INSECT ENDOCRINOLOGY

1> The Corpora Cardiaca (C.C), principal neurohaemal organ, are small paired structures, bluish in appearence (when viewed insitu), located just posterior to brain & is closely associated with dorsal aorta & oesophagus. It serves as a point of storage & release of materials brought to it by nervi corporis cardiaci.

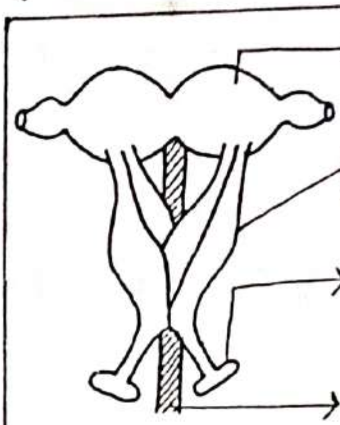


Fig. Insect neurohaemal organ

2> A large portion of c.c is made up of swollen axon terminals (extrinsic fibre) entering from the brain. The neurosecretory product may accumulate there or is released in haemolymph.

3> Intrinsic Neurosecretory cells (NSC) have been Identified in C.C by B. Scharrer (1963). The intrinsic cells have the endocrine activities similar to N.SC. Products of cardiacum NSC may be released from the surface of Neurohaemal organ (NHO) or may extend to other tissues (non-neural endocrine gland, eg-Corpora allata) by the way of axon (intrinsic fibre) originating from intrinsic NSC.

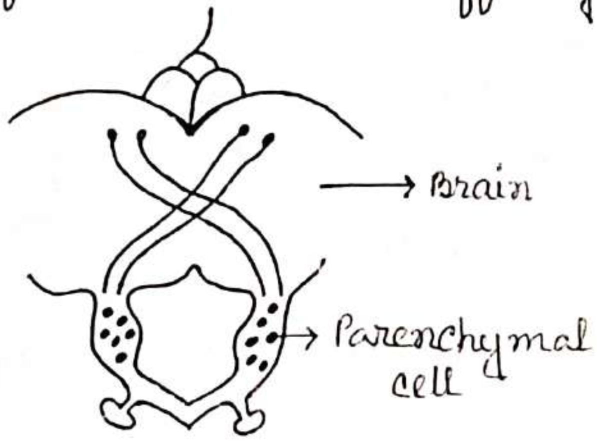
4> Each cardiacum is connected to its corresponding corpus allatum (C.a), a non-neural endocrine gland, by a single nerve composed of axon originating in brain which continues through cardiacum into the allatum

5> The C.C can act as an endocrine as well as a neurohaemal organ, due to possession of both extrinsic neurosecretory fibre. In other words, it acts as a storage organ of neurosecretion, as well as a release organ of secretion of NSC present in both

CNS & in itself. Thus, in insects, c.c is called as storage-release organ.

6) There are granules containing cells in c.c which are roughly equivalent to neurohypophyseal pituicytes.

7) It has two types of parenchymal cells - intrinsic, for production of neurohormones and chromophobe cells, for less colour affinity (no stain).



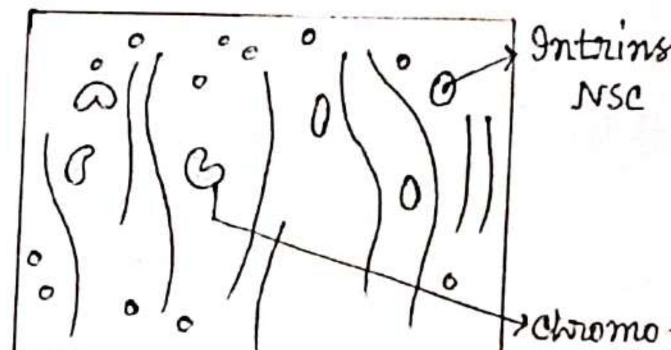


Fig. Corpora cardiacum (Large view)

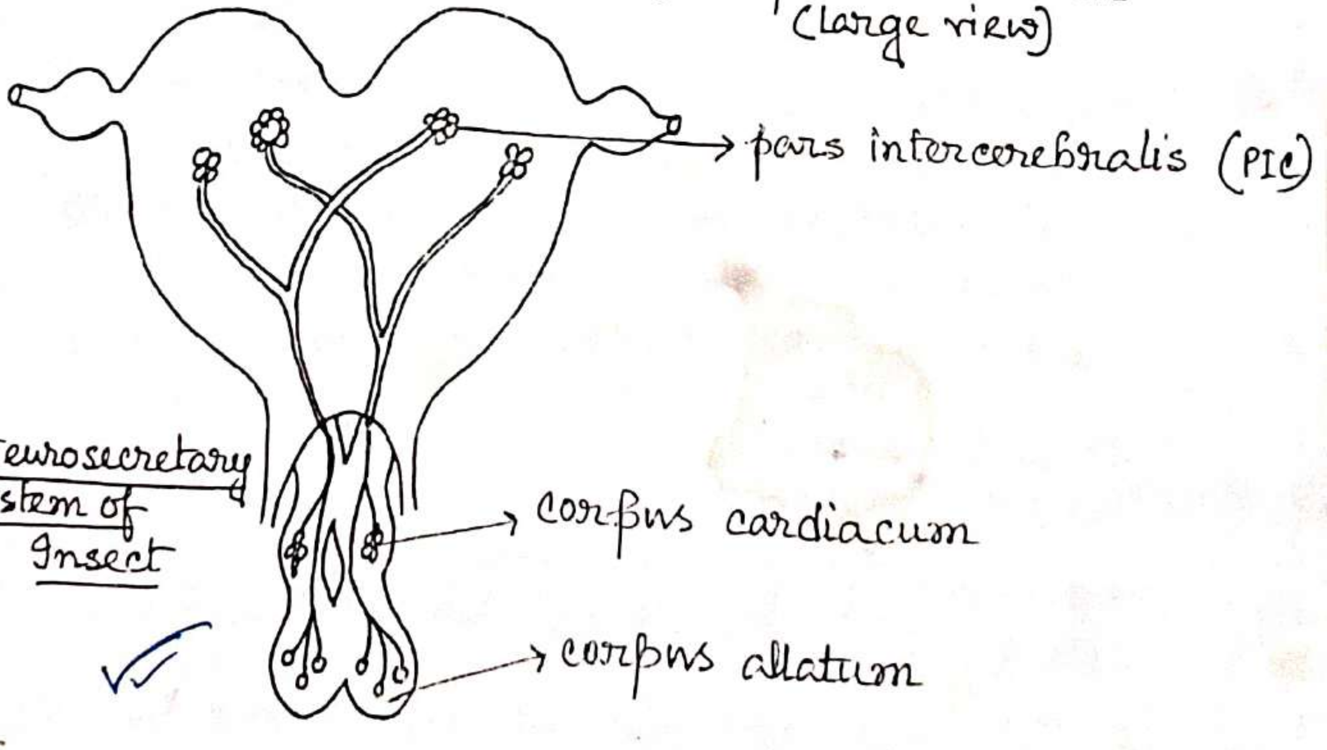


Fig. Neurosecretary system of Insect

N.B :-

C.C of one side receives the main extrinsic nerve from the NSC of the corresponding side, i.e, left C.C receives extrinsic neurosecretory originating in NSC

