it moves to the dorso-lateral side, combines, but does not fuse with the large lateral root bundle from within the ganglion serving the second pair of arms, and passes lateral to it (Figure 36). This may be the nerve innervating the tentacle muscles, since the eight brachial nerves receive fibres from both the brachial and pedal ganglia, but the tentacles receive only fibres from the pedal ganglion. The two other roots, 7b and c, remain ventral to the ganglion, then combine with the other pathways from within the neuropile of the brachial ganglion (Figure 37), and proceed anteriorly as the third and fourth pairs of brachial nerves.

Four pairs of root bundles, 8a, b, c, and d (Figures 37, 38) stem from the anterior half of the brachial ganglion in a symmetrical pattern. They arise on the dorsal surface at the periphery of the neuropile where the cell layer is thickest. They are circular, each with a diameter of about 120 μ. Each branch joins one of the brachial nerves on each side: root a with a brachial nerve 1, b with nerve 2, etc. They are the most dorsal of the three bundles which form each brachial nerve. The central part originates from fibres in the anterior part of the ganglion and the ventral portion is a contribution from the middle subesophageal mass.

Connections

The nerves of the lobe: the eight brachials, and the paired interbrachials, superior and inferior antorbitals, and

their root bundles, have been described above. The lobe also has extensive connections with both supra- and subesophageal centres of the brain.

(1) Connection with superior buccal lobe (see also pages 56, 65) Afferents and efferents may be present here.

(2) Connection with anterior pedal lobe. Numerous fibres pass both ways in the brachio-pedal connective. The fibres of root 1 begin and terminate within these two lobes but because of the fusion of fibres in the brachio-pedal connective, the other roots could not be followed.

(3 and 4) Connection with visceral and magnocellular lobes (see page 90). This is also a part of the brachio-pedal connective and is paired on each side. It contains fibres which pass to and from the visceral and magnocellular lobes and courses through the middle subesophageal mass in a well-defined bundle.

(5) Connection with posterior buccal lobe (see page 51). This is part of the cerebro-brachial connective which leaves the brachial ganglion at its dorso-posterior end from where it ascends almost vertically,lateral to the esophagus.

(6 and 7) Connections with subvertical and precommissural lobes, (see page 51). These axons pass in the cerebro-brachial connective enroute to the subvertical and precommissural lobes, but because the connective is joined by other fibres from the posterior buccal, its extent is difficult to determine (Figure 13).

(8) Connection with medial basal lobe. This is part of the brachio-pedal connective and passes through the anterior and lateral pedal lobes before reaching the medial basal.

Middle Subesophageal Mass

This has been called the pedal ganglion or lobe by most workers, and was described as the lateral lobe by Thore (1939). It is divided into four areas: the anterior pedal, anterior chromatophore, posterior pedal and lateral pedal lobes. Part of the magnocellular lobe is also located in this mass and the lobe will be described here.

The mass is separated from the brachial ganglion, although the two are connected by the massive brachio-pedal connective. It is joined broadly to the posterior subesophageal mass but is easily separated by a prominent curtain of collagenous connective tissue. Finally it is also connected to the cerebral ganglion through the anterior and posterior basal-subesophageal connectives which together form a continuous ring of nervous tissue around the foregut. In sagittal sections the mass is rectilinear in shape, about 5 mm long and 2 mm wide. The entire mass in relation to the rest of the brain can be seen in figures 1 and 2. It is the primary source of nerves arising directly from the central nervous system.

Anterior Pedal Lobe

This is the most extensive part of the middle subesophageal mass and is separated from the posterior pedal lobe

by the central pedal commissure. Laterally it is flanked by the paired lateral pedal and anterior chromatophore lobes. The lobe is rectilinear (Figures 42and 43) both in sagittal and frontal sections but widens posteriorly. In cross sections it is oval anteriorly but the dorsal surface invaginates in the centre to accommodate the circular esophagus. The lobe is bilaterally symmetrical and unpaired. Structurally it resembles the brachial ganglion with its pathways and root bundles, some of which pass through this lobe. Directly ventral to it and to the posterior pedal lobe there is a large blood sinus.

Cell layer

The cell layer exists only on the dorsal and ventral surfaces. It is thick on the ventral surface where it varies from 350 to 700 μ. On the dorsal surface it widens from 250 μ in the centre to 475 μ in the lateral protrusions of cells (Figure 46).

The neurons, arranged in the typical pattern of the large cells outside and smaller ones inside, resemble those of the brachial ganglion. Large neurons up to 110 μ in diameter, with nuclei of 28 μ containing one nucleolus have equal distribution in the dorsal and ventral cell rinds; the smallest cells are 7 μ in diameter. These cells are rounded and send a long, thick, tapering axon into the neuropile. The cells in the neuropile are usually single and only occasionally in groups of two or three. They are mostly round neuroglia associated with nerve fibres, particularly those leaving the lobe in the

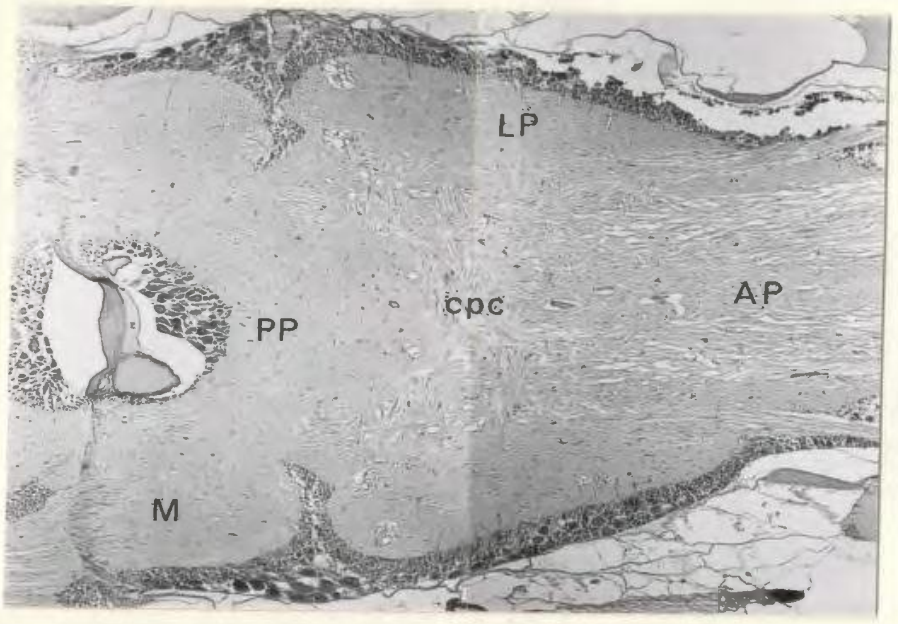


Figure 42. Middle subesophageal mass showing anterior, posterior, and lateral pedal lobes; frontal, x21, H and E, spec. 14.

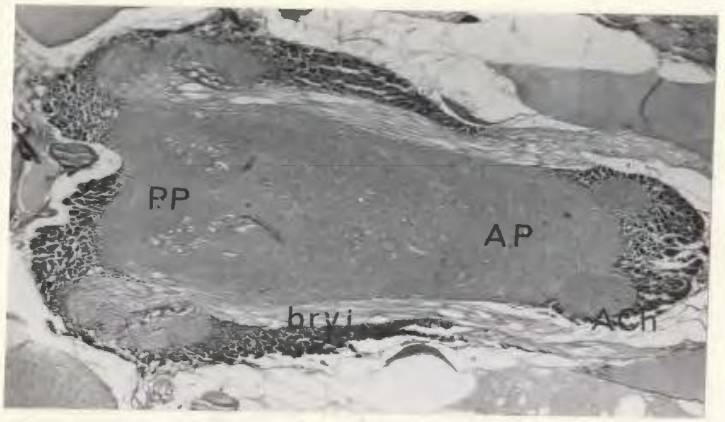


Figure 43. Middle subesophageal mass, more ventrally, showing anterior chromatophore lobe and the brachio-visceral connective; frontal, x17, H and E, spec. 14.

brachio-pedal connective, but some spingle-shaped bipolars are also present.

Neuropile and root bundles

The larger cells on the dorsal and ventral surfaces send axons through the thicker neuropile and these leave the lobe after anastomosing with axons from the higher motor centres and the central pedal commissure. Many of the large axons pass anteriorly through the centre of the lobe, some crossing over to the other side, forming the root bundles described below.

The central area of the anterior pedal lobe is less dense than the dorsal and ventral regions and the lateral pedal lobe (Figures 42, 45, 46). This is due to the convergence of the many root bundles which pass through it and also to the presence of the fibres of the central pedal commissure.

The root bundles are a prominent feature of this lobe but are less numerous than in the brachial ganglion. The bundles in the lateral pedal lobe will also be included here. As the brachio-pedal connective enters the anterior pedal lobe, the many tracts within it are grouped into three bundles, two large lateral ones from which stem roots 2, 3, and 5, and a much smaller central one which becomes root 1 (Figures 44,45,46,47). The connective at this level is about 1.8 mm deep and 0.6 mm wide.

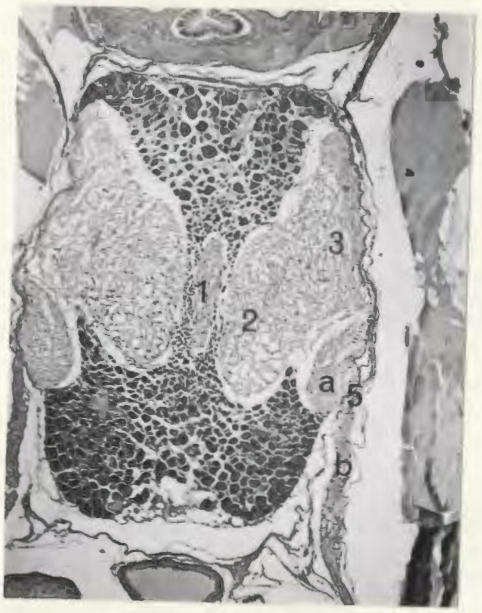
Root 1 is the only distinct single root in the connective and measures 120 by 400 μ. It passes directly through the centre of the lobe, where it gradually becomes smaller as its fibres separate into several very small bundles. It originates or ends

Figure 44. Anterior pedal lobe in anterior region; cross, x21, H and E, spec. 15.

Figure 45. Anterior pedal lobe showing root bundles; cross, x21, PAS, spec. 15.

Figure 46. Anterior pedal lobe; cross, x21, H and E, spec. 15.

Figure 47. Anterior pedal lobe showing brachio-visceral connective; cross, x21, H and E, spec. 15.



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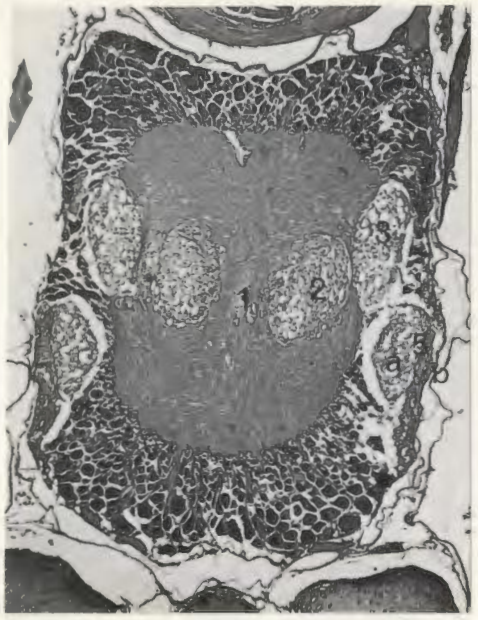

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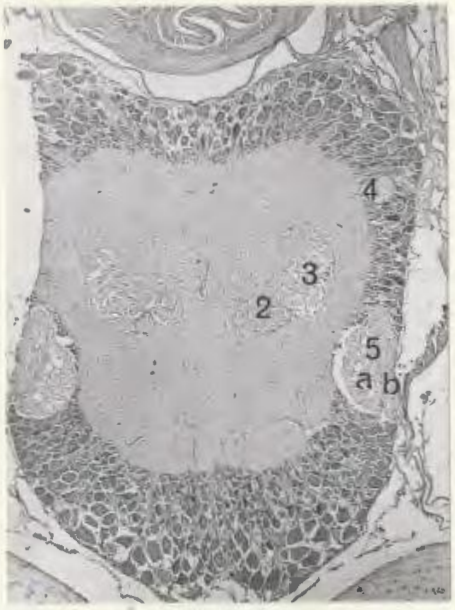

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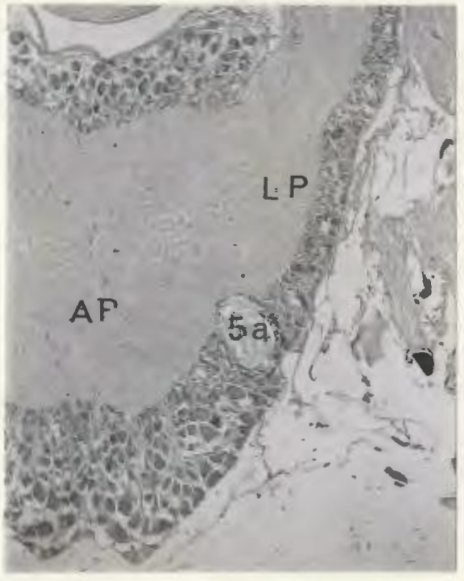

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in the anterior pedal lobe and anteriorly corresponds to root 1 of the brachial ganglion.

Root 2 is lateral to root 1 in the midline, is paired and measures 350 by 500 μ. It fuses with root 1 and some of its fibres may enter root 3 medially. It widens anteriorly in the connective but was not followed through to the brachial ganglion.

Root 3 is more distinct than root 2, is more compressed laterally and measures 550 by 225 μ. Anteriorly in the connective it fuses with branches from root bundles 2, 4,and 5. It is lateral and partly dorsal to root 2, and with the disappearance of the latter it moves medially (Figure 46) where it grows smaller and less distinct as it separates into several tracts which turn sharply dorsally around the esophagus to the posterior basal complex. The other fibres together with some from the medial bundles continue through the centre of the lobe, distinguished only by their loose packing, and extend posteriorly to the region of the central pedal commissure. The root could not be traced back to the brachial ganglion.

Root 4 is paired and measures a little more than 100 μ in diameter. It moves through the lateral pedal lobe and becomes enclosed in its posterior cell layer,whereupon it extends dorsally around the esophagus to its origin in the posterior basal complex. It is the lateral basal-anterior chromatophore connective.

Root 5 consists of tracts a and b, which are equal in size but have different origins and terminations. Tract a is more medial of the two and enters the pedal ganglion directly from the

brachio-pedal connective, but remains outside the anterior pedal lobe for some distance. In size and shape it resembles root 3. The tract continues dorsally, embedded in the cell layer, and represents the brachio-visceral connective (Figures 43,47) which was taken as part of the division between the anterior and lateral pedal lobes. Tract b is not included in the brachio-pedal connective. Within the anterior pedal lobe it is more dorsal than tract a and fuses with the neuropile of the lateral pedal lobe. It leaves the anterior pedal lobe a little more posterior and lateral to tract a, from which it can be distinguished by its more tightly packed nature, and continues ventrally as one of the nerves originating from the pedal lobes.

Connections

Some of the nerves originating from this lobe and passing anteriorly have already been described.

(1) Connection with lateral pedal lobe. The neuropiles are continuous laterally over a wide area (Figure 42), and fibres are exchanged both ways.

(2) Connection with posterior pedal lobe. The neuropiles are continuous posteriorly around and within the central pedal commissure (Figure 42). Exchange is also assumed to occur both ways.

(3) Connection with anterior chromatophore lobe. Their neuropiles are continuous antero-laterally (Figure 43).

(4) Connection with brachial ganglion (see page 68).

