CRUSTACEAN ENDOCRINOLOGY

Crustacean endocrinological studies commenced with the pioneer discovery of Koller (1920) that "an unknown factor from the eyestalk is a cause of color charge of the body". In crustaceans, the endocrine system is thoroughly investigated in Decapoda (shrimps, crayfishes, crabs, lobsters, prawns etc). The progress in crustacean endocrinology has been reviewed by some workers (Fingerman, 1992, 1997; Keller, 1992; Landau *et al.*, 1997, Reddy and Ramamurthy, 1999; Huberman, 2000).

A. STRUCTURE

The crustacean endocrine system, like that of other arthropods, consists of the nervous, epithelial and gonadal endocrine components (Fig.6.1):

(a) Neural Endocrine Components

(i) The X-organ - sinus gland complex.

(ii) The neurosecretory cells in the brain and other ganglia and related neurohaemal organs - the postcommissural and pericardial organs.

(iii) The stomatogastric neuroendocrine complex.

(b) Epithelial Endocrine Components

(i) The Y-organs.

(ii) The mandibular organs.

(iii) The androgenic glands.

(C) Gonadal Endocrine Components - The ovaries.

1. NEURAL ENDOCRINE COMPONENTS

1.1 The X Organ - Sinus Gland Complex (XO-SG) - It is the major endocrine centre in crustaceans. The eyestalk of the decapod crustaceans is a three segmented structure, consisting internally the optic ganglia (optic lobes) - basal medulla terminalis (MT), middle medulla interna (MI) and outer medulla externa (ME).

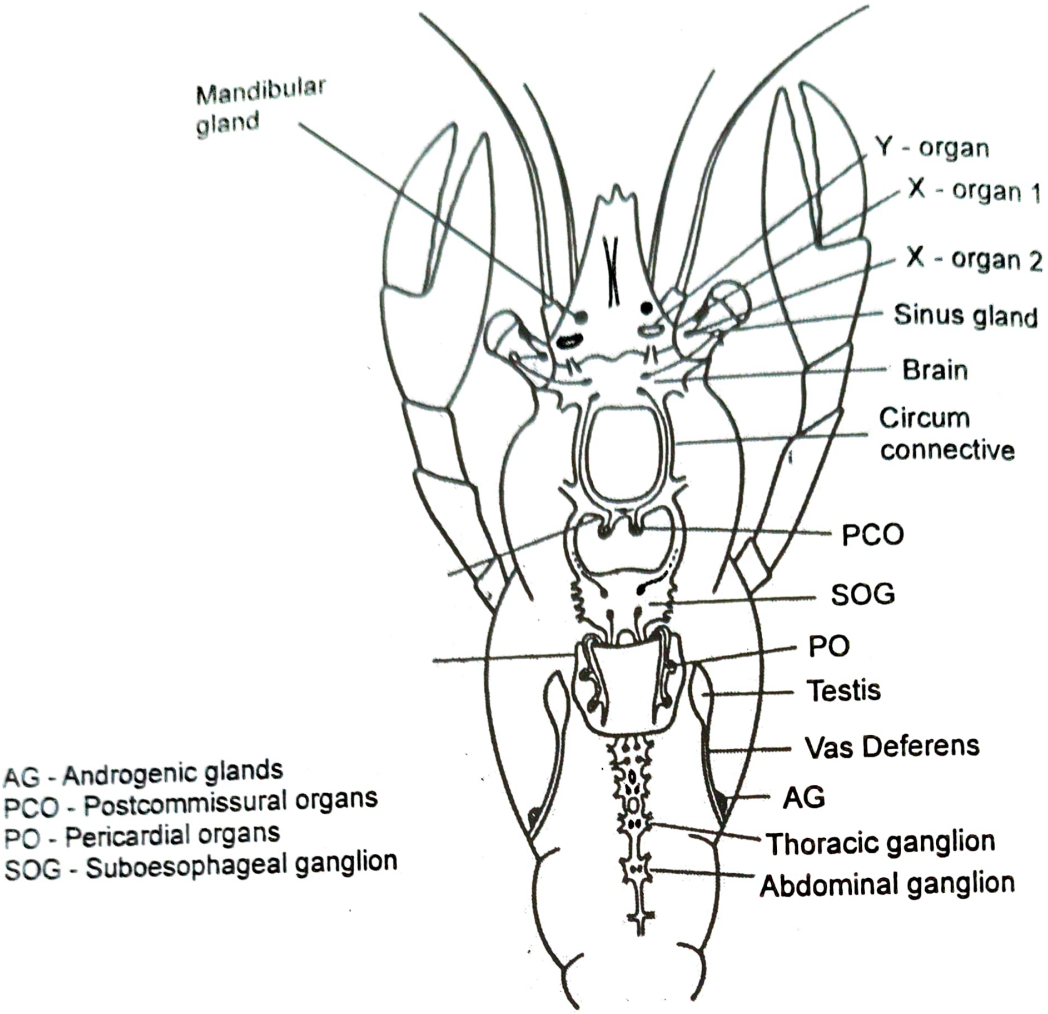
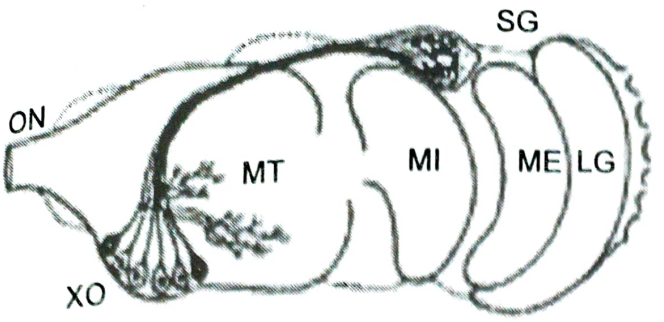


Fig. 6.1 : The endocrine system of a generalized male crustacean. (After Gorbman and Bern,1962)

(a) The X organ (XO) - In the brachyuran *Cancer,* the X organ (XO) and sinus gland (SG) are located at the proximo-ventral extremity of MT and in between the MI and ME respectively. The XO consists of a group of loosely packed neurosecretory cells (NSC) of variable shape, size and number. The neurosecretory cells in the XO can be classified into two to four types on the basis of staining and cytomorphological criteria (Fig.6.2).

In *Lysmata seticaudata,* some neurosecretory cells of the XO in MT send their axons to the SG while others to the sensory pore (SP). In this species, therefore, there are two neurohaemal organs (NHO) - the SG and SP on either side of ME - MI junction. The SG and SP are considered as the primary and secondary NHO respectively. In some crustaceans, like *Palaemon serratus,* there are two XO in MT and one XO in ME and all these XO send their axons to SG. Again some crustaceans, like *Gecarcinus lateralis,* consist of one XO in MT and another in the MI and all NSC send their axons to the SG. In crabs, the presence of large number of XO in the eye stalk, for example, four XO in MT, one in MI and one in ME ganglia in *Ocypoda platytarsis,* has been reported. Three to four XO are described in MT in *Carcinus maenas*. Besides XO axonal



LG - lamina ganglionaris,
MT - medulla terminalis,
MI - medulla interna,
ME - medulla externa,
XO - X organ,
SG - sinus gland,
ON - optic nerve

Fig. 6.2. The XO - SG complex in the crab, *Cancer* sp.

terminals, the SG receives the axons of cerebral medial and lateral groups of neurosecretory cells (MNC, LNC) also (Hignam and Hill 1979).

The NSC of the XO in the optic lobes and those found in the brain are primarily, the monopolor motor neurons. They are secondarily modified into the large glandular neurosecretory cells and well-equipped with the cytoplasmic organelles such as, the granular or rough endoplasmic reticulum, Golgi bodies, ribosomes, lysosomes, mitochondria and microtubules. Their axons often terminate into the SG. Their nuclei contain 2-3 large nucleoli and peripheral chromatin attached to the nuclear wall. The cell body and nucleus, both of NSC show concomitant changes in size and internal inclusion along with the neurosecretory cycle. The NSC cell bodies and axons are densely filled with the granular or colloidal neurosecretory material (NSM). The soma (perikarya) and axons of NSC stain distinctly with the chromalum haematoxyline phloxin (CHP), Aldehyde fuchsin (AF), Alcian blue (AB), Azan and performic acid - Victoria blue (PAVB) like (neurosecretory material specific) staining techniques and demonstrate NSM qualitatively as well as quantitatively in the cell bodies, axons and SG specifically. Similarly, the electron microscopic (EM) studies reveal the neurosecretory granules (NSG) in the NSC cell bodies, axons and SG quite distinctly. On the basis of cytomorphological variations such as shape, size, location, tinctorial (staining) properties and size of NSG under EM, the NSC can be classified into various cell-types such as the A,B,C and their subtypes like A_1, A_2 or B_1, B_2, C_1, C_2 etc.

(b) **The Sinus Glands (SG):** The SG are glistering white, somewhat inflorescent spherical or oval bodies located into close vicinity of the lamina ganglionaris or at the junction of MI and ME. It is externally covered with a thick neurolemma (outer sheath) while internally filled with bulbous axon terminals and connective tissue fibers. The NSC axons running from both, the X-organ and brain, are arranged in a radial fashion in the body of SG. They are densely filled with the NSM.

EM studies reveal that the SG is a compact mass of interdigitating

neurosecretory axon endings abutting upon the thin basal lamina of the central haemolymph lacunae. In the crab, *Carcinus maenas*, four types of axon endings vary in size and NSG population density. The axon profiles are surrounded by the astrocyte- like glial cells. The SG do into contain intrinsic NSC and exclusively function as the primary NHO. They store and release NSM from the NSC of the X-organs and brain. As the axonal endings are closely associated with the haemolymph lacunae in SG, various neurohormones are released directly into the haemolymph : The XO SG complex secretes the neurohormones:

i) Crustacean hyperglycemic hormone..... CHH
ii) Moult-inhibiting hormone..... MIH
iii) Gonad (vitellogenesis) - inhibiting hormone..... GIH/VIH
iv) Mandibular organ - inhibiting hormone, ... MOIH
v) Red pigment concentrating hormone..... DRPH
vi) Distal retinal pigment hormone..... DRPH

Besides these hormones, immunoreactivity of NSC of XO axons and SG with FMRF amide, serotonin, norepinephrin, substance- P and enkephalins has been demonstrated in various species of crustaceans.

1.2. Brain-Postcommissural Organ Complex (BR-PCO) - Posterior neurosecretory cells in the brain and their axons terminating into the neurohaemal organs, the PCO and constitute BR-PCO complex (Fig. 6.3).