1.2. The Corpora Cardiaca (Fig.7.1, 7.5). : Since the time of Lyonet (1762), the corpora cardiaca are described as the milky white paired bodies situated immediately behind the brain in between the anterior most part of aorta and the oesophagus in a number of insects. They were considered for a long time as the 'pharyngeal ganglia or oesophageal ganglia' of the stomodeal nervous system, while Hanström (1940) first recognized them as the cerebral neurohaemal organs. They may be oval, spherical or fusiform and are often fused medially and even with the corpora allata posteriorly. They are, however, situated on either side of the hypocerebral ganglion and sometimes connected with latter by the lateral nerves, the nervi cardiostomatogastrici. The corpora cardiaca develop

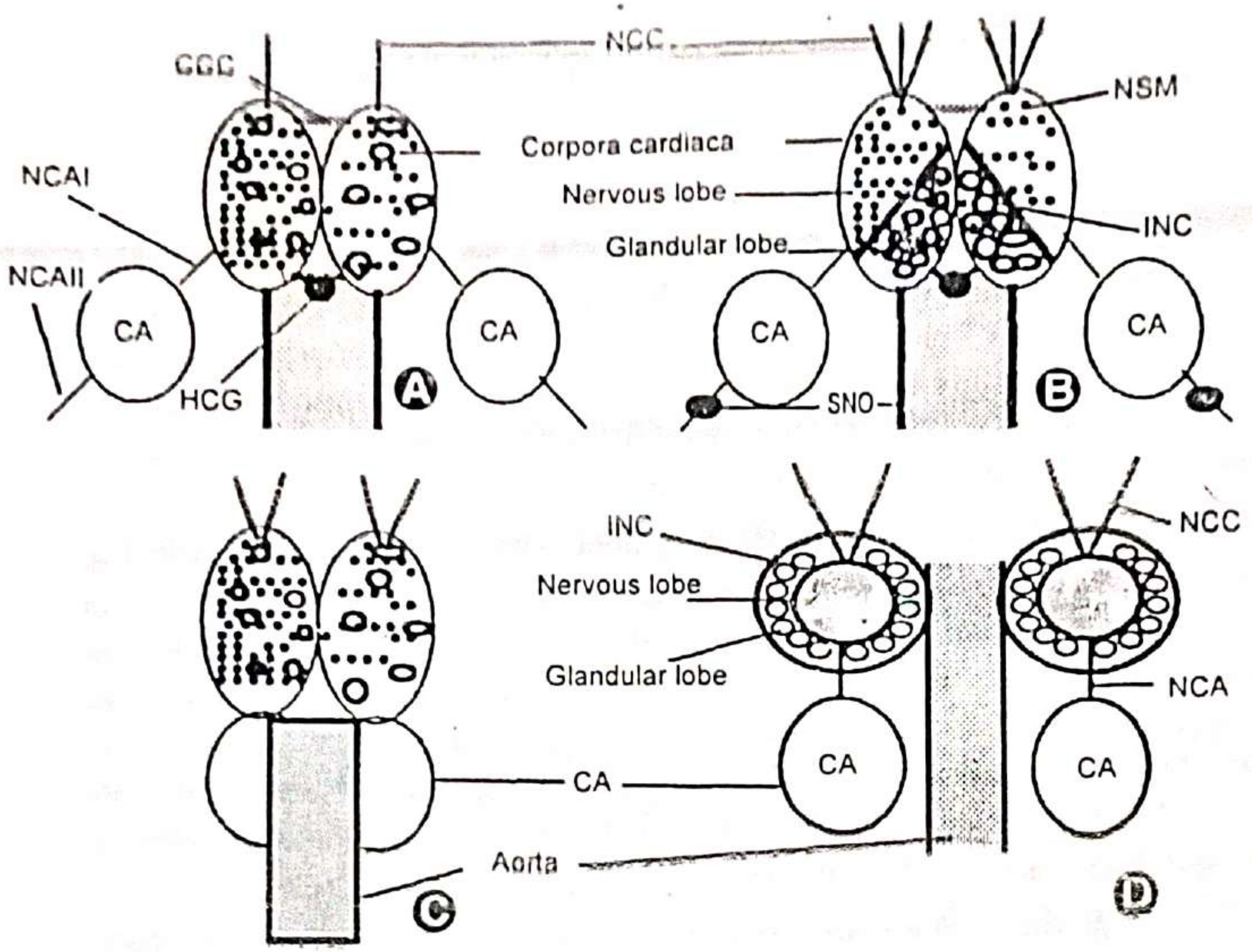


Fig.7.5 Anatomical organization and structure of the corpora cardiaca-allata-aorta complex : (A) diffused type in Odonata, (B) with glandular lobe in Dictyoptera, (C) with aorta as a primary neurohaemal organ and a single CA in Hemiptera and (D) with cortical glandular lobe and medullary nervous lobe in Coleoptera.

Abbr. INC - intrinsic neurosecretory cells, NCC - nervi corporis cardiaci, NCA - nervi corporis allati, CCC - CC commissure, HCG - hypocerebral ganglion. SNO - secondary neurohaemal organ, CA - corpora allata, NSM - neurosecretory material.

as the invaginations of the foregut during embryonic stage. They are innervated by a single pair of composite NCC or three pairs comprising NCC I, NCC II and NCC III fine nerves from the brain anteriorly and by a pair of nerves, the nervi corporis allati (NCA) from the ipsilateral corpora allata posteriorly.

Each corpus cardiacum consists of two parts; one nervous and another glandular. Both the parts are either separated completely (Dictyoptea, Orthoptera, Coleoptera) or diffused (Odonata). The nervous part of the CC contains extrinsic cerebral neurosecretory axon terminals exclusively. The glandular part, on the other hand, is composed of two types of intrinsic neurosecretory cells- the chromophobic without stained cytoplasmic inclusion and chromophilic with the stained material in their cell body. The corpora cardiaca, therefore, play dual functions as the neurohaemal organs as well as the endocrine organs.