(5) Connection with anterior basal lobe (see page 49).

Anterior Chromatophore Lobe

This lobe (Figures 48, 49, 50) is situated on the lateral anterior surface of the anterior pedal lobe and is divided into dorsal and ventral halves. The lobe is paired on each side of the brain. The centre is occupied by root bundles. The cell layer is thinner than that of the anterior pedal lobe and its neuropile denser. Posteriorly the neuropile merges with that of the anterior pedal. Because of its position, any fibres entering or leaving the lobe, with the exception of the brachial, must pass through the anterior or lateral pedal lobe first.

Cell layer

This covers the lobe dorsally, ventrally and laterally. On the dorsal side the rind is uniform at 200 - 250 μ, while elsewhere the variation in thickness is greater, from 125 μ on the lateral to 350 μ on the ventral surface. The neurons are similar to those of the anterior pedal lobe except that the larger ones are fewer; those on the lateral surface are the smallest. The large cells are mostly pear-shaped and send thick axons into the neuropile in bundles, as seen in the posterior chromatophore lobe. Large axons can also be seen passing through the neuropile, although in a less distinct pattern.

Neuropile

The large axons from both the dorsal and ventral halves pass posteriorly into the neuropile where they turn sharply to the anterior and probably leave the lobe in the brachio-pedal connective in a direction medial and ventral to the lobe. However, in

the ventral half this pattern was less distinct.

Connections

(1) Connection with anterior pedal lobe (see page 75).

(2) Connection with lateral basal lobe (see pages 45, 74). This is the major source of afferents from the higher motor centres. It enters the anterior chromatophore lobe in its posterior lateral corner. It is assumed that there are no efferents present in the pathway from the lateral basal lobe.

(3) The major efferents to the chromatophores probably are combined with the nerves arising from the anterior pedal lobe. It is also possible that fibres are exchanged with the brachial ganglion.

Posterior Pedal Lobe

The lobe is bilaterally symmetrical and unpaired, and contains many root bundles (Figures 1 and 42). It is covered laterally by the magnocellular lobe and here their neuropiles are continuous. Posteriorly it is connected with the posterior subesophageal mass and many fibres also pass here, but the junction is clearly defined. The lobe is therefore covered by cells dorsally, ventrally, and partly in the posterior end. More nerves arise from it than from the anterior pedal lobe. Between its cell layer and that of the posterior subesophageal mass runs the cephalic artery (Figure 42).

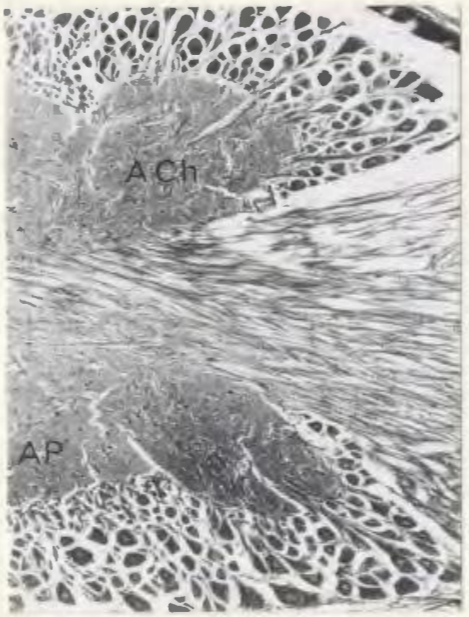
Cell layer

This shows more variation in thickness than that of

Figure 48. Anterior chromatophore lobe showing dorsal and ventral regions; sag., x35, Mallory triple, spec. 3.

Figure 49. Anterior chromatophore lobe showing left and right halves; frontal, x35, Heidenhain's Iron-Hematoxylin, spec. 5.

Figure 50. Anterior chromatophore lobe; cross, x21, Mallory triple, spec. 16.



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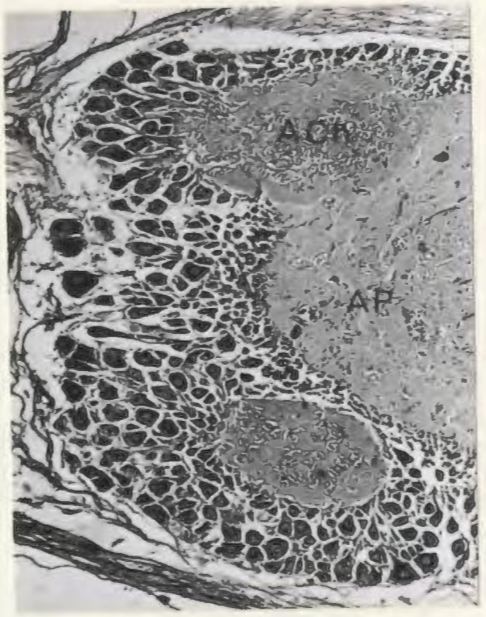

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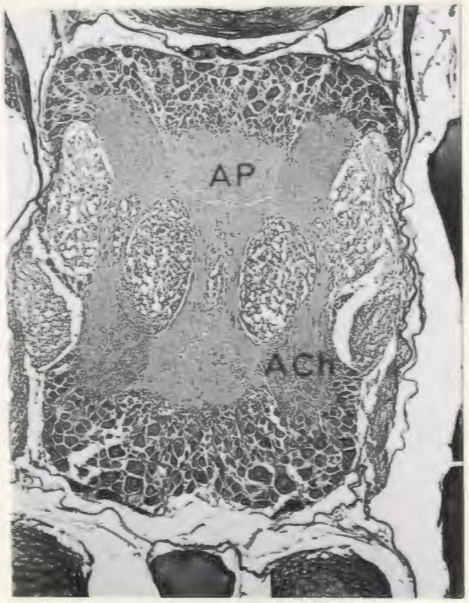

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the anterior pedal lobe, increasing from almost nothing where the blood sinus sinks in to 550 μ at the posterior dorsal corner. In cross sections there is a large indentation on the ventral surface caused by the blood sinus which extends nearly up to the neuropile. The dorsal layer is hollowed a little where the lobe lies under the esophagus, and its thickness varies from 350 to 175 μ. The posterior cell rind has a crescentic shape in sagittal sections and is 330 - 225 μ thick.

The neurons resemble those of the brachial and anterior pedal lobes in size and shape, with the larger cells on the outside of the cell rind. There are more small cells and fewer large ones here than in the anterior pedal lobe. The largest neuron seen was 90 μ. The cells are pear-shaped and send thick axons into the neuropile in bundles. No cell islands occur in the neuropile.

Neuropile, root bundles and nerves

The root bundles, listed with their corresponding nerves below, are a major feature of the neuropile. Most of them enter the four, and possibly more, nerves on the ventro-posterior surface of the lobe.

(1) The largest pair forms the anterior funnel nerves. This has two roots in the lobe, one near the central pedal commissure, and the other, which is more dorsal, further posteriorly (Figure 51).

It also receives a large root bundle from the visceral lobe, which enters the posterior surface of the nerve. The nerves, each measuring about 500 μ in diameter, pass through a single cavity in the cartilage (Figure 52) posterior to the anterior magnocellular commissure (Figure 56), and then turn sharply posteriorly. Associated with each nerve and located medial to it is a pair of thick-walled blood vessels, which join with the large blood sinus under the pedal lobe.

(2) A smaller single nerve, about 150 μ in diameter, emerges medial to both the anterior funnel nerves and the blood vessels (Figure 52). It corresponds to the median infundibular or funnel nerve described by Thore (1939). Its only root lies medial to those of the anterior funnel nerves, but arises more ventrally, posterior to the anterior magnocellular commissure.

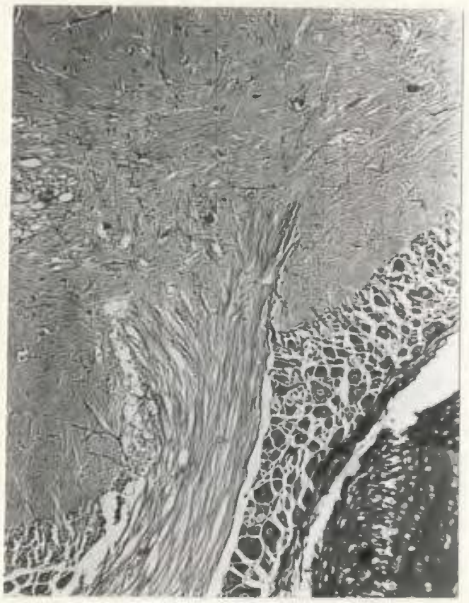
(3) A second pair of nerves probably corresponding to the medial pallial adductor nerves (Thore, 1939), leave as the lateral part of the anterior funnel nerves but turn laterally and penetrate through the cartilage near the posterior end of the optic lobes. Their root bundles can, however, be traced into the posterior pedal lobe where they are lateral to those of the anterior funnel nerves.

(4) The area of the origin and termination of the statocyst nerves is a paired triangular extension at the posterior lateral corner of the pedal lobe, directly in front of the magnocellular lobe. The nerves are paired and measure about 175 μ. The

Figure 51. Root bundle of anterior funnel nerve; sag., x35, Mallory triple, spec. 3.

Figure 52. Anterior and medial funnel nerves penetrating through cartilage; frontal, x21, Mallory triple, spec. 15.

Figure 53. Statocyst nerves; frontal, x28, H and E, spec. 15.



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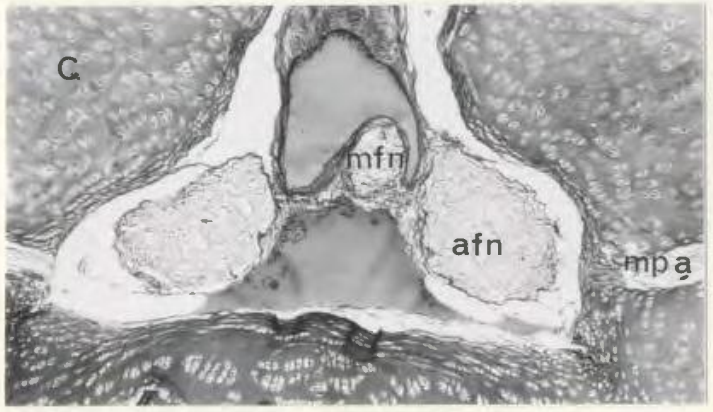

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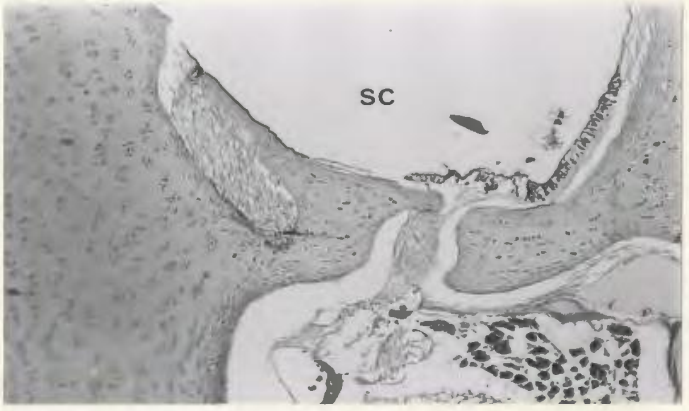

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structure and neurosensory cells of the statocyst of Octopus have been thoroughly described by Young (1960) and its function by Boycott (1960). A detailed study of the nerves was not undertaken here, but it was seen that each has two major branches (Figure 53). One branch stems from the dorsal region of the statocysts; its fibres pass between the row of cells lining the organ and the cartilage, and may form the outer plexus of axons surrounding the statocyst. The other branch is larger and more lateral, and is believed to contain afferents and efferents.

(5) Another group of paired nerves arises postero-laterally, and corresponds to the anterior and posterior head retractor nerves and the posterior occulomotor nerve in Octopus. The root bundle is oval, measures 650 by 300 μ, and arises deep within the lobe from several smaller bundles at the level of the anterior magnocellular commissure between the magnocellular, posterior and lateral pedal lobes, and many of its fibres originate near the central pedal commissure. It leaves the lobe postero-laterally between the posterior subesophageal mass and the cartilage, and then passes through the cartilage to innervate the muscles in the posterior head region lateral to the posterior salivary glands. A branch also penetrates through the cartilage in a more ventral direction and innervates the muscles of the ventral head region.

(6) A smaller nerve arises from each side of the dorso-lateral surface a little anterior to the head retractor nerves, which may

correspond to one of the branches of the superior ophthalmic nerve in Octopus. Its root lies in the division between the lateral pedal and the other pedal lobes, measures 200 by 120 μ, and originates in the lateral and anterior pedal lobes, dorsal to root 5.

There also seems to be an extensive interchange of fibres in the ventral portion of the posterior pedal lobe across the midline behind the central pedal commissure.

Connections

(1) Connection with lateral pedal lobe. Their neuropiles are continuous laterally, and the posterior pedal may come in contact with the fibres from the lateral pedal, particularly through the central pedal commissure. Exchange probably occurs both ways.

(2) Connection with anterior pedal lobe (see page 75).

(3) Connection with magnocellular lobe. Their neuropiles are continuous laterally (Figure 42).

(4) Connection with visceral lobe. Their neuropiles are continuous posteriorly, medial to the magnocellular lobe (Figure 42).

(5) Connection with medial basal lobe (see page 43). The fibres pass lateral to the esophagus and turn medially, probably towards the central pedal commissure.

(6) Connection with fin lobe. This originates within the anterior region of the posterior pedal lobe and passes posteriorly, medial to the fibres from the magnocellular lobe.

Lateral Pedal Lobe

This is paired and situated on the lateral surface of the middle subesophageal mass in the region where the sub- and supraesophageal masses join (Figure 42). Its anterior limit is the brachio-visceral connective and dorsally it extends around the esophagus to the medial basal lobe. Many of the incoming fibres to this and the other pedal lobes descend in the anterior and posterior basal-subesophageal connectives. It gives rise to fewer nerves than the other pedal lobes, and differs from the other subesophageal centres in that it does not contain the large root bundles which characterize their neuropiles.

Cell layer

This is present only laterally and posteriorly and is 2 - 3 cells thick. It is thickest (150 μ) in the mid region, becoming thinner anteriorly (90 μ) and posteriorly (60 μ). The cells vary regionally, the largest are found in the mid region and are smaller than in the other pedal lobes, measuring 60 μ with a nuclear diameter of 20 μ. The smaller cells inside the rind may be round or pear-shaped and usually send their axons singly into the neuropile. Further anteriorly the neurons get progressively smaller averaging 20 μ at the anterior end of the lobe. In the posterior end they are round, 5 - 10 μ in diameter and have thin axons. The larger pear-shaped motor neurons are at the periphery of the lobe or interspersed with the smaller neurons but rarely measure over 15 μ.

Neuropile

This is much denser than that of the anterior or posterior pedal lobes, due probably to the smaller number of axons passing through it. The larger cells on the surface of the lobe send their axons straight into the neuropile, although more ventrally near the brachio-visceral connective they pass more dorsally. Most of the fibres entering the lobe from the cerebral ganglion pass toward the centre, in the anterior pedal lobe, or to the medial half of the lateral pedal. Some root bundles originate in the area between this and the other pedal lobes.

Connections

(1) Connection with anterior pedal lobe (see page 75).

(2) Connection with posterior pedal lobe (see page 83).

(3) Connection with magnocellular lobe. Their neuropiles are continuous posteriorly over a limited area and fibres may run both ways between the lobes.

(4) Connection with anterior basal lobe (see page 49).

(5) Connection with medial basal lobe (see page 43).

(6) Connection with peduncle lobe. The fibres in the tract from the peduncle lobe enter the dorso-lateral region of the lateral pedal lobe and pass to the medial region near the central pedal commissure.