(a) **Cerebral neurosecretory Cells:** Crustacean brain is a bi-lobed structure, situated transversely in between the eye stalks. The brain is divided typically

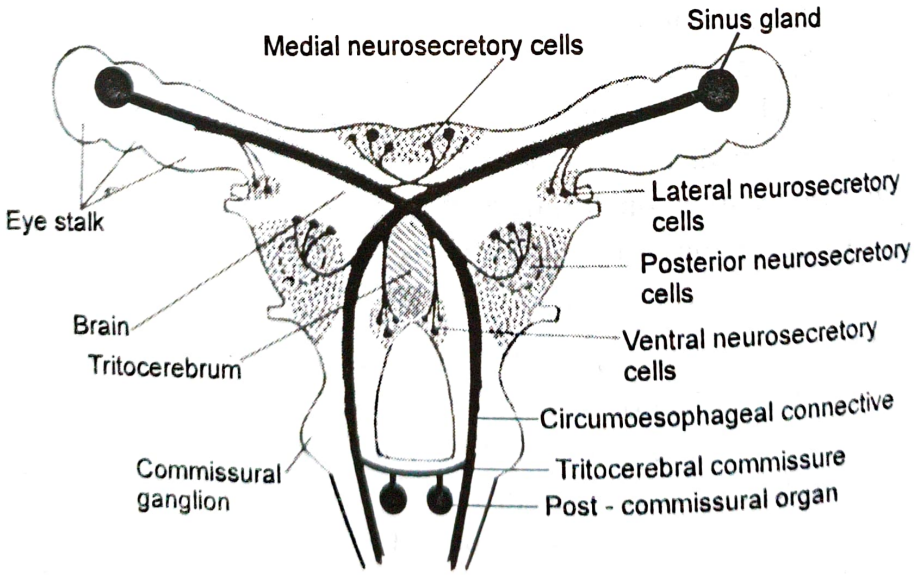


Fig. 6.3 Brain - post - commissural organ neuroendocrine complex (modified from Matsumoto, 1958).

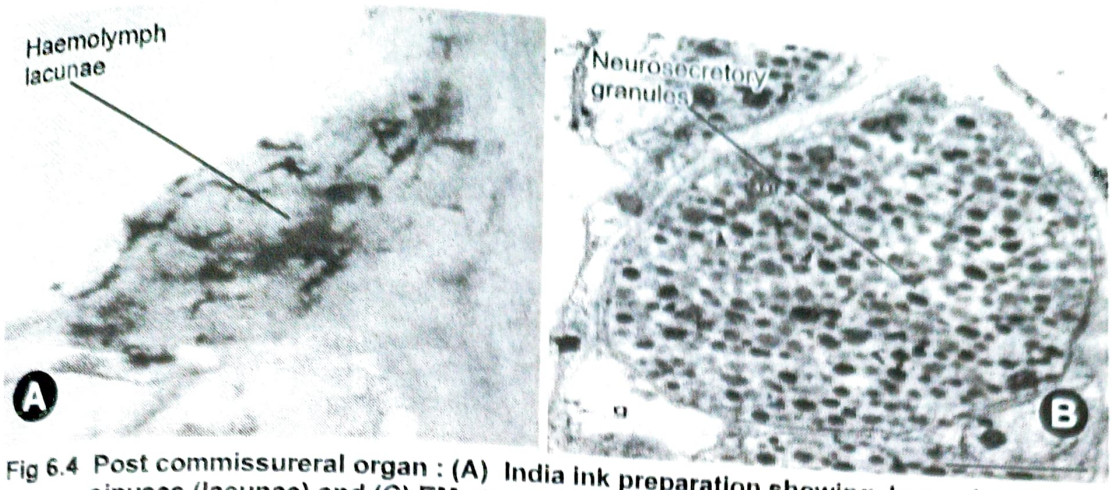


Fig 6.4 Post commissureral organ : (A) India ink preparation showing haemolymph sinuses (lacunae) and (C) EM structure showing neurosecretory granules (after skieb,1999).

into the protocerebrum (forebrain), deutocerebrum (midbrain) and tritocerebrum (hindbrain). Although, the presence of neurosecretory cells is reported all over the brain cortical region, the presence of well defined paired groups of the neurosecretory cells in the proto- and tritocerebral regions is repeatedly described in large number of decapod crustaceans.

The following three paired groups of NSC in the brain of crustaceans are reported:

(i) The medial neurosecretory cells (MNC) in mid-dorsal region on either side of the median furrow,

(ii) The lateral neurosecretory cells (LNC) in the lateral region of the protocerebrum;

(Iii) The posterior neurosecretory cells (PNC) in the posterial region of the Protocerebrum and

(iv) The ventral neurosecretary cells (VNC) in the tritocerebrum.

On the basis of histological, ultrastructural and immunocytochemical studies they are classified into two to four or even more cell-types. The MNC and LNC extend their axons to the SG while the PNC and VNC axons run to the post commissural organs (PCO). The VNC of the tritocerebrum and PCO, therefore, form a distinct neuroendocrine complex in Crustacea (Highnam and Hill, 1979).

(b) **Postcommissural Organs (PCO):** The PCO (Fig.6.4) lie near the oesophagus and they receive axonal terminals of VNC. The PCO emerge from the postoesophageal commissures that lie just posterior to the oesophagus (carlise and Knowles, 1953). They contain club-shaped plexes in antero-medial region and a dense aggregation of NSC axon endings. The axon terminals are of <1 to >15 μm in diameter. In the posterior plexes, the terminal

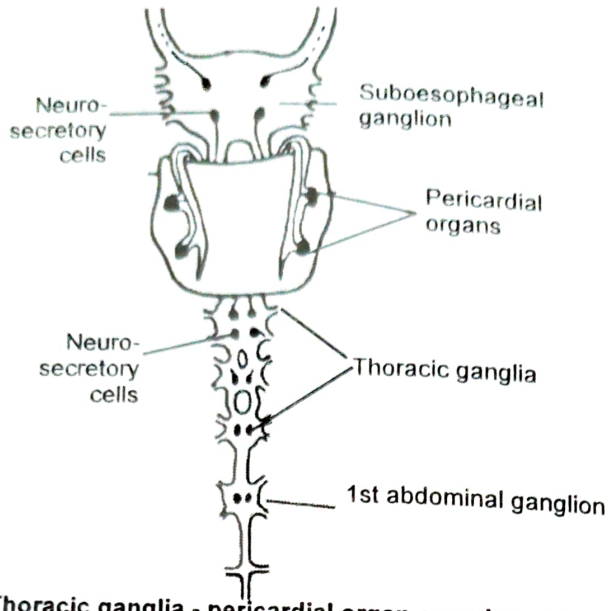


Fig. 6.5 Thoracic ganglia - pericardial organ complex-anatomy.

aggregates are often extensively fenestrated by the tubular areas (lacunae) filled with the haemolymph. The PCO wall consists of a thin neurolemma. The axon endings release their NSM in the haemolymph of lacunae directly.The BR-PCO complex is known to secrete various PDH, MSH and GSH. The crab, *Cancer borealis* is known to contain two tachykinin related peptides (TRP) besides chromatotropins such as pigment concentrating hormone (PCH) and pigment dispersing hormone (PDH). TRP is modulator which stimulates foregut musculature. The TRP is APSGFLGMR amide (*Cab*-TRP) (Messinger *et al*.,2005). The structure of GSH is, moreover not yet determined.

1.3.Thoracic Ganglia-Pericardial Organ Complex (TG-PO): It consists of the neurosecretory cells in the fused thoracic ganglia and their axons are terminating into the neurohaemal organs , the PO (Fig. 6.5).

(a) Thoracic Neurosetory Cells: There are five groups of neurosecretory cells in the thoracic ganglionic mass in decapod crustaceans. The thoracic neurosecretory cells are spread over the peripheral cortical region while their axons bypass the medullary glial mass and terminate into the pericardial organs.

(b) The pericardial Organs (PO): The structure of PO was initially studied by Smith (1947) and later on, Alexandrowicz (1953) and recognized them as the true NHO. The PO are the conspicuous paired nerve plexes like structures located in lateral regions of the pericardial cavity. The pericardial organs are located over openings of branchio-cardiac veins into the aorta (Fig 6.5, 6.6).

They are made up of the nerve fibers originating from the neuronal cell bodies lying within cortical region of the thoracic ganglionic mass and

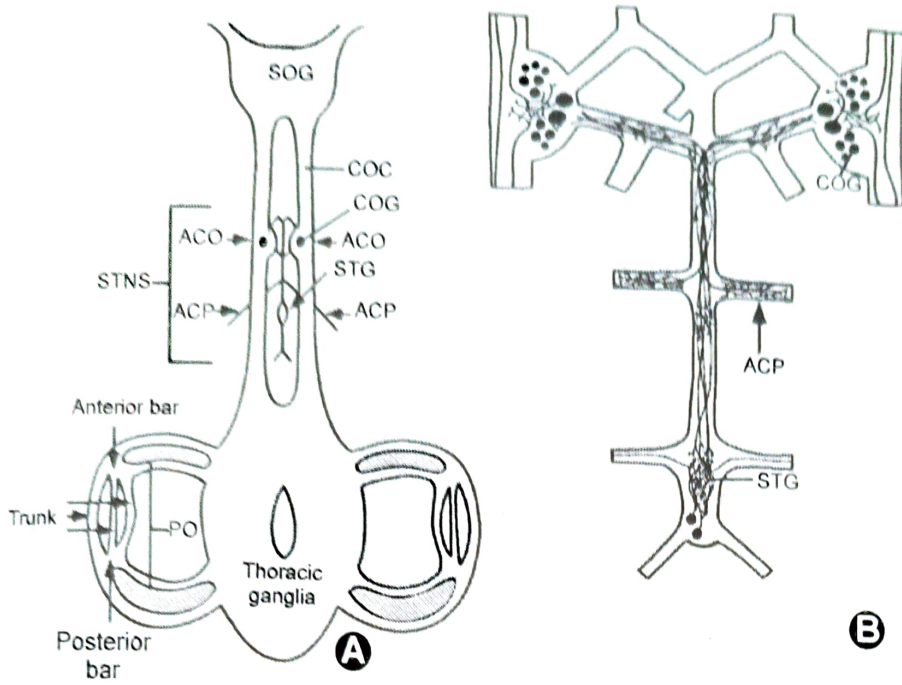


Fig. 6.6 **Stomatogastric neuroendocrine system of *Cancer* species : (A) anatomical position and (B) neurosecretory system (modified from Skiebe, 1999).**

ACO - anterior commissural organ
ACP - anterior cardiac plexus
COC the circumesophageal connectives,
COG - commissural ganglia
PO - pericardial organs
SOG - supraesophageal ganglion,
STG - stomatogastric ganglion
STNS - stomatogastric nervous system

projecting through the openings of the branchio-cardiac veins into the pericardium. The trunks of the PO consist of an inner core of nerve fibers, connective tissue and blood vessels and an outer cortex formed of neurosecretory axon terminals filled with NSM granules. A very fine (less than 1 µm thick) amorphous acellular epineurium forms an outer sheath of the PO. The PO are the largest NHO in the crustaceans. They contain thoracic NSC axon endings filled with variable size of NSM granules and on the basis of diameter of granules the thoracic NSC can be classified into 5 cell-types.