1.3. **The Corpora Allata** (Figs. 7.6). Müller (1828) first reported these structures as the "visceral ganglia" while Nebert (1913) made a comparative morphological study of the corpora allata and Wigglesworth (1934) recognized their endocrine function. They are paired spherical or oval bodies in most of the insects except Hemiptera, Dermaptera and adult dipteran flies where both the CA are fused and form a single median corpus allatum lying below (Hemiptera) or above (Diptera) the aorta.

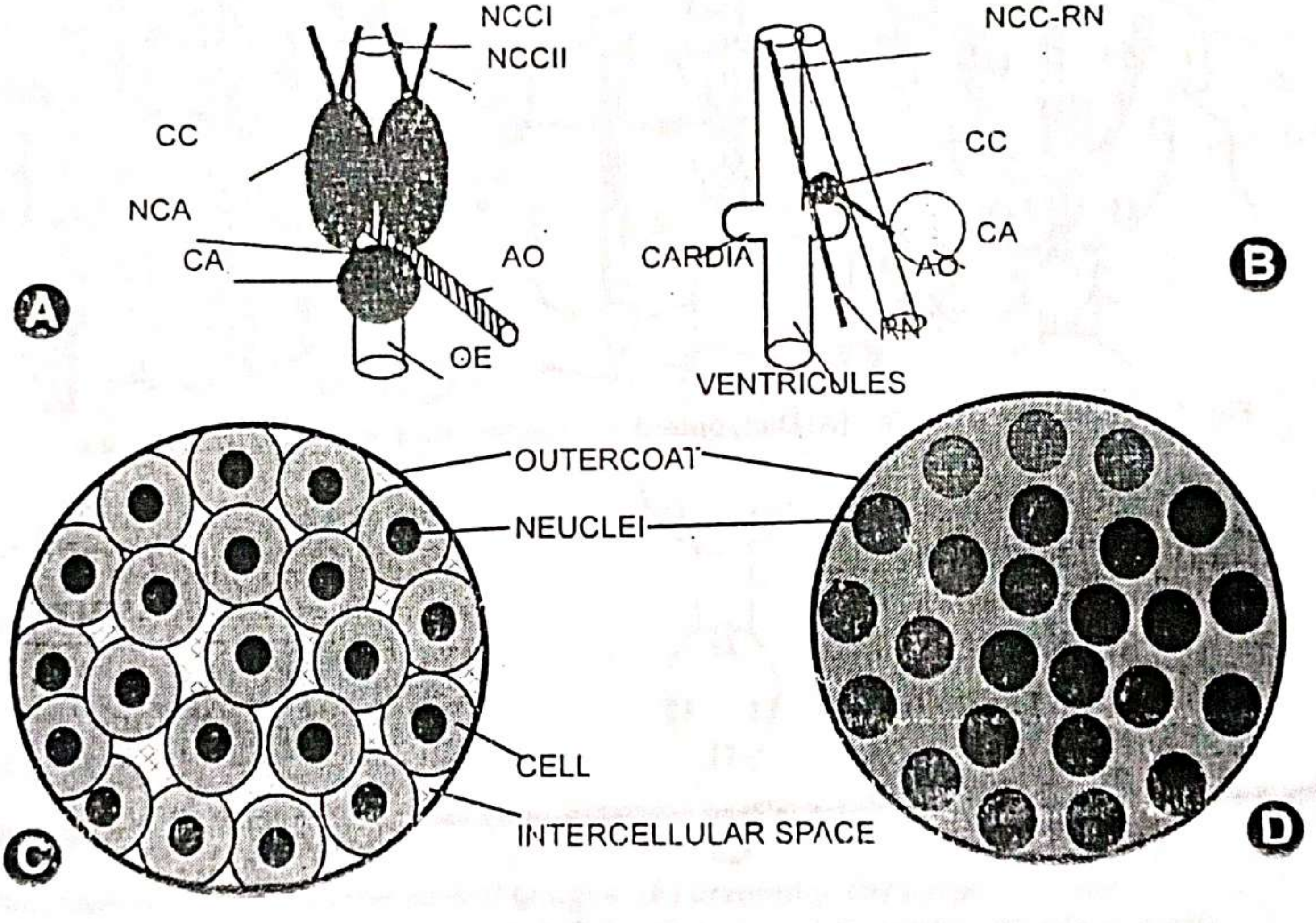


Fig.7.6 Schematic representation of the corpora cardiaca - allata complex in (A) Hemiptera, (B) adult housefly, (C) cellular structure and (D) syncytial structure of the corpora allata (for abbreviations refer earlier figures).

The corpora allata consist of small spherical epithelial cells internally and the connective tissue coat externally. The CA undergo cyclic changes mostly during metamorphosis and egg maturation during which they exhibit a syncytial and cellular structure. Some cells grow in size and contain giant and polyploid nuclei. Formation of intercellular spaces among the cells is well evident. The corpora allata are often smaller in the males than females and in latter they may increase by about 30 times in volume during vitellogenesis (yolk synthesis and deposition during oocyte development). They are innervated by the cerebral neurosecretory and ordinary nerve fibres externally or in some cases even internally. In some insects (Coleoptera) the CA may function as the secondary neurohaemal organs.

The hormone secreted by the CA is commonly called, the 'juvenile hormone' (JH) in both the immature and adult stages.

CORPORA ALLATA :-

1> Corpora allata (c.a) are bilaterally arranged structures in close proximity to c.c. The structure is connected with the latter by a single nerve, nervi corpora allati, but have no ontogenic relationship with c.c or anyother neurosecretory (NS) pathway.

Ecdysial Gland/Prothoracic Gland

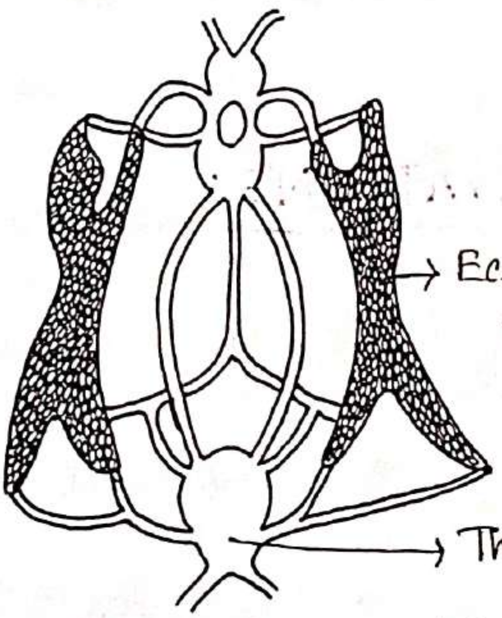


Fig. Ecdysial gland Location

1> The second non-neural endocrine gland of insects is ecdysial gland of ectodermal origin at base of second maxilla.