Magnocellular Lobe

The lobe in Illex is predominantly subesophageal and spreads dorsally only to the esophagus. It is paired and

lateral to all the other lobes with the exception of the peduncle complex and optic lobes. It consists of three regions and is in direct communication with other lobes. Its shape is irregular and not well defined (Figures 55, 60). At the posterior part of the middle subesophageal mass the two sides are connected by the ventrally situated anterior magnocellular commissure.

This lobe, unlike the other subesophageal centres, is not a lower motor centre. It differs structurally from the posterior subesophageal contres and the anterior and posterior pedal lobes in its connections, neuropile and cell layer. Young (1939) states that its position, just above the statocyst and at the meeting points of the optic, cerebral, middle and posterior subesophageal masses, and its shape make it ideally situated to receive and transmit impulses. Young also suggested that the lobe represents the expanded ventral and lateral parts of the periesophageal ring of the ancestral mollusc. The lobe contains the cells which give rise to the first order glant axons (Young, 1939).

Cell layer

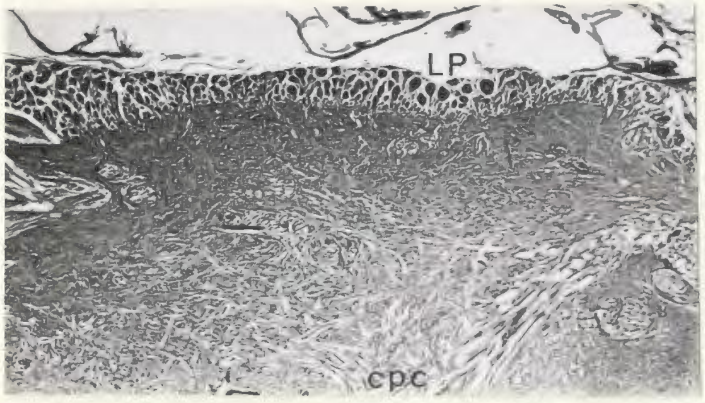
Each half consists of three divisions: a posterior part, lateral to the visceral lobe, with a lateral, ventral, dorsal, and posterior cell rind; a middle anterior part, covered with cells only laterally, with the large cells at the latero-ventral ends; a more dorsal anterior extension of the above part, which is circular in cross sections and covered laterally and

Figure 54. Lateral pedal lobe and region of central pedal commissure; frontal, x21, Mallory triple, spec. 5.

Figure 55. Magnocellular lobe; sag., x21, Alcian blue-chlorantine fast red, spec. 3.

Figure 56. Anterior magnocellular commissure; frontal, x28, Mallory triple, spec. 15.

Figure 57. Posterior magnocellular commissure: fusion of the first order giant axons; frontal, x35, Mallory triple, spec. 15.



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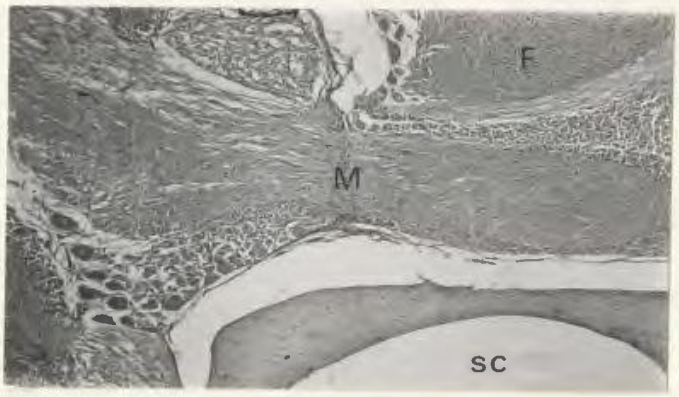

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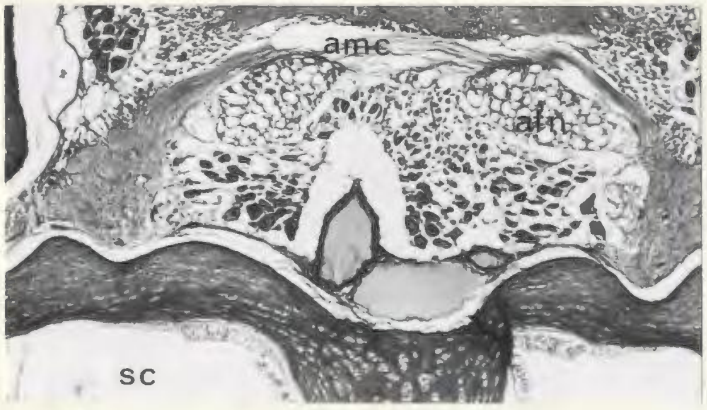

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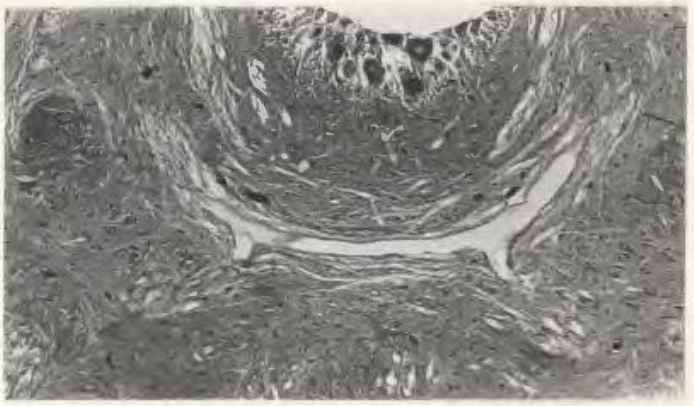

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partly medially by an incomplete cell rind.

The cells tend to be small particularly in the posterior region and are sharply contrasted with those of the fin and visceral lobes. Here the cell rind varies from 60 to 175 μ and is thickest laterally. Nearly all of the neurons are 6 - 25 μ with none over 40 μ. The greatest number of large cells are found in the mid-ventral portion of the lobe (Figure 55). The cells in the dorsal part of the ventral region are pear-shaped and send thick axons into the neuropile. The large cells here reach 90 μ and are located usually in a hollow where the dorsal and ventral regions meet and laterally along the ventral region. The giant cells giving rise to the first order giant axons were not seen and these fibres could not be traced back to any particular cell. Dorsally the cells are less than 25 μ corresponding in size to those of the posterior region. There are fewer cells in the neuropile of the dorsal and ventral regions than in the posterior region.

Neuropile

The majority of fibres in the ventral and posterior portions of the lobe follow an antero - posterior direction. In the ventral region the small cells send axons in a posterior direction. A cluster of large cells, protruding on the dorsal surface in front of the connective tissue send their large axons ventrally and toward the posterior end of the lobe. On the dorsal side the fibres pass mostly ventrally and then posteriorly under the tract from the posterior basal complex. The

characteristics of the neuropile are determined by the pathways within it and will be discussed in relation to the connections.

Connections

Because of the paired nature of the lobe all its connections are also paired.

(1) Anterior magnocellular commissure. This commissure joins the two lobes, is about 60 μ thick and 150 μ deep, and located near the ventral surface of the middle subesophageal mass at the anterior end of the ventral magnocellular lobe (Figure 56). Its fibres are loosely packed and there does not appear to be a great amount of exchange.

(2) Posterior magnocellular commissure. This commissure also joins the two lobes but is within the neuropile of the visceral lobe. The first order giant fibres (40 μ in diameter), which arise on each side of the magnocellular lobe and proceed posteriorly in the visceral lobe, cross in this connective and their axons fuse over a wide area (Figure 57). They do not extend beyond the visceral lobe but synapse with other large axons in the visceral lobe, which then travel to the stellate ganglion by way of the pallial nerve.

(3) Connection with optic lobes. The optic fibres enter the magnocellular lobe anteriorly and pass through it posteriorly, mostly in its medial half, but smaller branches run along its lateral wall. Many of the fibres penetrate the connective tissue wall and pass in the posterior magnocellular commissure.

(4) Connection with peduncle lobe. This starts at the top of the posterior region of the peduncle lobe, passes ventrally to the dorso-lateral corner of the magnocellular lobe and continues in the lateral region of the magnocellular lobe just inside the cell layer.

(5) Connection with brachial lobe (see pages 68 and 75). This is a smaller part of the brachio-visceral connective. The tract passes next to the anterior pedal lobe for one of third its length and then enters it and continues next to the cell layer. It is oval but becomes rounded near its destination and has a diameter of about 240 μ. (Figures 43, 47). It enters the lobe above the level of the ventral magnocellular commissure. Afferents and efferents are believed to be present.

(6) Connection with fin lobe. Their neuropiles are continuous over a small area in the posterior region at its dorso-medial surface but little exchange is believed to take place.

(7) Connection with visceral lobe. The major efferents of the magnocellular lobe pass here through the posterior magnocellular commissure and many end in the visceral lobe.

(8) Connection with posterior pedal lobe (see page 83).

(9) Connection with lateral pedal lobe (see page 85).

(10) Connection with medial basal lobe (see page 44). This occupies a wide area on the dorsal side of the lobe where their neuropiles are almost continuous. It enters the dorsal region anteriorly where it divides and sends many fibres back to the

posterior region.

(11) Connection with dorsal basal lobe (see page 40).

Posterior Subesophageal Mass

This has been described by most earlier workers as the visceral or palliovisceral ganglion or lobe. It is the broadest of the three subesophageal masses but in the antero-posterior direction it is the shortest (Figures 58, 59, 60 and 61). The entire mass is bilaterally symmetrical but some of its divisions are paired, and contains the fin, posterior chromatophore and visceral lobes. The vasomotor lobe may be represented by a posterior lobule located behind the visceral lobe. Also found here and probably associated with the vasomotor lobes is the neurosecretory tissue. On the dorsal surface the mass is hollowed slightly, conforming to the shape of the esophagus.

Fin Lobe

This lobe is the anterior part of Thore's (1939) accessory lobe. Its position and shape can be seen in figures 60 and 61. The lobe is a paired lower motor centre and extends ventrally only to the magnocellular lobe. Posteriorly it is bordered by the posterior chromatophore lobe, and medially its neuropile is continuous with that of the visceral lobe. The fin lobe has the highest percentage of large motor neurons of any lobe in the brain.

Cell layer

The cell layer is thickest at its lateral corners (up to 600 μ) and thinnest laterally (275 μ). There are few small

cells (less than 40 μ) in the lobe (Figure 61), their number not exceeding 15% of the total cell population, while the majority range from 50 to 125 μ, tending however to approach the latter size. They have a variety of shapes and each neuron is separated from the rest by a capsule of collagenous fibres. The nuclei are between 8 and 30 μ, with one, two, or three nucleoli. Where more than one nucleolus is present there is one large and two smaller ones, and one of the smaller is usually associated with the large one. In a cell of diameter slightly greater than 120 μ and a nuclear diameter of 30 μ , the largest nucleolus measures 8μ and the smaller two slightly more than 3μ.

Some of the large cells send axons 8 - 12 μ in diameter into the neuropile but these are much smaller than the first order giant axons. They are eosinophilic whereas the first order giant axons do not stain with any technique used. Two or three enter usually together and can easily be followed because of their thickness. Single small cells, some of them neurons, are randomly scattered throughout the neuropile: two were noted with a diameter of 9 μ and a thin tapering axon.

Neuropile

The large axons pass from the dorsal surface of the lobe into the neuropile mostly in a vertical direction, but also randomly through the neuropile intermingling and anastomosing with the incoming fibres. The axons then gather at the ventral surface and leave the lobe posteriorly (Figure 60). The neurons of the lateral surface send their axons out laterally. Most of

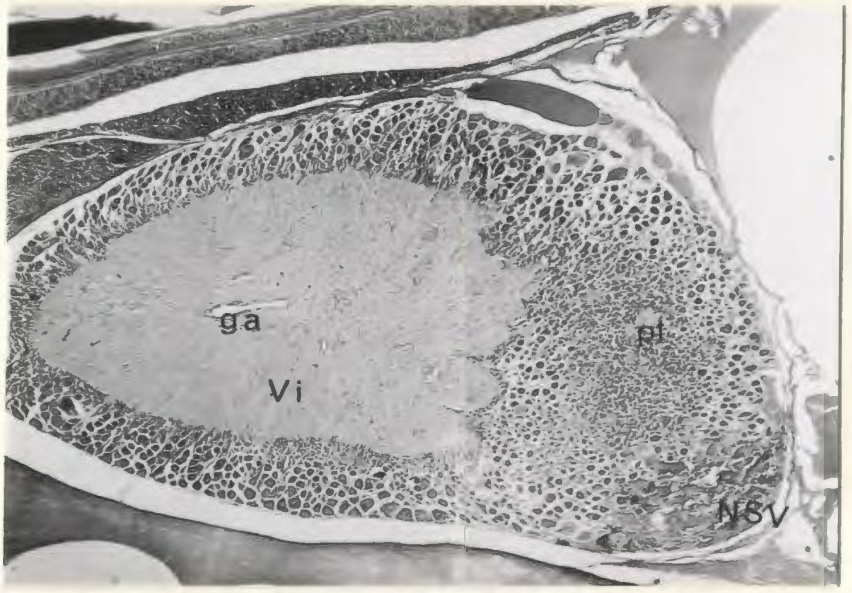


Figure 58. Posterior subesophageal mass showing visceral lobe; sag., x21, Masson's trichrome, spec. 3.

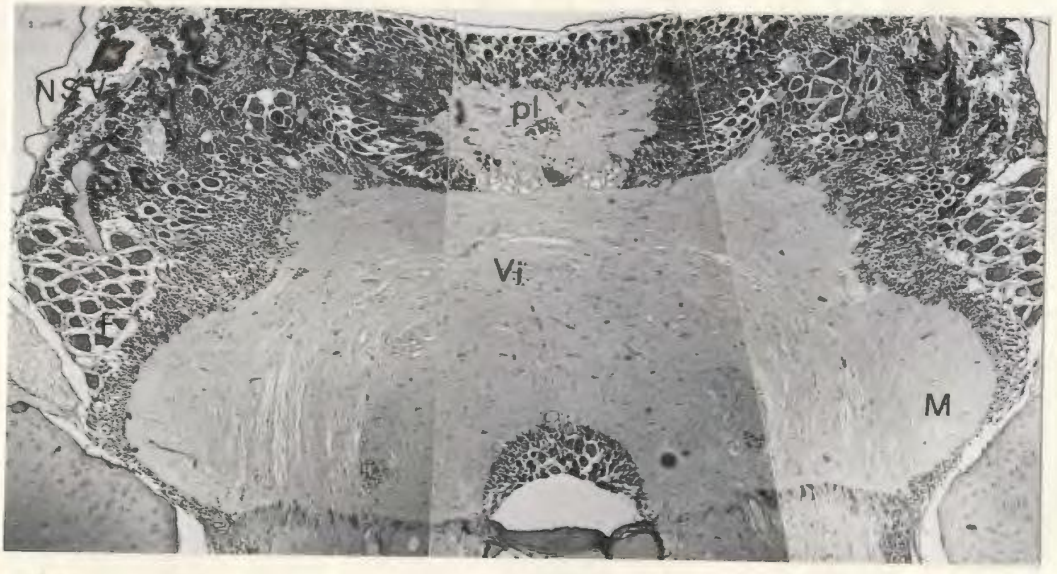


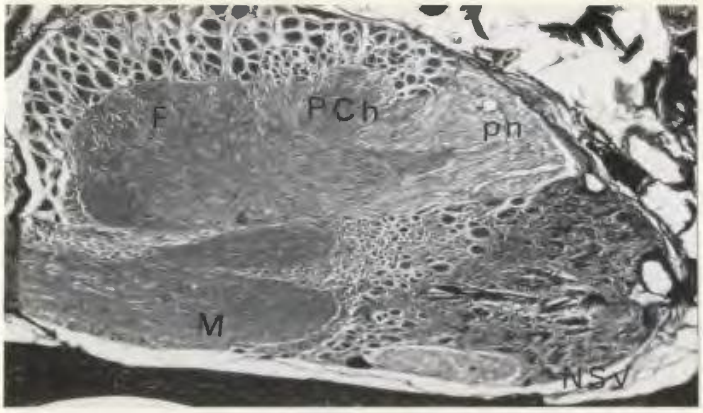
Figure 59. Posterior subesophageal mass showing visceral lobe, posterior lobule and neurosecretory tissue; frontal, x21, H and E, spec. 15.