They store and release various cardio-excitatory substances Serotonin, dopamine, octopamine and the crustacean cardiactive peptide (CCAP). Even <1% amount of the CCAP is fully effective on the heart.

1.4. Stomatogastric Neuroendocrine System: The stomatogastric nervous system (STNS) basically controls the movements of the foregut and the oesophagus of decapod crustaceans. The stomatogastric nervous system (STNS) consists of four ganglia, the paired commissural ganglia (COG), the oesophageal ganglion (OG) and the stomatogastric ganglion (STG), together with their connectives and motor nerves. The STNS is located between the brain and the suboesophageal ganglion (SOG), which are connected by the

circumoesophageal connective (coc) surrounding the oesophagus. The post-oesophageal commissure (POC) links both COC close to the SOG. The STG lies within the ophthalmic artery, which carries haemolymph containing hormones released by the pericardial organs to the brain (Skiebe, 1999). The cell bodies of NSC that release peptides as transmitters within the STG are found either within the STG or projecting to the STG, mostly from the CoG or the OG (Coleman *et al.*, 1992). Although numerous antibodies against peptides have been used, evidence for peptidergic cell bodies within the adult STG was found only for the FMRFamide and allatostatin families. Peptide neurohormones can be released either from neurohaemal organs or from local neurohaemal release zones located on the surface of nerves and connectives.(Fig. 6.6)

Two additional neuroendocrine sites, the anterior cardiac plexus (ACP) and the anterior commissural organ (ACO), are contained within the STNS. The ACPs are located on the anterior cardiac nerves (*acns*) and the ACO are located within the commissural ganglia (COG). In the anterior cardiac plexus (ACP) and the axons innervating it, MOIH-like labeling has been well evident. In addition, approximately a dozen somata have been labeled in each commissural ganglion (COG), and two in the stomatogastric ganglion (STG).

2. EPITHELIAL ENDOCRINE COMPONENTS

2.1. Y-Organs (YO): The paired Y organs of crustaceans are opalescent, lobulated epithelial glands embedded in the brown fatty tissue, ventrally at the base of antennules, just anterior to the brancheal chamber, close to the ventral carapace. They are well-distinct in all larval stages. They are ectodermal in origin

In the crab, *Cancer anthonyi*, at the first zoeal stage a pair of YO(Fig.6.7) are very minute structures. At this stage each YO consists of a

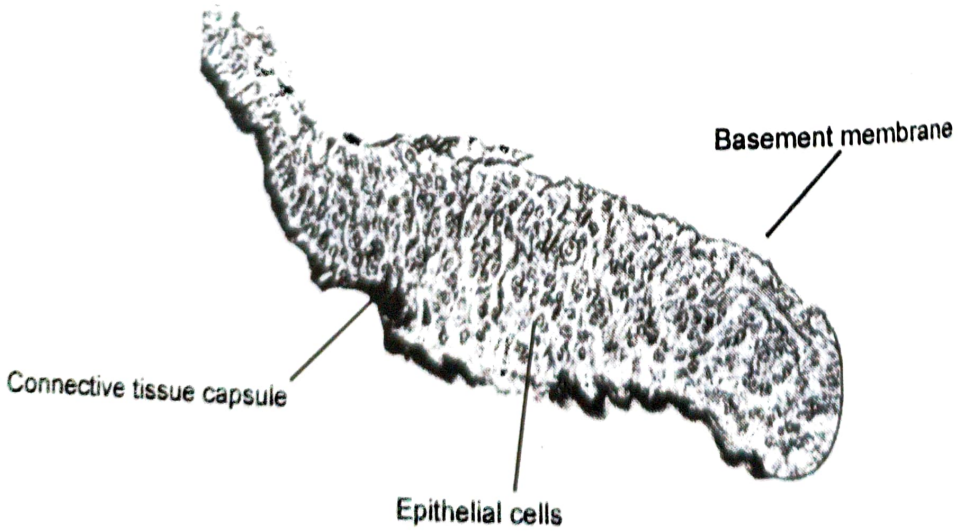


Fig. 6.7 The Y organ of the crab, *Cancer anthonyi*.

single cord of 6 to10 epithelial cells with darkly stained nuclei sparse cytoplasm and indistinct cell boundaries. As the development progresses, the glands become more complex due to extensive folding and intermingling. They retain an outer thick covering of connective tissue (capsule). The peripheral epithelial cells of the glands extend processes to the connective tissue capsule in order to conduct metabolic exchange with haemolymph. The processes contain mitochondria and are filled distally with electron dense material. The cytoplasm is scarce and contains vesicles, polymorphic mitochondria with tubular cristae and numerous free ribosomes and few rough endoplasmic reticulum and Golgi complexes. During transformation from inter-moult to pre-moult stage, the mitochondria, vesicles and electron dense particles in peripheral processes increase in number. The smooth endoplasmic reticulum and mitochondria are of tubular type characteristic of the steroid secreting cells.

In Crustacea, the Y organs undergo cyclical changes in all larval stages with the initiation of promoult (proecdysial) stage. The nuclei become large and cytoplasm contains large number of lipophilic droplets and granular inclusions. After ecdysis, the cells become vacuolated. The cell walls disappear and the glands change from cellular to syncytial structure at active stage while retain cellular structure at inactive or resting stage during the inter-moult period. They show hypertrophy frequently, at proecdysis.

The Y organs secrete moulting hormone, initially, the inactive from, α-ecdysone which is later on converted into the active form, β-ecdysone. The crustacean β-ecdysone was earlier called, the crustecdysone but is similar to the moulting hormone of insects, the β-ecdysone. The crustacean Y organs are therefore, analogous with the prothoracic glands (ecdysial glands) of the insects in structure, hormone secreted and function. They play active role during development as the moulting hormone (β-ecdysone) triggers the proecdysis through various molecular mechanisms facilitating moulting process. The activity of the Y organs is controlled negatively by the moult-inhibiting hormone (MIH) secreted by the X-organ-sinus gland complex. As a result secretion of ecdysone is inhibited and process of moulting is blocked.

2.2. Mandibular Organs (MO): The mandibular organs (Fig 6.8) were discovered first by Le Roux in 1968 and they were, later on, reported in a large number of decapod crustaceans. They are globular or spherical pale yellow, highly vascularized paired glands, located at the base of mandibular tendons in the cephalothorax. The MO are lying at anterior end of the paired chitinous tendons that extend from the mandibles to the dorsal carapace. Channels of haemolymph subdivided the glands into several epithelial cords. Each cord is composed of irregular shaped cells with eccentric nuclei. The nucleoplasm

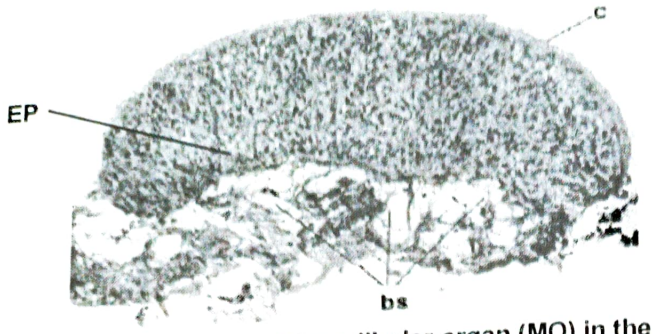


Fig. 6.8 Mandibular organ (MO) in the lobster .

contains patches of condensed chromatin attached with the nuclear envelope. The most distinguished cytological features of the MO signify presence of smooth endoplasmic reticulum, few Golgi bodies, numerous mitochondria and lipid droplets. The cytoplasm is often scanty and agranular. The cells undergo cyclical changes, secrete lipoidal methyl farnesoate (MF).

MF accelerates moult cycle in white strimp, *Penaeus setiferus*, and thus acts as moult promoting hormone. It stimulates protein synthesis in hepatopancreas, including that of vitellogenins (Homola *et.al.;* 1991) Sagi *et.al.*; 1993, 1994. Laufer *et al.;* 1993).

2.3. Androgenic Glands (AG): A distinct gland is attached near distal end at each vas deferens. They were first described by Cronin (1947) in the male blue crab, *Callinectes sapidum*. Seven years later, Charniaux-cotton (1954) confirmed their androgenic function (differentiation of secondary male sex characters, development of male gonads and regulation of spermatogenesis) in the amphipod, *Orchestia gammarella*. Similarly, Legrand (1955) also noticed their androgenic function in the isopad, *Armadillidium vulgare*. As these glands

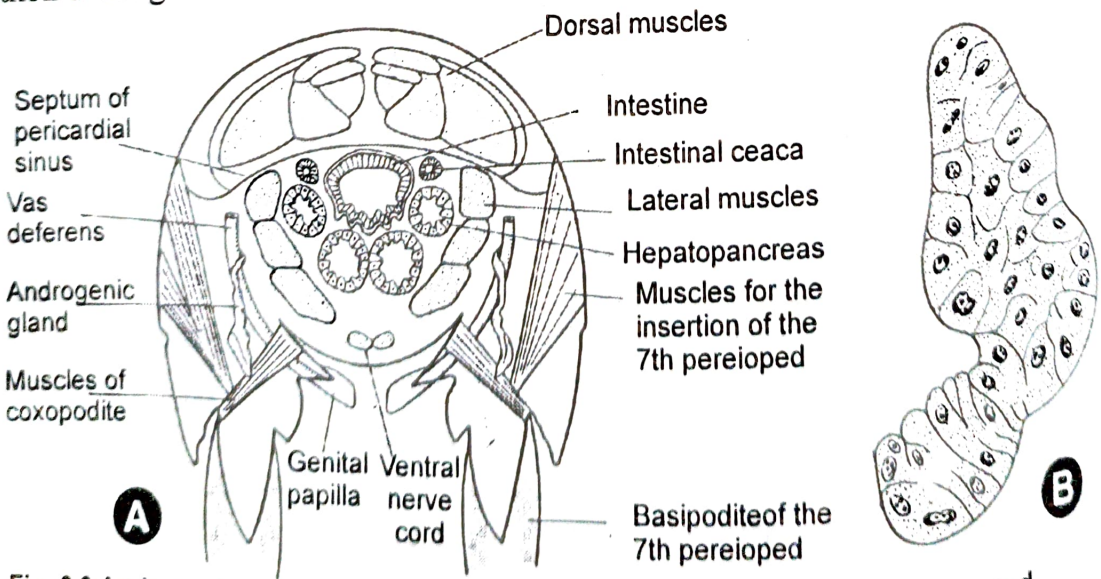


Fig. 6.9 Androgenic glands in *Orchestia gammarella*, : (A) anatomical position and (B) cellular structure (after Charniaux - cotton, 1958).

are found to be androgenic in function (determining masculine characters) are popularly known as the androgenic glands. In the male they start developing in undifferentiated young crustacean and gradually enlarge to form solid strands of epithelial cells, folded several times (Fig.6.9).