2> It is a paired structure & is found either in head or thorax.

3> The glands, whether present in head or thorax, are usually associated with tracheal system & are made up of a homogenous mixture of secretory cells, including a few haemocytes, tracheal, epithelial, & glial cells as well as scattered muscle fibres.

4> In certain groups of insects, gland is innervated by nerves from thoracic & suboesophageal ganglion, whereas, in some groups of insect, the gland is without any innervation.

5> E.M studies have suggested that stainable inclusions (NSMs) are present in nerves innervating ecdysial gland of cockroach, moths, flies. Two distinct types of granules have been observed suggesting that two separate responses may be under endocrine control.

6> The name of gland (ecdysial gland) emphasises its functional importance in the control of ecdysis or moulting. Activation of gland is dependent upon the release of an tropic hormone (ecdysiotropic hormone) from the neurosecretary axons of C.C.

N.B: More advanced the insect, the more diffused is the gland & more posterior will be its location (towards thorax).

Role of Neurohormones on Growth & Development of Insects :-

Insects grow towards maturity through stages or instars. At the end of each instars, moulting occurs when the old exoskeleton is shed with formation of a new skeleton of greater dimension for further growth. On reaching the adult form, moulting usually ceases except in few insect order. The final moult is very important in life cycle of insect since in this stage adult characters are formed with concurrent loss in specific juvenile characters.

"Metamorphosis" refers to this period of change from juvenile to adult moulting & metamorphosis (a collosal morphological change in structural organisation of juvenile stage to attain the adult stage) are two basic phenomena of growth and development in insects.

3 endocrine sources are actually orcheasting the growth & development of insects - the brain (NSC), ecdysial glands & C.A.

The NSC in the PIC region of brain produce ecdysiotropin (neurohormone) upon proper stimulation, which passes to neurohaemal organ by axon transport.

N.B :-

The initial stimulus for release of ecdysiotropin in Rhodnius prolixus originated in the propriroreceptors in the abdomen. Upon consumption of a large blood meal, the strech receptors are stimulated & the nerve impulses are sent to brain through ventral nerve cord & the NSC are then stimulated to release the hormone.

Upon the release of ecdysiotropin from neuro-haemal organ, it stimulates the ecdysial glands to synthesise & secrete the moult inducing hormone, ecdys[one]. Under influence of this hormone moulting process in th[e] Insects is initialised.

A coordination must take place between the moult hormone (ecdysone) & status quo hormone (Juvenile Hormone (JH) that maintains juvenile characters in larva) when the insect undergoes changes from juvenile to adult stage. C.A secretes JH (allatotropic hormone) in haemolymph. Both of these hormones (ecdysone & JH) interact on cuticle of larval stage & helps in moulting.

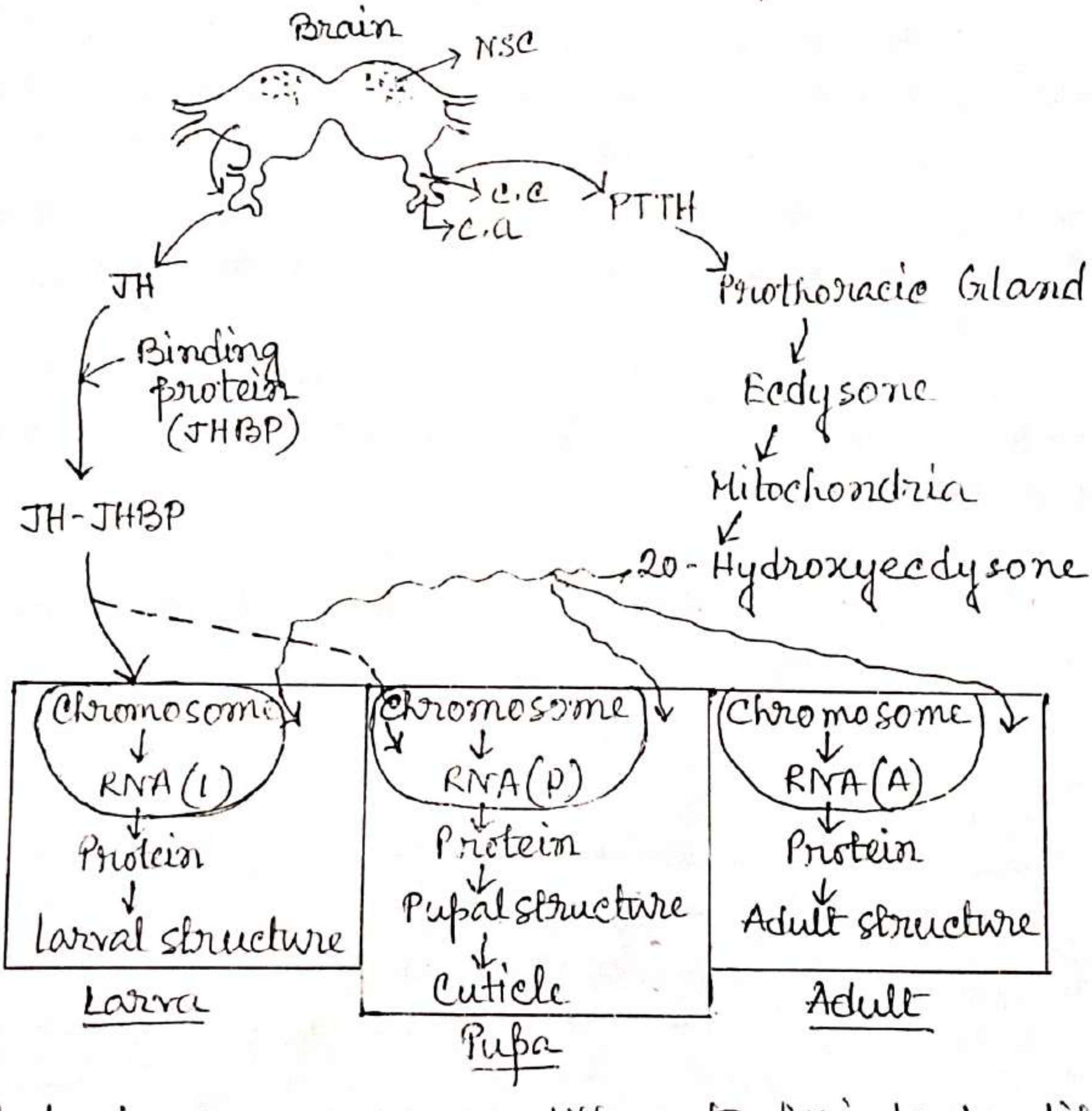


Fig. Endocrine influence of different periods in life to tobacco hornworm moth over its growth & development. It is assumed a critical concentration of Ecdysone & JH lead to transfer of appropriate nuclear information via m-RNA for formation of larval, pupal, adult stage.