Figure 60. Posterior subesophageal mass showing fin and posterior chromatophore lobes, magnocellular lobe and neurosecretory tissue; sag., x18, Mallory triple, spec. 3.

Figure 61. Posterior subesophageal mass showing fin, visceral and magnocellular lobes; cross, x18, H and E, spec. 16.

Figure 62. Dorsal region of posterior subesophageal mass showing fin, posterior chromatophore and visceral lobes; frontal, x21, H and E, spec. 15.

Figure 63. Junction of fin and posterior chromatophore lobes and the tract from the lateral basal lobe; sag., x35, Alcian blue-chlorantine fast red, spec. 15.



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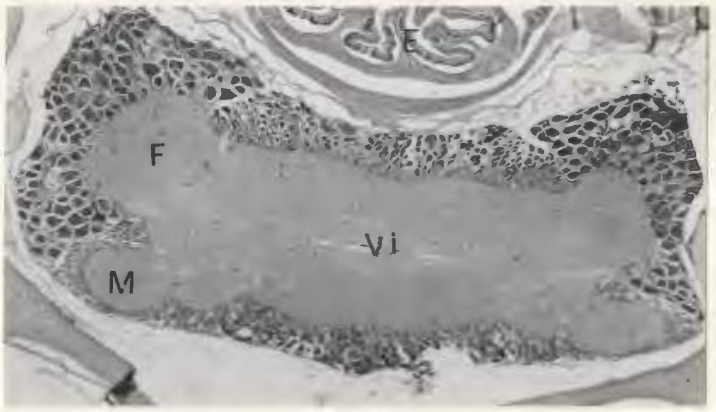

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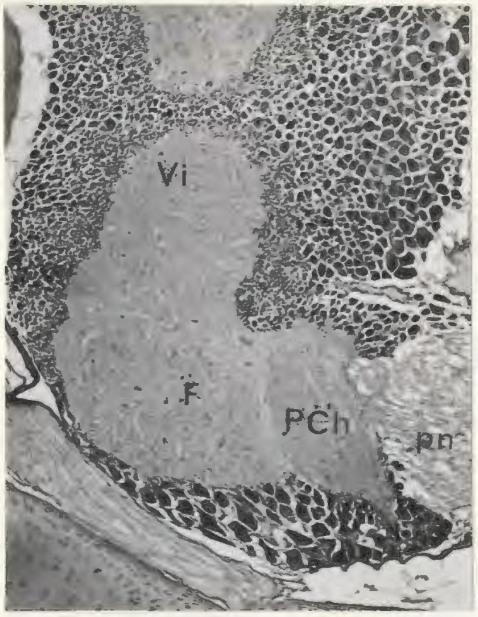

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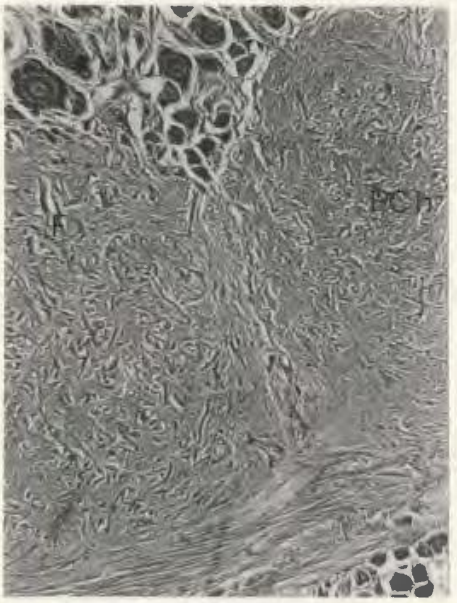

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the small fibres in the neuropile probably originate from outside the lobe and control the fins by synapsing with the large axons of the fin lobe neurons.

Connections

(1) Connection with posterior chromatophore lobe. Their neuropiles are continuous posteriorly (Figure 62) and a certain amount of exchange takes place particularly in the ventral region above the lateral basal - posterior chromatophore connective.

(2) Connection with magnocellular lobe (see page 90).

(3) Connection with visceral lobe. Their neuropiles are continuous medially. There is much more exchange here than between the visceral and posterior chromatophore lobes.

(4) Connection with posterior pedal lobe (see also page 83). This is probably the main source of afferents of the fin lobe. It passes posteriorly through or medial to the magnocellular lobe to the ventral portion of each fin lobe. It is more medial than the tract from the lateral basal to the posterior chromatophore lobe. Some of the fibres in this tract may arise from the medial basal lobe, or alternatively the fibres from the posterior pedal may synapse or anastomose with the latter, particularly in the region of the central pedal commissure, to relay the information from the higher motor centre.

(5) Pallial nerve. This is the major efferent pathway and is probably also the largest nerve of the animal. The large nerve

fibres gather in the ventral portion of the lobe, pass posteriorly under the posterior chromatophore lobe and in the ventral two-thirds of the nerve meet with fibres from the visceral lobe.

Posterior Chromatophore Lobe

This occupies the posterior region of Thore's accessory lobe and is considerably smaller than the fin lobe (Figure 62). Most of its posterior surface is the root of the pallial nerve (Figure 60), and ventrally it lies above the numerous fibres entering the nerve from the fin and visceral lobes. In shape it is similar to the fin lobe, that is circular or oval in all three planes.

Cell layer

It is thickest laterally (240 - 300 μ) and thinnest dorso-laterally. Its structural pattern is similar to that of the fin lobe but the cells are smaller. The largest neurons were 70 μ, with nuclei of 19 μ and axons 5 μ thick. The only other axons in this size range, with the exception of the first order giant fibres, originate from the large cells of the fin lobe. The smaller neurons range from 25 - 60 μ, are pear-shaped and also have large axons. Very few small cells are found only at the periphery of the neuropile. In the pallial nerve root bundle there are many glial cells with dark round nuclei.

Neuropile

The axons from the large cells follow a dorso-ventral direction to the bottom of the lobe where they turn posteriorly

and leave in the pallial nerve. Some appear to follow other directions, particularly toward the anterior ventral end, but it is assumed that all the fibres ultimately enter the pallial nerve. The neuropile is very similar to that of the anterior chromatophore and fin lobes. Numerous small fibres (undoubtedly from the incoming afferents) intermingle with these large axons.

Connections

(1) Connection with fin lobe (see page 95).

(2) Connection with visceral lobe. Their neuropiles are continuous medially but there appears to be little exchange.

(3) Connection with lateral basal lobe (see also page 45). This tract is lateral to the fibres from the fin lobe entering the pallial nerve and dorsal as well as partly lateral to the tracts from the medial basal to the magnocellular lobe. The connective is about 300 μ wide and 70 μ thick under the fin lobe. The fibres then proceed postero-dorsally into the anterior ventral corner of the posterior chromatophore lobe where they disperse in all planes and interweave with the large axons.

(4) Pallial nerve. This is the major source of efferents from the posterior chromatophore lobe. The axons leave from the ventro-posterior end of the lobe above the fibres of the fin lobe (Figure 59).

Visceral Lobe

The visceral lobe is the largest area in the posterior subesophageal mass. Its position and shape can be seen in figures 58, 59 and 61. The lobe is unpaired and bilaterally symmetrical. It is rather difficult to describe because of the

presence at its posterior end of a lobule with a distinct neuropile surrounded by many small cells (Figure 59), which may correspond to the dorsal and ventral vasomotor lobes in Octopus (Young, 1963a). Their neuropiles are continuous dorsally and ventrally though partly separated by a thin row of cells and by the posterior root bundle of the visceral nerve. The posterior lobule is medial to the neurosecretory tissue.

Cell layer

In cross sections a great difference is seen between the large cells of the fin lobe laterally, the small cells of the magnocellular lobe ventrally, and the cells of the visceral lobe between the two (Figure 61). The rind is about 300 μ thick dorsally and 240 μ ventrally and characteristically thins out evenly in both directions. The largest cells are grouped near the midline from where they diminish in size laterally with the smallest cells at the junction with the visceral and the fin lobes dorsally and with the magnocellular lobe ventrally. The cell size also decreases near the neuropile. The lateral neurons send their axons toward the midline of the lobe on both the dorsal and ventral surfaces. On the anterior surface the cell layer is crescent-shaped and contains both large and small cells, with the large ones in the midline. On the posterior surface the large cells are located posteriorly and laterally (Figure 59). The lateral cells reach 80 μ with a nuclear

diameter of 20 μ and are pear-shaped. Posteriorly the cells are smaller and round. Inside the groups of large cells there are small cells, many of which send axons into the posterior lobule. These cells are nearly all less than 12 μ with nuclei of 5 - 6 μ, but a few larger ones were observed. In the neuropile there are many neuroglia and also a few multipolars.

Neuropile

This is basically unspecialized but there are many distinct areas which correspond to root bundles.

The dorsal third of the lobe has only one notable feature — the thick axons which arise on the dorsal surface and pass ventrally to about half the depth of the lobe. Below this is the posterior magnocellular commissure. The incoming fibres cross and divide the neuropile into four regions: a small anterior area in which many fibres are directed circularly; two lateral regions into which enter many incoming fibres from other subesophageal lobes; a posterior area which contains the posterior dorsal lobule and an undifferentiated part of the visceral lobe. The interaxonic bridge of the first order giant axons is at this level (Figure 57). The posterior lobule becomes more distinct further posteriorly and here the incoming fibres from the magnocellular lobe have a circular appearance. Below the level of the fin lobe the posterior lobule is separated from the main part of the visceral lobe, while at the bottom of the posterior subesophageal mass they are united again. A cross section of the posterior part of the mass shows three regions, separated by a

narrow layer of cells, where all the fibres run in a lateral direction. The fibres of the root bundles will be described in relation to their nerves.

The posterior lobule can be seen in figures 58 and 60. The dorsal cell layer is thick and the axons of the most dorsal cells join into larger bundles which penetrate through the inner cells before entering the neuropile. Most of the fibres here are directed laterally as they are throughout the depth of the visceral lobe. There is considerable exchange of fibres between this and the main part of the visceral lobe ventrally between the root bundles of the vena cava and visceral nerves.

Nerves and connections

(1) Pallial nerve. The nerve is paired and arises posteriorly (Figure 62).The visceral lobe fibres are on the ventral and medial side of the nerve and probably stem from the larger cells at both the dorsal and ventral surface of the lobe.

(2) Visceral nerve. It is paired with the two branches arising close together from the ventro-posterior region of the lobe. It has two pairs of root bundles. The posterior pair originates between the indented layer of cells separating the main part of the visceral lobe from the posterior lobule. The anterior bundle does not arise as far dorsally and stems from a single bundle which divides into two further ventrally. The visceral nerve roots are posterior and medial to the posterior funnel nerve roots and appear smaller.

(3) Vena cava nerve. This paired nerve originates near the posterior root of the visceral nerve and some of the fibres probably stem from the vicinity of the posterior lobule. The main branch of the nerve is joined by tracts from the neuro-secretory tissue, which unite into larger bundles before joining and leaving the posterior subesophageal mass with the posterior funnel nerve. As it leaves the brain the vena cava nerve is about 70 by 120 μ and crosses over the posterior funnel nerve before joining it laterally.

(4) Posterior funnel nerve. The root bundle of this paired nerve stems from several small tracts scattered among the in-coming fibres from the magnocellular lobe at the level of the giant axons, which form one large root bundle on each side, then pass ventrally turning sharply posteriorly and emerge as the postero-lateral end of the lobe near the ventral surface where they are joined by the vena cava nerves.

(5) Collar nerve. Its root bundle originates in the posterior region, passes laterally, then emerges above the pallial nerve medially but crosses over and continues lateral to it.

(6) Connection with magnocellular lobe (see page 90).

(7) Connection with posterior pedal lobe (see page 83).

(8) Connection with brachial lobe (see pages 68 and 90).

(9) Connection with fin lobe (see page 95).

(10) Connection with posterior chromatophore lobe (see page 97).

Peduncle Complex

This area is dorsal and posterior to the optic tract on either side of the central nervous system (Figures 3 and 4). The complex is lateral to all parts of the brain with the exception of the optic lobes and connects with many lobes of the central brain medially and with the optic lobes laterally. They are also linked with each other by the large peduncle commissure which passes through the cerebral ganglion above the ventral optic commissure. The nervous structures contained here are the peduncle and olfactory lobes, but the optic gland is also regarded as a part of this complex.

Peduncle Lobe

Each occupies the lateral region of the peduncle complex and is difficult to separate from the olfactory lobe, which is dorsal and more posterior to it. The peduncle commissure passes almost horizontally through the central brain and is distinguished from the optic commissure in that the fibres in the latter proceed dorsally at a 45 degree angle, cross above the esophagus and continue ventrally along the other side. In the region of the peduncle commissure the lobe measures 1.5 mm. dorso-ventrally and 700 μ laterally. The lobe is oval (Figure 66) to the level of the peduncle commissure, beyond which two regions can be recognized: a ventral oval area named the basal zone and a dorsal area termed the spine in Octopus by Messenger (1967), which become more prominent posteriorly.

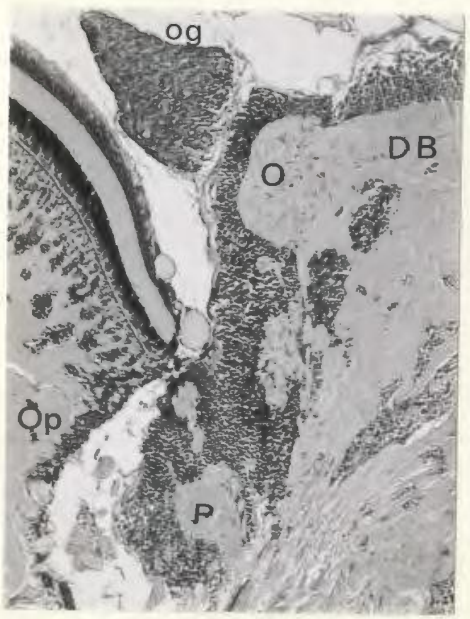
Cell layer

This is not uniform throughout the lobe. The rind is about 120 μ thick on the lateral surface and less than 40 μ on the medial surface of the basal zone, while in the region of the spine it is about 50 μ.

The neurons of the basal zone have a greater size range than in the spine, but no cells are larger than 25 μ. In the spine the cells do not exceed 10 μ and the majority are closer to 5 μ. A row of cells lies within the neuropile of the spine about 10μ from the cell layer (Figures 65 and 66), passing antero-posteriorly as well as dorso-ventrally. These cells measure 5 μ; each has one axon which divides into two branches, one proceeding toward the cell layer, and the other in the opposite direction to the neuropile. Aside from this there are many cells in the basal zone, which may occur singly or in large cell islands (Figure 66). Some of the islands contain more than two dozen cells which vary from 4 to 9 μ in size.