One end of androgenic gland is attached to the muscle of the last coxopodite while other end is lying over the vas deferens. It was earlier reported a terpenoid or sterioid, Farnesyl acetate or hedrahydroxy farnesyl acetone in *Carcinus meanas* (Ferezou, *et al;* (1978) but later on AGH is characterized as a peptide hormone. It is a glycosylated protein molecule (Martin *et al;* 1999).

B. CRUSTACEAN HORMONES

1. PEPTIDE HORMONES

1.1 **Red pigment concentrating hormone (RPCH) -** Fernlund and Josefsson, (1972) identified molecular structure of this hormone in the crustaceans, *Pandalusborealis* first and later on identical peptide has been characterized in three species of crabs, *Cancer magister, Carcinus maenas* and *Orconectes limosus* (Gaus *et al.*, 1990). It is an octapeptide -

PGlu-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH_2.

It is similar to insect insect decapeptide adipokinetic hormone (AKH) isolated from locust, *Locusta migratoria* (Stone *et al.*, 1976). These hormones jointly form AKH-RPCH family (Gäde *et al.,* 1997). It stimulates chromatophore /erythrophore pigment concentration.

1.2 **Distal retinal pigment hormone (DRPH) -** Ferlund (1976) first identified molecular structure of DRPH in *Pandalus borealis* as an octadecapeptide . It is also called α-PDH -

Asn-Ser-Gly-Met-Ile-Asn-Ser-Ile-Leu-Gly-Ile-Pro-Arg-Val-Met-Thr-Glu-Ala-NH_2

1.3 **Pigment dispersing hormone (PDH) -** All PDH molecules are octadecapeptides -

(A) **α - PDH** - It is also called the MSH or MDH (Melanophore stimulating/ Dispersing hormone -

Asn-Ser-Glu-Ile-Ile-Asn-Ser-Leu-Leu-Gly-Leu-Pro-Lys-Leu-Leu-Asn-Ala-NH_2

(B) **β-PDH** - It considered as the major β - PDH in eyestalks of *Uca pugilator* (Rao *et al;* 1985) -

Asn-Ser-Glu-Leu-Ile-Asn-Ser-Ile-Leu-Gly-Leu-Ile-Lys-Asn-Val-Met-Asn-Ala-NH_2.

It is also found in the crabs, *Cancer magister* (Klenholz *et al;* 1986); *Callinectes sapidus* (Mohrherr *et al;* 1990) and *Carcinus maenas* (Klein *et al.*,1992); crayfish *Pacifastacus leniusculus* (Rao and Riehm, 1993), and other species. It is also a major PDH in the shrimp *Penaeus aztecus* (Philips *et al.*, 1988).

1.4 Tachykinin related peptide (TRP) : It is a nonapeptide -

Cab-TRP : APSGFLGMR amide

1.5 Crustacean hyperglycaemic hormone (CHH) - It is a large protein molecule consisting of about 71 amino acid residues in *Homarus americanus* (Tensen *et al;* 1991; Kegel *et al;* 1989). It regulates release of glucose from the hepatopancreas. It is analogous with the vertebrate glucagon hormone and elevates the blood sugar level. Structure of CHH is given below (Fig. 6.10)

PGlu-Val-Phe-Asp-Gln-Ala-Cys-Lys-lys-Val-Tyr-Asp-Asn-Leu-Ile-Lys-Lys-Leu-Asn-Arg-Val-Cys-Glu-Asp-Cys-Tyr-Asn-Leu-Tyr-Arg-Lys-Pro-Phe-Val-Ala-Thr-Thr-Cys-Arg-Glu-Asn-Cys-Tyr-Ser-Asn-Arg-Val-Phe-Arg-Gln-Cys-Leu-Asp-Asp-Leu-Leu-Ile-Asn-Asn-Val-Glu-Tyr-Val-Tyr-Val-Ser-Asn-Val-Gln-Met-Met-OH

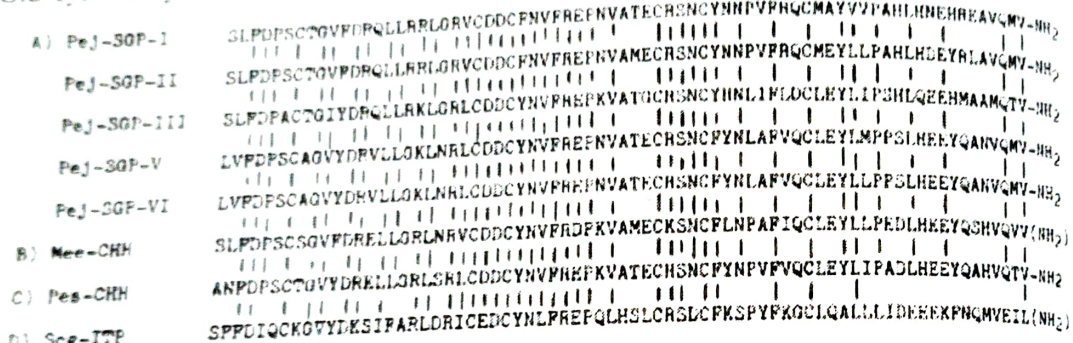


Fig.6.10 Amino acid sequences of CHH peptides : (A) Yang *et.al.*(1997), (B) Gu and Chan (1998), (C) Hubennan *et.al.* (2003), (D) meredith *et al* (1996) Pej. P. (*Marsupenaeusi japomcus* : Mee. *M. Ensis Pes. P.* (*Litopenaeus*) *scmmim* . *Scg. Sch. Gregaria* Ion Transport Peptide. In B and D. The amidared carboxyl-terminus has been deduced from the cDNA. Identical or similar (in charge or hydrophobicity) residues have been united by dashes.

1.6 Moult - inhibiting hormone (MIH) - It is also a large protein molecule similar to CHH and containing 72 amino acid residues in *Homarus americanus* (change *et al;* 1990) and 78 in *Carcinus maenas* (Webster, 1991) and number of amino acid residues have been reported upto 79 in other species. The protein molecule is characterized with 3 disulfide bonds in between 6 cysteine residues (Katayama *et al.*, 2003). Structure of MIH is given below (Fig.6.11).

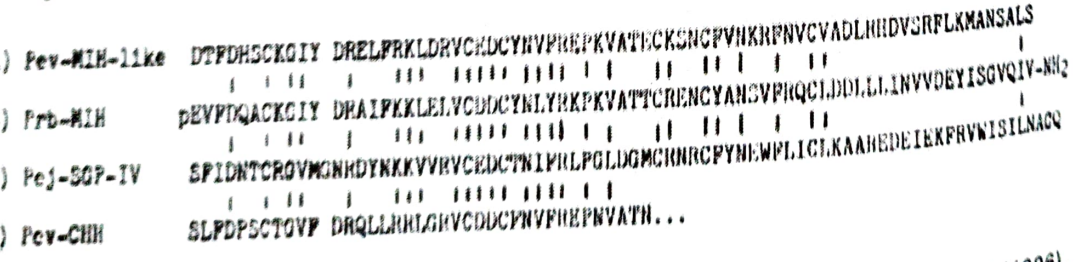


Fig. 6.11 Amino acid sequences of MIH peptides : (A) Sun (1994) (B) Aguilar *et.al.* (1996), Aguilar-Gaytan *et.al* (1997), (C) Yang et.al. (1996), (D) Sefiani et.al (1996). Pev. *P. (Litopenaeus) vannamer*, Prb, *Pro bowieri*, Pej. *P. (Marsupenaeus) japonicus*. Note that Pej-SGP-IV has an extra amino acid (Gly) at position 12 (Type II) Identical or similar (in charge or hydrophobicity) residues have been united by dashes.

1.7 Vitellogenesis -inhibiting hormone (VIH) - It is a protein molecule consisting of about 77 amino acid residues in *Homarus americanus* (Soyez *et.al.*, 1991). The CHH, MIH and GIH are similar to each other in most of the amino acid residue sequence representing same family of CHH. The molecular structure of VIH of some crustacean species is given below (Fig.12.6)

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Homga_VIH   MVTRVASGFSVQRVWLLLVIVVVLCGSVTQQASAWFTND-ECPGVMGNRDLYEKVAWVCND
Homam_VIH   MVTRVGSGFSVQRVWLLLVIVVVLCGSVTQQASAWFTND-ECPGVMGNRDLYEKVAWVCND
Nepno_VIH   MVTRVASGFSVQRVWLLLVIVVVLCGSVTQQASAWFTND-ECPGVMGNRDLYEKVAWVCND
Rimka_VIH   MVGQVNHDISVQRVLRLAALVISLLLITGTSARNLYDLDTECPGVMGNRDLYEKVVRVCDD
Penmo_VIH   ----------MKTWL------LLATLVVGASLANILDS-KCRGAMGNRDMYNKVERVCED
Meten_VIH   ----------MRTWLTFVAVMVWASLLVDESSAFSIDY-TCTGAMGNRDIYNKVSRVCDD
Macro_VIH   MASRLNQAFTLKKLTYVAIMMAVFGILLVDQTSARFLDD-ECRGVMGNRDLYEYVVRICDD
Armvu_VIH   ----------------------YNIPLGWGRRDMPG---CLGVLGNRDLYDDVSRICSD