The other is holometabolous development, in which the juvenile stages are markedly differ from adult by their appearance & pupae formation takes place. The changes that take place between different larval forms & between the last larval stage & pupal stage is known as moulting, & the changes that take place in pupa to attain adult structures, is known as metamorphosis.

eg →

Hatching egg —Moulting→ Different larval stages —Moulting→ Pupa —Metamorphosis→ Adult

Holometabolas development is observed in butterflies, Bombyx mori, moths etc.

Hemimetabolas development is an incomplete metamorphosis or, gradual metamorphosis, whereas, holometabolous development is complete metamorphosis.

Chemical Nature of INSECT HORMONE :-

The tests were performed on three main insect hormones –

1> The NH. eg, ecdysiotropin, synthesised by NSC of brain (PIC region) & which remains stored in NHO, the C.C.

2> The ecdysone, secretion of ecdysial gland.

3> The JH, synthesised within & released from C.A, the non neural endocrine gland (NEG).

1> Chemical Nature of NH :-

In insects, NH is known as brain hormone, or activation hormone, which is supposed to activate respective endocrine gland for physiological operations. The chemical nature of the NH (eg - ecdysiotropin) is debatable. It can be understood from following discussions :-

a> In year 1958, Tokyo scientists, kobayashi & kirimera, ren & extracted a large number of brains (~8500) from pupae of B. mori & found that extracted fraction is methanol or ether soluble material. From this point of view they claimed NH, obtained from pupal brain of B. mori is not peptide in nature, but has properties to that of cholesterol.

b> In 1961, Japanese scientist Ichikana & Ichizaki isolated brain hormone from B. mori & claimed from their test that it is a water-soluble, heat stable, non-dialysable substance & it must be polypeptide or protein and not a ether soluble material.

c> In 1962, Kabayashi et al. further reported that the brain hormone is cholesterol. In this case, they prepared 4 mg of crystals from 2,20,000 brains of Bombyx pupae.

d> In 1966, Tamazaki & Kabayashi, partially confirmed that the proteinaceous nature of active NH, but also said that, cholesterol is also a component of it.

e> In 1967, Ishigaki & Ichikawa, strengthened the proteinaceous nature of NH. An 8000 fold purification of this active was done by this group of scientist, & they claimed that the molecular weight of the active principle ranges 9000-31000.

2> Chemical Nature of Ecdysone:-

Another insect hormone is ecdysone, obtained from ecdysial gland or prothoracic gland. It is also known as growth and differentiation hormone. The hormone was first found out in 1930 & was known as moulting hormone, since it acts on Larval cuticle & induce moulting.

In 1954, German scientists, Butenandt & karlson for first time, isolated 25 mg of crystalline ecdysone from 500mg of silkworm pupae & have tested in Calliphora unit/ non-pupated individuals (pre-pupal stage).

They have shown that, if ·0075 mg of ecdysone (α-ecdysone) is injected in abdominal portion of a ligated prepupal individual of Calliphora sp. (ligation is made at 2/3 position of Calliphora pre-pupa, to cut off biologically the haemocoel connection of abdominal part from the anterior portion, so that, no ecdysone can circulate from anterior half to abdominal half). This injection will induce moulting in abdominal portion of about 50-70% in the treated pre-pupal stage of "Calliphora sp.". The moulting of anterior portion was normal, as it contains the ecdysial gland, the source of ecdysone.

They termed this quantity (·0075 μg) of α-ecdysone which causes 50-70% of the prepupal individual to undergo the process of successful moulting as "1 Calliphora unit".

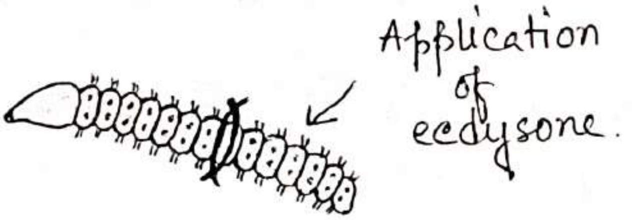


Fig. Ligated prepupal form

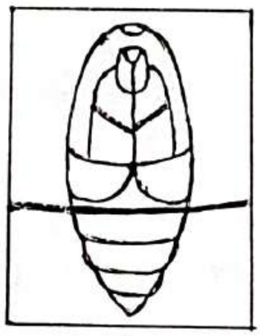


Fig. Pupation takes place normally

In 1963, karlson et al obtained 250 mg of crystalline ecdysone from 4 tons (dry weight of 1000 kg) of fresh silk worm pupae & reported that the material is a steroid having a molecular weight of 464.

In 1966, Karlson et al discovered that this active fraction is composed of two steroids α-ecdysone and its derivative β-ecdysone. The derivative is present in smaller quantities than the α-form, but is more active biologically than α-ecdysone.

Note:-

A number of ecdysone like materials have also been extracted from evergreen trees, weeds, ferns in America, Europe, Asia which are known as phytoecdysone, which are very close to chemical structure to the moulting hormone, ecdysone but functionally phytoecdysone is inhibiting to normal insect growth & development (Robbins et al, 1968).

3) Chemical Nature of Juvenile (Neotinin) Hormone:-

Corpora allata secretes this JH. The chemical nature of JH is identified from stored JH in 2 days old male Lepidoptera silk moth, Hylaphora cecropia, as haemolymph of this 2 days old male silk moth contains a high concentration of this active principle, about 30 folds greater than that of a female silk moth of the same age.

In 1956, Williams extracted the active principle from the haemolymph of 2 days old male Hylaphora cercopia with cold ethyl ether solution and described it as a heat stable non-sterol, unsaponifiable lipid. Since then, different authors in different time worked with this JH.

In 1967, Roller et al. discovered that the basic structure of this hormone is tripple isoprene unit of fernesol with a methyl ester derivative. This chemical structure is also obtained from a number of vertebrate and invertebrate tissues.