Neuropile

The neuropile of the spine differs from that of the basal zone in that it is a much finer network of fibres, most of which pass dorso-ventrally. The basal zone, which lies anterior to the spine has many fibres organized into well defined pathways passing in all directions through the lobe. The one prominent root bundle, originating from three or four branches in the central area of the lobe near the lateral cell rind, passes ventrally (the branches remain separated) to the parolfactory



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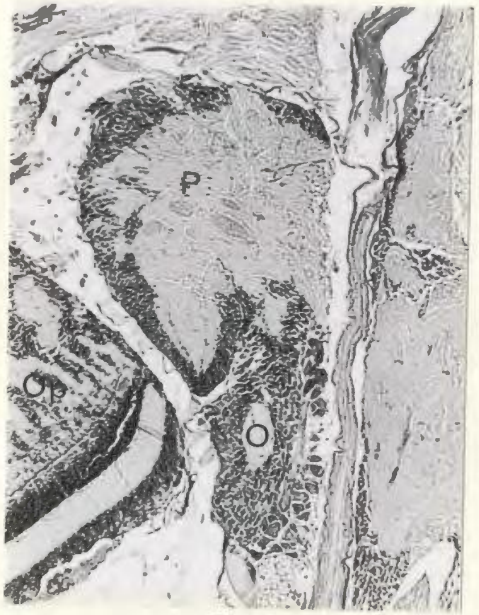

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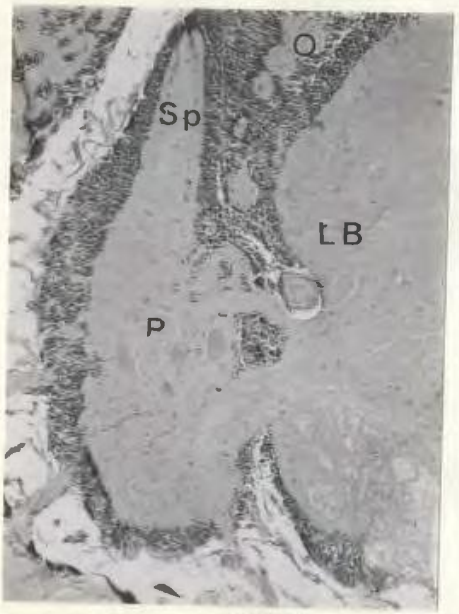

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Figure 64. Peduncle complex in dorsal region; frontal, x28, H and E. spec. 15.

Figure 65. Peduncle complex further ventral; frontal, x35, H and E, spec. 15.

Figure 66. Peduncle lobe showing basal region and spine; cross, x28, H and E, spec. 16.

vesicles, and is probably joined by fibres from the olfactory lobe.

Connections

(1) Peduncle commissure. This connects the two lobes dorsally. The fibres enter anteriorly and ventrally to the olfactory lobe. The commissure measures about 350 by 200 μ. The tract separates; one large bundle is situated ventrally along the periphery of the neuropile on the medial side, some fibres pass to the midline of the lobe where they disperse, and another prominent branch leads to the posterior of the basal zone and then turns ventrally. It is not known which of these fibres are afferent and efferent.

(2) Connection with optic lobes. There are many small tracts of 15 - 85 μ in diameter, which enter the lateral side of the peduncle lobe (Figure 66) whose fibres disperse through the lobe upon entering.

(3) Connection with olfactory lobe. Their neuropiles are continuous over a wide area and much exchange occurs.

(4) Connection with lateral pedal lobe (see page 90). This tract leaves the peduncle lobe in the lower region dorsal to the optic fibres before entering the magnocellular lobe, where it turns ventrally into the lateral pedal lobe.

(5) Connection with basal lobes (see pages 43, and 45).

Olfactory Lobe

This paired lobe occupies the medial and posterior regions of the peduncle complex and joins the dorsal basal lobe

in its postero-lateral corner (Figure 64). It is smaller than the peduncle lobe.

Cell layer

This is more irregular than that of the peduncle lobe. Laterally the cell layer is 150 μ and grows thinner dorsally where the cells are also smaller. There are no large cells in the lobe but the small cells of 5 μ are fewer than in the peduncle lobe. In the dorsal region the cells are from 5 to 10 μ and most have a fairly prominent axon. The cells are larger in the ventral half of the lobe. Many cell islands are found in the neuropile.

Neuropile

The fibres which originate in the lobe gather into many small bundles in the dorsal posterior lobule and then enter the neuropile where they remain for a considerable distance travelling mainly in a postero-anterior direction. Between these small bundles there is a very fine network of single axons which anastomose with them. In the dorsal region over a narrow area there are also many moderately large bundles between the peduncle and olfactory lobe which become prominent further ventrally.

Connections

(1) Connection with peduncle lobe (see page 105).

(2) Connection with dorsal basal lobe (see page 39).

(3) Peduncle commissure (see page 105). Because their neuropiles are continuous in the area of this commissure, it is believed that fibres from the olfactory lobe are also included in this connective.

SECRETORY STRUCTURES

Neurosecretory System of the Vena Cava

An examination of the brain in Illex illecebrosus revealed an extensive neurosecretory system in the posterior subesophageal mass (Figure 67) similar to that described in Eledone, Sepia and Octopus by Alexandrowicz (1964, 1965), and in Ommatostrephes and I. coindeti by Martin (1966).The neurosecretory tissue has been termed the 'neurosecretory system of the vena cava' (the NSV system) by Alexandrowicz (1964). No neurosecretion was found in any other part of the brain.

In I. illecebrosus the NSV system covers the entire postero-lateral region of the posterior subesophageal mass. The tissue is bisected posteriorly by the posterior lobule of the visceral lobe and united only over a narrow area ventrally where it surrounds the nerves leaving the lobe. Dorsally it is lateral to the pallial nerve but does not extend above the level of this or the collar nerve. In this area it consists of two large irregular blood sinuses joined across the midline by a small vessel surrounded anteriorly by one or two rows of cells. It appears that all the smaller blood vessels in the NSV system join the large sinus. Each half extends about 1 mm in depth and almost 1 mm in thickness laterally. Generally, it is clearly distinct from the other lobes.

A prominent feature of the NSV system is its extensive supply of blood sinuses. The larger vessels are found at the

posterior periphery while more anteriorly in its interior there are numerous smaller sinuses which are interconnected and surrounded by one or more rows of tightly packed cells.

Three types of neurons could be distinguished in the NSV system: small neurons, and two types of neurosecretory cells adjacent to the small blood sinuses: 'A' cells with chrom-hematoxylin positive granules, and 'B' cells with phloxine positive granules.

The cells which contain no neurosecretion are pear-shaped unipolars, 10 - 15 μ in diameter with round nuclei of 6 - 8 μ. They are found near the junction with the visceral lobe, not adjacent to blood sinuses, but may occur among the neurosecretory cells which surround the blood sinuses. They send their thin axons, combined into small bundles, along an irregular course to the posterior lateral areas where they gather into larger bundles before leaving the lobe with the visceral and posterior funnel nerves. These cells correspond to the 'small neurons' described by Martin (1966) in I. coindeti and Ommatostrephes.

The 'A' cells are elongate, although some appear round and measure 12 - 20 μ in length. They have round nuclei of 7 - 8 μ and axons which taper off from the cell body. They usually occur in small clusters, up to a dozen in each, concentrated in the interior of the tissue near the visceral lobe, although some are scattered. They are associated only with the small blood sinuses and capillaries which interconnect the larger

sinuses, and although they are often in the same area as 'B' cells, their axons always abut on a much smaller blood vessel than those of the latter. Their granules were stained by all three methods used: paraldehyde fuchsin (Figure 68), chrom-hematoxylin (Figure 69), and performic acid-Victoria blue (PAVB) (Figure 70). The PAVB reaction is not given by adjacent unoxidized control sections, which confirms the presence of cysteine; in uncounterstained sections the granules are dark blue. The axons of some cells stained with PAVB and paraldehyde fuchsin, indicating the passage of secretory products down the axon. It was estimated that specimen 6 contained over 30,700 cells with PAVB-positive granules. The granule distribution is probably due to fixation artifacts: they are either concentrated at one pole or scattered uniformly through the cytoplasm; in one cell there were two large granules at opposite ends of the cell with several smaller granules between them. The granules vary in size. The larger ones are often irregular in shape and may result from a fusion of two or more granules: one inclusion with a pale centre was over 4 μ. However, there appears to be a difference in the shapes of the secretory granules stained with chrom-hematoxylin and PAVB: the former are often circular and the latter angular.

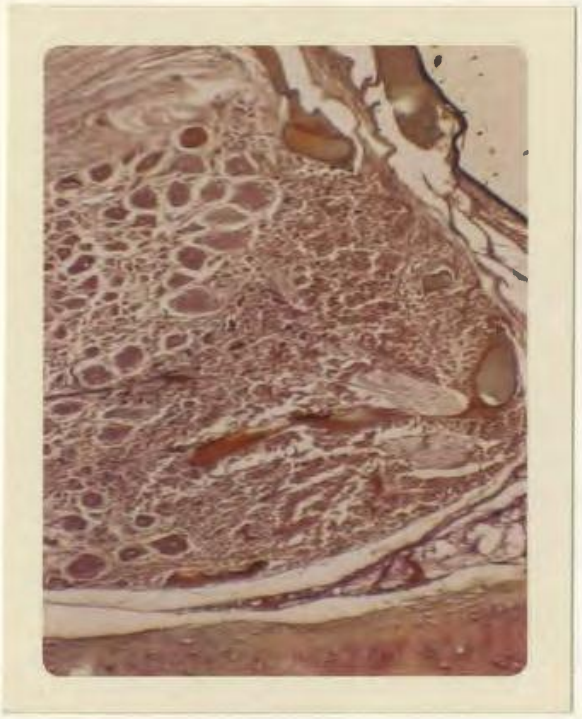
Type 'B' cells (Figure 71) are pear-shaped, about 10 μ long with a round or oval nucleus of 5 - 6 μ, distal to the wall of the blood sinus to which the cells appear attached by their tapered ends. They are concentrated at the periphery of the

Figure 67. Neurosecretory system of the vena cava; sag., x35, paraldehyde fuchsin-Halmi's stain, spec. 3.

Figure 68. Neurosecretory cells, type A; sag., x200, paraldehyde fuchsin-Halmi's stain, spec. 3.

Figure 69. Single neurosecretory cell (type A) with axon next to blood vessel; x700, Gomori's chrom-hematoxytin, spec. 6.

Figure 70. Inclusions in neurosecretory cells (type A); x700, PAVB, spec. 6.



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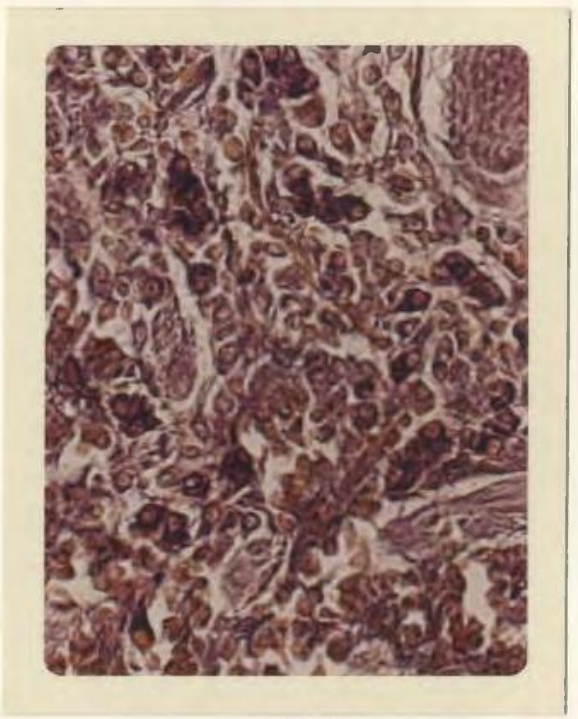

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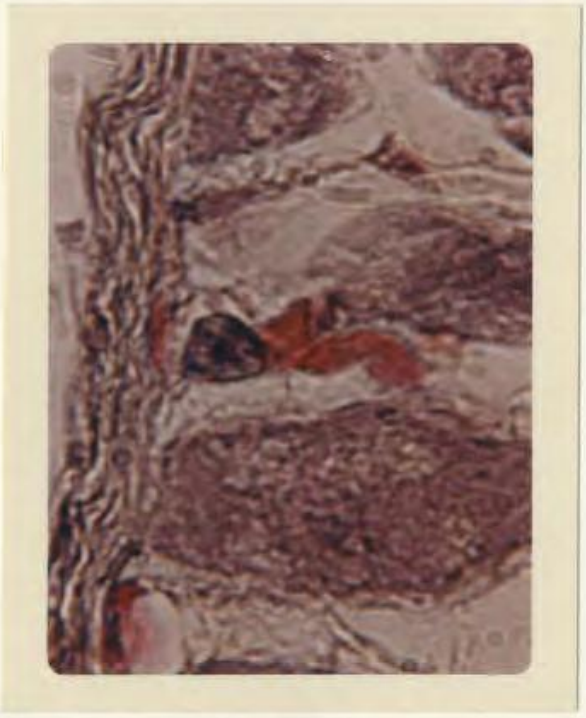

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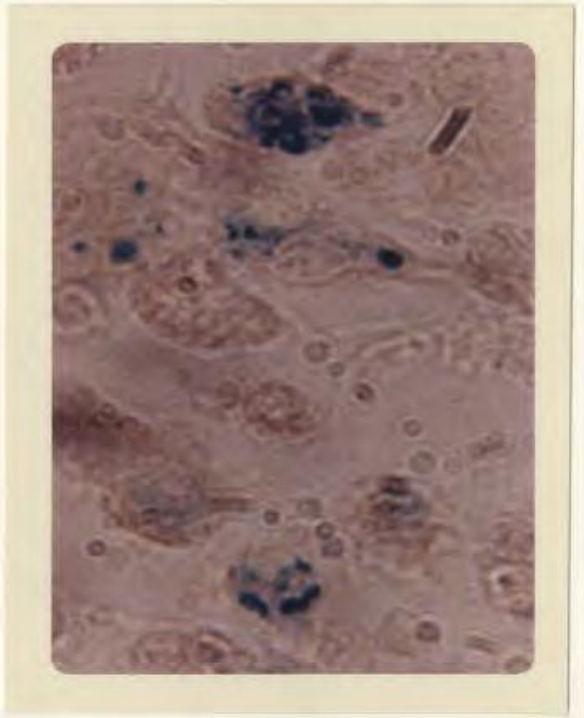

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NSV system in tightly packed rows surrounding the medium-sized blood sinuses. It appears that they release their granules into the sinuses where their thick processes filled with secretion touch their walls. These cells show the same features as the 'pear-shaped cells' in I. coindeti described by Martin (1966). They are smaller and more basophilic than the 'A' cells. The granules of 'B' cells stain with phloxine and under oil immersion appear fine (1 μ), and spherical, evenly distributed in the cytoplasm as well as in the axons. The granules were not seen in sections stained with paraldehyde fuchsin, counterstained with Halmi's stain, and may have been obscured by the intensive staining of the cytoplasm with orange G. They were unstained in oxidized and unoxidized sections with Victoria blue, and are more numerous than the 'A' cell granules.

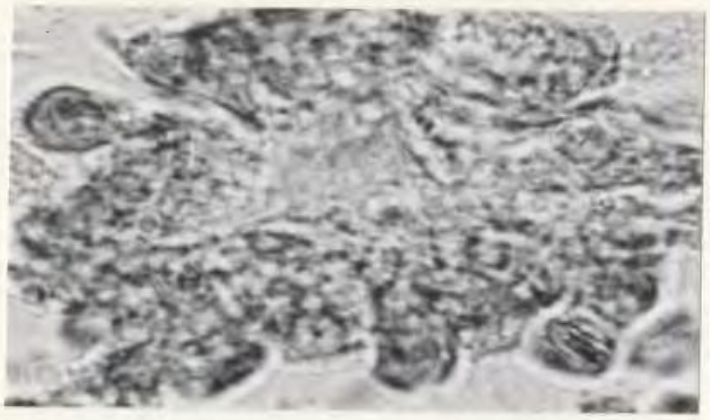
Optic Gland

This paired gland is adjacent to the central nervous system (Figure 64), but its structure is not nervous. It is posterior to the olfactory and dorsal basal lobes, receives a small nerve from the latter and lies at the dorso-posterior margin of the optic lobe. When fresh it is a small oval body (about 1 by 0.5 mm), contrasted by its yellow-orange colour against the white body and pale yellow of the optic lobes. The gland is surrounded by a capsule of loose precollagenous fibres (Cazal and Bogoraze, 1943) which is continuous with that of the dorsal basal lobe.