Homga_VIH   CANIFRNNDVGVMCKKDCFHTMDFLWCVYATERHGEIDQFRKWVSILRAGRK- 100%
Homam_VIH   CANIFRNNDVGVMCKKDCFHTMDFLWCVYATERHGEIDQFRKWVSILRAGRK- 99.1%
Nepno_VIH   CANIFRINDVGVKCKKDCFHNMDFLWCVYATERHGEIDQFRKWISILRAGRK- 96.4%
Rimka_VIH   CSNIFRENDVGTRCRKECFFNVDFLWCVYATERHGDVEQLNRWMSILRAGRK- 59.0%
Penmo_VIH   CTNIYRLPQLDGLCRNRCFNNQWFLMCLHSAKREAELEHFRLWISILNAGRPW 38.2%
Meten_VIH   CANIYRLPGLDGMCRNRCFNNFWFMICLRAAKREDEIDKFRVWISILNPGGAW 43.1%
Macro_VIH   CENLFRKSNVGSRCKKNCFYNEDFMWCVRATERTDELEHLNRAMSIIRVGRK- 45.5%
Armvu_VIH   CQNVFRDKNVESKCRSDCFSTSYFETCIMALDLAEKISDYKLHASILKE---- 34.1%
```

Fig. 6.12 Vitellogenesis inhibiting hormone (VIH) amino acid sequence comparison between crustacean species. *Homarus gammarus* (*Homga_VIH*), *Homarus americanus* (*Homam_VIH*), *Nephrops norvegicus* (*Nepno_VIH*), *Rimicaris kairei* (*Rimka_VIH*;), *Penaeus monodon* (*Penmo_VIH*), *Metapenaeus ensis* (*Meten_VIH*), *Macrobrachium rosenbergii* (*Macro_VIH*) and *Armadillidium vulgare* (*Armvu_VIH*; P83627). Asterisks indicate a single, fully conserved residue, colons indicate conservation of strong groups, stops indicate conservation of weak groups and dashes indicate no consensus.

1.8 Mandible Organ inhibiting hormone (MOIH) - Eyestalk ablation causes severe hypertrophy of the MO with ultrastructural changes in the nuclei, endoplasmic reticulum and mitochondria suggesting inhibitory effect of XO-SG complex on MO secretory activity (Laufer *et al.*, 1986, Tsukimura and Borst, 1992). In other words, XO-SG complex secrets mandibular organ inhibitory hormone exerting negative effect on the MO. The MOIH is a protein molecule consisting of 78 residues, (M.W. - 9235.6) with unblocked termini and three intrachain disulfide bridges, in the crab, *Cancer pagurus* (Wainwright *et a.,l* 1996). It shows 60% similarity with MIH, CHH representing subsection member of CHH family.

Structure of mandibular organ-inhibiting hormone in the crab, *Cancer pagurus* is given below (Wainwright *et.al.* 1996) :

RRINNPQQNPIGNRAMYEKVDWICKDCA NIFRKDGLLNNCRSNCFYNTEFLNC

IDATENTRNKEQLEQWAAILGAGWN

1.9 Crustacean cardioactive Peptide (CCAP) - It was isolated and identified first in the crab, *Carcinus* and later on, in several decapods. Immunocytochemical (ICC) studies revealed its synthesis in some neurosecretory cells in the thoracic ganglion and accumulation in the pericardial organs. ICC also shows cardiac ganglion as another source of the hormone. Similar to vasopressin-oxytocin, it is a nonapeptide and the Cystein amino acid residues of position 3 and 9 are linked by disulfide bond (Stangier et al; 1987) -

Pro-Phe-Cys-Asn-Ala-Phe-Thr-Gly-Cys-NH_2

Second CCAP found in crab is proctolin- like peptide(Sullivan, 2005). Experimental studies reveal that CCAP hormone accelerates heartbeat amplitude in all the decapods studied so far. The CCAP is also recognized through ICC studies in the midgut endocrine cells in *Drosophila* and other insects and demonstrated the functions, such as, contraction of the gut muscles inducing peristaltic movement and release of the digestive enzyme amylase.

1.10 Androgenic Gland Hormone (AGH) : glycosylated dimeric peptide (protein) AGH is a glycosylated protein molecule of 8.7 Da (Sagi and Khalaila, 2001). According to Sagi and Khalaila (2001) A and B chain form a hormone molecule while C chain acts as a connecting link. A and B chains are linked together by two inter and two intra-disulfide bonds. There are two amino-acids Lys and Ary (KR) in between the chains. The molecule of AGH, thus resembles with super-family of insulin. It contains, in fact 73 amino acid residues (A=29+B=44) and a prohormone peptide of about 44+46+29=119 amino acid residues (Fig. 6.13).

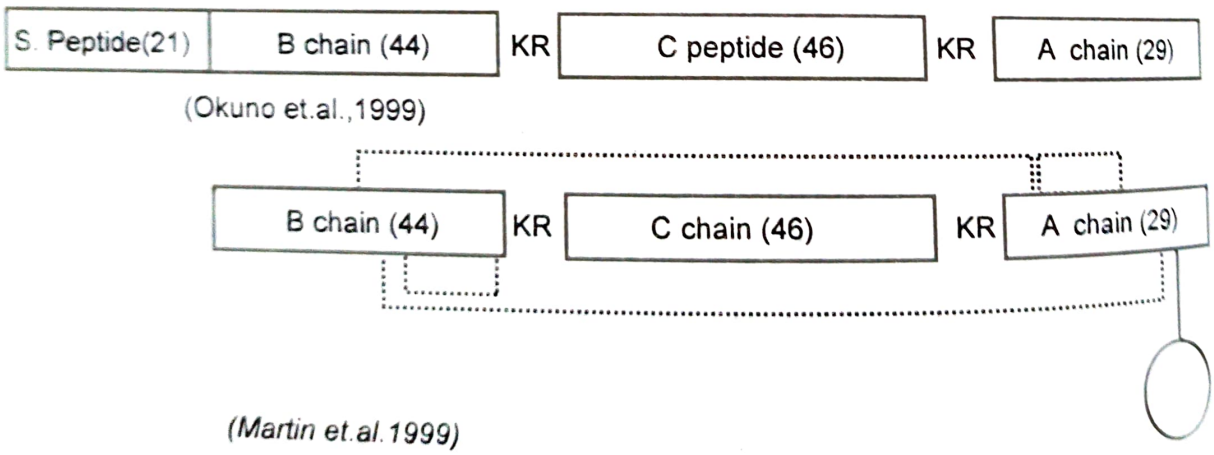

Fig 6.13 Structure of the AGH of *Armadillidium valgare*.

Abbr., - KR - lys-arg, intra-and inter-chain disulfide bridges shown by - lines, 0 indicating a glycan moiety.

B chain (44 aa) - *KR* - **C chain** (46 aa) *KR* - **A chain** (29aa) + Glycan Moity.

2. LIPOIDAL HORMONE - METHYL FARNESOATE (MF)

Hormone of mandibular organ (gland) is identified as the sesquiterpenoid, methyl farnesoate (MF) in various crustaceans (Laufer *et.al.*; 1987). It is almost similar in chemical structure with insect. JH III but without an epoxide group. It is formed from Farnesoic acid. Haemolymph MF binding protein transports MF. It functions as ecdysiotropin and exerts stimulatory effect on the YO at the onset of premoult and as a gonadotropin or ovarian stimulating hormone in *M. rusenbergii* prawns.

The Biosynthetic Pathway Of Methyl Farnesoate

The biosynthetic pathway of MF (Fig.6.14) appears to be similar to the general mevalonate pathway for acyclic isoprenoids (Goldstein and Brown, 1990). Indeed, many of these steps have been demonstrated for the synthesis of juvenile hormone (Schooley and Baker, 1985; Goodman, 1990). The initial steps of this pathway involve the synthesis of mevalonate from acetate via HMG-CoA synthase and HMG-CoA reductase, followed by its conversion to isopentyl pyrophosphate. Three units of isopentyl pyrophosphate (one of which isomerizes to 3,3′-dimethylallyl pyrophosphate) are condensed to form farnesyl pyrophosphate, which is hydrolyzed to farnesol and oxidized in two steps to form farnesoic acid. The farnesoic acid is then converted to MF by farnesoic acid *O*-methyl transferase (MeT).

Farnesoic acid

Methyl farnesoate

A

Acetyl-CoA

↓ Thiotase, HMG-CoA synthase, HMG-CoA reductase

Mevalonic acid

↓ Mevalonate kinase, 5 - phospho mevalonate kinase, 5 - phospho mevalonate decarboxylase

Isopentyl pyrophosphate

↓ Prenyl trnsferase

Farnesyl pyrophosphate

↓ Phosphatase pyrophosphate, Farnesol dehydrogenase, Farnesal dehydrogenase

B

Fanesoic acid

↓ Farnesoic acid O-methyl transferase (MeT)

Methyl farnesoate

Fig.6.14 Mandibular gland hormone : (A) Structure and (B) synthesis.

3. STEROIDAL HORMONES

3.1 Ecdysone - The Y organs secrete the moulting hormone - α-ecdysone (2β,3β,14α, 22R, 25-pentahydroxy-5β-cholest 7-en-6-one) which is later on hydroxylated to beta-ecdysone (2β, 3β,14αa, 20R, 22R, 25- hexahydroxy-5β-cholest- 7- en-6-one), an active hormonal molecule (Huber and Hoppe, 1965; King and Siddall, 1969). It is an ecdysteroid formed from the parental cholesterol molecule. Although the crustacean hormone was initially called cruseecdysone or ecdysterone, the chemical structure of crustacean and insect ecdysone is one and the same, singularly representing 20-hydroxyecdysone. Detail account is given in the chapter - 7. Three crustacean ecdysteroids are reported . β-ecdysone is also isolated from the ovary and eggs of various

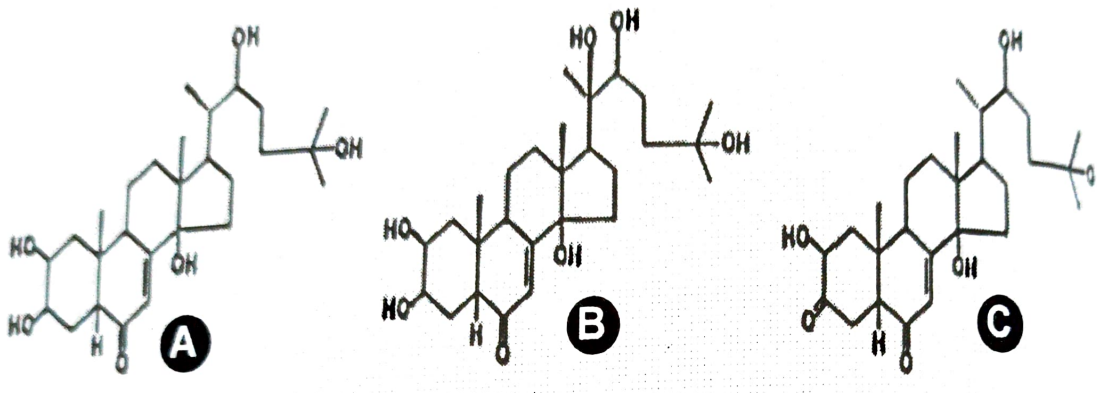

Fig. 6.15 Structure of crustacean ecdysteroids : (A) ecdysone, (B) 20-OH-ecdysone and (C) 3-dehydroecdysone. (Modified from Chang, 1997).

3.2. Vertebrate Steroid Hormones - Gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring (SIM) was employed to examine the steroid content of tissue extracts from the decapod crustacean *Nephrops norvegicus* (Fairs *et* al., 1989). These authors confirmed the presence of 17β-estradiol in the eggs and haemolymph, and found 5α-dihydrotestosterone, testosterone and pregnenolone in ovarian tissue. Pregnenolone and estradiol-17β have also been identified in the tissues of the brine shrimp *Artemia* sp. by GC-MS (Novak *et* al., 1990). Similar results were reported for *Penaeus monodon* (Fairs *et* al., 1990) in which GC/MS with SIM revealed both 17β-estradiol and estrone to be present in the ovarian tissue during vitellogenesis. Testosterone was also detected in mature ovarian tissue. Using HPLC and RIA methods, progesterone-like and estradiol-like substances were detected in ovaries, hepatopancreas and body fluids of the soldier crab, *Mictyris brevidactylus* (Shih, 1997).