Account of Post-embryonic growth & development of Holometabolous Insects :-

Most of pterygote insects are classified into 2 broad groups according to their growth and development. These are :-

1. Holometabolous Development :-

it is complete metamorphosis in which juvenile stages are markedly different from adult by their appearence and pupal formation takes place. These stages during the course of metamorphosis involve - Larva, pupa, adult. eg: Butterfly.

2> Hemi-Metabolous Development :-

It is the incomplete metamorphosis, in which juvenile has not deviated greatly from the characteristics of adult's features. These stages of development involve several nymphal stages.

eg - Cockroach, Grasshopper, termites etc.

Defination of Metamorphosis & Moulting :-

Moulting:- The changes that take place between various larval forms and the last larval form to pupal form is known as moulting.

Metamorphosis:- Metamorphosis is the term which refers to the period of changes that take place from juvenile to adult stage, i.e, the final moulting.

The process involves the growth & development of holometabolous insects:-

1> Moulting & metamorphosis are basic phenomena concerned to growth & development of insects. The endocrine mechanism regulates the growth & development of all insects. Three of such endocrine structures that are found in insects are :-

i> neurosecretory cells of brain
ii> ecdysial gland
iii> Corpora allata (c.a)

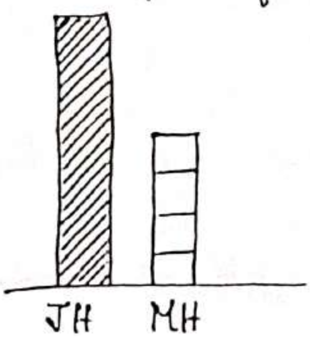
2> The activated NSC of pars intercerebralis (PIC), upon proper stimulation, produce brain hormone, ecdysiotropin which passes to neurohaemal organ - corpora cardiaca by axon transport. In corpora cardiaca, the ecdysiotropin is stored and then from it, ecdysiotropin is liberated into blood.

3) The ecdysiotropin released from C.C stimulates ecdysial gland to synthesise and secrete the very important moult inducing hormone (MH), named as ecdysone. Under influence of MH, moulting process in the insect is initiated.

4) A 3rd hormone, Juvenile hormone (JH) from C.a is released into the haemolymph, then the tissues will not become adult or in other words, JH suppresses pupal differentiation.

5) Hormonal level in blood during development :-

i) A varying concentration of JH & required amount of MH (ecdysone) are required for each moult. When a high concentration of JH is present, prior to each moult, the resulting organism will be immature.



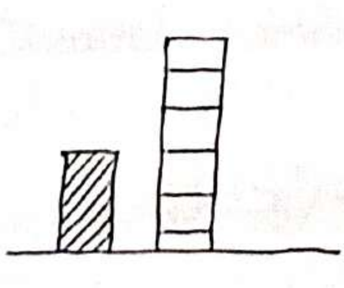
Larval stage

JH >> MH => Larva retaining its position

Larva $\xrightarrow{\text{moult}}$ Larva

Remark :- JH causes status or stability during resting stage of larva, so, JH is called statusquo hormone.

ii) During growth and development or in other words, during moulting process, the above situation is just reversed, i.e. JH is at a minimum concentration, or, completely absent in adult stage.

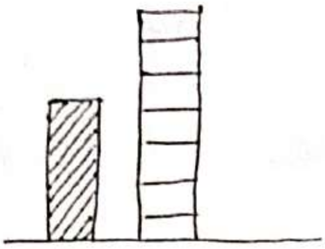


Adult stage

JH << MH => During growth & Development phase in adult stage.

Pupa ⟶ Adult

iii) During larval to pupal moult, MH is at highest peak and JH will not maintain its status, i.e, JH will be present in less amount.



MH >> JH → pupal stage

larva $\xrightarrow{\text{Moult}}$ pupa

Diapause:-

a) Definition:-

The occurrence of an extended interruption in the normal progress of growth and development in insect, is commonly known as diapause, Sometime, it is referred to as a condition of arrested growth either in immature (embryo, larva, pupa) or adult insect.

Diapause, is a condition of reduced metabolism which is reflected through various physiological specialization. In this way, it is set apart from quiescence, which is more temporary or transitary reduction in metabolism which occur in response to actions to adverse environmental factors.

b) Physiological characteristic features associated with diapause are as follow:-

i) Decrease in oxidative metabolism.

ii) Loss of body water.

iii) Responsiveness to external stimuli.

iv) Fat accumulation in the body.

v) An increase in insects ability to withstand environmental stressess with hypertrophy of fat tissue.

c) Environmental stimuli cause the diapause :-

Normally, diapause is due to overwintering condition for insects. The various stages are largely responsieve to environmental signals, eg - photoperiod, temperature, humidity or nutrition. These stimuli influence neural endocrine system, usually the NSC in protocerebrum, which in turn control activities of various endocrine glands, stimulating or inhibiting organs.

d) Example :-

When the developing embryos are placed under a long photoperiod & high temperature, the females produced from these embryos will deposit eggs which will enter into a period of diapause.

Hasegawa (1957) was first to show that the immediate stimulus for development of diapause eggs of B. mori is a neurosecretion, known as, Diapause hormone (DH). DH is synthesised & release from NSCs available in suboesophagial ganglia in the female.

This hormone is released at a suitable time and act upon all eggs, while, they are still in genital tract.

Transplantation of suboesophageal ganglia with active NSCs into pupae which are reared in short photoperiod and low temperature condition causes mature female to lay diapause eggs.

It is proved that the influence of brain over suboesophageal ganglia appears to be that of nervous inhibition, which controls the release of DH (Fukuda, 1962)

e) Hormonal control :-

i) Insects posses a group of light and temperature sensitive secretory cells on hind gut (proctodeum) which under the proper environmental stimuli are stimulated, either directly or indirectly.

ii) The proctodeum releases the hormone, proctodone, in haemolymph.

iii) This secretion then activates NSCs of brain to release ecdysiotropin.

iv) Ecdysiotropin activates the ecdysial gland to produce ecdysone & this terminates diapause.

External Stimuli

↓

Cells in proctodeum

↓ Proctodone

NSC in brain

↓ Ecdysiotropin

Ecdysial gland

↓

Terminalis diapause

Fig. Pattern followed by European Corn borer Larva, Ostrinia nubilalis. in terminating larval diapause

BURSICON:-

1) This hormone is normally produced by brain, sometime, it is produced by abdominal ganglia.