Figure 71. Neurosecretory cells, type B; x1000, phloxine, spec. 3.

Figure 72. Optic gland; frontal, x80, PAS, spec. 5.

Figure 73. Optic gland cells; x700, Heidenhain's Iron-Hematoxylin, spec. 5.



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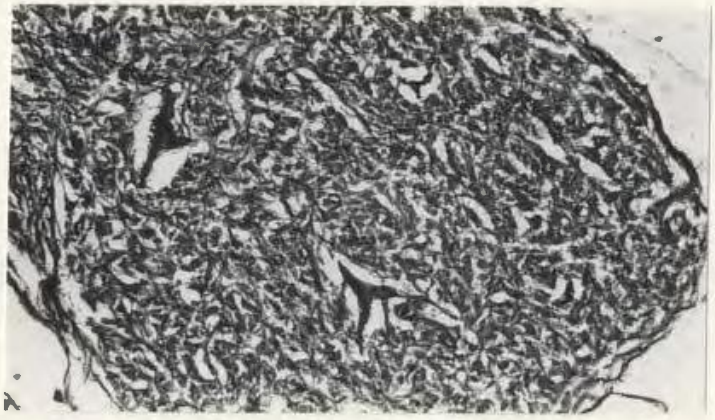

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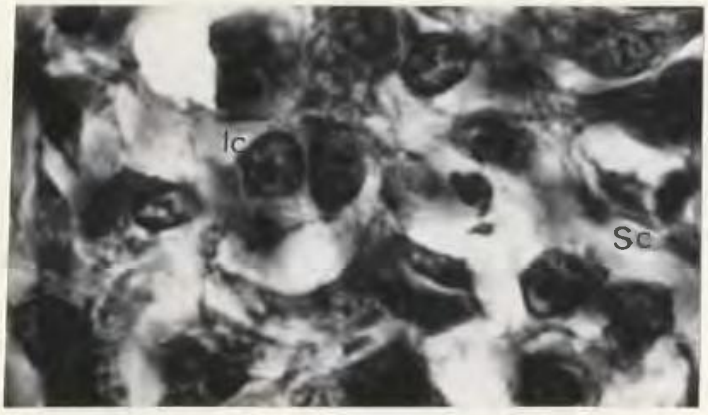

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A prominent feature of the gland is its rich network of blood vessels (Figure 72). On its ventro-medial surface a large vessel, over 150 μ in diameter, is connected to numerous small blood vessels (as little as 3 μ) which penetrate the gland.

Two types of cells are present in the gland (Figure 73). The large cells measure about 12 μ and have large, distinct round or oval nuclei of 6 - 8 μ, each with a large nucleolus of 2 μ and many dark, basic staining granules usually located near the periphery of the nuclear membrane. Apart from this, both nucleus and cytoplasm are relatively clear. These cells, following silver impregnation and gold toning, show several extensively branched thick and thin processes in intimate contact with the walls of the larger blood vessels and capillaries which at times appear to penetrate through the walls, although the fibres seen inside blood vessels may actually pass directly above or below them.

A second type of cell is smaller and more oval, measuring about 6 - 7 μ with a round or oval nucleus of about 3 - 4 μ which is more basophilic than the above type. These cells are also closely associated with the capillary network in the gland. Their nuclei have a granular appearance which is partially obscured by the intensity of basophilia. These cells may also have processes similar to those of the large cells. The staining reactions of both types of cells will be described in the next section.

Parolfactory Vesicles

These glands were first discovered by Thore (1939) in Illex coindeti and are very similar in structure to the epistellar bodies of octopods (Young,1936; Cazal and Bogoraze, 1944, 1949; and Boycott and Young,1956).

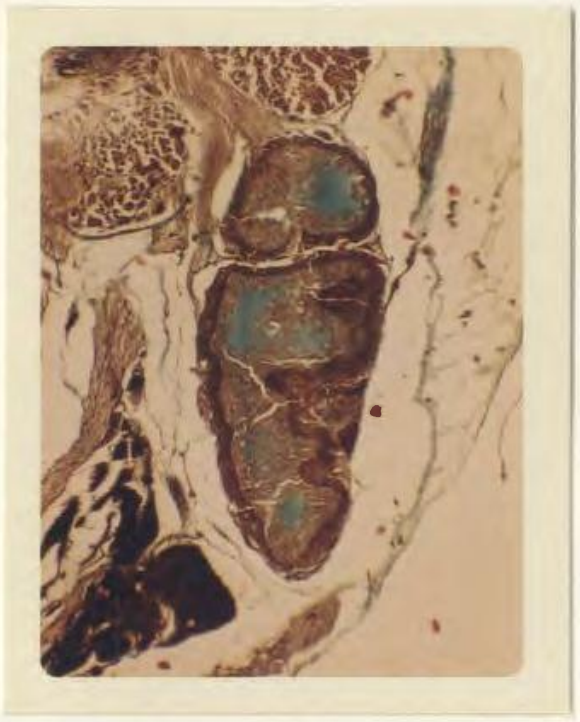
There are two pairs of parolfactory vesicles, one large and the other considerably smaller. When fresh they are orange in colour. The larger pair (Figures 74, 75, 76) is ventral and entirely subesophageal: it lies beneath the optic tract and the peduncle complex mostly below the level of the subesophageal ganglia, and its axis is dorso-ventral. The smaller pair is supraesophageal and anterior to the larger pair above the optic tract and peduncle complex, and is oriented antero-posteriorly. In specimen 6 one of the larger vesicles measured 2.4 x .45 x .45 mm, and the smaller one .725 x .35 x .325 mm. In a medium-sized specimen (No. 14, 178 mm) the larger vesicle was only 2mm long. Individual differences were noted in the size and shape of the organ.

The larger vesicle receives a nerve from the peduncle and possibly the olfactory lobe, which passes ventrally along the anterior surface of the vesicle, where it branches laterally in the mid-region. Two distinct nerves penetrate the cell layer of the small vesicle separately, with one branch passing dorsally into the posterior end of the vesicle, and an anterior branch along its ventral surface.

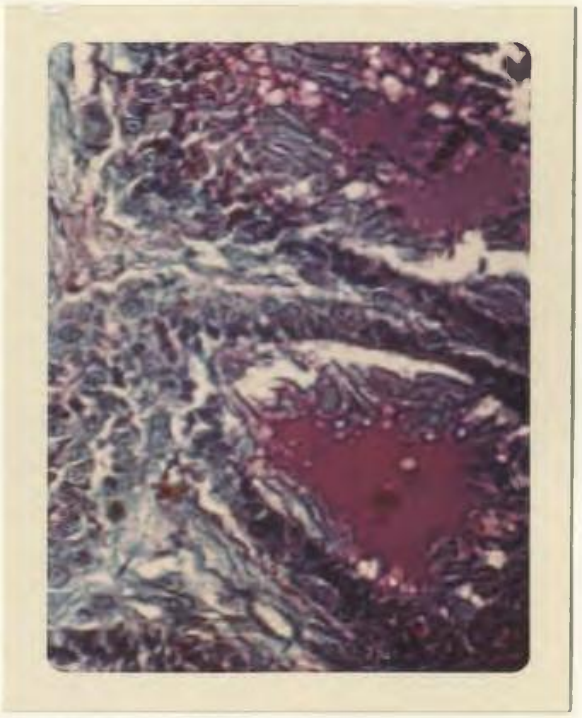
Figure 74. Parolfactory vesicle showing follicular structure and innervation; sag., x 35, Masson's trichrome, spec. 3.

Figure 75. Parolfactory vesicle showing cells, protrusions and colloid; sag., x200, PAS, spec. 6.

Figure 76. Parolfactory vesicle showing positive reaction for protein with alloxan-Schiff; frontal, x100, spec. 5.



74



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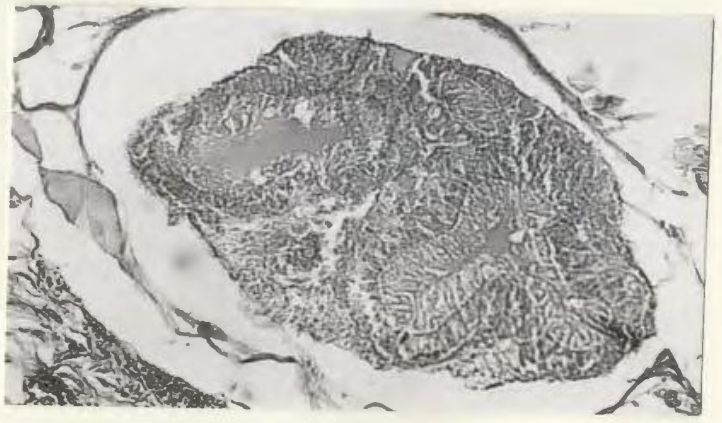

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Both pairs of vesicles consist of frequently interconnected follicles filled with a mass of colloid (Figure 74). Each follicle superficially resembles a lobe of the brain, except that its cells surround an internal body of colloid. The small pair of glands has only three or four follicles, of which one is long and oval while the other two or three are all less than 100 μ in diameter and are usually elongated. In the larger pair the follicles are more irregular in shape and variable in size. There are 8 - 14 follicles and some may reach 900 μ in length. Each is partially enclosed by a capsule of collagenous connective tissue and blood vessels adjacent to the outer cell layer. The layer of follicular cells surrounding the colloid is usually 25 - 30 μ thick. The cells are small, very tightly packed, and showed little detail in the preparations examined.

Two types of follicular cells were recognized (Figure 75). The proximal columnar cells (10 x 5 μ) next to the colloid have large oval nuclei (7 x 3 μ) or in fewer cases smaller and less oval (3 x 5 μ) nuclei, which are more basophilic than those of the distal cells and resemble the small cells of the optic gland. The distal cells lie directly beyond the inner layer of proximal columnar cells at the periphery of the follicle. They are somewhat pear-shaped or triangular and have processes which penetrate through the layer of proximal cells into the lumen of the follicle. They are large (15 - 20 μ), with nuclei (6 - 10μ)

containing numerous dark granules (1 μ), demonstrated with hematoxylin, which resemble granules seen in the nuclei of the large cells in the optic gland. The protrusions in the follicles (Figure 75) reach 3 - 4 μ in width upon penetrating the colloid and may widen up to 8 μ. The depth to which they penetrate varies but probably does not exceed 30μ.

The cytoplasmic granules in both types of cells stain less intensely with gallocyanin than neurons of a similar size. The material present in the lumen of the follicle is mainly colloid with several vacuoles up to 15 μ in diameter, generally located near the periphery in association with the protrusions from the distal cells. They may be air bubbles or lipid droplets but their nature could not be determined. The colloid of the vesicle is usually homogenous and its staining reactions will be described in the following section.

HISTOCHEMISTRY

The results of the histochemical tests performed are presented in table 2. Many structures outside of the central nervous system, such as cartilage, blood plasma, white body, salivary glands and the cuticle of the esophagus, were intensely stained with certain methods and were valuable as positive controls for the reagents.

Optic Gland

The small cells contained PAS-positive granules which either formed a ring around the nucleus or were united in a mass. In some cases the granules formed a long string, but as these cells are in close association with the capillaries, the extent of the PAS-positive material was difficult to determine. The intensity of the staining was reduced only slightly after diastase digestion but was almost completely eliminated after acetylation. The large cells had no PAS-positive material. The alcian blue test for acid mucopolysaccharides and the toluidine blue for metachromasia were negative in both types of cells.

With alloxan-Schiff the cytoplasm of the small cells stained more deeply than that of the larger, indicating a greater concentration of protein. The Millon reaction as well as the DMAB-nitrite test for indole groups gave a slight reaction in both cell types, although not a precise localization. The stainable DNA content was greater in the small than in the large cells and the RNA content, shown with gallocyanin, was moderate in both but less than in neurons of similar sizes.

Table 2

Results of Histochemical Tests

	PAS	Controls diast.	Controls acetyl.	Alcian blue (acid mucop.)	Toluidine blue (metachromasia)	Alloxan-Schiff (protein)
Optic Gland:						
Large cells (cytoplasm)	-	-	-	-	-	+
Small cells (cytoplasm)	++	+	-	-	-	++
Parolfactory Vesicles:						
Colloid	+++	+++	-	-	-	++
Large cells	++	++	-	-	-	+
Protrusions	±	±	-	-	-	+
Small cells	±	±	-	-	-	++
Neuropile	-	-	-	-	-	+
Neurons (cytoplasm):						
Large	++	++	-	-	+++ (Nissl bodies blue)	+++ (and nucleolus)
Medium	+++	+++	-	-	++ (Nissl bodies blue)	+++ (and nucleolus)
Small	±	-	-	-	+ (Nissl bodies blue)	++ (and nucleolus)
Cartilage matrix	+++	++	-	++	+++	+
Blood plasma	+++	++	-	-	-	+++
Esophagus:						
Cuticle	+++	++	-	-	-	+
Mucosal epithelium	-	-	-	-	-	+
Submandibular gland:						
Cells (cytoplasm)	+++	++	-	-	-	++
Colloid	+++	++	±	++	+++	-
White body	±	-	-	-	-	+++

Table 2

Results of Histochemical Tests

	Millon (tyrosine)	DMAB-Nitrite (tryptophan)	Feulgen (DNA)	Gallocyanin (RNA)	Sudan black B Luxol fast blue
Optic Gland		+			
Large cells (cytoplasm)	+		+	++	=
Small cells (cytoplasm)	+	++	++	++	+
Parolfactory Vesicles:					
Colloid	-		-	-	+
Large cells	+		-	++	-
Protrusions	+		-	-	+
Small cells	+		++	++	±
Neuropile	+	+	-	-	+
Neurons (cytoplasm):					
Large	+ (and nucleolus)	+ (and nucleolus)	+	+++	-
Medium	+ (and nucleolus)	+ (and nucleolus)	+	+++	-
Small	-	-	++	+++	-
Cartilage matrix	-	-	-	-	++
Blood plasma	+++	+++	-	-	±
Esophagus:					
Cuticle	-	-	-	-	+
Mucosal epithelium	++		+	+++	-
Submandibular gland:					
Cells (cytoplasm)	-		++	+++	
Colloid	-		-	-	
White body	+	+++	+++	+++	+

Table 2

Results of Histochemical Tests

	PAVB (cysteine)	control	Chrom-hematox. (neurosecretion)	P.F. (neurosecretion)
Optic Gland:				
Large cells (cytoplasm)	-	-	lt. blue	lt. violet-orange
Small cells (cytoplasm)	-	-	pink	lt. violet-orange
Parolfactory Vesicles:				
Colloid	±	-	-	orange
Large cells	-	-	lt. pink	orange
Protrusions	+	±	-	lt. violet
Small cells	-	-	lt. pink	orange
Neuropile	-	-	-	lt. violet
Neurons				
Large	-	-	lt. blue	lt. violet-orange
Medium	-	-	lt. blue	lt. violet-orange
Small	-	-	red	orange
Cartilage matrix	+++	+++	blue	violet
Blood plasma	-	-	red	orange
Esophagus:				
Cuticle	+++	+	lt. blue	orange
Mucosal epithelium	-	-	pink	orange
Submandibular gland:				
Cells	-	-		violet
Colloid	++	++		violet
White body	-	-	red	orange

Table 2

Results of Histochemical Tests

	Lead sulphide (acid phosphatase)	Eosin	Mallory triple	Masson's trichrome
Optic Gland:				
Large cells (cytoplasm)		-	violet	brown
Small cells (cytoplasm)		-	violet	brown
Parolfactory vesicles:				
Colloid		+++	blue	green
Large cells		-	violet	brown
Protrusions		+++	violet	brown
Small cells		-	violet	brown
Neuropile	-	++	violet	brown
Neurons:				
Large	++		violet	brown
Medium	++		violet	brown
Small	++		violet	brown
Cartilage matrix	-		blue	green
Blood plasma	-	+++	violet	brown
Esophagus:				
Cuticle	-	+++	blue	green
Mucosal epithelium	+++		violet	brown
Submandibular gland:				
Cells	++	-	violet	brown
Colloid			blue	
White body	+++	-	red	brown

No neurosecretory material was found in the gland. With paraldehyde fuchsin and orange G the cytoplasm of most cells stained light violet or orange. With chrom-hematoxylin the nuclei of the large cells contained granules, usually less than 1 μ, darkly stained in contrast to the light coloration of the cell. These were not shown with PAVB and may represent nucleolar granules.