Levels of estradiol-17β and progesterone vary significantly during vitellogenesis in the shrimp *Penaeus monodon* with peak levels of progesterone

Table 6.1 Hormones in Crustacea

S.No.	Hormones	Abbr.	Chemical	Endocrine Organs
1.	Crustacean hyperglycemic hormone.....	CHH	Protein	XO SG, PCO
2.	Moult inhibiting hormone.....	MIH	Protein	XO SG
3.	Gonad inhibiting hormone	GIH/VIH	Protein	XO SG
4.	Mandibular organ inhibiting hormone	MOIH	Protein	XO SG, STG
5.	Red pigment concentrating hormone...	DRPH	Octapeptide	XO SG
6.	Distal retinal pigment hormone.....	DRPH (PDH)	Octadecapeptide	XO SG
7.	Pigment dispersing hormone	PDH	Octadecapeptide	BR-PCO, CNS
8.	Tachykinin like peptide	TRP	Nonapeptide	PCO
9.	Crustacean cardioactive Peptide	CCAP	Nonapeptide	TH - PO
10.	Androgenic Gland Hormone	AGH	Glycosyl protein	AG
11.	methyl Farnesoate	MF	Terpenoid	MO
12.	Ecdysone	Ecdysone	Ecdysteroid	YO / ovary

(For abbravations refer text)

correlating with levels of vitellogenin (Quinitio *et* al., 1994). In contrast to the positive results of these studies, Koskela *et* al. (1992) failed to observe any effects of exogenous 17β-estradiol or 17α-hydroxyprogesterone on moult cycle duration or ovarian development in the tiger prawn, *Penaeus esculentus*. In a review of the occurrence of ecdysteroids and vertebrate-type steroids among the invertebrates, including arthropods, Lafont (1991) concludes that, as is the case for insects, no firm evidence yet exists to indicate the role of vertebrate-type steroids within crustacean tissues. A similar conclusion is reached by Fingerman *et* al. (1993).

C. FUNCTIONS

Next to insects, structure and functions of endocrine system are well understood in the crustaceans among Arthropoda and even in Invertebrata. The endocrine system is well developed in Crustacea and secretes large number of hormones which regulate development, reproduction, metabolism and various physiological processes such as, heart excitation, stomodeal contraction, retinal adaptation, colour change, behaviour etc.

1. HORMONAL CONTROL OF DEVELOPMENT

The postembryonic development of crustaceans is indirect, i.e., embryo develops into adult stage after passing through a series of successive immature forms, the larvae, the process is called, the metamorphosis. Each preceding larval stage develops into a succeeding larval stage through casting

off old integument (particularly cuticle), the process is called, the moulting. In Crustacea, moulting process is continued even at adult stage and moulting phase alternates with the reproductive phase. At each moulting cycle, the epidermis secretes new cuticle on one hand and cast off old cuticle. It is a complex process, net result of involvement of large number of genes (Kuballa et al., 2011).

1.1 Moulting Cycle: It is a cyclic process that occurs in all arthropods, from insects to crustaceans, and is essential for growth, reproduction and metamorphosis. The crustacean moulting cycle encompasses the period between two successive moults and has been subdivided into 4 major stages: (1) intermoult (anecdysis), (2) pre-moult (proecdysis), (3) moult (ecdysis), and (4) post-moult (metecdysis). The intermoult period is the longest stage of the moulting cycle, during which muscle regeneration and the accumulation of energy reserves such as glycogen and lipids occurs. Pre-moult witnesses the atrophy of somatic muscle, the resorption of the old exoskeleton, and the formation of a new exoskeleton in preparation for the onset of ecdysis. Ecdysis, or the moult itself, involves the shedding of the exoskeleton through a rapid uptake of water from the environment, causing the exoskeleton to rupture. Further water uptake occurs during post-moult facilitating the expansion of the new, still soft, exoskeleton; this expansion is essential for the growth of the animal. Exoskeletal hardening, via sclerotization and mineralisation, then takes place (Fig. 6.16).

1.2 Hormones involved in Moulting : Moulting process is controlled by a well synchronized hormonal regulatory mechanism. The moulting process controlling hormones are the moulting hormone or ecdysone secreted by the Y organs (YO), moult inhibiting hormone (MIH) secreted by the X organ-sinus gland (XO-SG) complex in the eye-stalk and Methyl farnesoate (MF) secreted

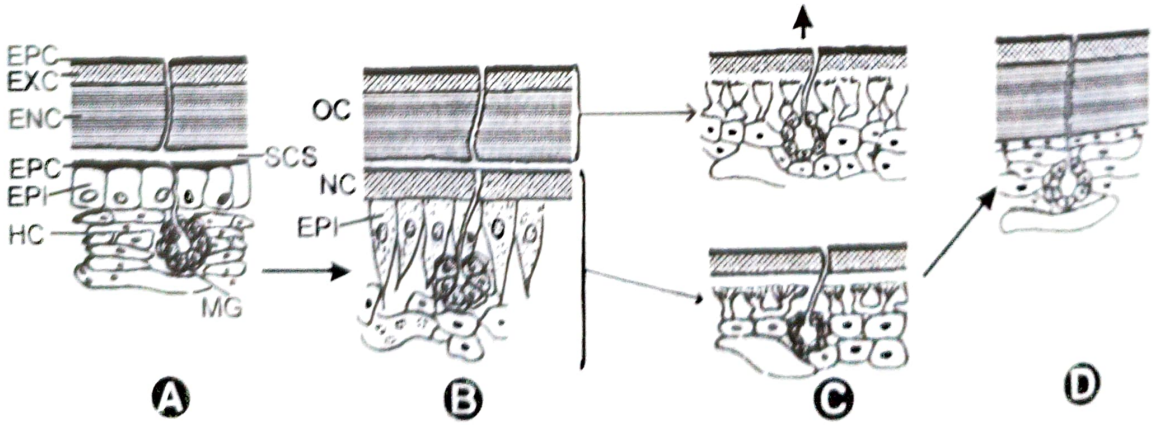

Fig.6.16 Moulting cycle of a generalized crustacean : (A) proecdysis/ premoult, (B) ecdysis / moult, (C) Metecdysis / postmoult and (D) intermoult stages.

Abbr - EPC - epicuticle, ENC- encocuticle EPI - epidermis EXC - exocuticle
HC - haemocytes MG - mucus gland OC - old cuticle NC - new cuticle
SCS - sub cuticular space

The mandibular organs secrete MF in crustaceans. It is similar to JH III of insect except absence of epoxy group. Tobe *et al.* (1980) however, proposed homology in between the corpora allata in insects and mandibular organs in crustaceans on the basis of their similar ectodermal origin, epithelial glandular structure and chemical nature of hormone. Recent studies reveal function of MF in moulting. The MF stimulates Y-organs during premoult moult period of a moulting cycle (Change, 1993) and thus functions as an ecdysiotropin. Besides these metamorphic hormones, there is sufficient evidence of active role of CHH during moulting in the decapods.

1.3 Regulatory Mechanism : Recently Kuballa and Elizur (2007) narrated the hormonal regulation of metamorphosis in Crustacea. (Fig. 6.17)

Positive Regulatory mechanism : At the outset of premoult phases, the nervous stimuli, environmental and intrinsic factor stimulate MO. As a result, MO secret MF which activates YO and YO release α-ecdysone in the haemolymph (blood). Then α-ecdysone is converted into β-ecdysone. β-ecdysone first initiates DNA amplification and induces mitosis in the epidermal cells. Epidermis looses contact of cuticular surface and formation of subcuticular spaces takes place. This process is called, apolysis. Besides apolysis, ecdysone stimulates protein and lipid synthesis and increase in glycogen content in the epidermal cells. Thereafter, β-ecdysone stimulates gene of the cells of hepatopancreas. The hepatopancreas starts synthesis of the moulting protein, cryptocynin. These processes are continued during the moult

by the mandibular organs (MO). Another hormone, gonad inhibiting hormone (GIH) of XO-SG complex become active and inhibits the gonadal growth during moulting period. After large number of studies conducted through various experimental techniques, it is now well evident that the MIH, as its name applies, inactivates the secretory activity of Y organs and inhibits synthesis of ecdysone during intermoult. The MIH, therefore, exerts negative action on the Y organs (repressive control). Whenever the MIH concentration is reduced to almost neglegible concentration, then only the Y organs secrete ecdysone that induces moulting process, called premoult induction (Watson *et al.*, 1989; Change, 1993), This inhibitory effect of MIH on moulting is known since long back (1920) through eye-stalk extirpation experiments (Soumoff and O,Connor 1982). Mattson and Spaziani, 1985) perhaps, first reported MIH causing inhibition of ecdysone secretion from the Y organs. The eye-stalk extirpation, moreover, causes precocious moulting due to highly elevated ecdysteroid level in the blood.

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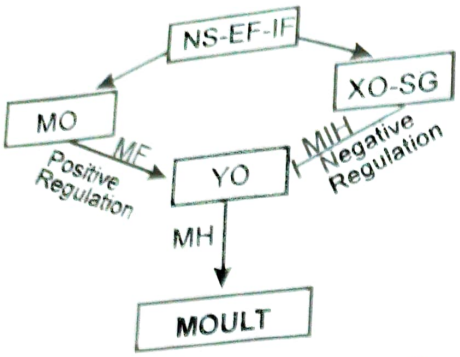

Abbr.: NS nervous system, EF- environmental factors, IF- intrinsic factors, MO mandibular organ, YO- Y-organs, XO-SG X-organ sinus gland complex, MF methyl farnesoate, MH - moulting hormone (ecdysone), MIH moult-inhibiting hormone. Stimulatory effect of MF on protein synthesis has been reported in hypodermis in a crab, *Gecarcinus lateralis* (Paulson and Skinner, 1988). → - stimulation, ⊣ inhibition.

Fig. 6.17 Hormonal regulation of moulting cycle.

YO → ECDYSONE → HP + HP → DNA + RNA + PROTEIN SYNTHESIS.