2) Purified extracts of this hormone are stable upto temperature of 55°c but are inactivated by repeated freezing. High salt concentration, extremes of pH, short chain alcohols all inhibit Bursicon activity. Bursicon is inactivated by proteolytic enzyme.

3) Bursicon is a protein hormone of molecular weight of ~ 40,000 and is not species specific.

4) Bursicon is responsible for tanning, darkening and hardening of exoskeleton of newly molted insects.

5) How bursicon acts?

In blowflies, the afferent stimulus originates probably in abdomen, and then travel to brain by ventral nerve cord. There is a trigger in release of Bursicon into haemolymph.

6) Chemical activities :-

Bursicon increases permeability of blood cells to tyrosine and is involved in the hydroxylation of tyrosine to DOPA. Furthermore, Bursicon produces the substract for action of DOPA Carboxylase, and then this enzyme is induced probably by ecdysone. Bursicon influences formation of protein which will react with tanning.)

Role of NH on Reproduction :-

1) Majority of adult insects reproduce in a cyclical manner during its life span. The maturation of gonads in insects is under control of NH. The integrated control of reproduction in insects involves a sequence of events. Stimuli are directed into the CNS, where NSCs are influenced to produce are change in hormonal output of an endocrine organ.

2) The 3 aspects of neuro-endocrine control of reproduction are -

a) Stimuli (extrinsic/intrinsic) that influence brain.
b) The role of brain (NSC) in controlling the C.A by secretion of allatotropic hormone.
c) The mode of action of hormone secreted from C.A (eg-gonadotropin) on target organ or cells (oocytes).

3) Vitellogenesis or synthesis or incorporation of yolk protein in oocytes is an important factor for maturation of egg (oocytes) in female insects and which

is orchestrated through endocrine regulation.

4) These aspects have been studied in a Diptera, Calliphora erythrocephala (blow fly), feeds on animal protein.

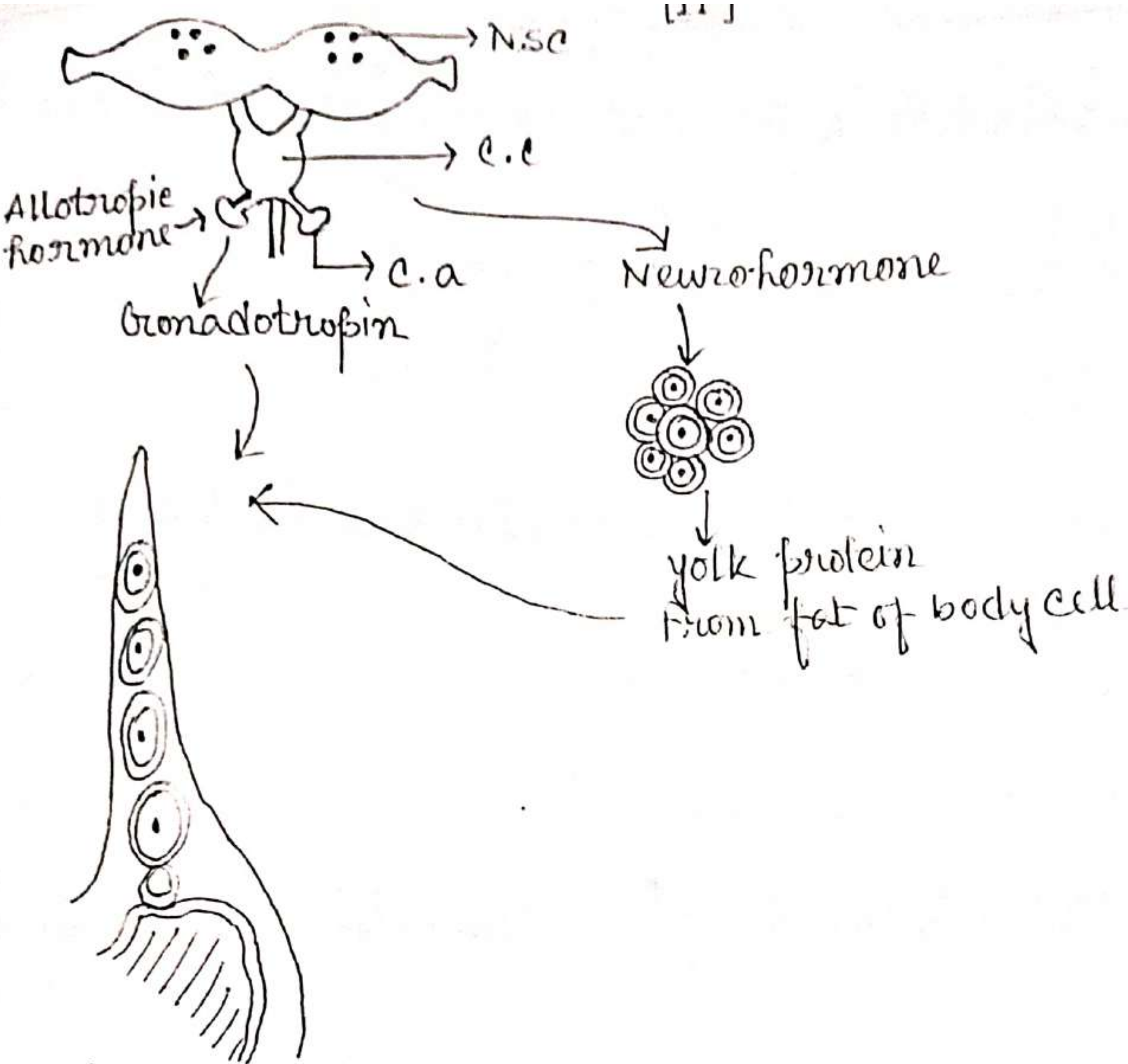
a) Quality & quantity of food (the intrinsic factor) taken by these insects help in synthesis of yolk protein in haemolymph.

b) The dietary protein stimulates the NSC, in these insects, to bring an increase in protease enzyme which digest the protein that appears in haemolymph which directly stimulates c.a to release gonadotropin which then acts on the oocytes in the ovary to incorporate protein yolk.

c) If gonadotropin is not present at right time, due to absence (ablation) of c.a., vitellogenesis won't take place.

d) Implant of C.a from donor into the ablated insect again sets up secretion of gonadotropin & consequently vitellogenesis takes place.