Parolfactory Vesicles

The colloid of the parolfactory vesicles stained with eosin (H and E), aniline blue (Mallory triple), light green SF (Masson's trichrome) and orange G (paraldehyde fuchsin-Halmi's stain). It was not stained with paraldehyde fuchsin, PAVB, chrom-hematoxylin or phloxine which rules out the possibility that it may contain neurosecretory material. Its intense staining with PAS was unaffected by diastase digestion but completely eliminated by acetylation. The colloid was not metachromatic and did not stain with alcian blue. It was moderately stained with alloxan-Schiff which indicates the presence of proteins, but not as deeply as the cytoplasm of the follicular cells. The Millon reaction appeared negative. With Sudan black B no reaction was observed but a weakly positive patchy staining was shown by Luxol fast blue in paraffin sections.

The small proximal cells contained PAS-positive material, which usually formed a homogenous mass along the

border of the lumen. Many of the larger distal cells had PAS-positive granules distributed evenly throughout the cytoplasm. These granules varied in size and were considerably larger than those seen in the protrusions. The PAS staining of these particles was not affected by diastase digestion but eliminated by acetylation.

The protrusions from the larger distal cells into the follicular lumen were stained brown with Masson's trichrome. A slight reaction was given by PAVB, particularly along the sides of the protrusions. The staining also appeared to a lesser extent in adjacent unoxidized sections, so it could not be fully attributed to sulphydryl or disulfide groups. With paraldehyde fuchsin these protrusions stained violet with varying intensity, but not as intensely as the granules in the neurosecretory cells of the NSV system. These processes, however, were not stained with chrom-hematoxylin. The PAS-positive substance seen at the edges of the protrusions was probably part of the colloid, but some was also present within the protrusions in the form of very small granules forming a thin thread along their centre, from where they enter the colloid down to its tip. It was unaffected by diastase digestion but eliminated by acetylation. The protrusions were negative to staining methods for metachromasia and acid mucopolysaccharides but gave a weak positive reaction with alloxan-Schiff and the Millon reagent, which indicate low protein content. In paraffin sections Luxol fast blue gave slight positive staining.

No neurosecretory material was detected in either one of the two follicular cell types. Under high magnification some dark staining granules were seen in the nuclei of the distal cells stained with chrom-hematoxylin and paraldehyde fuchsin, but these were also visible after some routine stains and probably were nucleolar granules.

Neuropile and Neurons

In the neuropile only the small blood vessels and connective tissue were stained with PAS. The neuropile was also unstained with alcian and toluidine blue. It was stained lightly with alloxan-Schiff and also with the DMAB-nitrite and the Millon reagents. Sudan black B stained the neuropile slightly indicating that very little lipid was present in the axons, or that the material failed to survive dehydration and Tissuemat embedding.

The neurons stained to a varying degree with PAS, depending on their size. The small neurons in the vertical lobe, perhaps because of their scarce cytoplasm, did not show any staining. The medium-sized neurons had the greatest amount of cytoplasmic PAS-positive material which varied in different regions of the brain, with the largest concentration in those of the visceral lobe. These particles were usually amassed near the nuclear and cell membranes with some granules in between them. The large cells had a similar distribution of PAS-positive material and their nucleoli also stained slightly. The reaction in these cells was eliminated by acetylation but

unaffected by diastase digestion. The same particles were not stained with alcian or toluidine blue, but the latter stained the Nissl granules deep blue. Both cytoplasm and nucleoli stained intensely though not uniformly with alloxan-Schiff, while the nucleus remained unstained. The cytoplasm and nucleoli of the larger neurons also stained slightly with DMAB-nitrite and the Millon reagents. Sudan black B gave no reaction. All neurons showed a strong nuclear reaction for acid phosphatase while the fibres of the central nervous system did not stain with the exception of the optic lobe fibres, which along with the cells stained black and thus could be easily recognized. The nuclear staining and probably also that of the optic lobe axons may be diffusion artifacts. The other methods for enzymes - alkaline phosphatase, non-specific esterase and 5-nucleotidase gave negative results.

Other Structures

The cartilage matrix stained intensely with PAS; this was abolished by acetylation but unaffected by diastase. It demonstrated strong metachromasia with toluidine blue and stained deeply with alcian blue. The cartilage stained intensely with PAVB, very weakly with alloxan-Schiff, visible mainly along the edges of the mass, but not with the DMAB-nitrite or Millon reagents. The PAVB staining was also seen in unoxidized control sections, indicating that it is not due to cysteine but to sulfate or other acid groups present in the cartilage, which are also responsible for its strong metachromatic and alcian blue staining. The cartilage showed no staining with Sudan black B.

The blood plasma also stained intensely with PAS. This was unaffected by diastase digestion but eliminated by acetylation. It contained no metachromatic substances or acid mucopolysaccharides. With alloxan-Schiff it stained more intensely than the colloid of the parolfactory vesicles,and the Millon and the DMAB-nitrite tests also gave strong reaction. The plasma itself was not stained with PAVB, but the walls of the thicker blood vessels were and this was probably due to the presence of elastic fibres, usually arranged in two layers. There was no staining with the lipid stains.

The cuticle of the esophagus gave an intense reaction with PAS which was unaffected by diastase digestion but eliminated by acetylation. It stained blue with Mallory triple, green with Masson's trichrome, reddish-violet with paraldehyde fuchsin and dark blue with chrom-hematoxylin. The dark blue staining with PAVB was greatly reduced in adjacent unoxidized sections. There was no reaction with alcian blue or the metachromatic stain. It stained very pale pink with alloxan-Schiff, much less than the columnar epithelial cells. It did not stain with DMAB-nitrate and the Millon reagent, which indicated a very low content of protein, but gave a weak reaction for the lipid methods. Acid phosphatase was concentrated in the distal border of the epithelial cells and also appeared in the nuclei, though not in the proximal cytoplasm, and slightly in the epithelial basement membrane. However, the most intense acid phosphatase reaction was seen in the white body which stained entirely black, but remained colourless in control sections.

The colloid of the submandibular gland was stained blue with Mallory triple. Both the cell cytoplasm and the colloid were PAS-positive; the reaction was reduced slightly after diastase digestion,and almost completely eliminated by acetylation. Only the mucoid was metachromatic (reddish-violet with 0.5% aqueous toluidine blue) and also stained moderately with alcian blue but not with alloxan-Schiff or Millon reagents. It stained deep purple with paraldehyde fuchsin, and moderately with PAVB in both oxidized and unoxidized sections indicating that it is sulphated but does not contain much cysteine. The cell cytoplasm stained weakly with alloxan-Schiff demonstrating the presence of little protein.

DISCUSSION

Bullock (1965) states that the morphological plan of the nervous system in cephalopods is basically the same as in other molluscs. He points out, however, that they differ greatly superficially and several new ganglia have been added.

The central nervous system in Illex illecebrosus is similar to those of other coleoid cephalopods but differs in its degree of decentralization. All are built on the same plan. The same broad divisions are found and homologus parts in different cephalopods have the same functions (Wells, 1962).

A classification of the brain centres was first established for Sepia officinalis by Sanders and Young (1940) and described for several species of octopods and decapods by Wirz (1959), who interpreted the relative size of the lobes and centres as a reflection of the modes of life among different cephalopod groups. Boycott's (1961) physiology-oriented classification differs from that of Sanders and Young. He recognized five basic centres in Sepia: lower, intermediate, and higher motor centres, silent areas and primary sensory centres.

Most of the connections within the brains of cephalopods tabulated by Bullock (1965) were also found in I. illecebrosus. Although some of the connecting pathways reported by other workers were not seen, such as that between the peduncle and anterior basal lobes, they are nevertheless assumed to exist.

The cerebral ganglion is the major supraesophageal ganglion in coleoid cephalopods. Its shape, position and structure in I. illecebrosus is similar to that in I. coindeti and differs very little from that of other decapods such as Loligo and Sepia, but major differences are observed on comparison with that of Octopus. Thus, in Octopus the superior buccal is fused to the cerebral ganglion (Young, 1965a) while in Illex the two are widely separated.

The lobe which occupies the anterior portion of the cerebral ganglion in I. illecebrosus is the posterior buccal, known to earlier workers (Hillig, 1912; Richter, 1913) as the inferior frontal lobe in decapods. The lobe is well defined in octopods. Thore (1939) suggested that this was actually the posterior part of the buccal lobe rather than the inferior frontal lobe because the arrangement of the neuropile as well as the cell sizes and types of the rind indicated that it was a motor lobe. Thore's "pars posterior" must therefore correspond to the posterior buccal lobe of octopods which represents a ventral extension of the subfrontal lobe (Young, 1965b) and was described by Thore as the "pons buccalo-frontalis inferior" in Octopus, and the "pars anterior" is the superior buccal ganglion. He did not discuss the inferior buccal ganglion. Young (1963a) suggested that the posterior buccal lobe of decapods may represent the posterior buccal, subfrontal and inferior frontal lobes of octopods, but in view of their inferior tactile learning ability and discrimination these functions in decapods may have been taken over by another higher or silent

area in greatly reduced form. Boycott (1961) demonstrated the motor function of the posterior buccal lobe by producing slight movements of the buccal mass upon electrical stimulation of this centre. The neurons of this lobe are the largest within the cerebral ganglion and quite unlike the small sensory cells described by Young (1963a) in the subfrontal and inferior frontal lobes. Further learning experiments on decapods are needed to reach an understanding of the functions of the brain centres. In Octopus the posterior buccal lobe is paired (Young, 1965b) while in Illex it is bilaterally symmetrical but unpaired. An embryological study of the central nervous system is needed to determine whether the posterior buccal lobe of Illex and other decapods is homologous to that of octopods. The connective which leaves the posterior buccal lobe postero-ventrally and passes within the cell layer of the anterior basal lobe seems to correspond to the palliovisceral-posterior buccal tract described in Octopus by Young (1965b).

In the vertical lobe of Illex Thore (1939) also noted that the dorsal surface was free of neurons and that they were concentrated anteriorly and posteriorly. In Sepia and Loligo there are many cells on the dorsal surface (Thore, 1939). The surface of the vertical lobe in Illex is smooth whereas in Octopus it is folded into five gyri (two lateral, two medial, one median), each with its own distinct neuropile but an exchange of fibres (Thore, 1939, Young, 1964a).. Thore believed the entire vertical lobe of decapods to be homologous to the median gyrus of Octopus, but this idea has gained little support. The

parallel neuropile of the vertical lobe was described in Sepia (Owsjannikow and Kowalevsky, 1867; Thore,ibid) and in Illex coindeti (Thore,ibid). Thore also noted that the row of cell islands outside the parallel neuropile seen in Illex was not present in Octopus. The methods used in this study did not demonstrate clearly the patterns of axons in the neuropile, but Young (1964) concluded that the fibres leaving the lobe are the processes of the large neurons while the processes of the smaller cells end within the vertical lobe. The small cells have a single trunk which divides into a tuft of equal branches in the neuropile. Because of their complexity the connections of these fibres are not well understood. The vertical lobe is regarded as the highest centre of the brain (Bullock, 1965). Boycott (1961) described it as a silent area in Sepia upon electrical stimulation and Sanders and Young (1940) as an association centre inferring a learning and memory function which was later verified in Octopus. Its connections are restricted to other silent areas of the brain.

The shape and position of the unpaired superior frontal lobe in Illex correspond to those of other decapods (Thore, 1939). The division of its cell population was not noted by Thore in the decapods he examined. In Octopus there is one median and two lateral superior frontal lobes, which differ structurally and have different connections (Young, 1964 a, b), but these are not found in decapods (Young, 1964b; Boycott, 1961). The median region in Illex is not homologus to the median superior frontal

lobe of Octopus, since in Octopus the median lobe contains the smaller neurons (Young, 1963a) which are located in the lateral regions in Illex. Young has noted that the superior frontal lobe of decapods corresponds only to the lateral superior frontal lobes of Octopus. In I. illecebrosus two connections with the posterior buccal lobe were seen on the left side but only one on the right, while in I. coindeti Thore (ibid) reported several pairs, but saw only two pairs in Sepia and Loligo. The superior frontal lobe also falls into the class of silent areas defined by Boycott (1961) and receives many fibres from the optic lobes which come in contact here with those of the vertical lobe.

The anterior basal lobe in I. illecebrosus corresponds to the central lobe of Thore (1939). Its position and shape are similar to those of other decapods but on comparison with published figures it appears to be more tapered at the postero-ventral end and larger on the postero-anterior axis. It is relatively larger than the homologus lobe in Octopus. Thore (ibid) directly applied his description of this lobe in Sepia to I. coindeti and also noted the unusual distribution of cells on its anterior surface in Sepia. Nearly all its connections are to motor centres. A large connective with the peduncle lobe described in Octopus (Messenger, 1967) was not found in I. illecebrosus, although a bundle of fibres in the anterior basal lobe may correspond to it. The neurons of the lobe are medium-sized and characteristic of a higher motor centre, and their grouping by size in different areas may indicate divisions

of function within this lobe.

The posterior basal lobe was described as the occipital lobe by Thore (1939). In Sepia it was divided into six lobes by Boycott (1961) but these divisions are not well differentiated in Illex and in many cases share neuropiles. The complex in Illex is similar to that of Sepia and the differences are essentially only quantitative (Thore, ibid). It contains some of the higher motor centres of the brain but other areas are silent (Boycott, 1961), indicating clear divisions of function. The silent areas are usually the most dorsal and probably work in conjunction with the vertical and superior frontal lobes.

The superior buccal ganglion was found to be unpaired and bilaterally symmetrical in I. illecebrosus although Richter (1913) reported it to be paired. It is also unpaired in Octopus (Young, 1965a) but located in a different position. Thore (1939) suggested that this part of the brain may have been displaced by the great development of the optic lobes,but whether this decentralization is the more primitive or a more specialized condition is still a matter of opinion. Even the brain of Nautilus is more concentrated than that of pelagic decapods such as Illex (Young, 1965c). It is probably more correct to assume that decentralization results from the elongation and narrowing of the body which would tend to make the squid a more efficient and active swimmer. This would mean that the decentralization of the pelagic decapod brain and the condensation and greater development of the octopod brain are both specializations, but working in opposite

directions, and each would be better suited to the mode of life of the respective animal. Microscopically the ganglion is unspecialized and its neuropile is without prominent features (Bullock, 1965). Its morphology in Illex has been described and diagrammed by Richter (1913). Several of the connections observed by Young (1965a) in Octopus were not seen in Illex, but it is possible that many of the nerves associated with the esophagus, which were not followed to their origins or terminations, may correspond to those described in Octopus.

The inferior buccal ganglion is paired in Octopus (Young, 1965a), and Richter (1913) found it to be paired in I. coindeti and several other oegopsid squid. However, the present histological preparations show the ganglion to be unpaired in I. illecebrosus which may, however, result from complete fusion of two halves during early ontogeny. The anterior group of nerves described as the lingual corresponds to the "nervi linguales" of Richter and probably to several of the anterior nerves described by Young (1965a) in Octopus.