XO-SG → CHH → EC + HP + MUSCLE → GLYCOGEN CONTENT REDUCTION.

XO-SG → GIH → GONAD DEVELOPMENT INHIBITION.

Negative Regulatory Mechanism : With the beginning of postmoult phase, nervous stimuli, environmental and intrinsic factors stimulate XO-SG complex. They discharge MIH in the blood. MIH inhibits secretory activity of the YO and as a result β-ecdysone secretion is blocked. Gradually, concentration of β-ecdysone drops down and become negligible. The XO-SG complex remains active during intermoult phase.

2. HORMONAL CONTROL OF REPRODUCTION

2.1 Reproductive System: In Crustacea, sexes are generally separate and male and female can be distinguished from their secondary sex characters, pleopods, the first paired pleopods are larger in males than in females.

Female reproductive system consists of a pair of ovaries on stomach and hepatopancreas in cephalothorax, oviducts run ventrally and open into the gonopores independently and an external spermatophore reception area. The gonopores are encircled with a large tuft of setae forming ovulation passage.

Male reproductive system consists of a pair of milky white, elongated multilobulated testes near pericardial area and vasa deferentia opening through individual gonopore on either side of the base of the 5th pair of pleopods. Vasa diferentia differentiate distally into the broad seminal vesicles.

Gonadal development is suppressed prior to adolescence and occurs during intermoult/ reproductive period at the adult stage. Egg development passes through pre, early, late vitellogenic stages leading to a final maturation stage. Spermatogenesis is a continuous process begins with differentiation of spermatogonia and after passing through spermatocytes, spermatids, terminates with formation of spermatozoa. In Crustacea, sperm are transported in the form of spermatophores.

2.2 Hormones involved in Female Reproduction: (Fig. 6.18) In female crustaceans, at least two antagonistic neuroneurohormones are known to play vital role, one the gonad (vitellogenesis) inhibing hormone (GIH/VIH) of XO-SG complex and other, the gonad-stimulating hormone (factor) (GSH/GSF) from the BR-PCO complex (Eastman-Reks and Fingerman, 1984). According to Wongsawang *et al.*, (2005) eyestalk is a source of both, GIH/VIH and GSH. Long back, Panouse (1944) noticed removal of eyestalk causing rapid increase of size of ovary and precocious egg deposition demonstrating eyestalk as a site of GIH. This hormone primarily inhibits vitellogenesis by inactivating vitellogenin (vg) synthesis or Vg gene activity (Treerattrakool *et al.*, 2008). It is now well established that the GIH arrests gonadal development during moult period and GSF stimulates gonadal development during reproductive period.

Crustacean hyperglycemic hormone (CHH) is almost alike GIH and MOIH in molecular structure (all three belong to same family) and known to inhibit protein synthesis in ovary and yolk protein, vitellogen synthesis in hepatopancreas in the shrimps, *Marsupenaeus japonicus, P. semisulcatus, matapenaeus ensis* and crabs *Carcinus maenas, Cancer pagurus, Callinectes sapidus* etc.

Besides, neurohormones, MF from MO plays active role in ovarian maturation. During reproductive period MF functions as a gonadotropin and stimulates ovarian development by inducing protein synthesis in the previtellogenic oocytes (Soroka *et al.*, 1993). MF enhancing ovarian maturation is well evident in some crustaceans - *Penaeus vannamei* (Laufer *et al.*, 1997), *Procambarus clarkia* (Laufer *et al.*, 1998) and *Oziotelphusa senex* (Reddy and Ramamurthi, 1998).

Recent investigations explored key role of the β- ecdysone in female reproduction, particularly, maturation of ovary (synthesis and deposition of vitellogenins) and now-a-days it is referred as the ovarian hormone. It therefore performs dual functions - initially secreted as a moulting hormone from YO during premoult-moult period and later on, as an ovarian hormone during reproductive period (Wongsawang *et al.*, 2005; Brown *et al.*, 2009). Presence of β- ecdysone in the ovaries and eggs of several crustaceans has been noticed (Nagaraju, 2011). According to the crustacean endocrinologists, 20-hydroxyecdysone(β-ecdysone)is physiologically analogous to the vertebrate-estrogens. It is known to stimulate Vg 1 gene expression in hepatopancreas explants *in vitro* in the shrimp, *Metapenaeus ensis* (Tiu *et al.*, 2006).

A cluster of information on involvement of several other substances in crustacean ovarian maturation is now available such as, 17-estradiol and progesterone like vertebrate steroids, ovarian prostaglandins - E2 (PGE2), F2

(PGF2) and D2 (PGD2), neurotransmitters like- serotonin (5-HT), octopamine (OA), dopamine (DA) etc. (Fingerman, 1997) and opioid peptides particularly, met- enkephalin and leu-enkephalin (Fingerman *et al.*, 1985).

2.3 Hormones involved in Male Reproduction: Since the discovery of androgenic gland (Cronin, 1947) in the crab, *Callinectes sapidus* and first report regarding its androgenic function in the isopod, *Armadillidium vulgare* (Charniaux-Cotton, 1954), later workers confirmed regulation of male differentiation (masculinization differentiation of primary and secondary sexual characteristics) and induction of spermatogenesis in large number of crustaceans (Sagi et al., 1997). A complete sex reversal was achieved by andrectomy of males at an early immature stage resulting in female differentiation of primary (internal reproductive system) and secondary female sex characters in the prawn, *Macrobrachium . rosenbergii* (Sagi *et al.*, 1990). Similarly androgenic gland implantation into early stage immature female prawn, *M. rosenbergii* leads to the development of testis, sperm ducts and male gonopores (primary male sex characters) and masculine, copulatory appendages (secondary male sex characters) as well (Nagamine *et al.*, 1980). Experimental data suggest inhibition of vitellogenesis after administration of the AGH also (Sagi and Khalaila, (2001). Androgenic gland, however functions independently, i.e., without under influence of any other hormone, neurotransmitter or neuromodulator.

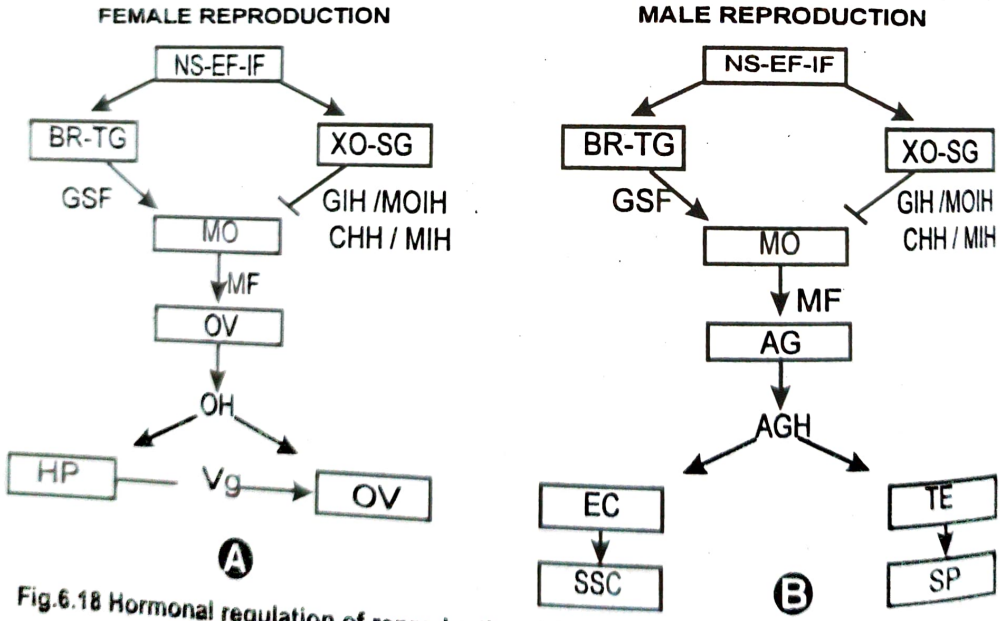

Fig.6.18 Hormonal regulation of reproduction: (A) female reproduction and (B) male reproduction.

Abbr.: NS nervous system, EF- environmental factors, IF- intrinsic factors, BR-TG brain, thoracic ganglia, MO mandibular organ, XO-SG X-organ sinus gland complex, MF methyl farnesoate, MIH moult-inhibiting hormone. AG androgen gland, AGH androgen gland hormone, GIH gonad inhibiting hormone, CHH crustacean hyperglycemic hormone, GSF gonad stimulating factor, OV ovary, OH ovarian hormone, HP hepatopancreas, EC epidermal cells, TE testis, SP spermatogenesis.

The eyestalk GIH however, inhibits while GIF stimulates a secretory activity of AG and control spermatogenesis process in testis. Whenever haemolymph concentration of GIH is high and that of GSF and AGH is low, the spermatogenesis and development of testis is arrestedin the crab, Purutelphusa hydrodromus (Gupta, 1989). Chang and Sagi (2008) confirmed stimulation of spermatogenesis by AGH. Existence of GSF is claimed by some workers (Aiken and Waddy, 1980), and NSC of brain and thoracic ganglia has been suggested as a source (Kulkarni et al., 1981, Joshi and Khanna 1984)but molecular structure of a hormone is still obscure. Joshi and Khanna (1984) moreover, noticed hypertrophy of AG after injection of thoracic ganglia extract in *Poemon koolooenseduring* spermatogonial quiescence. In the light of some reports available, role of other hormones such as MF, steroids, opoids and neurotransmitters in male reproduction can not be ruled out, though at present the adequate evidence is lacking.

3. HORMONAL CONTROL OF COLOUR CHANGE