5) In other insects, the site of yolk protein synthesis is fat body cells. The NSCs being stimulated by extrinsic/intrinsic stimuli (eg - environmental stimuli, photoperiod, humidity, food supply etc) secrete the neurohormone. The C.C release this hormone in haemolymph, which acts upon fat body cells, that come down to haemolymph. The C.a being stimulated by allotropic hormone produces gonadotropin in haemolymph that urge upon developing oocyte to incorporate yolk protein.



vitellogenesis

Sequence of Vitellogenesis in <u>Calliphora</u> sp.

protein Diet

↓ stimulation

NSC in brain

↓ NH cause release of

protease in gut

↓ increase of haemolymph protein

Activation of C.a

↓ Gonadotropin

ovary

↓

vitellogenesis takes place

Short Note

Complete Metamorphosis	Incomplete metamorphosis.
1. It occurs in holometabolous insects.	1. It occurs in the hemimetabolous insects.
2. Life cycle stages comprises of Egg, Larva, Pupa & Adult.	2. Life cycle stages comprises of Egg, Nymph, adult.
3. The larval insects show stemmata, a visual organ. Eg: Mosquitoe, Housefly (Diptera), Lepidoptera. etc	3. Stemmata is absent. Eg: Dragonflies (odonata) etc.

Metamorphosis: It is a developmental process by which immature larval insects are transformed into adult insects.

~~Metamorphi~~ Metamorphosis is intimately regulated by Neuro-endocrine regulation. They are:

1) PTTH: (Prothoracicotrophic hormone)

It is secreted from the median neurosecretory cells of brain. It stimulates the prothoracic gland to secrete MH. (moulting hormone).

2) MH : (Moulting Hormone / Ecdysone)

It is secreted from the prothoracic glands under stimulation from PTTH. It has various functions:

i). It enhances the moulting process after the end of each instar. This transformation requires an increase of RNA, proteins, mitochondria & endoplasmic reticulum.

ii). It stimulates Adenyl cyclase function in epidermal tissues, suggesting a role of cAMP in moulting hormone action.

3. JH : (Juvenile hormone). It is secreted from the corpora allata & promotes larval character development & prevents transformation into adult. Both JH & MH (moulting hormone) are secreted during the larval instars. But JH is not secreted or less secreted at the last larval instars so that the insect can transform into pupa & finally into adult.

4. Eclosion : It is secreted from the median neurosecretory cells of the brain. It directly acts on CNS and :

i). helps in shedding of old cuticle after moulting.

ii) helps to increase the plasticity of wing cuticle.

iii). It plays an important role in programmed degeneration of ecdysial muscles.

5. Bursicon : It is secreted from various areas, depending upon the species of insect. It

PTO

helps to promote sclerotisation & tanning of cuticle. It is also known as tanning hormone.

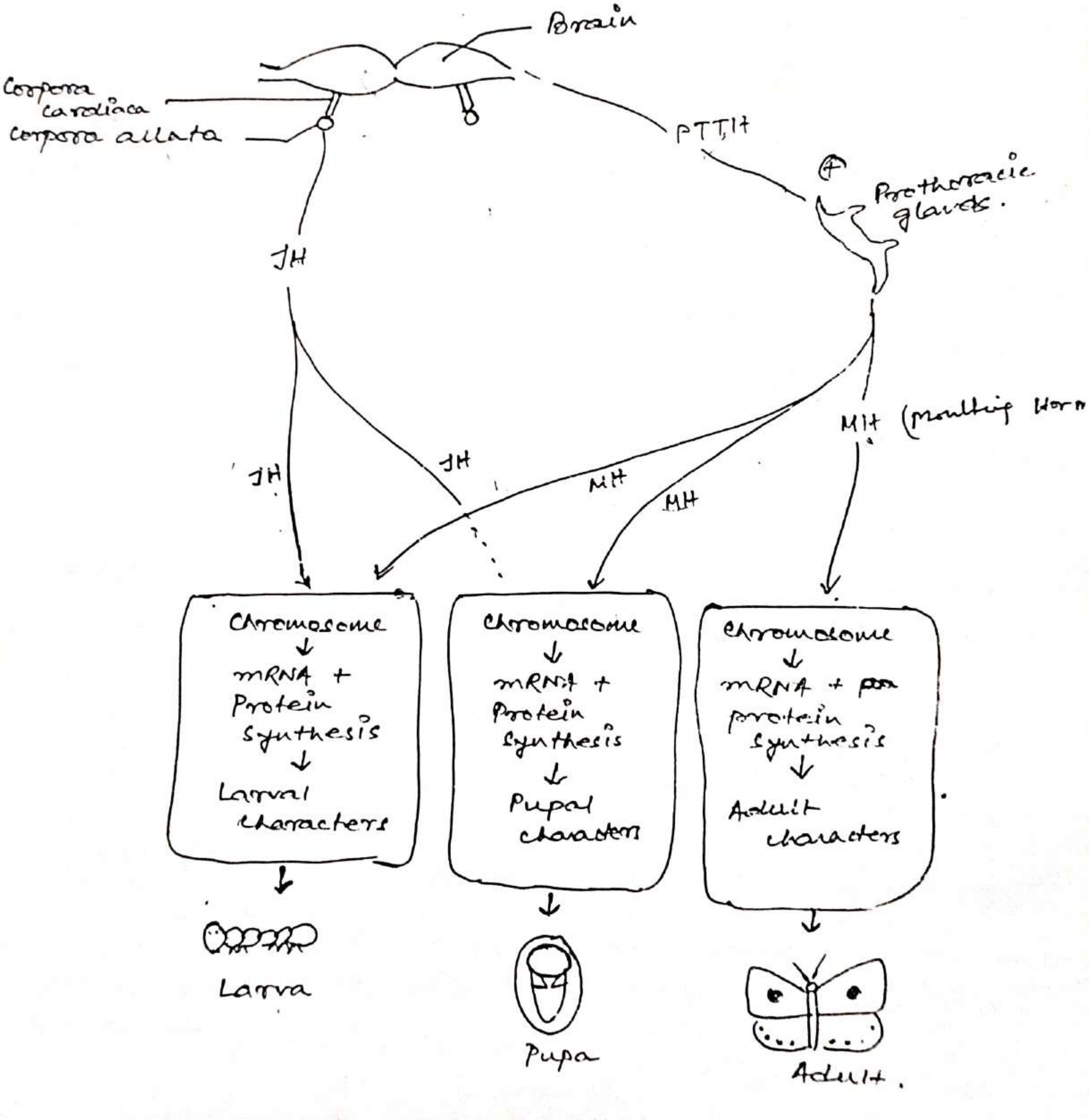


Fig : Neuro endocrine regulation of Metamorphosis.