In Illex the anterior subesophageal mass is located much further anteriorly than in the case of octopods (Thore, 1939), but in this respect Sepia resembles Octopus. Consequently many nerve fibres and bundles cross this relatively long distance between the anterior and middle subesophageal masses.

The position of the brachial ganglion in Illex in relation to the rest of the brain is comparable with that of other oegopsid squid (Richter, 1913). In the brachial ganglion of Octopus, Young (1963a) distinguished pre- and post-brachial lobes, but no such divisions were found in Illex. The ganglion is unpaired and bilaterally symmetrical in Illex. In Octopus a connective from the dorsal surface of the ganglion passes above the esophagus and forms a ring (Bullock, 1965), but this was not observed in Illex. The ganglion is considerably better developed in Illex than in Sepia and has a greater neuropile mass (Thore, 1939). The neurons in Octopus vary greatly in size and their distribution in size classes estimated by Young (1963a) resembles that found in Illex. Bullock (1965) suggests that in cephalopods the brachial ganglion may represent a new ganglion associated exclusively with the arms. The eight brachial nerves in Illex arise from only three definite root bundles, but it is probable that some of the roots contain two ill-defined branches, as in other cephalopods the nerves arise from five root bundles (Bullock, 1965). The arm nerves in Illex have the same roots as in Sepia (Thore ibid): the eight brachial nerves receive fibres from both the brachial and pedal ganglia, while the tentacle nerves receive fibres from the pedal ganglion only. This is supported by the findings of Boycott (1961), who showed that the brachial ganglion controls only the arms, whereas the anterior pedal lobe both the arms and tentacles.

Young (1963a) speculates that the roles of the two ganglia may be divided so that the brachial controls the detailed movements of individual arms, while the pedal directs their unified action in activities such as swimming. He also suggested that many of the small neurons in the anterior region of the brachial ganglion may be concerned with the coding of chemotactile information from the arms. Boycott (ibid) classified this ganglion as an intermediate motor centre.

The middle subesophageal mass was described as the lateral lobe by Thore (1939), and in Illex contains the same divisions described by Boycott (1961) for Sepia. The anterior chromatophore lobe, regarded by Boycott as not well differentiated in decapods, could be seen clearly in Illex in all three planes. It resembles the corresponding ganglion in Sepia and its connections are the same (Thore, ibid). Thore noted the medial unpaired connective between the anterior and middle subesophageal masses in I. coindeti which corresponds to root 1 described here, and believed that its elongation was a secondary characteristic. In its ventro-posterior corner in octopods there is a paired lobe described by Thore (ibid) as the anterior funnel or infundibular lobe which gives rise to the anterior funnel nerve. Thore stated that this was less differentiated in decapods, but Bullock (1965) proposed that the infundibular lobe of octopods may be homologous to the ventral magnocellular lobe in decapods. Thore did not

describe the magnocellular lobe as such but recognized the region. This ganglion is the major source of cranial nerves which were studied in oegopsid squid by Richter (1913) and in Octopus, Sepia and Illex by Thore (ibid). Although the nerves were not followed here to their destinations, homologies were determined by comparison with the work of Richter and Thore. The anterior funnel nerve has roots in the posterior pedal and visceral lobes as described by Thore. The branch which leaves the pedal lobe with the anterior funnel nerve and turns medially may correspond to the medial pallial adductor nerve of Thore. The median funnel nerve found between the two anterior funnel nerves was not observed by Richter (1913). Only two branches of the statocyst nerve were seen in Illex although Thore (ibid) reported three. The innervation of this organ is very complex and was described in Octopus by Young (1960). Many fibres of the statocyst nerve end near the central pedal commissure whose fibres according to Boycott (1960) come in contact with the afferents, and thus through the information received from the statocysts the movements caused by the pedal lobe can be interpreted. The commissure separates the anterior and posterior pedal lobes and most of the fibres of the commissure come from the lateral pedal or the supra-esophageal centres and do not leave the ganglion (Boycott, ibid). The other major nerves arising from the pedal lobes appear to correspond to the anterior and posterior head retractor nerves and the posterior occulomotor nerve whose roots are much like those

diagrammed for Sepia by Thore (ibid). The magnocellular lobe is the source of the first order giant fibres in decapods and has two commissures (Young, 1939). Young states that giant fibres from the magnocellular lobe fuse in the posterior magnocellular commissure and synapse with fibres in the visceral lobe.

The peduncle complex in decapods is more closely associated with the central brain than in octopods, resembling the situation seen in the larval Octopus (Messenger, 1967). In Illex the olfactory lobe begins as a lateral extension of the dorsal basal lobe. Here the optic and peduncle commissures are not situated as in Sepia (Boycott, 1961) where they begin more dorsally, or in Octopus where they originate dorsal to the esophagus (Messenger, ibid). This variation may be due to the shape of the peduncle complex: in Illex it is elongated, while in octopods it is flattened dorso-ventrally, and also more elongated than in Sepia (Messenger, ibid).

In the posterior subesophageal mass of decapods Thore (1939) saw two distinct lobes, which he called the visceral and accessory. In the accessory lobe he distinguished two parts - the side and medial gyri. The side gyrus would correspond to the posterior chromatophore lobe, and the medial, where the cells were considerably larger, to the fin lobe. In Illex the side gyrus has moved to occupy the posterior region of the accessory lobe. The fin lobe is absent in octopods (Young, 1963a), as might be expected in animals with no fins. The cells of the fin lobe are the largest in the brain and show little gradation in size. Their

axons leave the lobe in the pallial nerve, and since the fins are the most distant from the brain of all organs, these large axons are capable of very rapid impulse conduction to integrate the movements of the animal. The fibres from the posterior chromatophore lobe innervate the chromatophores in the posterior part of the body directly. The vasomotor lobes, quite distinct in octopods where they are represented by a group of six lobules at the back of the posterior subesophageal mass (including a dorsal, a ventral and two lateral pairs) (Boycott, 1961 and Young, 1963a), are much less discrete in decapods. In Illex the posterior lobule, wedged between the neurosecretory tissue and the remainder of the posterior subesophageal mass, may represent the fused dorsal and ventral vasomotor lobules. The root bundles in the visceral lobe are similar to those described previously in I. coindeti by Thore (ibid), but the large connective from the peduncle lobe was not found here.

The plan of the neurosecretory system in Illex differs from that of Eledone and Octopus (Alexandrowicz, 1964, 1965), mainly in the arrangement of nerves leaving the posterior end of the posterior subesophageal mass in these octopods, and resembles that of Sepia (Alexandrowicz, 1965). In Illex the NSV tissue surrounds the roots of the posterior funnel and vena cava nerves and also the visceral nerves. However, the system has been studied in oegopsid squid by Richter (1913). Its extent is similar to that described by Martin (1966) in I. coindeti and Ommatostrephes, but does not seem to be as extensive

in the central posterior region. In I. illecebrosus its structure is identical to that described by Martin (ibid). However, in the present work two types of neurosecretory cells are identified, whereas Martin recognized only one. Cell A which contains cytoplasmic granules stainable with paraldehyde fuchsin, chrom-hematoxylin and PAVB was also described by Martin. Cell B contains smaller cytoplasmic granules stained by phloxine. The A cells are rounded or irregularly shaped and each sends an axon to a nearby capillary or small blood sinus. The B cells are pear-shaped and surround the blood sinuses. Both types of cells with their secretory granules can be seen in Martin's figures since the B cell granules stain also with aniline blue (Mallory triple). It is probable that two or more products are formed in such an extensive neurosecretory system. Certain types of neurosecretion are stained by the acid component of the stain (i.e. phloxine, orange-G), rather than by the alkaline paraldehyde fuchsin, chrom-hematoxylin and PAVB.

The concentration of the neurosecretory cells into a definite tissue reflects that in higher animals specific areas tend to be secretory; some examples are the pars nervosa of vertebrates, sinus gland of crustaceans and corpus cardiacum of insects (Hagadorn,1966). A feature shared by neurosecretory systems and cells is their close proximity to a rich blood vessel network. In Illex one of the notable aspects of the system is its extensive network of small blood sinuses which are sometimes encircled by

axons of the neurosecretory cells. Another outstanding feature is the small size of the neurosecretory cells. In most other animals they are among the largest cells. An electron microscopic study is needed to determine the structure of the granules in these cells, and whether the cells in the juxta-ganglionic region of the inferior buccal ganglion of Illex also contain similar granules, in view of the fact that neurosecretory granules have been reported in Octopus (Barber, 1967).

The structure of the optic gland in Illex is similar to that described for Octopus. Cazal and Bogoraze (1943, 1949) regarded the cells of the gland as modified neurons, but Boycott and Young (1956) showed that it was not nervous. Wells and Wells (1959) demonstrated its endocrine function in Octopus by severing the optic gland nerve and observing an increase in the cell size which was evidently an indication of secretory activity and subsequent maturation of the gonads. The gland, therefore, controls reproduction. The rich network of blood vessels and the close association of the cell processes with the vessels also would suggest an endocrine or secretory function for the organ in I. illecebrosus. Only two distinct types of cells were found in I. illecebrosus as in Octopus (Wells and Wells,1959), but Haefelfinger (1954) recognized three in I. coindeti. Both types of cells in I. illecebrosus were smaller than those measured in Octopus by Cazal and Bogoraze but this may reflect the sexual immaturity of the specimens studied. The carbohydrate material in the cytoplasm

of the small cells may be related to secretion but in Octopus only the larger cells were experimentally linked with gonad development and therefore their secretion must be of different nature.

The two pairs of parolfactory vesicles in I. illecebrosus resemble those described by Haefelfinger (1954) in I. coindeti. Haefelfinger believed that it was constructed as an endocrine gland, but Nishioka, Yasumasu and Bern (1966), and Nishioka et al. (1966) demonstrated that the parolfactory vesicles of decapods and the epistellar bodies of octopods are photoreceptors with a higher concentration of rhodopsin and Vitamin A than is present in the retina. Haefelfinger described two cell types which are quite similar to those found here in I. illecebrosus, but Nishioka, Hagadorn and Bern (1962), with the aid of an electron microscope, distinguished four types in the epistellar body of Octopus. It is possible that these also exist in the parolfactory vesicles of I. illecebrosus, although they are difficult to resolve because of their tight packing. The follicular arrangement is not quite like that diagrammed by Haefelfinger, but this may be due to sub-specific or individual differences. Haefelfinger found no innervation for the smaller pair of vesicles, which is described in I. illecebrosus.

The colloid of the parolfactory vesicles probably has a high carbohydrate content as indicated by a positive PAS reaction unaffected by diastase digestion, but fails to stain with neurosecretory stains. The large quantity of Vitamin A demonstrated in

the vesicles by Nishioka, Yasumasu and Bern (1966), would not be responsible for the PAS-positive staining as it would be removed during processing. The PAS-positive material within the protrusions of the larger distal cells into the lumen of the follicle may be a secretory product. The slight paraldehyde fuchsin reaction given by the extensions of the cells into the lumen was shown to be caused by a brush border on the surface of the protrusions (Nishioka et al.,1966). The weak positive reaction given with PAVB indicates small traces of cysteine and some other weaker reducing groups. The alloxan-Schiff reaction indicated the presence of protein but since it was weaker than that given by blood, the substance in the colloid must be a mucoprotein or a glycoprotein.

SUMMARY

The general features of the central nervous system described here for Illex illecebrosus illecebrosus do not differ substantially from those of other decapod cephalopods. The brain consists of four main ganglia, one supraesophageal and three subesophageal, which are subdivided into lobes, probably representing functional divisions. Associated with these are several other more remote ganglia and lobes.

The cerebral ganglion is supraesophageal and contains nine lobes, some of which are poorly defined. The vertical lobe is the most dorsal and is unpaired. Its neurons are small and the lobe has few connections. The neuropile of the superior frontal lobe contains well defined optic bundles and numerous radial fibres, and two distinct types of neurons. It has thin connections with the posterior buccal lobe and three pairs of connections with the subvertical lobe. The neurons of the subvertical lobe are arranged in cell islands and the lobe is continuous with the precommissural ventrally and connects with several other lobes of the cerebral ganglion. The precommissural lobe is anterior to the ventral optic commissure, extends around the esophagus and sends fibres to the anterior and lateral pedal lobes. The dorsal basal lobe is continuous with the medial basal ventrally and contains many cell islands. It connects with the optic lobes, peduncle complex and other areas of the cerebral ganglion and gives rise to the optic gland nerve. The medial basal lobe connects with other divisions of the posterior basal

complex and sends many fibres to the lateral and posterior pedal lobes. The lateral basal lobe is a paired extension of the medial basal and its main efferents go to the chromatophore lobes. The anterior basal lobe is anterior to the precommissural lobe. Its structure is complex and both its neuropile and cell layer exhibit distinct features. It extends laterally around the esophagus sending fibres to the lateral and anterior pedal lobes. The posterior buccal lobe is at the anterior extremity of the cerebral ganglion and contains its largest neurons. It connects with the superior buccal and brachial ganglia.

The nervous centres which lie anterior to the cerebral ganglion are the superior and inferior buccal ganglia. The superior buccal ganglion is supraesophageal, flattened dorso-ventrally and has a very thin cell layer. It is the source of the labial nerves and is joined to the cerebral ganglion by the cerebro-buccal connective. The inferior buccal ganglion lies ventral to the esophagus just below the superior buccal and is associated with the juxta-ganglionic tissue posteriorly. Its only connection with the brain is through the superior buccal ganglion.

The anterior subesophageal mass or brachial ganglion lies under the inferior buccal, has an irregular cell layer and contains many large neurons. It has many root bundles and gives rise to the brachial nerves. It connects with several lobes.

The middle subesophageal mass lies directly below the cerebral ganglion and is subdivided into five lobes. The anterior pedal lobe occupies the anterior two thirds of the mass and has a

dorsal and ventral cell layer containing large neurons. It gives rise to several nerves and connects with other pedal lobes. The paired anterior chromatophore lobe is located on the lateral anterior walls of the anterior pedal lobe and is subdivided into dorsal and ventral halves. The posterior pedal lobe, covered laterally by the magnocellular lobe, gives rise to several nerves and contains several root bundles. The lateral pedal lobe is paired and constitutes the region where the supra- and subesophageal masses join. The cell layer is present only laterally and the neurons become smaller at the anterior and posterior margins. The paired magnocellular lobe is irregular and communicates with several lobes. It has two commissures and its neurons vary regionally in size.

The posterior subesophageal mass is divided into three separate lobes. The paired fin lobe comprises its antero-lateral region. It contains the greatest number of the largest neurons with very large axons. The posterior chromatophore lobe is also paired and its efferents enter the pallial nerve. The visceral lobe is the largest, its neurons show gradation in size laterally, and it gives rise to several nerves.

The peduncle complex is above and posterior to the optic tract and consists of the peduncle and olfactory lobes. The peduncle lobe is larger and contains small cells. It gives rise to a large commissure which passes through the brain above the optic commissure. The olfactory lobe is paired and begins as a lateral extension of the dorsal basal lobe. It is more

irregular than the peduncle lobe and its cells are slightly larger.

Neurosecretory cells are concentrated in a distinct area at the lateral posterior ends of the posterior subesophageal mass. Three cell types are present: pear-shaped neurons containing granules stained by phloxine which are concentrated around large sinuses, neurons containing granules stained by chromhematoxylin located near small sinuses, and small neurons with no secretory granules.

The optic gland is paired, has a rich capillary network and cells of two types with no secretory granules.

Two pairs of parolfactory vesicles lie on each side of the brain. The larger pair contains 8 - 14 and the smaller 3 - 4 follicles filled with colloid. The follicles may be only partly partitioned by the surrounding cell layer which consists of two types of cells. The small proximal follicular cells are tightly packed and the larger distal follicular cells send processes through the proximal layer into the colloid. The colloid is probably a mucoprotein or glycoprotein.

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