3.1 Colour Change Phenomenon - Integumental colour change and eye adaptation due to movement of pigment granules in the chromatophores and retinula cells in accordance with circadian rhythms (dark and light) is a common phenomenon in crustaceans. This is reversible colour change mechanism results from either dispersion or concentration of pigment granules within the chromatophores and retinal (retinula and other ommatidial) pigment cells.

In Crustacea, chromatophores may contain one (monochromatic), two (dichromatic) or several (polychromatic) pigments. Monochromatic chromatophores contain only a single coloured pigments, red, blue, yellow, white and black pigments. Prawn and shrimps generally contain polychromatic chromatophores while Anomura, Brachyura, Isopods and Stomatopoda contain mono and dichromatic chromatophores.

In crabs colour change of the body is diurnal rhythmic activity it becomes dark during day-time and pale during night. The rhythmic colour change is found to be due to pigment movements in the monochromatic black chromatophores predominantly. Dark colour of body occurs due to dispersal while pale colour developd due to concentration of pigment (melanin) granules in the chromatophores (melanophores). In initial experiments it was noticed that if eyestalks of a crab removed, it resulted into appearance of permanently pale body colour (blanching) due to concentration of pigment in the black chromatophores and also suggesting the eye stalks as a source of chromatophorotropins.

Colour change in crustacean is of great significance. It provides

defense, thermoregulation and mating and parental care behaviour. As a dark colour of body resembles with sea bottom and the blanching camouflages with sea surface in both conditions, an animal is protected from predator.

3.2 Role of Chromatophorotropins - Crustacean endocrinology is emerged out with the pioneer discovery of hormonal control of colour change (Koller, 1927; Perkins, 1928). The XO- SG and neurosecretory cells in various ganglia of nervous system secrete some pigmentary effector hormones (chromatophorotropins) which stimulate epidermal chromatophores and distal pigment cells in the eye (Fingerman, 1988; Fingermann *et al.*, 1994). These hormones cause light or dark adapted pigment translocation in extraretinular ommatidial cells and dispersion or concentration of pigment granules in chromatophores as well. The pigmentary effector hormones however, have antagonistic action and can be divided in two groups 1. pigment concentrating hormones causing chromatophoral pigment dispersion and ommatidial light adaptation and 2. pigment dispersing hormones inducing chromatophoral pigment concentration and ommatidial dark adaptation (Rao, 1985; Fingermann, 1988).

3.2.1 Red Pigment Concentrating Hormone (RPCH) The RCPH elicits chromatophore pigment concentration in shrimps. RCPH induces dark-adaptational movement of screening pigment in the distal eye pigment cells (Garfias, *et al.*, 1995). It also plays extra pigmentary role as stimulation of methyl farnesoate secretion from mandibular organs, (Laufer *et al.*, 1987) and neuromodulation (Dickinson *et al.*, 1997). The RCPH causes pigment concentration in Decapods variably, *e.g.*, in erythrophores alone in *Uca pugilator, Cambarellus shufeldtii*, in leucocytes and erythrocytes in *Palaemon squilla. Penaeus aztecus* or in melanophores, leucophores and erythrophores in *Crangon crangon*. RPCH induces pigment concentration in all four types of chromatophores - melanophores, leucophores, erythrophores and xanthophores in *Penaeus japonicus* (Yang *et al.*, 1999).

3.2.2 Pigment Dispersing Hormones (PDH) The PDH are octadecapeptides called light adapting distal retinal pigment hormone (DRPH/ α-PDH). Several PDH variants are reported in individual species of crustaceans.

All forms of PDH are specifically characterized with only one function, i.e., pigment dispersion in chromatophores. PDH, besides triggering pigment dispersion in chromatophores, induces in addition, light adaptational screening pigment movements in the eye and called distal retinal pigment hormone (DRPH) also (Kleinholz, 1975). Highly effective PDH in eyestalks of *Uca pugilator* is the β-PDH (Rao *et al.*, 1985). It has been found that the β-PDH is most potent while α-PDH is list potent in inducing pigment dispersion. All PDH forms belong to a single PDH peptide family.

RPCH → CHROMATOPHORES → PIGMENT CONCENTRATION → BLANCHING → MO → MF

PDH → CHROMATOPHORES → PIGMENT DISPERSION → DARKENING

3.3 Control of Retinal Pigment Movement - It is further stated that the light and dark phases of diurnal cycle stimulate compound eyes which via central nervous system control the release of dispercing hormones (PDH) or concentrating hormone (RPCH).

In the light adapted eye, the distal and proximal pigments prevent the rhabdom (imafge formation site of retina) from receiving excess light and the reflecting pigment cells move beneath the basement membrane. In the dark adapted eye, on the contrary, the distal and proximal segments do not cover the rhabdome and reflecting pigment moves externally to the basement membrane so that rhabdom receives maximum quantity of light from own as well as adjacent corneas. In Palaemonetes, the eyestalk extract injection causes migration of the distal pigment towards the light adapted position and after a period of about 4 6 hours it returns to a former position. The injection of brain extract produces a light adapting response.(Fig.6.19)

RPCH → DISTAL EYE PIGMENT CEELLS → UPWARD PIGMENT MIGRATION → DARKADAPTTTATION

PDH→ DISTAL EYE PIGMENT CEELLS → DOWNWARD PIGMENT MIGRATION → LIGHT ADAPTTTATION

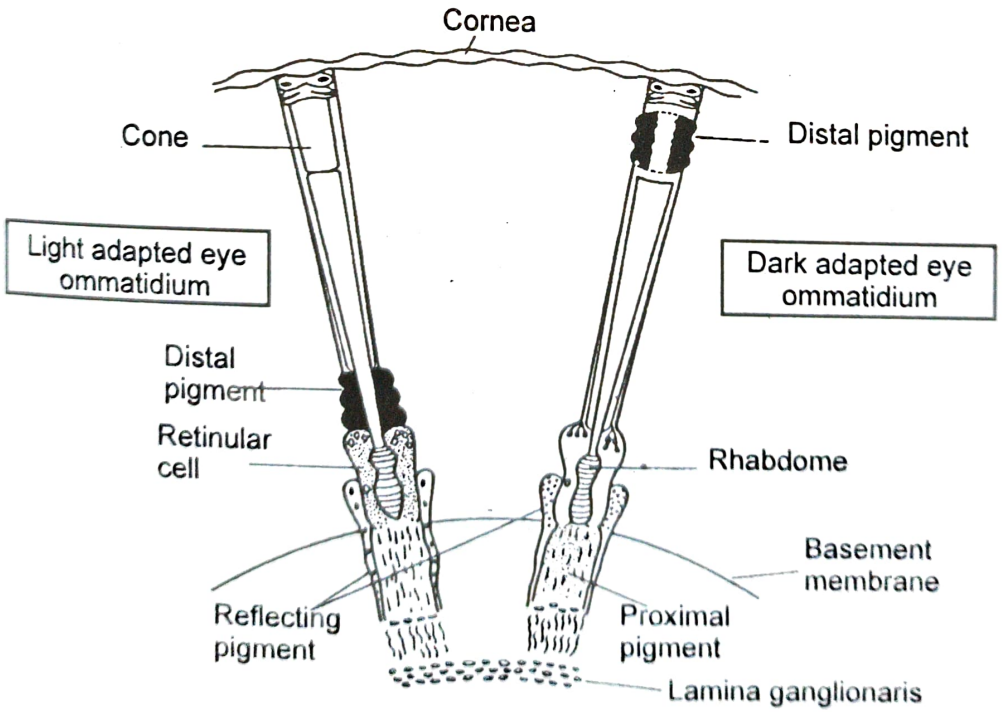

Fig. 6.19 Ommatidium of a crustacean eye in the light adapted and the adapted condition (After carlisle and Knowles, 1959)

4. HORMONAL CONTROL OF HEART CONTRACTION

In Crustacea heart is large sac-like single chambered structure and innervated by the dorsal long cardiac ganglion (pace maker) and a single dorsal nerve. It extends inhibitory and acceleratory nerve fibres which terminate into nerve plexus and become a part of the pericardial organ. The pericardial organs lie across the opening of the branchio-cardiac veins so they release crustacean cardio- active peptide (CCAP) in the blood directly. The CCAP hormone is a small nonapeptide molecule and it accelerates amplitude of the isolated heart and enhances the frequency of beating (Fig. 6.20). The rhythmic contractions of the heart of the blue crab *Callinectes sapidus*. are neurogenic, driven by rhythmic motor patterns generated by the cardiac ganglion (CG). The CG contains only nine neurons: four local premotor interneurons that are thought to act as pacemakers and five motor neurons that send bursts of spikes out of the ganglion to contract the single-chamber heart. Through mutual electrical coupling, all of the motor neurons fire in precise synchrony (Fort *et al.* 2004).

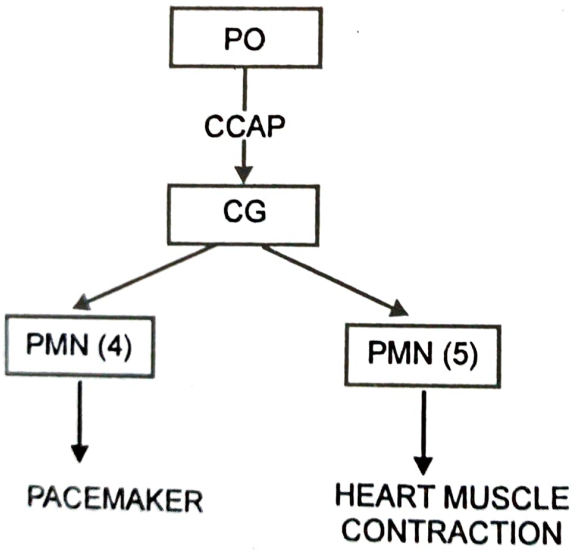

Fig.6.20 Hormonal regulation of heart by the CCAP (after Fort *et.al.* 2004). Abbr. - CCAP - Crustacean cardiactive peptide, CG - Cardiac ganglion, PMN - Premotor neurons, PO - Pericardial organ.

Schematic summary of the actions of CCAP on the *Callinectes* cardiac system. Increasing density of background shading from *left* to *right* symbolizes increasing concentration of CCAP. As the CCAP concentration increases different parts of the system are progressively modulated, with progressive changes in the contraction waveform. At low concentrations, CCAP acts peripherally on the heart muscle to increase contraction amplitude. At higher concentrations, CCAP acts also centrally on the cardiac ganglion (CG) to increase spike burst and contraction frequency. These primary actions of CCAP then have secondary consequences mediated by the feedforward and feedback mechanisms of the system.

□□□